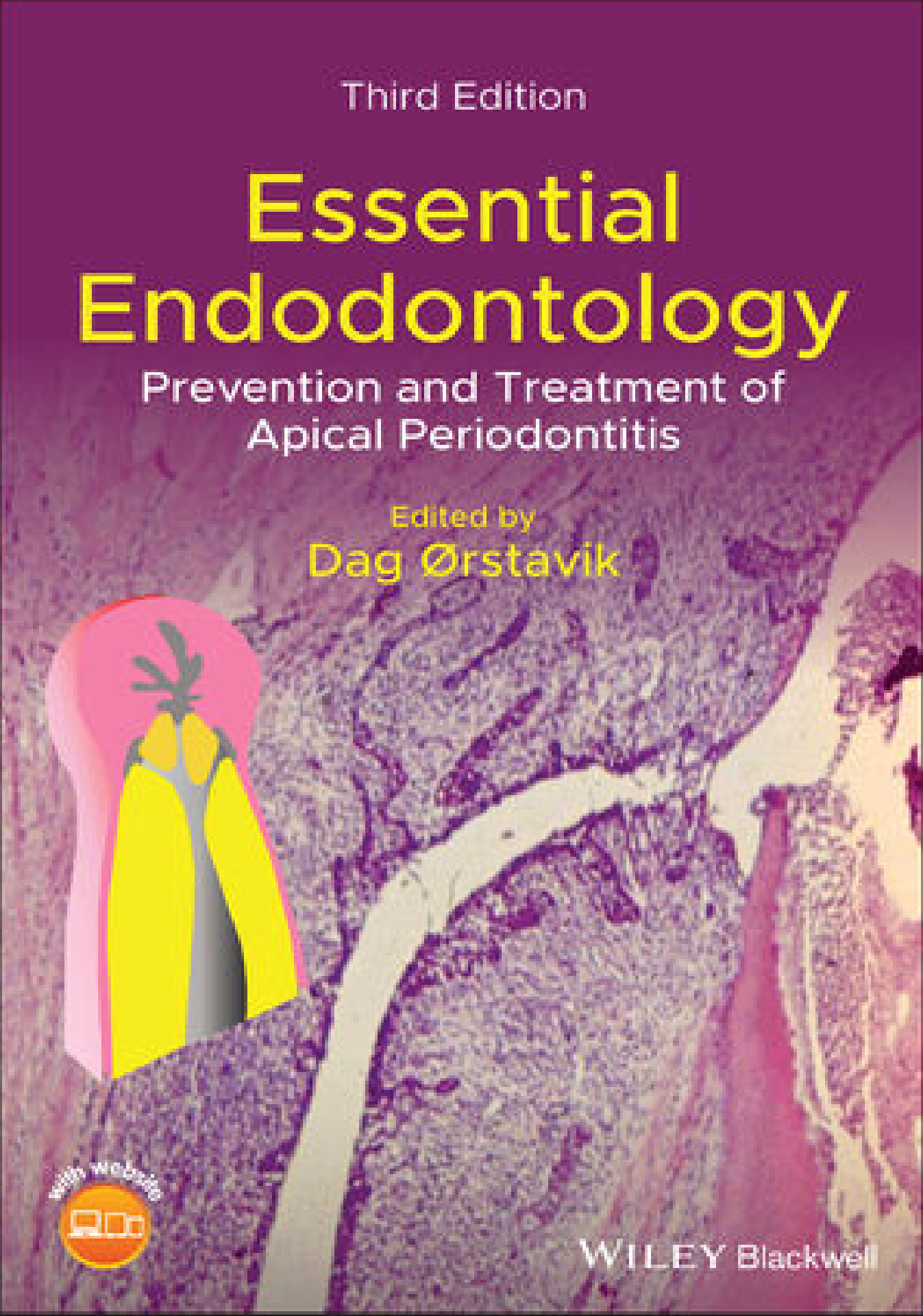
Third Edition

Essential Endodontology

Prevention and Treatment of Apical Periodontitis

Edited by

Dag Ørstavik



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Foreword

Two infections affect the survival of teeth: those of the gingival/periodontal and pulpal/apical periodontal tissues. The pain and loss of function that come with severe forms of either disease may also severely impair the quality of life in affected individuals. Infections of the pulp and periapical tissues belong to the domain of endodontology. While other conditions and diseases form important part of the discipline, treatment of teeth with pulpitis or apical periodontitis by root fillings or apical surgery constitute by far the most important part.

Essential Endodontology seeks to integrate basic, biological, and microbiological knowledge of apical periodontitis with diagnostic and treatment practices. The emphasis of the book remains the same as before. It focuses on the biology and clinical features of endodontology's most important disease in order to promote ever better approaches to its diagnosis, prevention, and therapy.

One might ask if there is still a need for textbooks of this kind. Any student or practitioner can access the most advanced, novel techniques and methods, as well as scientific publications, directly on social media or from public databases. However, one may argue that there is an even greater need for the more advanced, basic text today than before. The figure illustrates the explosion in the number of publications related to endodontics in recent years. In the decade leading up to the second edition in 2008, the number of new endodontic publications was up by 38 per cent from the decade before. In the next 10 years, the increase was 125 per cent, totaling 14,685 publications.

It is clear that the total scientific contributions to the discipline now far outnumbers

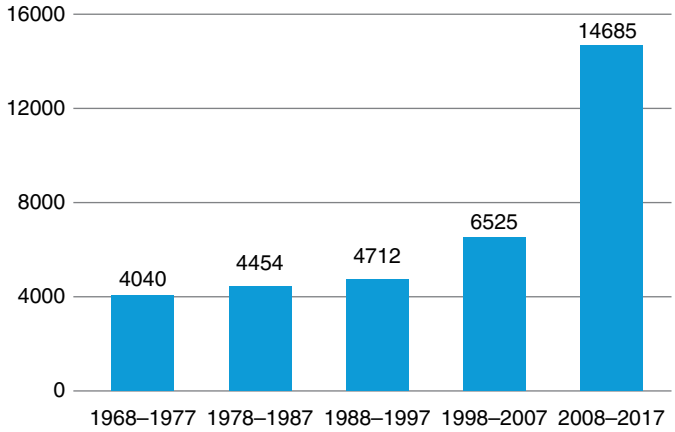

Figure: The number of publications listed in PubMed (US National Library of Medicine, National Institutes of Health) with the search term "endodontics" during the past five decades.

what any researcher, scientist, or clinician can possibly read or absorb. It is also impossible for a novice in the field to navigate in an area where the quality of available information will be highly variable. Thus, a compressed basis of knowledge provided by experts in their fields is essential as a starting point for further studies, and provides a backbone of knowledge and insights. The target audience for the book remains postgraduate students, teachers, and researchers focusing on endodontology. *Essential Endodontology* will also serve as a supplement for undergraduate students of endodontics.

The 2008 edition was hardly a year out in print before the co-editor of both previous editions, Thomas R. Pitt Ford, passed away. His contributions to the previous two editions were a *sine qua non* for their completion, and his professional and personal qualities were sorely missed in the preparation of this third edition. I hope that the reader will find the spirit from the previous editions prevailing in the present, and recognize the focus on quality and depth that was Tom Pitt Ford's hallmark.

Dag Ørstavik

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The website contains downloadable figures from the book.

1

Apical Periodontitis

Microbial Infection and Host Responses

Dag Ørstavik

1.1 Introduction

Endodontology includes pulp and periapical biology and pathology. As a clinical discipline, however, endodontics mainly deals with treatment of the root canal and the placement of a root filling, or treatment by surgical endodontics. The technical procedures associated with treatment focus on the particular problems of asepsis and disinfection of the pulp canal system. Treatment measures to preserve pulp vitality are a shared responsibility with conservative dentistry, and include specific techniques in dental traumatology. Recent research has shown the importance of asepsis and disinfection procedures also for treatment of pulps exposed by caries or trauma, extending classical endodontic treatment principles to the management of deep caries (see Chapter 9).

For vital teeth requiring partial or full pulp removal, the initial diagnoses and the difficulties associated with treatment may be related to the state of the pulp, but the purpose of treatment is no longer the preservation of the pulp but the prevention and/or elimination of infection in the root canal system. The ultimate biological aim of this treatment is to *prevent apical periodontitis*. For teeth with infected/necrotic pulpal with an established apical disease process, the biological aim is to *cure apical periodontitis*. Of the endodontic diseases, apical periodontitis is therefore prominent as it is a primary indication for root canal treatment and because it is by far the most common sequel when treatment is inadequate or fails (Figure 1.1). Even the measures taken to preserve pulp vitality may be viewed as ultimately preventing root canal infection and the development of apical periodontitis.

The importance of microbes in the initiation, development and persistence of apical periodontitis has been thoroughly documented (see Chapter 4). The emphasis in this book is on the infectious etiology of apical periodontitis and on the aseptic and antiseptic principles applied during treatment. Furthermore, new research findings have impact on aspects of diagnosis, treatment, prognosis and evaluation of outcome in endodontics. It is therefore important to use the acquired knowledge to build treatment principles logically, and to show how all these fundamental aspects can be applied in clinical practice.

1.2 Terminology

Both pulp and pulp-periodontal diseases have been subject to many classification systems with variable terminology. Periodontitis caused by infection of the pulp canal system has been termed apical periodontitis, apical granuloma/cyst, periapical osteitis and

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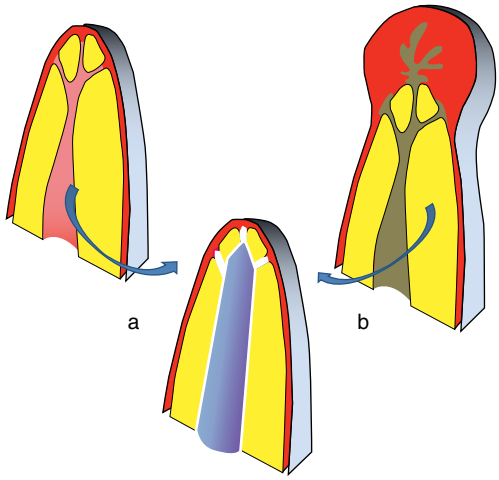

Figure 1.1 Pulp extirpation (a) prevents and root canal disinfection (b) cures apical periodontitis. Both need a root filling of the entire pulpal space.

Table 1.1 Classification of apical periodontitis [18].

AAE	ICD-10
Symptomatic apical periodontitis SAP[1]	K04.4 Acute apical periodontitis of pulpal origin[2]
Asymptomatic apical periodontitis AAP	K04.5 Chronic apical periodontitis
Chronic apical abscess	K04.6 Periapical abscess with sinus[3]
Acute apical abscess	K04.7 Periapical abscess without sinus
Condensing osteitis[4]	
Radicular cyst	K04.8 Radicular cyst

[1] presents with a broad range of symptoms
[2] presents with strong pain
[3] further subdivided in relation to sinus tract location on surfaces
[4] may be seen as a variant of AAP or Chronic apical periodontitis

periradicular periodontitis, among other terms. Sub-classifications have been acute/chronic, exacerbating/Phoenix abscess and symptomatic/asymptomatic, among others [18]. The two most accepted classification schemes are presented in Table 1.1. These are quite similar, but symptomatic teeth according to the AAE classification may include more cases than teeth with acute apical periodontitis according to the ICD. The latter term is for cases presenting with subjective needs for immediate treatment, while symptomatic teeth may include teeth that only slightly affect the patients and that are diagnosed by chairside testing (see Chapter 7). The term "chronic" is useful for prognostication and follow-up studies: symptomatic or not, it implies the presence of a radiolucent lesion, which is a major predictor for treatment success [24]. The term "symptomatic" confirms that there are objective signs verifying the diagnosis.

Apical periodontitis includes dental abscess, granuloma and radicular cyst as manifestations of the same basic disease. The balance between the virulence and extent of infection on the one hand and the body's response on the other, determines whether the condition is symptomatic/acute versus asymptomatic/chronic. The historical emphasis on the differential diagnosis of a cyst versus a granuloma has been abandoned. This is due to the fact that radiographs, even from CBCT, are not very sensitive in discriminating between cysts and granulomas [6]; they share the same etiology and basic disease processes (Chapters 3 and 4); and their treatment and prognosis are also similar (Chapters 11 and 12). However, so-called true cysts separated from the root canal infection that initiated them may show impaired healing [27] and require surgical removal, but there are no means for diagnosing such cases without scrupulous histological investigation of surgical biopsies [32].

Terminology should not be considered a straitjacket for authors or clinicians. Therefore, variants of the terms and references to other diagnostic schemes, in this book and other texts, are inevitable, and can even be desirable. However, given that insurance companies and other third parties require codes or terms for reimbursements, and legal issues dictate clear basic diagnoses as basis for treatment, selection and proper usage of a recognized classification scheme is mandatory.

1.3 Pulp Infection and Periapical Inflammation

The oral cavity is an extension of the skin/mucosal barrier to the external environment. In the digestive tract, it may be viewed as the first battleground for the body's efforts to maintain homeostasis and keep infection away from the vulnerable interior parts of the body. Infection occurs when pathogenic or opportunistic microorganisms infiltrate or penetrate the body surface. In the oral/dental sphere, the body surface is either the mucosa or the enamel/dentine coverage of underlying soft tissue. Endodontic treatment aims to re-establish the muco-cutaneo-odonto-barrier with a complete seal from the coronal to the apical end of the treated root, whereas voids or leaks in the restoration may present an opportunity for bacteria to establish themselves close and eventually ingress into the body's interior. The emphasis on coronal as much as apical leakage of bacteria and bacterial products reflects this line of reasoning.

The evolution of permanent teeth in a dentition with multiple functions is integral to the evolution of animals [40], not least primates and man. However, the structure of these teeth is such that if fracture occurs, microorganisms may enter the body and establish a foothold in the exposed dentinal and pulpal tissues. Unless protective mechanisms were developed, such infections would be life-threatening and presented a strong survival disadvantage in the young [40]. Employing and modifying general mechanisms of inflammation, apical periodontitis evolved to combat and contain the infections in the compromised dental pulp spreading through its ramifications and the tubules of dentin (Figure 1.2). While defining the disease, apical periodontitis works therefore to our advantage; it is the underlying infection that is the cause for concern.

The protection by tissue responses comes at a cost, however. Clinical symptoms that accompany the inflammation may be distressing to the patient, and the granuloma or cyst are not always effective in containing the invading microbes. The pain sometimes following the inflammation of the pulp and periapical tissues can be excruciating and is a testimony to the potential danger of the infection. This pain is also the starting point for human attempts to combat dental disease. Thus, acute pulpal and periapical inflammation were the among the first targets of the dental profession.

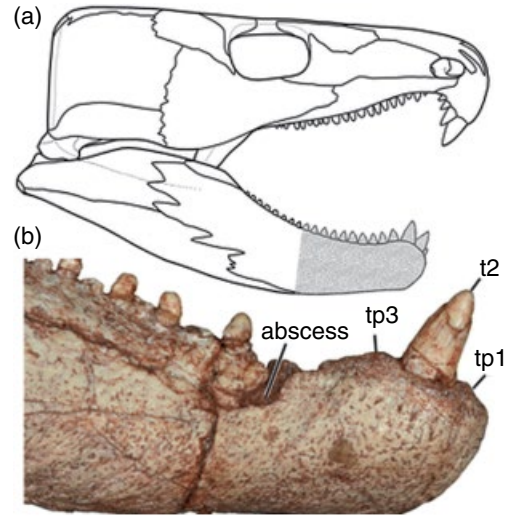

Figure 1.2 Evidence of dental and mandibular pathology in Labidosaurus hamatus, a basal reptile from the Lower Permian of Oklahoma. (a) Skull reconstruction in right lateral view. (b) right hemi-mandible in lateral view. Reproduced with permission from [40].

Teeth, cheek cells, tongue crypts, tonsillar irregularities, gingival sulci and other anatomical structures are safe havens for microbial populations of the mouth. From these areas, microbes of varying virulence may emigrate and cause infections such as tonsillitis, gingivitis, pericoronitis, marginal periodontitis, dental caries, pulpitis and apical periodontitis. Whereas physiological and mechanical cleansing activities tend to reduce the level of microorganisms in the mouth, environmental factors sometimes favor infection rather than its prevention. Current research on oral microbial communities emphasizes the

concept of biofilm formation and development, with particular physiological, genetic and pathogenic properties of the organisms expressed as consequences of the conditions within the biofilm (see Chapter 4).

Caries has been the dominant dental infection for decades, and infection and inflammation of the pulp and periapical tissues are often an extension of the dental caries process. Researchers studied the occurrence and epidemiology of apical periodontitis as part of caries investigations. The infectious nature and the possibilities for spreading and complications of apical periodontitis should form the basis for independent surveys of public health consequences of endodontic diseases.

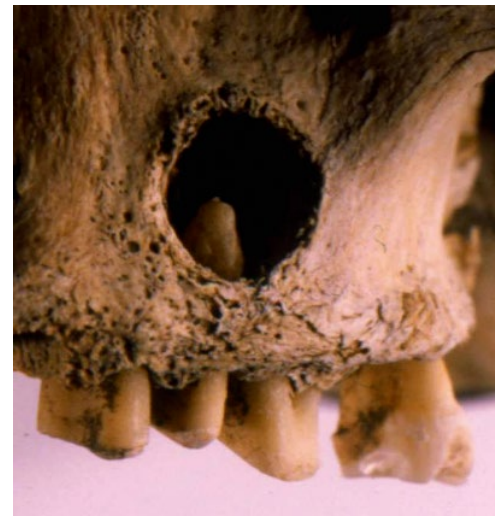

Figure 1.3 Apical periodontitis in an upper premolar of a woman's skull found in Iceland and dating to the 12th century. Trauma or wear caused exposure of the pulp with infection and lesion development.

1.4 Biological and Clinical Significance of Apical Periodontitis

1.4.1 Apical Periodontitis as an Infection

Root canal infections and apical periodontitis is common today and is a frequent finding in skulls from archaeological investigations (Figure 1.3). In the pre-antibiotic era, infections of the pulp and periapical tissues were potentially serious and needed close monitoring. In the early days of antibiotics, it was found that most of these infections were readily susceptible to penicillin, and therefore the spread of infection to regional spaces was often controllable by antibiotics. Today, it is recognized that pulp infection may be caused by organisms of different virulence (see Chapter 4), and that control of the infection is not always easily accomplished.

The flora of the mouth fortunately has relatively few pathogenic organisms, which usually have low virulence. Most are opportunistic, causing disease only in mixed infections or in hosts compromised by other diseases. However, organisms that are not normally pathogenic in the oral cavity may exhibit features of virulence if allowed access to the pulp or periapical tissues. Studies of the infected pulp have shown the presence of oral bacteria that normally inhabit the mouth, which do not normally cause disease. The apical periodontitis response to pulp infection may be viewed as a way of taming and coping with expressions of virulence by the infecting organisms. Thus, the pain frequently encountered in the early stages of disease development usually subsides in response to the tissue reactions. Furthermore, the initial expansion of the lesion of apical periodontitis is soon followed by periods of quiescence, possibly even regression or at least consolidation of the lesion. This dynamic process is accompanied in time by changes in the composition of the flora recoverable from the root canal.

Some forms of apical periodontitis have been associated with particular species dominating in the pulp canal flora. However, evidence from molecular analysis implies that endodontic infections may be more opportunistic than specific, and include many more species than previously thought (Chapter 4). Research into microbiological causes and interactions in apical periodontitis are imperative for improvements in diagnosis

and treatment. Particularly, this would apply to the so-called "therapy-resistant" cases of apical periodontitis, in which infection persists despite apparently adequate root canal treatment, and to retreatment cases. Modern microbiological techniques have demonstrated an almost endless complexity and variability of the endodontic infections [19, 53], opening new avenues for research and expanding our understanding of the disease.

1.4.2 Infection Control

The outcome of endodontic treatment is dependent on use of an aseptic technique and antiseptic measures to prevent and/or eliminate infection. However, the critical role of infection control may not always be given the prominence it deserves. The transmission of hepatitis viruses has been an issue for a long time, and there is concern about prion transmission via contaminated instruments. The sterilization procedures for contaminated endodontic instruments have limitations, so there is a strong tendency towards applying single-use instruments. Most contemporary, machine-operated instruments are designed for single use, a practice that benefits the local treatment and prevents cross-infections.

1.4.3 Microbial Specificity and Host Defense

The host responses to root canal infection have been the subject of much research. There is great similarity between the pathogenic processes in marginal and apical periodontitis, many of the findings in periodontal research have direct relevance to apical periodontitis. Our understanding of the immunological processes involved in the development of apical periodontitis is expanding (Chapter 3). Microbiological variability and virulence factors in infected root canals have been demonstrated, and the bacterial flora may vary with the clinical condition of the tooth involved (persistent infection, therapy-resistant infection) (Chapters 4 and 11). Thus, different strategies of antimicrobial measures may be possible and even desirable depending on the microbiological diagnosis in a given case.

Reports of apical periodontitis with particularly aggressive microbes are fortunately very rare. Root canal infections with bacteria causing necrotizing fasciitis have been reported with very serious, even life-threatening consequences [48], and bacteria with resistance to common antibiotics may pose a problem, particularly in patients with impaired immune system [5]. However, it is important to remember that incomplete and inadequate root canal treatment can lead to infections requiring hospitalization and extensive medical treatment [17].

1.4.4 Endodontic Infection and General Health

The focal infection theory has been a source of both frustration and inspiration in dental practice and research. Both irrelevant and sometimes incorrect arguments and concepts were used to dictate an unnecessary wave of tooth extractions in healthy individuals for decades. Unsubstantiated opinions on the subject restricted clinical developments in the field of endodontics for a very long time. The controversy, however, has also sparked important new discoveries, and it is, even today, an important part of the frame of reference in studies of endodontic microbiology and host defense mechanisms.

1.4.4.1 Influence of General Health on Apical Periodontitis

Apical periodontitis and other disease processes may mutually affect each other. The root canal infection meets a response that is defined by the host's condition and dependent on genetic and constitutional factors, including systemic diseases. This variable tissue response may limit or allow expansion of the apical lesion, and it may promote or impair healing responses during and after treatment of the infection.

Diabetes is the classical example: it causes a general, reduced defense against infections and diabetic patients may have more and larger lesions [41, 45].

Smoking has a general, adverse effect on infection defenses and affects marginal periodontitis and wound healing negatively; it may also affect the incidence and healing rate of apical periodontitis [25, 36], but the effect may be weak or questionable [42], and confounding factors (age, marginal periodontitis) make conclusions about its effect difficult [25].

There is speculation that infection by the *varicella zoster* virus may be causally associated with root resorption and development of apical periodontitis [35, 47], but the evidence is very limited and inconclusive [22]. Similarly, other viral infections have been implicated in the pathogenesis of otherwise bacterially initiated apical periodontitis [20, 21, 26, 29]

Sickle cell anemia may cause pulp necrosis, preferentially in the mandible, apparently in the absence of microbial infection [12]. Subsequent infection causes classic apical periodontitis. The mechanisms for this increased susceptibility are poorly understood, but patients express high levels of genes for inflammatory cytokines [13]

Systemic medicaments influencing the immune and host response status in patients will influence the biological processes associated with apical periodontitis as well [9]. Moreover, as study designs and research methodologies become more sophisticated, dental diseases are found to be linked with diseases in other locations. Patients with inflammatory bowel disease have a higher prevalence of apical periodontitis and their lesions are larger [37], as they are in diabetic patients with poor glycemic control. Immunosuppressive medicaments generally, or the diseases for which they are used, may not influence healing after endodontic treatment significantly [2, 33]. Other medicaments may favor healing; one study found that statin intake improved the incidence of healing after endodontic therapy [1].

There is a well-establish relationship of marginal periodontitis and preeclampsia and preterm births [34], which is traditionally linked with a raised level of inflammatory blood markers [10, 50]. Similarly, preeclampsia occurs more frequently in the presence of apical periodontitis [23].

Antimicrobial and pain-relieving medicaments have their place in treatment of apical periodontitis. However, when applied for other indications, they may mask symptoms [38], possibly impair body defenses [52] and the microbial population may develop resistance to the antibiotics.

Patients treated with immunosuppressants or who otherwise have compromised immune systems need special consideration. A number of the blood dyscrasias, notably leukemias, are associated with potentially serious sequels to apical periodontitis: infection spreads easily and may require extensive antimicrobial therapy. *The irradiated patient* is a special case: the incidence of osteoradionecrosis [8] after oral surgical procedures places high demands on effective, conservative treatment of endodontic conditions. Similarly, patients on bisphosphonate therapy by intravenous injections may be at risk for tissue necrosis after surgical endodontics [31]. Case reports of complications from endodontic therapy in patients with reduced resistance [5, 46] point to the importance of meticulous and complete endodontic treatment in such patients.

1.4.4.2 Apical Periodontitis Affecting Other Tissues and Organs

Distant and systemic consequences of apical periodontitis is the other side of the coin.

Sinusitis may be induced by root canal infections of maxillary molars or, in very few instances, second premolars [49]. In the lower jaw, inflammation may cause paresthesia of the mandibular or mental nerve. These complications normally subside after successful treatment of the affected tooth [51].

Generally, any disease for which bacteremia poses an additional hazard is of concern in endodontics. Particularly, a history of infective endocarditis, congenital heart disease, rheumatic heart fever or the presence of an artificial heart valve or other susceptible vascular implants may necessitate the

implementation of an antibiotic regimen in conjunction with the endodontic procedures. The magnitude of risk for cardiovascular complications due to bacteremia of dental origin is low and the need to controlling minor and chronic oral infections, including apical periodontitis [39], may be questioned [11]. The formalization of guidelines for antibiotic prophylactic needs by physicians and dentists to ensure safety for patients at risk has made decision making easier [28].

Atherosclerosis is central to the development of cardiovascular disease (CVD) [14]. The arterial plaques may be sites of colonization by microbes circulating during transient bacteremia, and oral infections may thus be a risk factor for CVD [44]. Specifically, apical periodontitis has been associated with an increased incidence of cardiovascular disease events [3, 4, 15, 16, 43]. Research into this association is complicated by a lack of strict criteria for assessing the nature of the periapical infection; radiographic observations give only the status at the time it is taken and cannot discriminate between ongoing infection and a healing lesion. Root-filled teeth, with or without a lesion, may represent a history of pulpitis or apical periodontitis. Pooling all root filled teeth with untreated teeth with apical periodontitis in an individual has been used as a measure of an "endodontic burden"; this is independently associated with CVD [16]. However, viewed isolated from other factors, root-filled teeth are associated with reduced incidence of cardiovascular disease [30]. The chain of events that may give apical periodontitis a role in CVD development is purely conjectural at this stage. However, the blood levels of several cytokines and other compounds associated with CVD are elevated in patients with apical periodontitis [3].

1.4.5 Tooth Loss and Replacement

Untreated apical periodontitis represents a chronic infection of the oral tissues at locations close to many important tissues. While these infections may remain quiescent for decades, they may also develop and spread with serious consequences for the individual. In the face of the risks of such chronic infection from involved teeth, their extraction and replacement by implants has been put forward and discussed as a viable alternative to endodontic treatment. The variable success rates (by strict criteria) of treatment procedures for the cure of apical periodontitis (Chapter 5) are sometimes used as an argument in favor of implants. However, what little evidence is available does not indicate a lower survival rate of endodontically treated teeth [7], and the superiority of tooth preservation compared to its replacement should be stated as a biological principle of preference. The challenge from other treatment options to endodontics as a discipline should act as a driving force to produce more scientifically solid evidence for the modalities of cure and prevention applied to our disease of interest, namely apical periodontitis.

1.5 Concluding Remarks

Pulp and periapical inflammation, the associated pain and the consequences of root canal infection remain significant aspects of dentistry today. New knowledge and insights provide better treatment opportunities and stimulate further research activities. The prevention and control of apical periodontitis has a solid scientific base, but the many variations in the clinical manifestations of the disease still leave technical and biological problems that need to be solved. Technological advances in treatment have made possible effective treatment of teeth that were previously considered untreatable, and further developments in microbiology, host biology and image technology are certain to improve the scientific foundation of endodontology in the near future.

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2

Dentin-Pulp and Periodontal Anatomy and Physiology

Leo Tjäderhane and Susanna Paju

2.1 Introduction

Although dentin and pulp are fundamentally different in that dentin is a mineralized tissue and the pulp is a soft tissue, they are developmentally interdependent and remain anatomically and functionally closely integrated throughout the life of the tooth. Thus, the two tissues are often referred to as the dentin-pulp complex.

Dentin and pulp develop from embryonic connective ectomesenchymal cells from the cranial neural crest during the bell stage of the tooth development (Figure 2.1). The inner dental epithelium of the "bell" encases the condensed mesenchyme. The epithelial-ectomesenchymal interactions initiate the differentiation of the first odontoblasts at the periphery of the dental papilla, and the rest of the mesenchyme will form into future pulp. The differentiating odontoblasts start the secretion of dentin proteins and initiate enamel matrix secretion by ameloblasts [138].

When root formation initiates after crown morphogenesis, Hertwig's epithelial root sheath (HERS) develops from the epithelium at the cuff of the enamel organ. When the HERS grows apically, the adjacent dental papilla cells differentiate into odontoblasts to form root dentin. HERS is critical for root dentin formation: if HERS is disrupted, the dental papilla cells fail to differentiate. On the other hand, cross talk between differentiating odontoblasts and HERS is also necessary for appropriate root formation. The HERS fragmentation allows dental follicle cells to contact the root dentin surface and to differentiate into cementoblasts to form cementum. Also, some of the HERS cells may undergo transition to become cementoblasts. Dental follicle cells secrete collagen fibers that are embedded into the cementum matrix and form the periodontal ligament. Parts of HERS remain in the pulp and in the periodontal connective tissue (Figure 2.2) as epithelial cell rests of Malassez [88, 112, 221]. The formation of lateral root canals and an apical delta of accessory canals rather than a single apical foramen may be a normal variant or it may be due to disturbances of HERS.

The soft tissue of the dental pulp communicates directly with the periodontal ligament (PDL) through the apical foramen or foramina. Sometimes the apical area consists of a delta of accessory canals with several communications between the pulp and the PDL. The rest of the PDL is separated from the pulp-dentin organ by the cementum. Periodontal ligament fibers are embedded in the cementum and alveolar bone as Sharpey's fibers, and attach the teeth to the alveolar bone (Figure 2.3).

2.2 Dentin

Dentin is the largest structural component of human tooth. Dentin provides support to enamel, preventing enamel fractures during

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

Companion website: www.wiley.com/go/orstavik/essentialendodontology

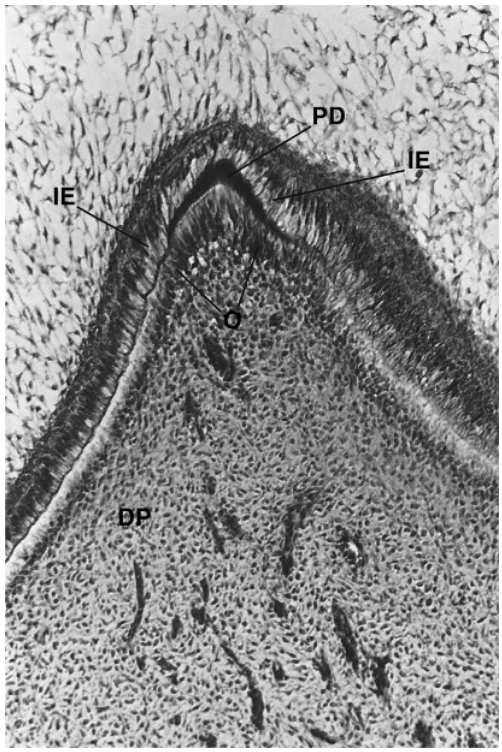


Figure 2.1 Initiation of dentinogenesis at the bell stage of tooth development (DP, dental papilla; O, odontoblasts; PD, predentin; IE, inner dental epithelium).

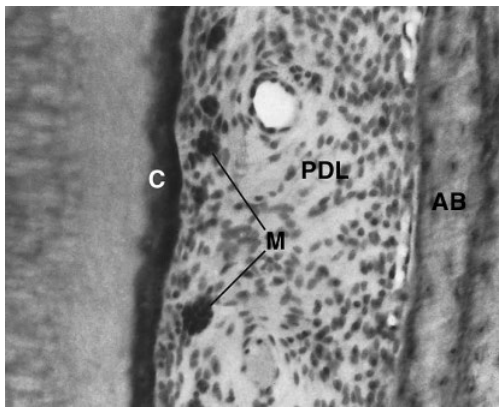


Figure 2.2 Section through the periodontal ligament (PDL) showing Malassez's epithelial rests (M) adjacent to cementum (C) (AB, alveolar bone).

occlusal loading. It also protects the pulp from potentially harmful stimuli and participates in the overall protection of the continuum of the hard and soft tissue often referred as the dentin-pulp complex. Dentin in different locations of a tooth may qualitatively differ from each other, which enables it to meet the requirements in that specific location.

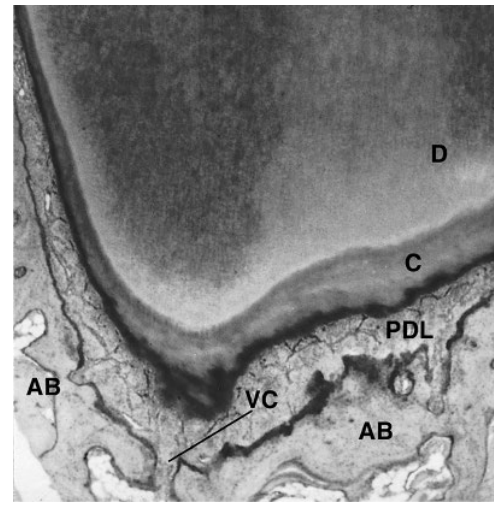


Figure 2.3 The apical portion of a tooth showing alveolar bone (AB), periodontal ligament (PDL), cementum (C), dentin (D), Volkmann's canal (VC).

Dentin is mineralized connective tissue, nanocrystalline-reinforced collagen biocomposite, with unique properties that provide teeth with mechanical strength under heavy occlusal forces. About 70 w-% (55 vol-%) is minerals and 20 w-% (30 vol-%) organic components, the rest being water. However, since the structure of dentin varies within a tooth, these values are only average [208]. About 90% of dentinal organic matrix is highly cross-linked type I collagen, the rest being non-collagenous proteins such as proteoglycans and other proteins, growth factors and enzymes, and small amount of lipids. The mineral is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), but contains impurities (CO_3, Mg, Na, K, Cl) and fluoride, and should thus be called biological apatite [208].

The major part of dentin is intertubular, formed by the dentin-forming odontoblasts at the dentin-pulp border. Almost the entire dentin organic component is located in intertubular dentin. While forming dentin, odontoblasts leave behind dentinal tubules, in which peritubular dentin is later formed.

Peritubular dentin formation leads to a slow occlusion of tubules. Since peritubular dentin is highly mineralized, the mineral-organic matrix ratio increases from the dentin-pup border towards the dentin-enamel junction, and with age [208, 209].

2.2.1 Dentin Formation

2.2.1.1 Odontoblasts, Predentin and Mineralization Front

Odontoblasts are the outermost cells of the pulp, separated from the rest of the pulp tissue (pulp proper) by a cell-poor layer of Weil. During and immediately after the differentiation the odontoblasts organize into a distinguished odontoblast cell layer, and the mineralization of organic matrix completes the mantle dentin formation [138] (Figure 2.4). In the coronal part of the tooth, the morphological features and cell membrane polarization are unique among collagen-synthesizing cells [39, 205]. Odontoblasts are terminally differentiated post-mitotic cells, meaning that they have withdrawn from the cell cycle and cannot be replaced by cell division [39, 209]. Coronal odontoblasts are highly polarized both morphologically [39] and by cell membrane polarity [205] and organized in a pseudostratified palisade, while in root they form a single cell layer [39]. The cell body is located on a pulpal wall of dentin and odontoblast processes inserted into dentinal tubules (Figure 2.5). The cell body is 20–40 µm tall, depending on dentinogenic activity, and contains a large nucleus at the basal portion of the cell, Golgi apparatus, rough endoplastic reticulum, several mitochondria and other intracellular structures [39]. Adjacent odontoblasts are attached with extensive tight junctions forming a stable barrier between cell bodies but may be disrupted e.g. as a response to trauma or caries [23, 40, 209]. The cytoplasmic odontoblast process penetrates into mineralized dentin tubules. It has a 0.5–1 µm main trunk and thinner lateral branches through which the processes may be connected with each other [27, 39, 209] (Figure 2.6). The odontoblast processes are suggested to detect the integrity of the region, acting as a receptor field. Any stimulation is transmitted to the cell body, inducing responses that aim to maintain the tooth integrity. At the same time, the processes withdraw, leaving the tubules empty [127], which in ground section is seen as so-called dead tracts. The extent of odontoblast processes into dentinal tubules is still a matter of debate due to the conflicting results obtained with different research methods and by the possible species differences. In rat molars, odontoblast processes extend all the way to the DEJ [127]. In human teeth, most studies indicate that the odontoblast cell processes would not extend far from the dentin-pulp border (200–700 µm) [27, 209].

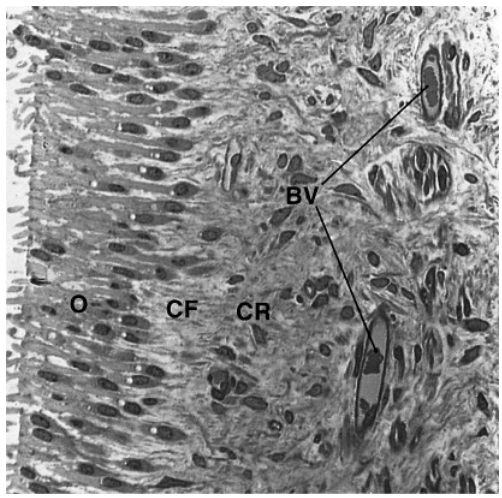


Figure 2.4 Tall, columnar odontoblasts (O), the relatively cell-free zone (CF) and the relatively cell-rich zone (CR) in the dental pulp ((BV), blood vessels).

The 10–30 µm layer of unmineralized predentin is located between odontoblasts and mineralized dentin (Figure 2.5). This is where the dentin organic matrix is organized [14] before the controlled mineralization at the mineralization front to form intertubular dentin. The backbone of the organic matrix is type I collagen, whereas non-collagenous proteins – glycoproteins, proteoglycans and enzymes – control the matrix maturation and mineralization (Figure 2.5). The mineralization

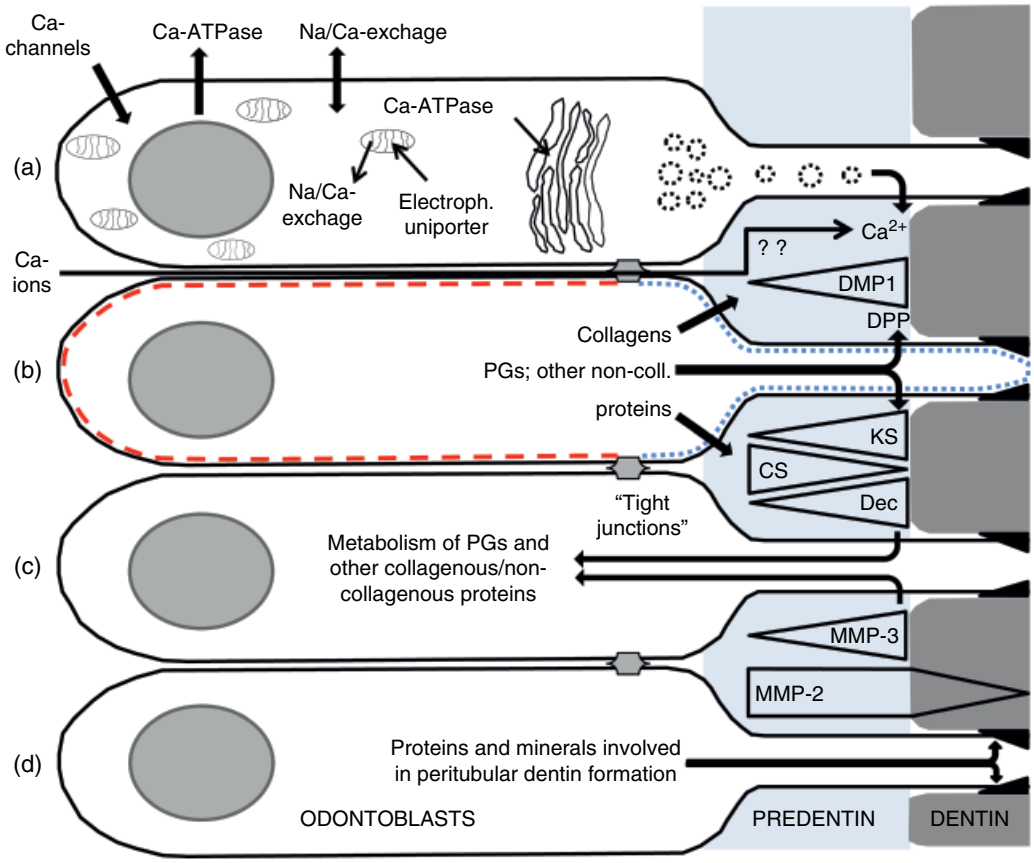


Figure 2.5 Schematic description of odontoblast function in dentin formation. Factors involved in dentin matrix formation, maturation, and mineralization. (a) Ca^{2+} ion transport and handling in the odontoblasts to provide calcium for mineralization and to maintain physiological cytosolic Ca^{2+} concentration. Intravesicular Ca-ATPase-dependent accumulation of Ca^{2+} is needed for the controlled transport in the mineralization front. Some calcium may also enter via an intercellular route despite the presence of tight junctions at least in cases of odontoblast cell layer integrity disruption (arrow with question marks). (b) Dentin organic matrix components are processed by odontoblasts and secreted into predentin at precise locations (e.g. dentin phosphoprotein, DPP, directly at or close to the mineralization front). Differential presence of Dentin matrix protein-1 (DMP1), proteoglycans (PGs, e.g. decorin [Dec]) or their side-chains (keratin sulfate [KS], chondroitin sulfate [CS]) indicate enzymatic modifications of the proteins in the predentin for controlled mineralization. Enzymes such as matrix metalloproteinases (e.g. MMP-2 and -3) participate in protein processing during predentin maturation. Cell membrane is highly polarized into basolateral process (blue dotted line) and apical cell body membrane (red dashed line), divided by tight junctions [205]. (c) Odontoblasts also have a transport system that excludes the unwanted proteins and degradation products from predentin. (d) Odontoblasts are also responsible for peritubular dentin formation. Modified from [209].

of dentinal collagen happens via proteoglycan-collagen interaction in the collagen gap zone (intrafibrillar mineralization) [43, 209]. Interestingly, matrix vesicles that are responsible for immature bone and calcifying cartilage mineralization are involved in mantle dentin and reparative dentin but not in primary or secondary dentin mineralization [193]. The mineralization front is often considered to be linear, but actually mineralized globular protrusions called calcospherites are common [209, 213].

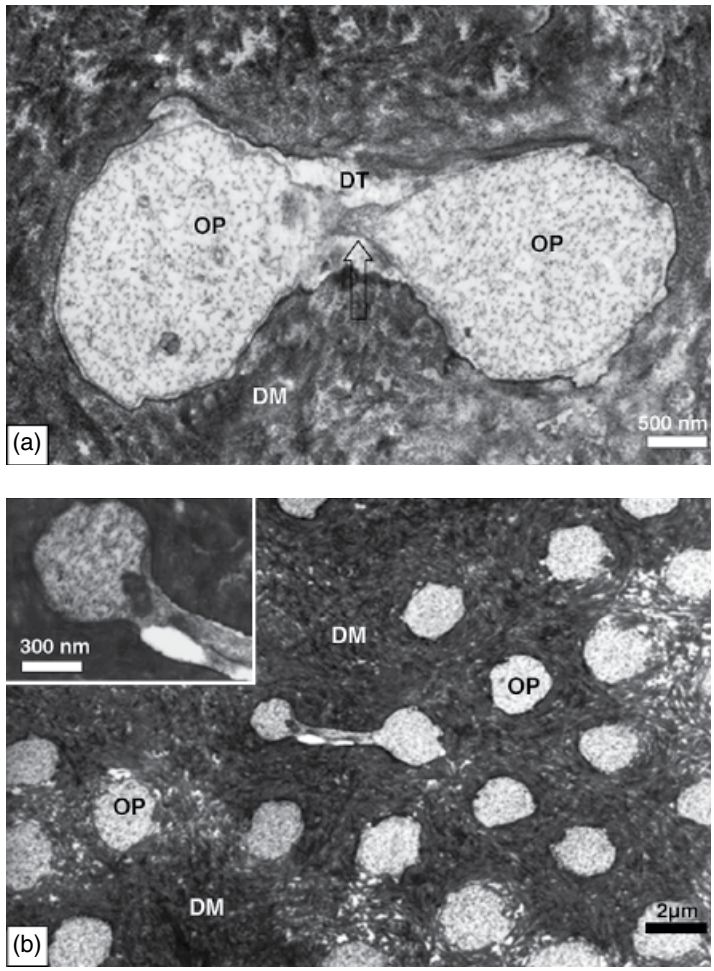


Figure 2.6 Odontoblasts processes (OP) with presence of intercellular connections (empty arrow) filling the the dentinal tubules enveloped by intertubular dentinal matrix (DM) (original magnification (a) 10,000×; (b) 2500×; inset 20,000×). (Reproduced from [27] with permission from *Tissue and Cell*.)

2.2.2 Dentin Structure

Dentin can also be divided according to the time of formation into: dentin-enamel junction (DEJ); mantle dentin; primary and secondary dentin; and tertiary dentin, which is further divided into reactionary or reparative dentin, according to the structure and the cells responsible for formation.

2.2.2.1 DEJ and Mantle Dentin

In humans, DEJ is a 7–15 µm wide wavy, scalloped structure [59, 131, 169, 213] that is different from both enamel and dentin [59]. The scalloped form of the interface is believed to improve the mechanical attachment of enamel to dentin. Mantle dentin is 5–30 µm thick layer of the outermost dentin. The matrix is formed during and immediately after the odontoblast terminal differentiation, contains organic remnants of dental papilla, and the mechanisms of mineralization is different from that at the mineralization front [193, 209]. Instead of large tubules, small ramifications of each tubule are present in mantle dentin (Figure 2.7). Unlike the rest of dentin, mantle dentin contains type III collagen (so-called von Korff fibers).

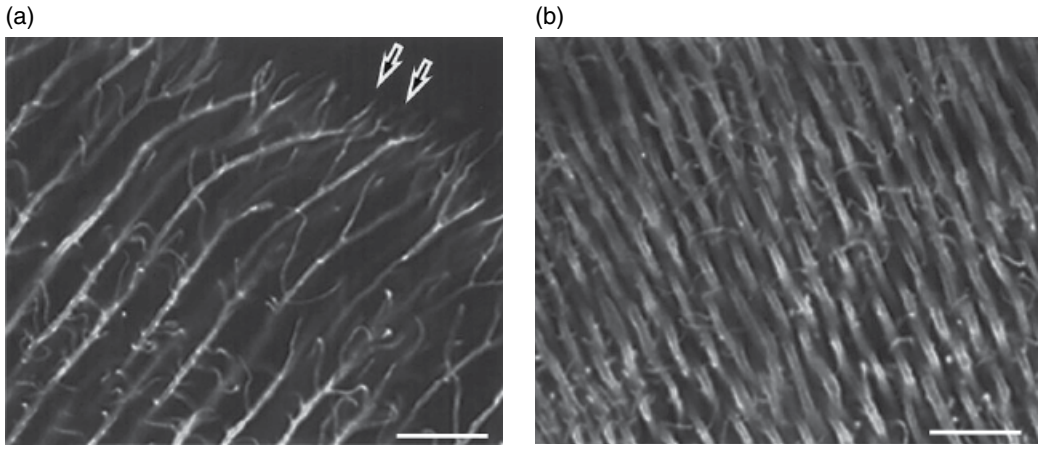


Figure 2.7 (a) Intensive branching of human tooth dentinal tubules close to DEJ (arrows). (b) Intensive branching of dentinal tubules in the middle part of dentin (bars: 20 μm). (Reproduced from [101] with permission from *Anatomy and Embryology*.)

There appears to be a gradual change of the mineralization rate from the mantle dentin towards the pulp [197], which may create up to 500 μm "resilience zone" necessary to prevent fractures under high occlusal forces [197, 208, 231, 232].

2.2.2.2 Primary and Secondary Dentin

Primary dentin formation (primary dentinogenesis) occurs during the formation and growth of the bulk of the crown and root, forming the main portion of dentin. After it, dentin formation continues as secondary dentin at approximately 1/10 of the rate [208]. The exact time for the "end" of primary dentinogenesis is vague, and actually primary dentin formation slows down gradually [100]. The difference between primary and secondary dentin even in histological or electron microscopy images is often difficult, and has no clinical relevance. Secondary dentin formation continues throughout life, leading to gradual obliteration of the pulp chamber and root canals [208].

2.2.2.3 Dentinal Tubules and Peritubular Dentin

Dentin tubularity contributes e.g. to the mechanical properties [11–13, 129] and behavior in dentin bonding [202]. Generally speaking, the tubules extend from the DEJ at right angles and run smoothly S-shaped course to the dentin-pulp border, but the direction may be different immediately beneath enamel [232]. Tubule orientation may also be different between the dental arches [232], which may affect the mechanical response to loading of teeth in occlusion [208, 232]. The density of the tubules varies depending on the location in the tooth, but is always highest in the dentin-pulp border and reduces towards the DEJ [142] (Figure 2.8). The number of tubules slowly decreases towards the apex, and in the root dentin and especially in the apical area, extensive branching occurs [77, 129, 142, 143]. In coronal area, it is highest and the direction is straighter under the cusps, where also the odontoblast processes [212, 229] and dense nerve innervation [23] penetrate deeper into the tubules. This may relate to the sensing of external irritation and contribute to the regulation of dentin-pulp complex defensive reactions.

Peritubular (intratubular) dentin forms in a regular circular manner on the walls of the dentinal tubules (Figure 2.9). This highly mineralized structure results with an age-related reduction in tubular lumen diameter, even complete occlusion of the tubule.

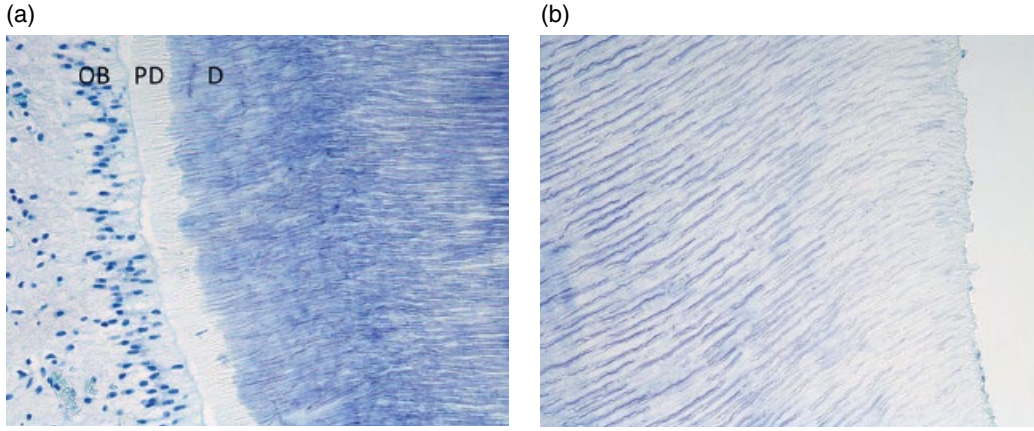


Figure 2.8 Dense tubularity close to the dentin-pulp border (a) and much sparser close to the DEJ (b). Toluidine blue staining of a human third molar from a young patient. OB: odontoblasts; PD: predentin; D: dentin.

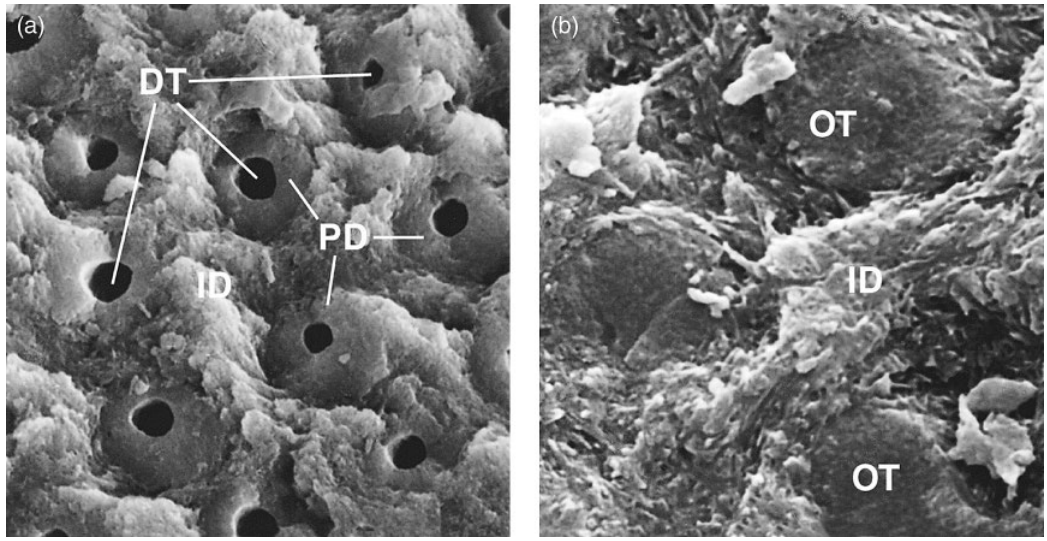


Figure 2.9 Fractured dentin showing intertubular dentin (ID), peritubular dentin (PD) and dentinal tubules (DT): (a) newly erupted tooth; (b) obturated dentinal tubules (OT) in a tooth from an old individual.

This is called dentin sclerosis. The tubules may also be occluded by mineral crystals due to reprecipitation or from the mineral ions from the dentinal fluid in cases of extensive wear or caries. Often this phenomenon is also called dentin sclerosis, although "reactive (dentin) sclerosis" would be a more appropriate term [208]. Peritubular dentin is often heterogeneous, and it is perforated by tubular branches and several small fenestrations [70], which allow dentinal fluid and its components to pass across the peritubular dentin.

2.2.2.4 Tertiary Dentin

Tertiary dentin is formed as a response to external irritation, including physiological and pathological wear and erosion, trauma, caries (in case of which both the lesion size and activity may affect [16]) and cavity preparation, and chemical irritation. The growth factors and other bioactive molecules

present in mineralized dentin and liberated during caries or wear are believed to initiate and control the tertiary dentin formation and structure [187]. Tertiary dentin increases the mineralized barrier thickness between oral microbes and other irritants and pulp tissue, aiming to retain the pulp tissue vital and non-infected. The form and regularity of tertiary dentin depends on the intensity and duration of the stimulus. There are two kinds of tertiary dentin: reactionary dentin, formed by original odontoblasts, and reparative dentin, formed by newly differentiated replacement odontoblasts [16, 138, 171, 173, 209] (Figure 2.10). Reactionary dentin is tubular and relatively similar to secondary dentin in structure, while reparative dentin (also called fibrodentin or even "calcified scar tissue" [16, 138, 171, 209]) is usually atubular or poorly tubularized and may present in variable forms (Figure 2.11). Reparative dentin is believed to be relatively impermeable, forming a barrier between tubular dentin and pulp tissue.

2.2.2.5 Root Dentin

Root dentin bears some distinct differences to coronal dentin. Right under cement, the granular layer of Tomes represents coronal mantle dentin with thin canaliculi and poorly fused globules. The granules contain uncalcified or poorly calcified collagen fiber bundles, and has been suggested to function as a "resilience zone" similar to mantle dentin [102]. As mentioned above, tubular density in root dentin is lower than in coronal dentin, especially in the most apical part [77, 129, 142, 143] (Figure 2.12). Age-related root tubular sclerosis starts from the apical region and advances coronally [149, 216], influencing root dentin permeability [164, 198]. Also, other regional differences occur, as buccal and lingual root canal dentin has patent tubules, while the mesial and distal dentin can be completely occluded [164, 198] (Figure 2.13). These tubular patency/occlusion patterns may correspond to stress distributions under occlusal loading [208], and affect both the bacterial penetration and disinfection [164, 198].

The apical part has also relatively large number of accessory root canals and apical branching (apical delta) and cementum-like lining the apical root canal wall [208] (Figure 2.14). The percentage of apical delta varies between 5.7% (maxillary anterior teeth) to 16.5% (mandibular molars), with the average number of canals being 4 (range 3–18) and about 87% having vertical

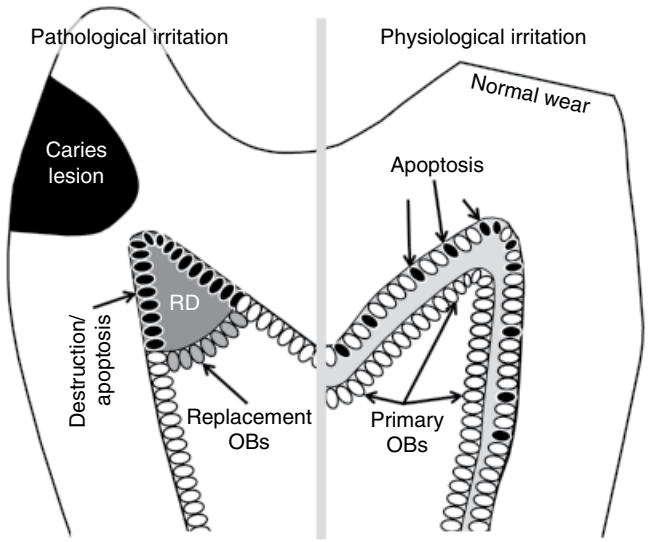


Figure 2.10 Intensive irritation (e.g. deep caries lesion) induces local odontoblast destruction and apoptosis and differentiation of replacement odontoblasts forming reparative dentin (RD). Normal wear or other mild irritation induces reactionary dentin formation by primary odontoblasts. Crowding of the odontoblasts causes apoptosis of selected cells (black cells). Modified from [209].

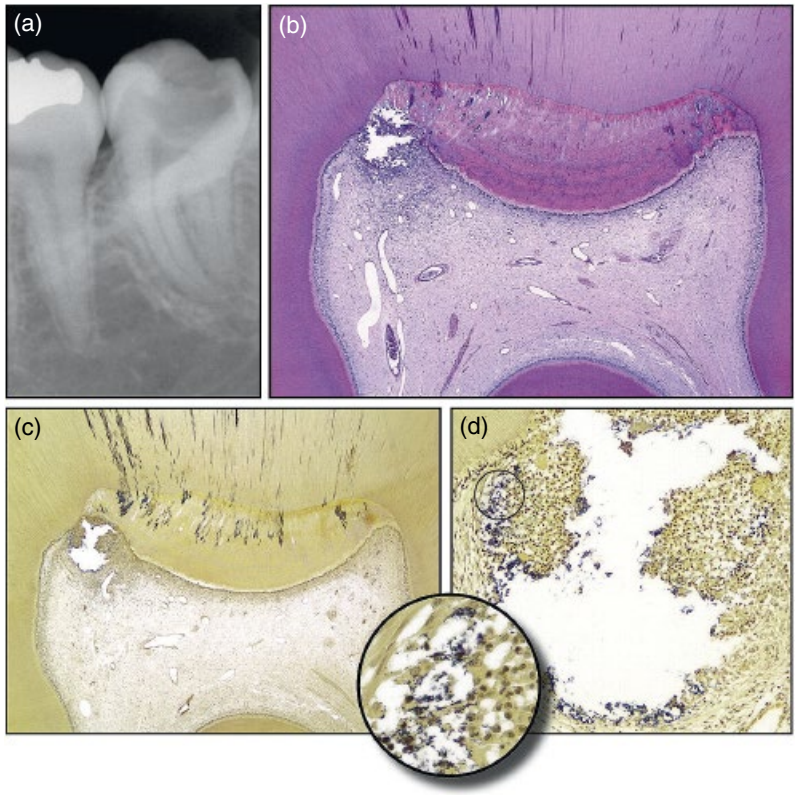


Figure 2.11 (a) A radiograph of a lower molar tooth with deep occlusal caries. (b) Tertiary dentin, consisting of both reactionary and reparative type. A limited area of necrosis is present in the mesial pulp horn (hematoxylin-eosin, original magnification 16×). (c) A section close to that in B (Taylor's modified Brown and Brenn, original magnification 16×). (d) A detailed view of the local microabscess. Bacteria surrounded by inflammatory PMNs on the right, fibroblasts on the left (original magnification 100×; inset 400×). (Reproduced from [172] with permission from *Journal of Endodontics*.)

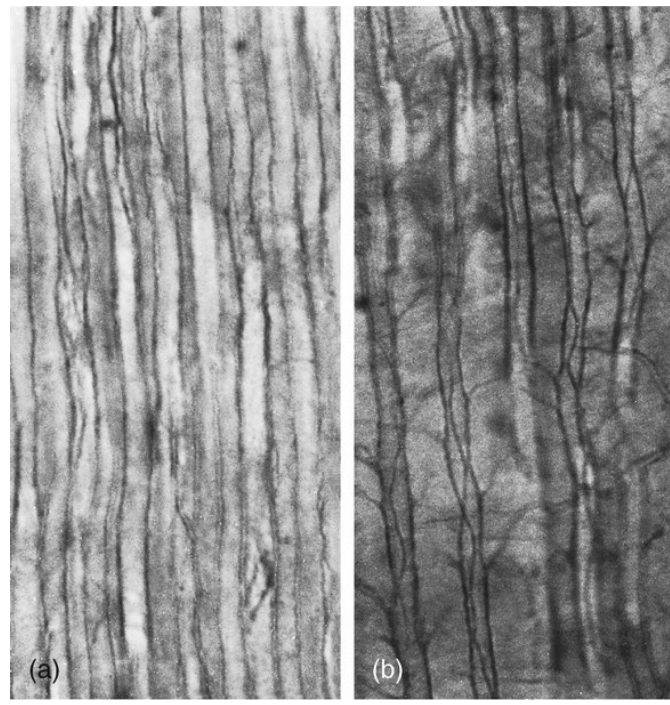


Figure 2.12 Longitudinal view of dentinal tubules: (a) in the crown; (b) in the root. The tubules are further apart in the root than in the crown and numerous fine branches are found in the root. Hematoxylin and eosin stained sections.

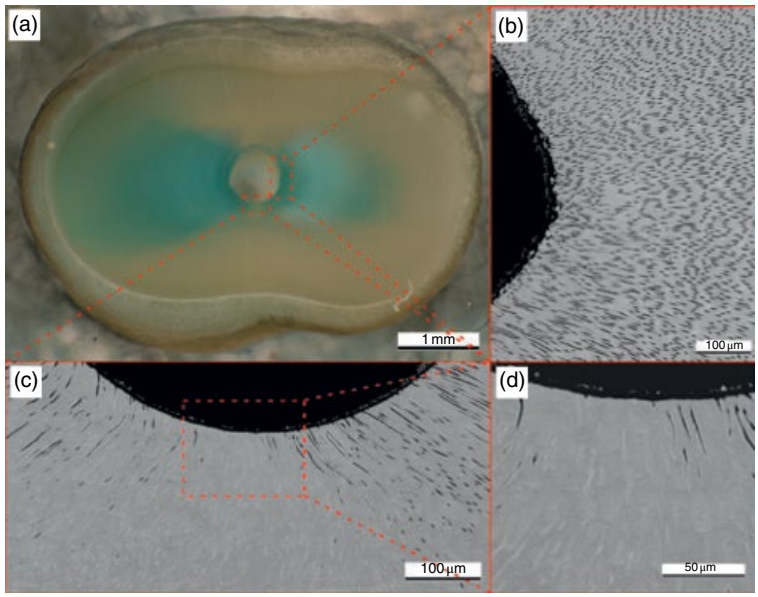


Figure 2.13 Cross-section of a tooth showing the typical bucco-lingual dye penetration (a). (b–d) Backscattered electron micrograph of areas with (b) and without (c, d) dye penetration. Patent dentinal tubules in dye-penetrated area and marked tubular sclerosis in approximal, non-dyed areas (c, d). Original magnifications: (a) 16×; (b), (c) 1000×; (d) 3000×. (Reproduced from [164] with permission from *Journal of Endodontics*.)

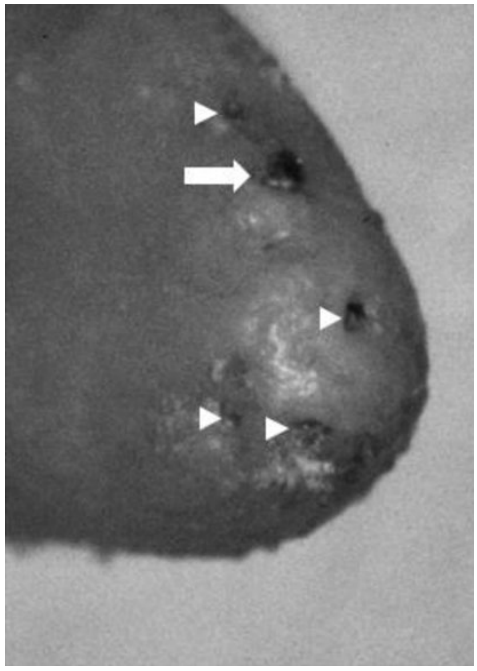
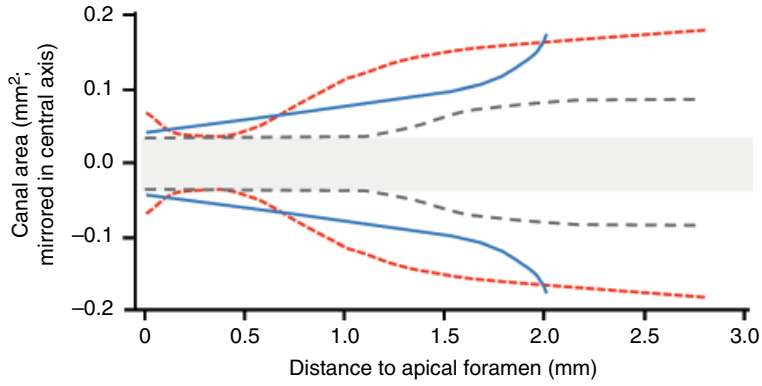


Figure 2.14 Human canine root tip with the major foramen (arrow) and four accessory foramina (arrowheads) large enough to easily fit ISO 10 instrument.

extension of 3 mm or less [60]. The traditional form of single narrow apical constrict was questioned in a recent micro-CT study, identifying long (≥ 1 mm) parallel form as the most common, and also a tapered form with no clear constrict as relatively frequent in all types of teeth [179] (Figure 2.15).

2.2.3 Dentinal Fluid

The space between the odontoblast process and tubule wall is filled with dentinal fluid. The odontoblast cell layer forms a functional barrier which mostly restrains the passage of fluid, ions and other molecules along the extracellular pathway, and at least in teeth without tissue damage (e.g. caries, cavity preparation, abrasion), dentinal fluid content is believed to be strictly under odontoblast control [209] (Figure 2.5). Dentin also contains several serum proteins, at least albumin, IgG, transferrin, fetuin-A and superoxide dismutase 3 (SOD3) [135], believed to be present mainly in dentinal tubules. With the exception of



Legend	Shape	Anterior	Premolar	Molar
	Traditional	20 (7–46)	6 (1–24)	14 (6–30)
	Taper	12 (3–37)	8 (2–28)	12 (5–28)
	Parallel	68 (43–86)	86 (65–95)	74 (57–86)

Figure 2.15 Cross-sectional apical root canal areas with different forms of the apical constriction, and the distribution (percentage with 95% confidence intervals) in different tooth groups. Each point represents a canal cross-section. X-axis: distance to apical foramen in mm, Y-axis: canal area in mm^2, mirrored at the zero axis. Modified from [179].

transferrin [160] they are not expressed by the odontoblasts. Therefore, serum proteins have a passage to dentinal fluid, even in intact teeth. However, the presence of SOD3 [165] and even 100 to 200-fold higher concentration of fetuin-A in dentin compared to serum [200] strongly indicate active transport systems by the odontoblasts [209].

Some evidence exists that physiological dentinal fluid flow may be controlled by endocrine system. A factor called parotid hormone is suggested to affect dentinal fluid flow rate. This hormone, secreted under hypothalamus control has been isolated from bovine, rat and porcine parotid glands and is present in plasma [175, 209]. A synthetic parotid hormone has equal biological activity to respective parotid gland-purified hormone in enhancing intradentinal fluid movement [233].

Dentinal fluid has a distinct role on the stress-strain distribution within the bulk dentin, increasing resilience (the capacity to absorb energy elastically upon loading) and toughness (the ability to resist fracture) [109]. In carious teeth, dentinal fluid is considered a protective factor through the occlusion of dentinal tubules (especially in slowly progressing, chronic lesions) [141] and as part of the innate response of the dentin-pulp complex with the deposition of intratubular immunoglobulins [73]. Both the quality and the quantity of immunoglobulins seem to vary according to caries depth and intensity even in uninfected tubules [73]. However, it is important to realize that inward flow also occurs all the way to the pulp [23, 117] even through enamel, at least in young teeth [23]. The study with different size microspheres demonstrated the size-dependence of penetration, the larger ones (0.2–1 µm, the size of small microbes) in the inner third of dentin and the smallest (0.02–0.04 µm) even in the pulp [117]. Thus, outward fluid flow is not capable of "washing out" the noxious stimuli from the tubules. Dentinal fluid also affects the success of adhesive restorative procedures. Increased dentinal wetness, due to increased size of dentinal tubules and fluid flow, makes successful bonding in deep cavities (close to pulp) more difficult

than to superficial dentin [166]. Dentinal fluid may cause degradation of hydrophilic adhesives, but also increase the collagen degradation rate in the hybrid layer, both leading to decrease in bond strength durability [202].

2.3 Pulp Tissue and its Homeostasis

The pulp tissue – sometimes called pulp proper – is loose connective tissue with type I and III collagens. Cells and structures are embedded in a gelatinous ground substance, containing mainly of chondroitin sulfates, hyaluronates and proteoglycans and interstitial fluid. The cells depend on the interstitial fluid as a mean for nutrient and oxygen transportation and elimination of metabolic waste products (Figure 2.16). Nerves and blood vessels enter the pulp through the apical foramen or foramina (Figure 2.17). They run close together until the main branching takes place in the coronal pulp and final, profuse branching in the odontoblast/sub-odontoblast region (Figure 2.18).

2.3.1 Pulp Cells

The main cell population in the pulp tissue are fibroblasts. Immediately under the odontoblast layer there is relatively cell-free layer (of Weil) rich in tenascin and fibronectin but low amounts of type III collagen [134]. Below that is the cell-rich layer with dense population of fibroblasts (Figure 2.4). The distribution of the fibroblasts in the rest of the pulp is less dense and relatively uniform. The pulp also contains mesenchymal stem cell-like dental stem cells with self-renewal capacity and multidifferentiation potential [18]. Pericytes are perivascular stellate cells forming a discontinuous layer in close contact with the endothelial cells surrounding capillaries and a continuous layer around microvessels [176]. They are classically considered as regulators of angiogenesis and blood pressure. Nowadays, pericytes (or their precursors) are recognized to have mesenchymal stem cell characteristics,

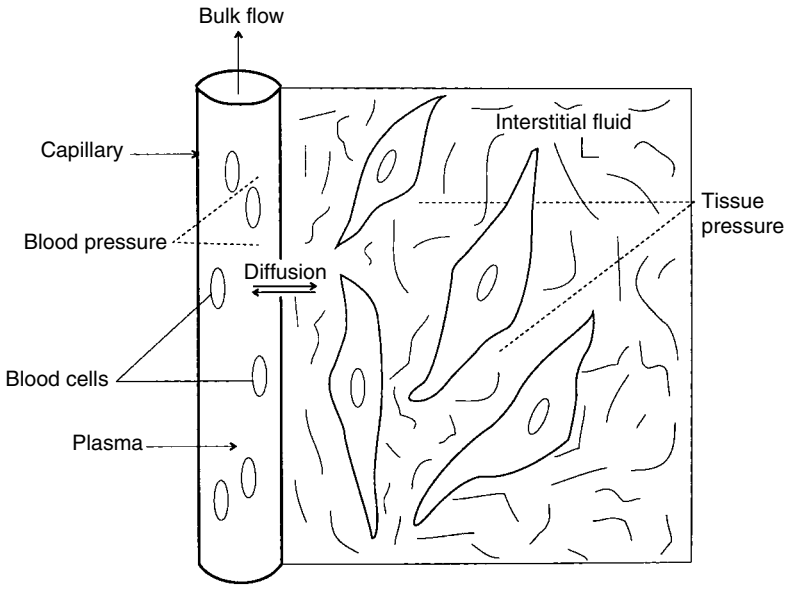


Figure 2.16 Schematic diagram illustrating capillary, cells and interstitial fluid. Blood is brought to the capillaries by bulk flow, and diffusion links plasma and interstitial fluid. The cells are surrounded by interstitial fluid acting as an extension of the plasma.

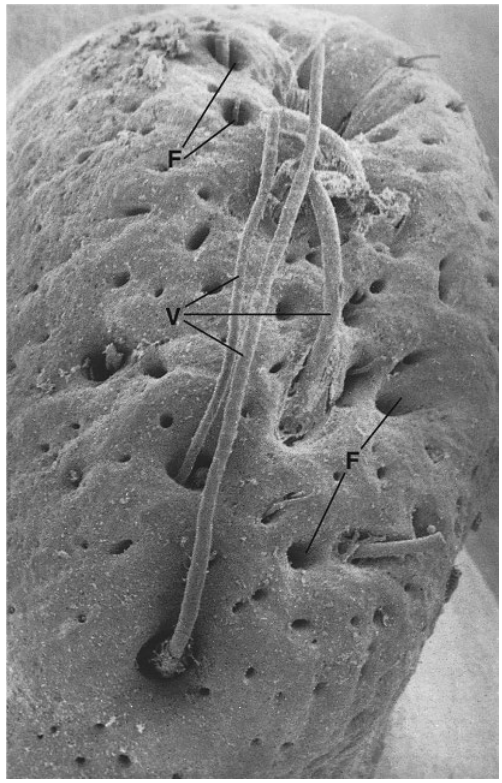


Figure 2.17 Vessels (V) entering/leaving the pulp through numerous apical foramina (F) in a dog tooth. The number of foramina is not representative for human teeth. (Reproduced from [192] with permission from *Journal of Endodontics*.)

including multipotentiality. They are selectively capable of differentiating into adipocytes and hard-tissue-forming cells osteoblasts and chondrocytes [95, 176] also in dental pulp [162, 230], possibly along with other mesenchymal stem cells of a nonpericyte origin [55].

2.3.2 Blood and Lymph Vessels

Like in any tissues, blood flow is required in the pulp to bring oxygen and nutrients to the cells, and to remove carbon dioxide and metabolic waste products. The pulp circulation is supplied by the maxillary artery, dividing into dental arteries and further arterioles that enter the teeth via apical foramina and through lateral canals. Arterioles are centrally located, and some of them pass directly to the coronal pulp while others supply the root pulp. The blood drains into venules, which largely follow the same course as the arterioles and a triad of arteriole, venule and nerve is often found in central pulp (Figure 2.19). The vasculature differs between the crown and the root. In the root, blood vessels penetrate the apical area of the pulp and form tiny branches. In crown area, capillaries form a subodontoblastic plexus of successive individual glomerular structures that each supply 100–150 µm of subodontoblastic and odontoblastic

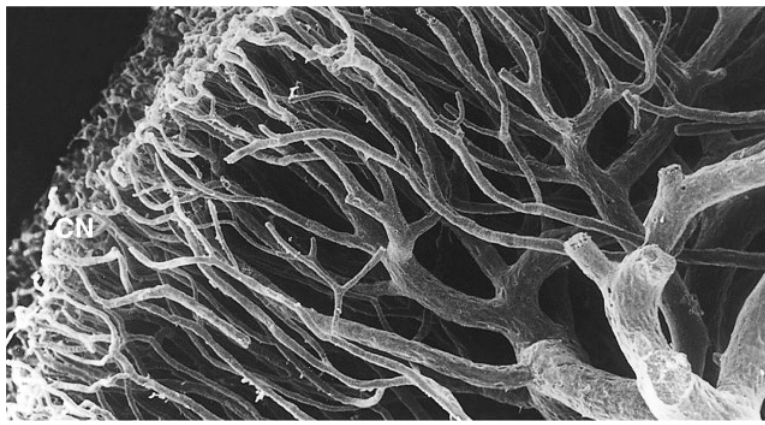


Figure 2.18 Blood vessels in the pulp. Note terminal capillary network (CN) subjacent to the predentin. (Reproduced from [192] with permission from *Journal of Endodontics*.)

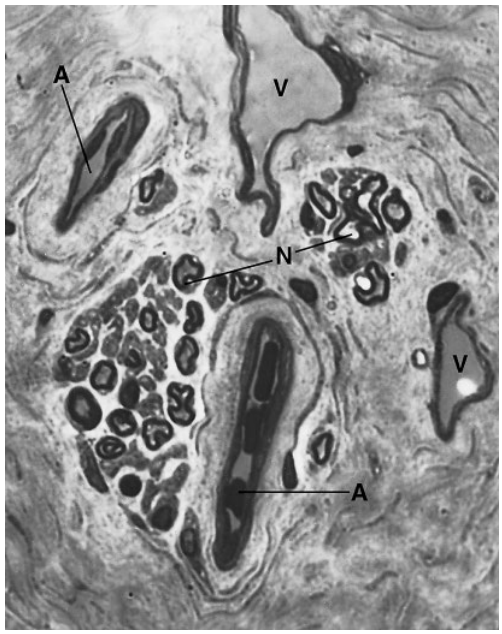


Figure 2.19 Area from the central part of the pulp showing the triad arteriole (A), venule (V) and nerve (N).

areas [90]. In young teeth with rapid dentinogenesis, capillaries enter the odontoblast cell layer to ensure their nutrition. Pulp capillaries are relatively thin-walled, may be discontinuous and fenestrated [28, 52, 90]. Pericytes are embedded within the capillary basement membrane, where they may migrate and undergo transition to a fibroblastic phenotype [28], modulate inflammatory events (e.g. leakage of plasma proteins) and may be involved with calcification of blood vessels [186] and thus be related to pulp stone formation. The blood vessels of pulp are innervated by sensory and by sympathetic nerve fibers (Figure 2.20) [23, 81].

The pulp tissue interstitial fluid has lower colloidal osmotic pressure than blood plasma, favoring capillary absorption. This helps to retain low tissue pressure, which is essential for the proper function of the blood vessels in the dentin-encased low-compliance environment. Surprisingly, the presence of lymphatics in dental pulp still remains controversial. The

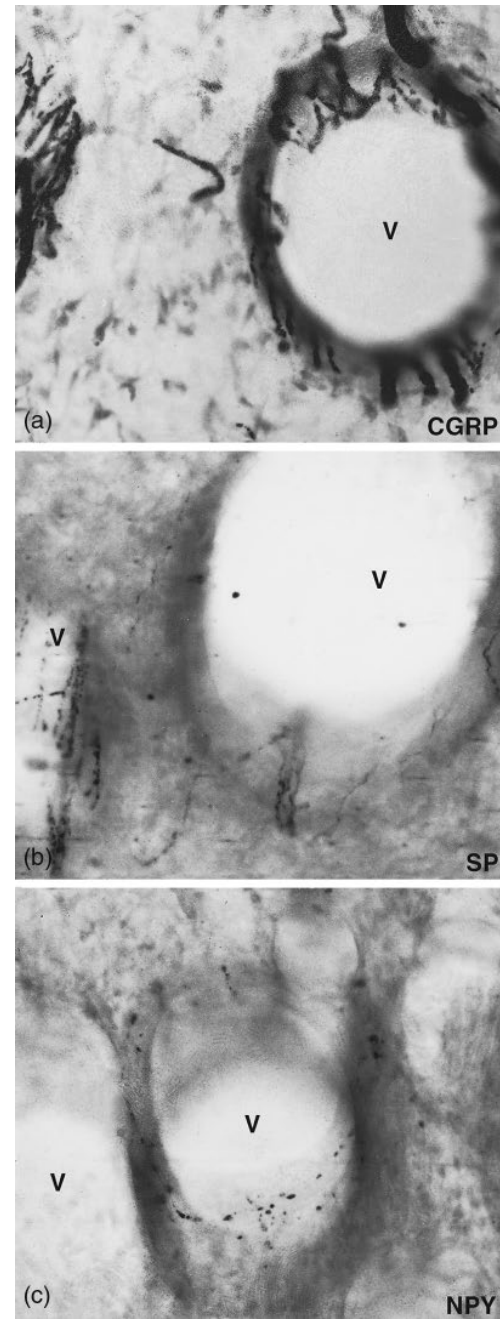


Figure 2.20 Serial cross-sections of vessels (V) from cat canine pulp. Network of sensory nerve fibers containing the neuropeptides CGRP (a), substance P (b) and neuropeptide Y (c) in the vessel walls. (Reproduced from [81] with permission from *Acta Odontologica Scandinavica*.)

earlier studies indicating pulp lymphatic capillaries have lately been disputed especially in studies using specific lymphatic markers [64, 119, 133, 218].

2.3.3 Nerves

Both myelinated and unmyelinated nerves are present in the pulp (Figure 2.21), majority of them being sensory. The sensory innervation of the pulp is very effective, terminating mostly in the odontoblast layer, predentin, and inner 0.1 mm of mineralized coronal dentin, where they run in close proximity of the odontoblast processes [27, 41] (Figure 2.22). There are at least six dental sensory nerve fiber types with specific distribution to focus on particular regions of blood vessels, coronal pulp, and dentin (Table 2.1). The sensory fibers are especially dense near the pulp horn tips, where sensitivity is also greatest, and gradually decrease towards the dentin-enamel junction (DEJ). Only few nerve endings are present in root pulp and

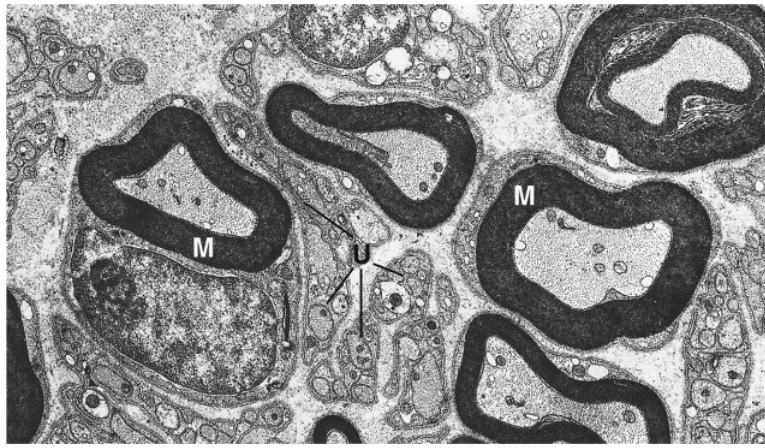


Figure 2.21 Electron micrograph illustrating details of a nerve from the central part of a pulp with myelinated (M) and unmyelineated (U) nerve fibers. (Reproduced from [41] with permission from *Acta Odontologica Scandinavica*.)

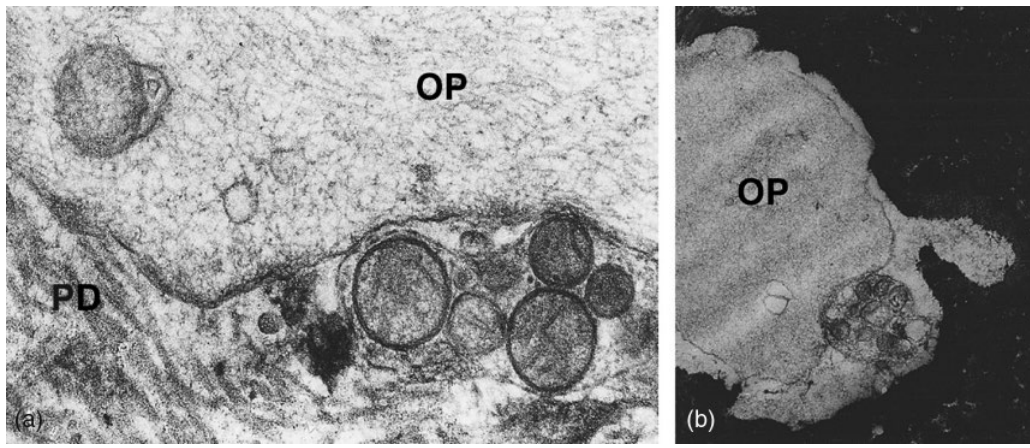


Figure 2.22 Nerve fibers in the periodontoblastic space (a) in the predentin (PD) and (b) in dentin and close contact with the odontoblast processes (OP). (Reproduced from [41] with permission from *Acta Odontologica Scandinavica*).

Table 2.1 The types, roles, mechanisms of activation, and sites of terminal endings of pulpal nerves.

Fiber type	Sensation	Activation	Terminal sites
Sensory			
A-beta	"Prepain", sharp pain	Mechanical: vibration, dentin fluid movement Electric (low voltage) Chemical: mustard oil, serotonin	Primary: Predentin, OBs, Secondary: dentin, pulp
A-delta fast	"Prepain", sharp pain	Intense cold Mechanical: dentin fluid movement Electric (mid-voltage) Chemical: mustard oil, serotonin	Primary: dentin, predentin, OBs Secondary: pulp
A-delta slow	Ache	Intense cold Electric (high voltage) Pulp damage (ATP) Chemical: capsaisin	Primary: pulp Secondary: blood vessels
C-fiber – polymodal	Ache	Intense heat Electric (high voltage) Pulp damage (ATP)	Primary: pulp Secondary: blood vessels
C-fiber – silent	Ache	Chemical: capsaisin, histamine, bradykinin	Primary: pulp Secondary: blood vessels
C-fibers	Ache	Electric (high voltage) Tissue damage (?)	Primary: OBs, pulp, blood vessels
Sympathetic			
C-fiber		Sympathetic activation, inflammatory mediators	Primary: blood vessels, pulp

dentin-pulp border [23]. Pulp innervation is closely related to the microvasculature [90] where the blood vessels are innervated by sensory and by sympathetic fibers, and a few other sympathetic fibers are located in cervical pulp [23, 90]. Nociceptive nerve fibers alert of damage and cause reflex withdrawal that limits the intensity of the initial injury. They also facilitate repair via amplifications of inflammatory, immune and healing mechanisms. Pulpal peripheral nerve fibers secrete a variety neuropeptides that activate receptors on the plasma membrane on target cells and affect tissue homeostasis, blood flow, immune cell function, inflammation, and healing. This phenomenon is called neurogenic inflammation, and occurs in the absence of direct chemical, thermal or microbial irritation [19, 23, 30]. Nerve fibers also adjust their own functions, cytochemistry, and structure to fit the tissue conditions [23, 65]. On the other hand, adrenergic agonists – better known for their vasoconstriction effect – may directly inhibit of dental nociceptor afferents [31, 76] and environmental conditions, such as pH, can regulate the nociceptive afferent activity, and may be significant in the clinical development and amelioration of dental pain [69]. These examples demonstrate bi-directional tissue-nerve interactions and neuroplasticity especially prominent in nociceptive sensory fibers, the major component of dental innervation (Figure 2.20) [23].

2.3.4 Pulp Stones

Pulp stones are discrete or diffuse pulp calcifications. One tooth may contain one or several pulp stones with varying size in coronal or in radicular pulp. The exact cause of pulp calcifications remains largely unknown.

External irritation (caries, attrition) certainly may induce pulpal calcifications, but pulp stones also appear in teeth with no apparent cause (e.g. impacted third molars). There seems to be an increase in prevalence with age, especially with the cumulative effect of external irritation [67]. The age-related pulp calcifications have been related to the blood vessels and nerve fibers. Structurally, there are "true" pulp stones, lined with odontoblasts (or rather odontoblast-like cells) and containing dentinal tubules; and "false" pulp stones, which are more or less atubular calcifications, also described as dystrophic calcification [171]. The distinction between the "true" and "false" pulp stones may be artificial, as both tubular and atubular dentin can be present in a single pulp stone (Figure 2.23). Large pulp stones in pulp chamber may obstruct the canal orifices, and in root canal they may complicate access to the apical canal [67, 208]. Apart from creating problems with endodontic procedures, pulp stones do not seem to have any other significance [67].

2.4 Pulp Inflammation

The encasement of the pulp within dentin and enamel creates a low-compliance environment that is unique in human body in terms of inflammatory tissue response. As in any other tissue, external irritation regardless of its nature (chemical, mechanical or thermal) induces a local inflammatory reaction characterized by the dilation of the vessels and decrease in the blood flow resistance (Figure 2.24). Vasodilation and the early recruitment of immune cells are mainly regulated by the sensory nerves via the release of the vasoactive neuropeptides (Table 2.1; Figure 2.25) [30]. The pertinent role of sensory nerves was demonstrated in studies where denervation caused a significant reduction of immunocompetent cells [57] and dramatically advanced pulp necrosis [24] after pulp exposure. Vasodilation together with lower resistance cause an increase in intravascular pressure and capillary blood flow, leading to leukocyte extravasation and filtration of the serum proteins and fluid into the tissue, mainly in the subodontoblastic area [201]. The increase in vascular permeability and accumulation of the proteins can happen quite rapidly, and it is clearly observable already four hours after the cavity preparation [201]. The vascular reactions aim to provide inflammatory cells and to eliminate microbial toxins and metabolic waste products from the area. However, if the external irritation exceed certain threshold level (e.g. continues and intensifying microbial stimuli from advancing caries lesion), it is possible that the pulpal reaction does not limit to the restricted area. Because of the protein and fluid filtration and the increase in cell content, the tissue becomes edematous and tissue pressure increases. In almost all other tissues this would lead to swelling, but in a pulpal low-compliance environment the tissue pressure may increase to the level that exceeds venular pressure, causing the compression of the venules (Figure 2.24). This is followed by increased flow resistance and concomitant decrease in blood flow, because the venous drainage is impeded. The slower blood flow causes aggregation of red and other blood cells and local elevation of the blood viscosity, which further reduces blood flow. The following local hypoxia, increase in metabolic waste products and carbon dioxide, and decrease in pH lead to vasodilation of the adjacent vascular structures, thus leading to the spreading of inflammation (Figure 2.24). The local inflammatory reaction will lead to local necrosis (local pulpal abscess) sometimes called necrobiosis, where part of the pulp necrotic and infected and the rest is irreversibly inflamed [2, 115] (Figure 2.11). Matrix metalloproteinases (MMPs), the enzymes degrading collagen and other extracellular matrix proteins and produced by odontoblasts [206, 207, 211] and especially by the inflammatory cells (PMN-leukocytes, macrophages and plasma cells), aim to confine the spreading of infection. Both chemical [204] and genetic [136, 220]

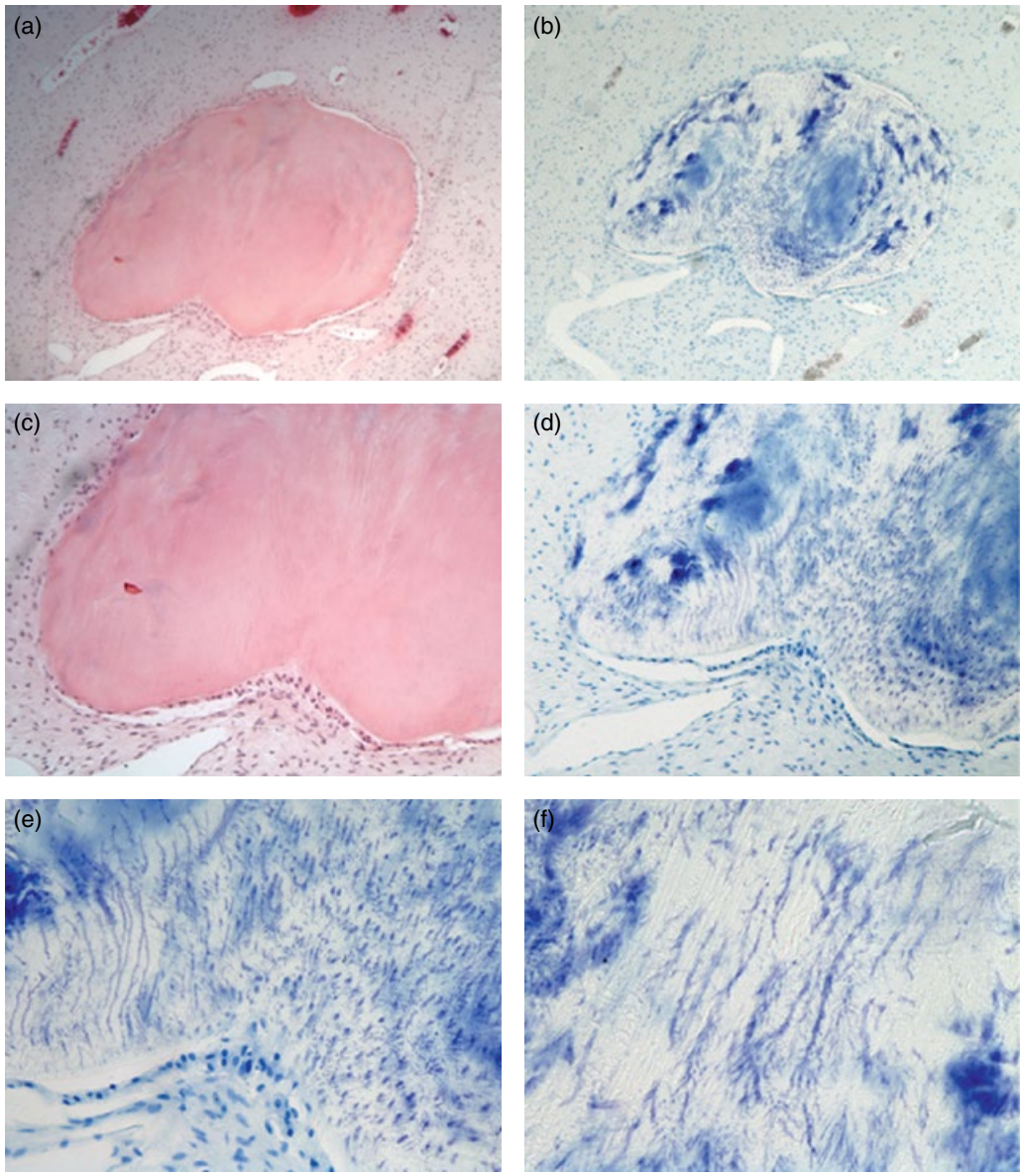


Figure 2.23 Approximately 3-mm human dental pulp stone, stained with hematoxylin-eosin (HE) (a, c) and Toluidine blue (b, d–f) staining. (a, b) Toluidine blue demonstrates heterogeneous structure compared to the solid appearance with HE staining. (c) Higher magnification of the lower left corner of (a). Odontoblast-like cells line the lower border of the pulp stone, while the left side is devoid of cells. (d) Same area stained with Toluidine blue demonstrates tubular structure at the site of the odontoblast-like cells, while the area without cells is free of tubules. (e) Higher magnification of (d). Well-formed longitudinally cut tubules are on the left side, while on the right side less organized tubules cut across the tubule direction. (f) In another part of the pulp stone, tubules appear sparse and with numerous fine branches and microbranches. (Reproduced from [208] with permission from *Endodontic Topics*.)

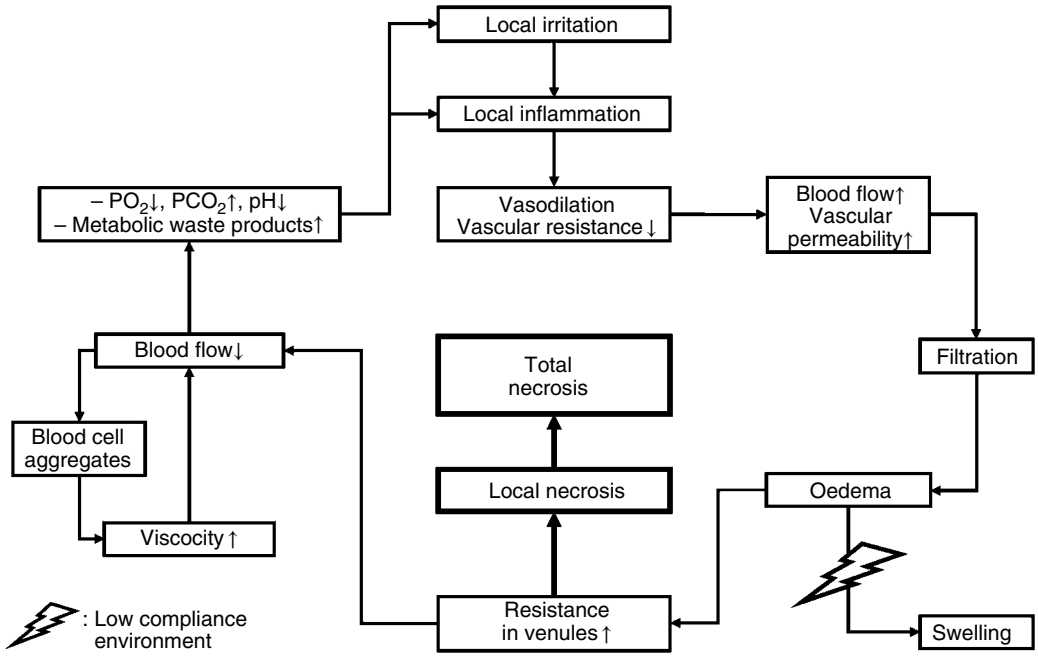


Figure 2.24 Localized inflammatory reactions in the pulp by e.g. caries, while the adjacent tissue remains intact. At the site of inflammation, inflammatory mediators lead to changes in vascular size and blood flow. If the reaction continues, the vicious circle leads first to a local and finally to total necrosis. (Modified from [201].)

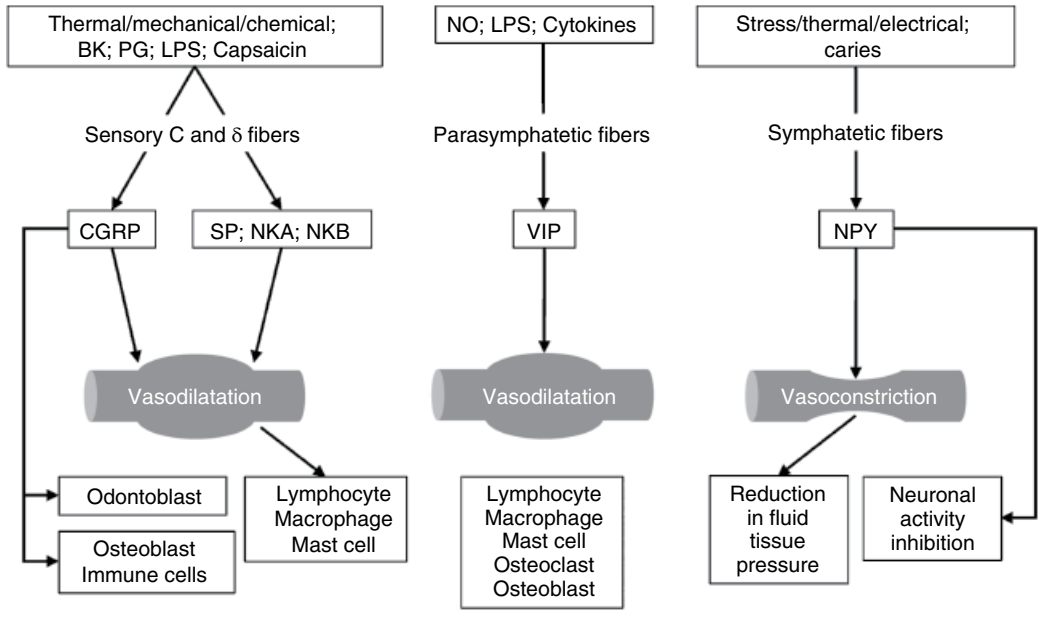


Figure 2.25 Neuropeptide release and their role in neurogenic inflammation. Different irritations and inflammatory mediators such as bradykinin (BK) and prostaglandins (PG), lipopolysaccharides (LPS) and capsaicin result in release of calcitonin gene-related peptide (CGRP) and tachykinins substance P (SP), neurokinin A and B (NKA and NKB, respectively) from C and A-delta sensory fibers. In addition to vasodilation, they activate immune cells and odontoblasts. Vasoactive intestinal polypeptide (VIP) is released from parasympathetic fibers stimulated by nitric oxide (NO), LPS and cytokines, generating vasodilation and exerting immunomodulatory effects on different immune cells. Sympathetic fibers release neuropeptide Y (NPY), resulting with vasoconstriction and reduced fluid tissue pressure. NPY also inhibits neuronal activity in normal conditions. Adapted from [30].

impairment of MMP function leads to increased apical pathogenesis. However, uncontrolled enzyme activity may also lead to increased tissue destruction [4, 72, 183, 211, 219]. If this vicious circle continues, it will slowly spread and lead into total necrosis (Figure 2.24).

Pulp vasculature is equipped with means to control the spread of inflammatory reaction and necrosis. Arterial U-loops and arteriovenous anastomoses (AVAs) may redirect some of the arterial blood flow away from the inflamed area (Figure 2.26). There are also arterial branching sites with constrictions or sphincters that can control the blood flow into the arterioles leading to the inflamed area. The controlled blood flow may control the level of tissue pressure in a manner that will allow sufficient function of the vasculature in and around the inflammatory area [201]. Neuropeptide Y (NPY), released from sympathetic fibers, generates vasoconstriction, which also will lead to reduced fluid tissue pressure [30].

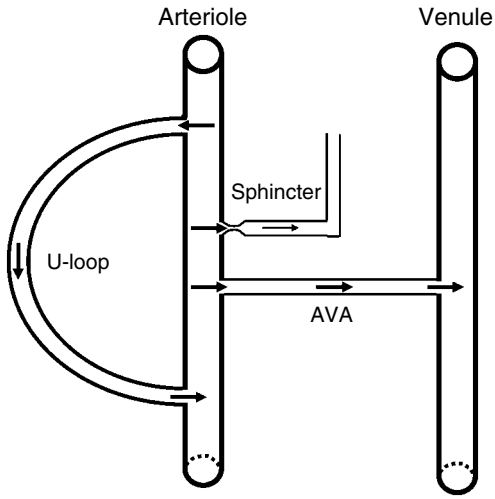


Figure 2.26 Pulp vascular structures that regulate the blood flow. U-loop and arteriovenous anastomosis (AVA) can be opened to redirect the blood flow away from the area of inflammation to reduce the interstitial tissue pressure. Smooth muscle constrictions in the branching of the arterioles can restrict or close the diameter of the arteriole. Modified from [201].

Pulpal healing and regeneration processes require angiogenesis. Pro- and anti-angiogenic factors liberated from dentin or pulp cells, neuropeptides and hypoxia control pulp vascularization [3, 10, 71, 174, 218], and inflammation leads to angiogenic capillary sprouting.

2.4.1 Immune Cells in Pulp

Human dental pulp contains resident immunocompetent cells that participate in the maintenance of tissue homeostasis and are able to mount innate adaptive immune responses against approaching infection [73, 74]. Inflammatory mediators initiating these responses are released from carious dentin or resident pulp cells [38, 187, 189]. The control of the resolution or advance of the initial inflammation is further regulated by a complex network of inflammatory chemokines, directing the trafficking of immune cells, and cytokines that regulate immune and inflammatory responses [73, 74, 82]. They are produced either by the odontoblasts [73, 74, 85, 116, 159], pulp cells [38, 73, 74], or (mostly) by the immune cells attracted to the site of inflammation. The effects of mediators are temporal context dependent: if inflammation is resolved, low levels of proinflammatory mediators may then promote tissue repair, whereas in chronic inflammation repair mechanisms become inhibited [38].

Immature dendritic cells (DCs) and T cells are important in immunosurveillance as part of the innate response to caries. Class II major histocompatibility complex (MHC)-positive immature DCs are located especially in the subodontoblastic but also in the odontoblast-predentin area [40, 52, 153], some of them extending their cytoplasmic processes into dentinal tubules [153, 154]. They detect microbial antigens, initiate maturation, and then migrate to regional lymph nodes to present them to naïve T cells. *Streptococcus mutans* can rapidly transform monocytes into mature DCs within 24 hours *in vitro* [75], which may contribute to the

local maturation of DCs in inflamed pulps. As a reaction to caries, subodontoblastic DCs infiltrate the odontoblast layer and invade reactionary dentin [40] attracted by TGF-beta liberated from dentin or secreted by the odontoblasts [54]. During maturation, DCs produce pro-inflammatory cytokines and chemokines that recruit circulating immature DCs, DC precursors and T cells to inflamed tissues [114].

The predominant T-cell type in healthy pulp is the memory CD8+ T cell [54, 61, 94], which have higher migratory capacity across endothelial cells than CD4+T cells. Its functions in the normal pulp remain undefined, but in general an immunosurveillance role of CD8+T cells has been proposed [110]. Natural killer (NK) cells are found in the bloodstream and can respond to inflammatory chemokines by extravasating into inflammatory sites. A very small population of NK cells may be present in healthy pulp, where also they may participate in immunosurveillance [61]. Regulatory T cells (T-reg) are absent [20] or present only in very low amounts [61] in healthy human pulp.

Neutrophils and macrophages are professional phagocytes in innate immune responses. Tissue macrophages are generally derived from circulating monocytes and show a high degree of heterogeneity, which is influenced by their microenvironment [73]. Small number of macrophages [93] and even smaller number of neutrophils [61] may be present in the pulp of an intact tooth. The quantity of macrophages increases reasonably early under caries lesion, when caries is limited to the DEJ or outer dentin [93, 94]. Macrophages may be activated in the early stage of pulpitis to protect the dental pulp by increasing vascular permeability, and to remove foreign antigens and damaged tissue caused by proteases [73, 94]. Activated macrophages are effective in eliminating pathogens in both innate and adaptive immune responses, important in tissue homeostasis through the clearance of senescent cells, and in remodeling and repair of tissue after inflammation. Neutrophils may not be important in early and reversible pulpitis, as they are few in pulpal tissues under shallow caries, but the numbers increase when caries approaches the pulp [94].

2.4.2 Odontoblasts as Immunological Cells

In dentin-pulp complex, odontoblasts represent the first line of cellular defense against external irritants, and odontoblasts have several means to participate in the initiation and development of dentin-pulp complex inflammatory and/or immune responses [53, 68, 116, 217]. Odontoblasts express Toll-like receptors (TLRs), which are a group of transmembrane glycoproteins that recognize microbial and viral particles, fungal proteins, and viral and bacterial RNAs and DNAs [194]. TLRs initiate the early activation of innate immune responses such as the recruitment of inflammatory cells, production of antimicrobial peptides, and maturation of dendritic cells [53], and may participate in reactionary dentin formation [33]. Out of the 10 TLRs identified in humans, TLR1–9 genes are expressed in mature human odontoblasts or human cultured odontoblast-like cells [48, 53, 158], with TLR2, -4 and -8 (recognizing lipoteichoic acid [LTA] from Gram-positive bacteria, lipopolysaccharide [LPS] from Gram-positive bacteria, and viral RNA, respectively) being most studied [53, 98, 147, 158, 217] (Figure 2.27). In principle, odontoblast-expressed TLRs would be capable of recognizing practically all essential microbial components.

Defensins are a group of small (3–5 kDa) peptides with broad-spectrum antimicrobial, immune cell chemotactic and bacterial toxin inactivation activities [97]. Seventeen defensins in two subfamilies, α- and the β-defensins, are found in humans [97]. Human β-defensins (hBD) -1 and -2 are expressed by the odontoblasts both *in vivo* and *in vitro* [46, 47] and suggested to participate in the innate host defense of human dental pulp [46]. hBD-2 is a host-derived ligand for TLR4 [15], and the inhibition of central TLR

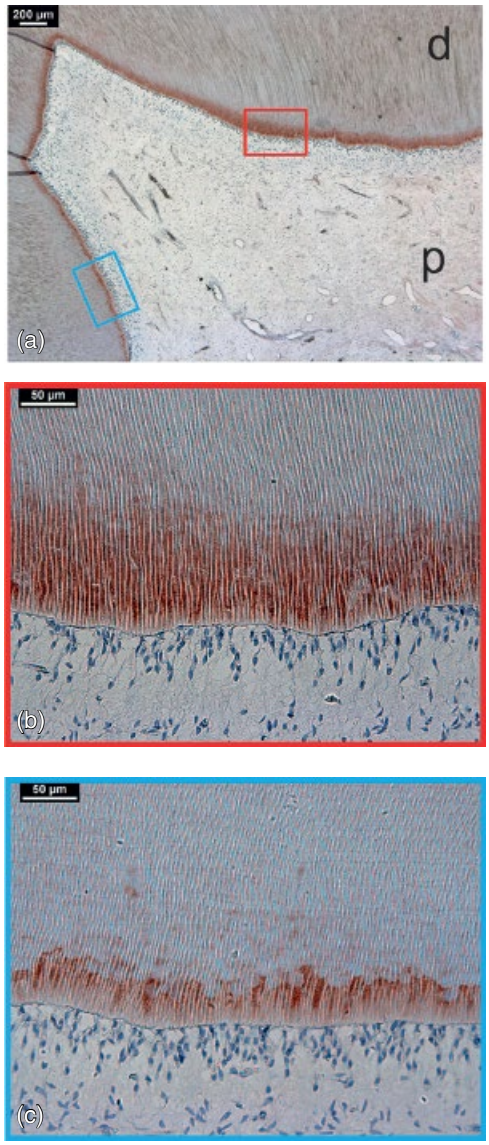


Figure 2.27 Immunohistochemical staining of TLR8 in human third molar. (a) An intensive staining is present in the dentin-pulp border in all parts of the tooth, with varying staining patterns between different areas. (b) Occlusal dentin-pulp border (higher magnification of the area marked with red square in (a)). (c) Approximal wall of the pulp chamber (higher magnification of the area marked with cyan square in (a)). (Reproduced from [158] with permission from *International Endodontic Journal*.)

signaling [80] blocks the *S. mutans*-induced increase in IL-6 and -8 gene expression in odontoblast-like cells *in vitro* [45]. Odontoblast-derived hBDs may thus also participate in the auto- or intracrine regulation of odontoblast immunodefense.

Proteinase-activated receptors (PARs) are G protein-coupled receptors that undergo irreversible proteolytic activation by proteases. They participate in controlling a wide range of biological processes, such as inflammation, hemostasis, thrombosis, and embryonic development [156], and in skeletal growth and bone repair [62]. PAR-2 is present in dental pulp fibroblast-like cells [121, 145], and PAR-1 and -2 are present in human odontoblasts [9]. The expression is significantly increased in response to caries both in the odontoblasts [9] and in pulp tissue [121, 145], indicating a regulatory role in reparative dentin formation and/or in pulp inflammation.

Even though odontoblasts appear to have a distinct role in the regulation of the initiation and progress of the dentin-pulp complex inflammatory response, more research is needed before any clinical treatment approaches are justified. For example, the most potential reparative dentinogenesis-inducing dentinal growth factor TGF-β affects inflammatory interleukin production in mature human odontoblasts [159], inhibit TLR2 and TLR4 expression, and decreases odontoblast-like cell responses to caries pathogens *in vitro* [86]. Much of the data come from *in vitro* studies, which differ significantly from the clinical reality. The complexity of the dentin-pulp complex calls for detailed *in vivo* or in situ studies, with approaches as close as possible to clinical reality.

2.5 Pulp Nociception and Hypersensitivity

Psychological studies of humans show only three main perceived sensations from teeth [23]. There is an initial poorly defined low-stimulus "pre-pain" sensation based on fast-conducting A-beta and A-delta fibers. That sensation shifts to sharp pain at higher stimulation intensities. In addition, there are dull ache sensations related to C-fiber and

probably slow A-delta signaling. Dental nociceptive neurons may also have properties unique from other tissues [36]. An unusual feature of dental innervation is the sensitivity of all types of nerve fibers to intense cooling of the tooth [23]. Several differences distinguish pain in teeth from other tissues. Especially in pulpitis, slight thermal or air stimuli that elsewhere are felt as cold, hot or light "breeze" can easily evoke pain in teeth. The low-compliance environment of the pulp and nerve sprouting and changes in neuropeptide expression of dental afferent neurons may lead to increased pain sensitivity [181]. Thus, stimulations of tooth by any type of stimulus result in a painful sensation, unlike in other tissues in the body [23, 36].

Although the sensory nerves even in completely healthy pulp may deliver pain sensation, inflammation intensifies those sensations. Nociceptor sprouting has been characterized as an early neural reaction to dentin injury, related to reactionary but not reparative dentinogenesis [23, 40]. Analyses of single nerve fibers in animals show expansion of dentinal receptive fields for A-fiber activation [150] as well as central plasticity [181] under those conditions.

Dentinal hypersensitivity is due to the activation of nociceptive trigeminal ganglion neurons that innervate dental pulp (i.e., dental primary afferents). The exact mechanism of this activation is not perfectly clear. The hydrodynamic mechanisms, in which tubular fluid movements are mechanically detected by nerve endings near the dentin-pulp border, has been widely accepted and dominating theory behind dental nociception. However, accumulative evidence of other potential mechanisms has led to other possible theories: neural theory, with nerve endings in dentinal tubules directly responding to external stimuli; and odontoblast transducer theory, where odontoblasts themselves may act as pain transducers [36, 126] (Figure 2.28).

Instead of mere mechanical stretching, mechanoreceptors of nociceptive afferents in dentinal tubules offer a potential mechanism in the pain delivery mechanism in hydrodynamic theory (Figure 2.28). Even normal

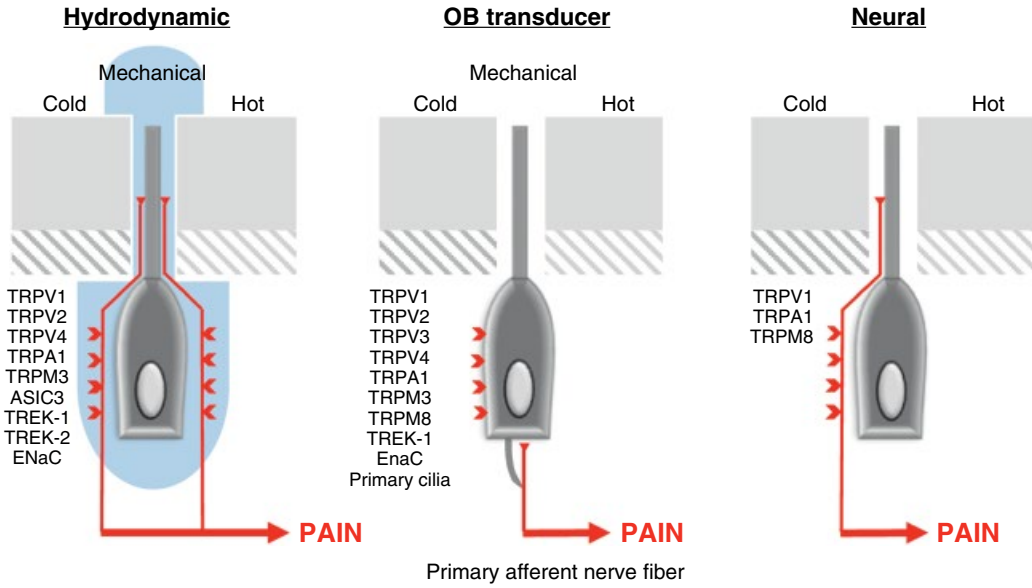


Figure 2.28 Molecular mechanisms of hydrodynamic, odontoblast transducer and neural theories of dental nociception. Fluid movement initiated by diverse external stimuli eventually activates mechanoreceptors in dental primary afferents. In neural theory, nerve afferents in the dentinal tubule are directly activated by external stimuli. Candidates of mechano- and thermosensitive molecules are listed. Adapted from [36].

chewing forces may create sufficient fluid flow to excite putative mechanoreceptors [163], especially in horizontal loading [191]. Especially transient receptor potential vanilloid 1 and 2 (TRPV1, TRPV2), transient receptor potential ankyrin 1 (TRPA1), and transient receptor potential melastatin 8 (TRPM8), expressed in dental afferents [36, 105, 106], are good candidates. The significant increase of TRPA1-positive axons in the painful irreversible pulpitis [106] and the role of TRPA1 in perception of cold [103] further indicates the importance of this receptor in dental hypersensitivity.

The expression of voltage-gated Ca^{2+}, Na^{+} and K^{+} channels, Ca^{2+}-activated K^{+} channels, store-operated calcium channels and Na^{+}/Ca^{2+} exchanger [7, 8, 26, 89, 120, 125, 126, 182, 222] indicate the excitability of odontoblasts. Indeed, action potentials can be evoked in odontoblasts by electrical stimulation *in vitro* [8, 91, 126]. Human odontoblasts also have electrical coupling, allowing information transmission between large number of neighboring cells after electric stimulation. This way the receptive field to external irritation may be significantly larger that the area of irritation [91]. Human odontoblasts express at least TRPV1, TRPV4, TRPA1, TRPM8, mechanosensitive TWIK-Related K+ channel (TREK-1), pH-sensing epithelian Na^{+} (EnaC) channels and cannabinoid receptor CB1 [35, 49, 50, 106, 125, 168, 188, 195]. Since synaptic structures between odontoblasts and nerve afferents do not exist, ATP [37, 49, 118, 182] and glutamate [35, 107, 152] have been proposed as signal mediators between odontoblasts and neural afferents. Purinergic P2X ATP receptors [6, 99, 105] and mGluR5 glutamate receptors [107, 152] are present in nerve fibers in the pulp, subodontoblastic plexus of Raschkow, odontoblast layer and even in dentinal tubules.

Another possible mechanism for sensory function for the odontoblasts is primary cilia. Primary cilia are single non-motile flagellar organelles present on nearly all vertebrate cells, where they serve a diverse set of signaling functions [63]. Primary cilia also function as flow sensors in osteoblasts, osteocytes, and chondrocytes [128, 223]. In bone cells, cilia are responsible for changes in cellular activity [128], suggesting a role in bone remodeling. Odontoblasts express primary cilia [39, 123, 199], where they may participate in odontoblast polarization and terminal differentiation and in signals that influence cell movement toward the pulp [39, 63, 123]. The cilia may respond to mechanical stimulus from dentinal fluid movements [124] or other signals from either dentin or pulp extracellular milieu or both. In odontoblasts, primary cilia locate in apical pole of cells *in vivo* [39, 199], as is the case also with bone cells [128]. The putative sensor function, the intimate relationship between odontoblast primary cilia and nerve fibers (Figure 2.29), and the capacity to generate action potentials indicate the possible role of odontoblast primary cilia in tooth pain transmission [199].

The neural theory is based on the dental afferent expression of receptors that take part in the transduction of a specific stimulus to electrical impulses [36]. In coronal dentin, especially in the pulp horn area, afferent nerves penetrate into dentinal tubules to approximately 100 μm depth [23, 27]. The presence of nociceptive and thermosensitive TRP receptors in dental afferent neurons would facilitate the direct pain sensation without need for hydrodynamic mechanical or odontoblast-transduced stimulus (Figure 2.28).

2.6 Age-related Changes in Dentin-pulp Complex

Even though the capacity for dentin formation remains throughout life, even odontoblasts show signs of aging. The first signs occur during the transition between primary and secondary dentinogenesis, when dramatic changes occur in the odontoblast phenotype [39] (Figure 2.30), related to differential transcriptional activity [185] and increase of

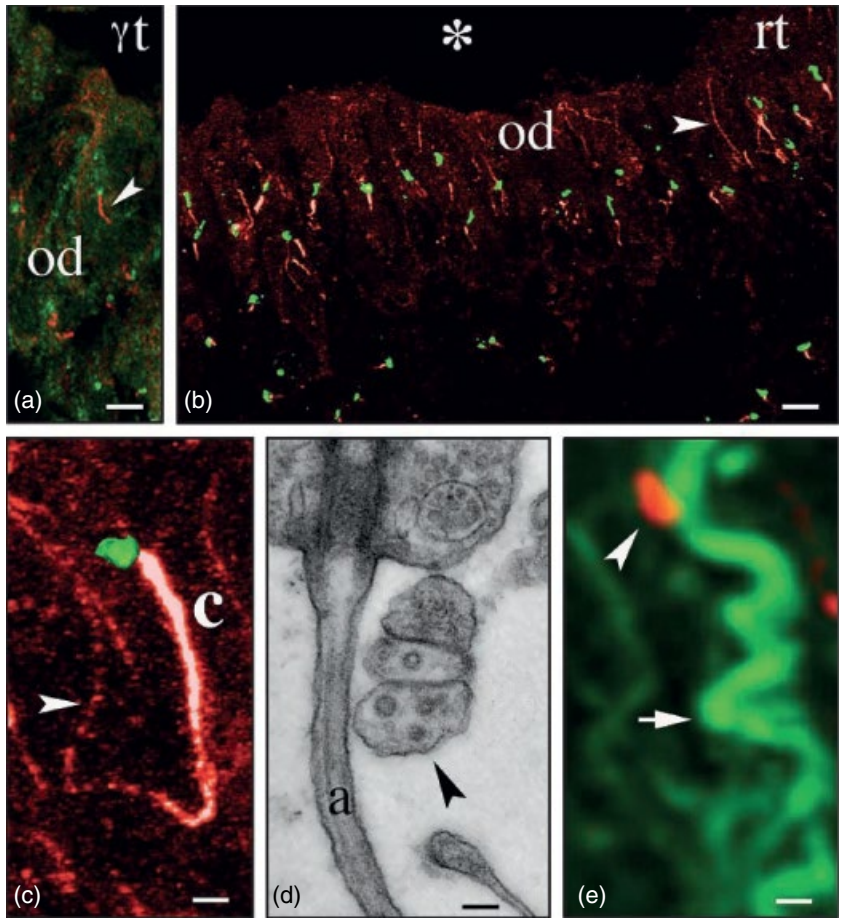


Figure 2.29 Primary cilium in human odontoblasts *in vivo*, confocal laser microscopy (a, b, c, e) and TEM analysis (d). (a) Arrow indicates odontoblast (od) primary cilium axoneme (bar: 10 μm). (b) Green color indicates rootlet in cilium basal body and pinkish-red the cilium; arrow shows nerve fiber; p: pulp core, *: dentin (bar: 10 μm). (c) Close relationship between primary cilium axoneme ("tail") (c) and a nerve fiber (arrow); green indicates cilium basal body at the odontoblast cell membrane (bar: 1.25 μm). (d) TEM image of a primary cilium stemming via the basal body from the odontoblast in close contact with a nerve-like structure (arrow) (bar: 0.20 μm). (e) Intimate contact between a ciliary structure (red; arrowhead) and a nerve fiber (green; arrow) (bar: 0.65 μm). (Reproduced from [199] with permission from *Journal of Dental Research*.)

odontoblast apoptosis due crowding [56, 137]. Mature human odontoblasts also develop an age-related autophagic-lysosomal system with autophagic vacuoles to facilitate the turnover and degradation of cellular components [39]. Autophagy is a housekeeping process with an "anti-aging" function active in most long-lived cells, consisting of self-digestive pathways mediated by lysosomes to maintain cellular homeostasis. In odontoblasts, autophagic activity has been proposed as essential survival mechanisms. Autophagy could also constitute an alternative mechanism of cell death, named "autophagic type II programmed cell death" (Type II PCD) [39].

As the pulp ages and reparative dentin gradually accumulates, pulp tissue tends to become more fibrous and the number of the odontoblasts and fibroblasts decreases [42, 87, 146]. Reduction of both the odontoblasts and fibroblasts is more pronounced in root than in coronal pulp [42, 146]. The capillary

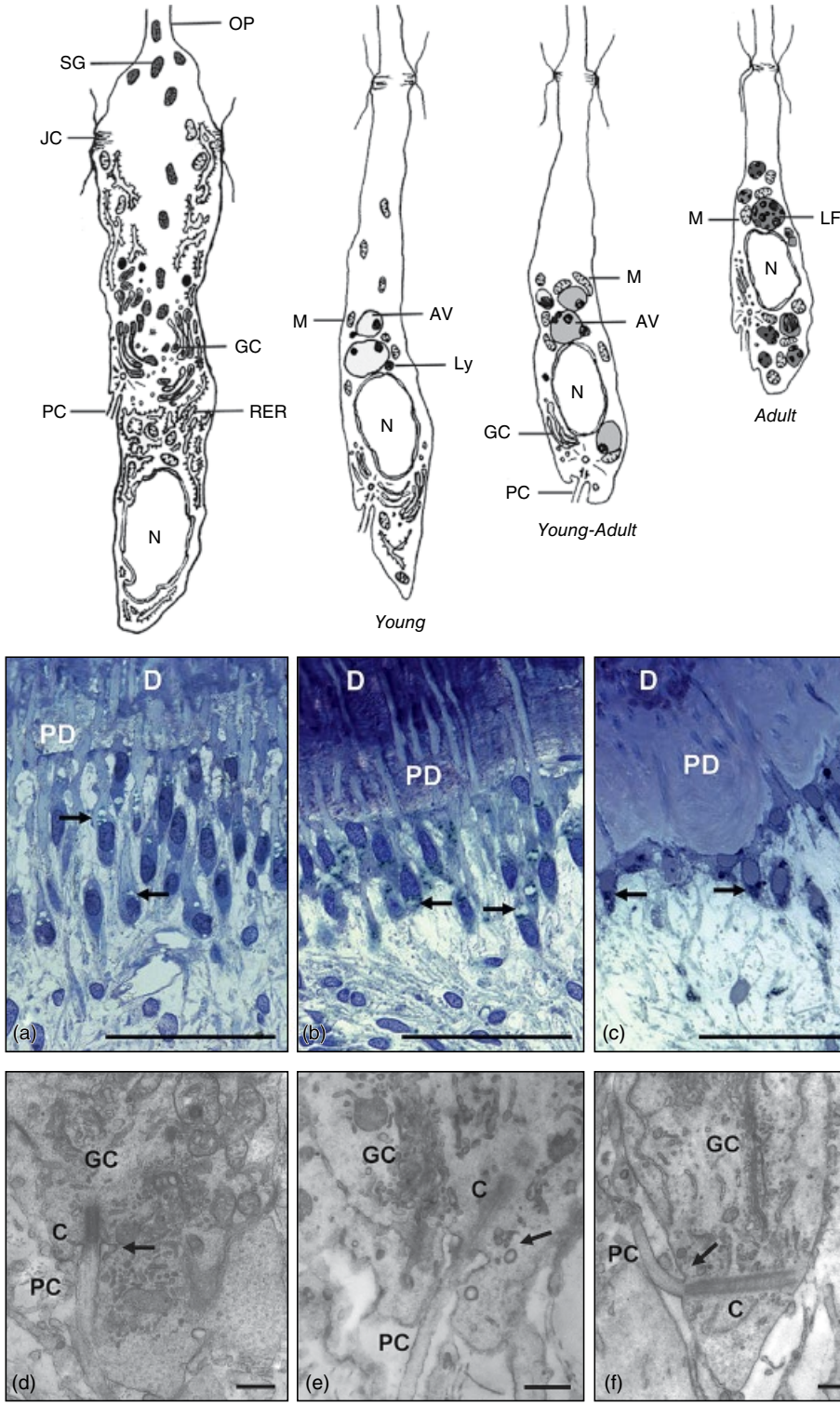


Figure 2.30 Upper panel: Schematic presentation of the age effects on odontoblast morphology. (a–c) Light microscope images of the coronal odontoblastic layer from 15-year (a), 25-year (b) and 75-year (c) patients' teeth. Odontoblasts in young and young-adult individuals are columnar cells with large autophagic vacuoles (a, b, arrows), while in older teeth odontoblasts become shorter and flattened with dense deposits accumulated within autophagic vacuoles (c, arrows). (d–f) TEM images of the primary cilium (PC) with the respective ages, showing that PC is a persistent structure in odontoblasts. C, centrosome; D, dentin; GC, Golgi complex; PD, predentin. Scale bars: 50 μm (a–c) and 0.5 μm (d–f). (Reproduced from [39] with permission from *Journal of Dental Research*.)

endothelium shows morphological and cytoskeletal changes, including increased transendothelial transport [52]. The innervation decreases and shifts its cytochemistry [23]. A comprehensive comparison of gene expression between young and old teeth indicated reduced expression of the functional gene groups involved with cell and tissue development, cell growth and proliferation, and hematological and immune system development and function in aged teeth. Conversely, the expression of genes involved with apoptosis were increased [210]. Overall, these changes may reflect a reduced ability of regeneration and repair in case of tissue destruction.

2.6.1 Age-related Changes in Dentin

In terms of clinical endodontology, the most important age-related change in dentin-pulp complex is the gradual obliteration of the pulp chamber and root canals even in intact teeth [5, 130, 146, 167] because of the slow secondary dentin formation (Figure 2.31). Relative increase in dentin thickness is higher in the root than in coronal area [146]. External irritation by caries, restorative procedures etc. may naturally speed up the process significantly. In incisors, canines, and premolars the obliteration initiates from the coronal direction, but in molars also the pulp chamber floor dentin frequently grows towards the roof. The obliteration may make the location of the root canals difficult [170] and the initial root canal preparation and creation of the glide path more challenging.

The effects of aging on dentin mechanical properties have been debated for decades, but the more recent studies strongly indicate that aging induces changes in the strength and resilience of mineralized dentin. While dentin tensile strength may increase with the occlusion of tubules [129], it occurs with the cost of reduced flexural strength [184]. The most important aspect is the increased mineral-to-collagen ratio due to the peritubular dentin occlusion of dentinal tubules [11, 144, 184] in aged dentin, which increases the hardness, especially in outer dentin [144]. As a result, the fatigue crack growth exponent is about 40% lower [13], the endurance strength about 48% lower [12], and the fatigue crack propagation over 100 times faster [13] in old than in young dentin. Dentin flexure strength reduces approximately 20 MPa/decade, and correlates well with tubular occlusion [11, 184] (Figure 2.32). The tubules in root dentin are similarly occluded [198] (Figure 2.33).

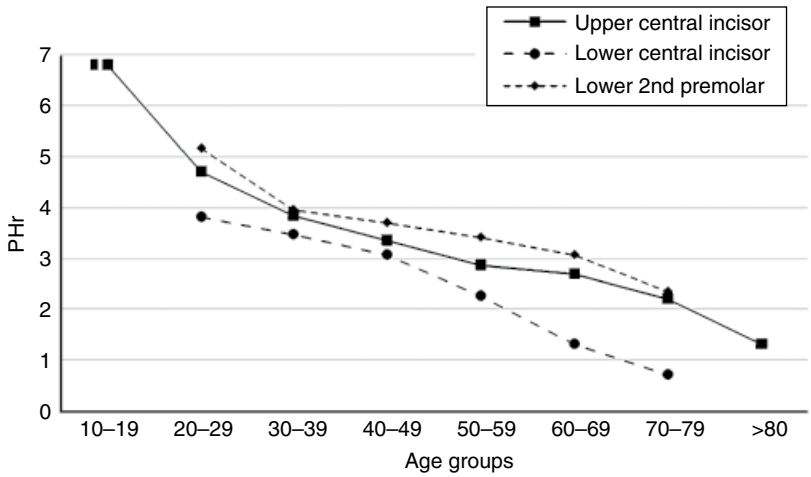


Figure 2.31 Reduction of the pulp space with age. PHr is the pulp volume percentage of the total and hard tissue volume. Data adapted from [5] and [167].

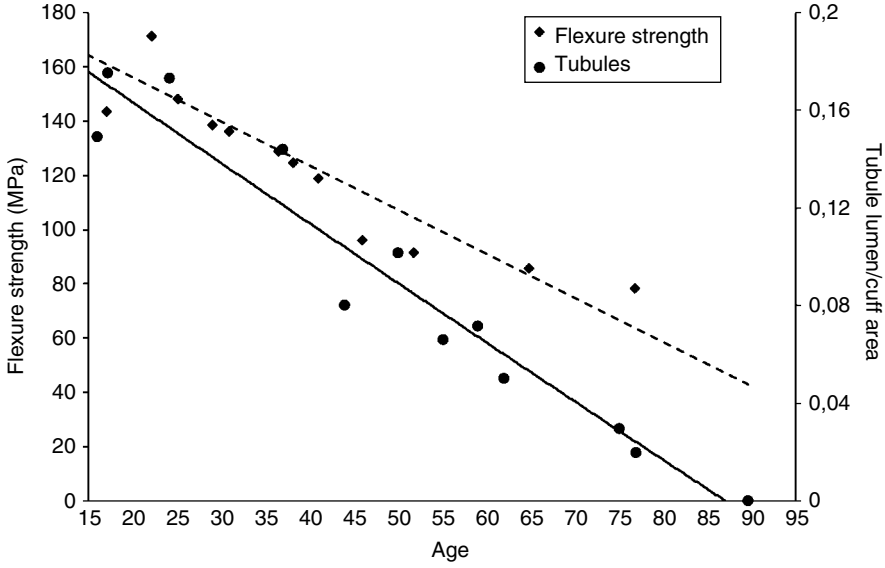


Figure 2.32 The change in dentin tubular lumen dimensions and the influence of age on the dentin strength in human third molars. "Cuff" indicates peritubular dentin. Data adapted from [11].

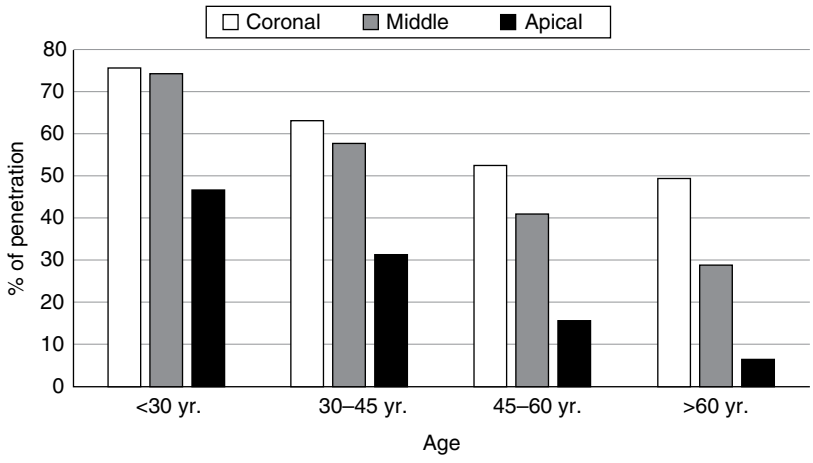


Figure 2.33 Relative mean dye penetration (in percentage of complete dentin area) after extensive incubation of Methylene blue in instrumented root canals. Data adapted from [198].

Human root dentin may have higher flexural strength than coronal dentin [51, 184], but is similarly reduced with age [184, 228] along with increase in mineral content and hardness [226].

Age-related changes in the organic components seem also to contribute to the dentin mechanical properties [12, 184, 228]. Aged dentin is more cross-linked [228] and has high levels of pentosidine cross-links of collagen [184]. This non-enzymatic advanced glycosylation end-product (AGE) – cross-linking between individual collagen molecules stiffens the collagen matrix, making it more fragile, and participates in low bone strength in osteoporosis patients [177, 215]. Also in human dentin, high AGE levels significantly reduce dentin flexural strength and contributes to the lower mechanical strength of aged dentin both experimentally [139] and *in vivo* [184].

Other changes in dentin organic matrix with age may also occur: decrease and loss of matrix-degrading enzymes has already been demonstrated [132, 151, 196], which may implicate also changes in their substrates, namely collagen and non-collagenous proteins.

2.6.2 Caries-affected Dentin

Caries damage develops and increases with age and has clinical significance in relation to tooth restoration. Minimally invasive restorative dentistry aims to avoid unnecessary removal of tooth structure, and cavity preparation is limited to the removal of caries-infected dentin, leaving the restoration to be adhesive-bonded to caries-affected dentin. Caries-affected dentin has lower mineral content and altered structure and composition of dentin organic components, including collagen. These changes reduce dentin hardness, stiffness, tensile strength, modulus of elasticity and shrinkage during drying [203]. As a result, dentin in and under the composite-dentin interface is more susceptible to cohesive failures due to the polymerization stress and occlusal forces [202]. Even short exposure of dentin to lactic acid (the most important acid produced by cariogenic bacteria [34]) significantly reduces dentin fatigue strength, increases the crack extension rate, and reduces the fatigue crack growth resistance [44, 155]. Since fatigue crack is a precursor to unstable fracture, lactic acid exposure increases the probability of restored tooth fracture at lower occlusal forces [155]. Especially the endodontically treated teeth are more prone to fractures because of the weaker structure due to loss of tissue, but also because the incremental crack extension occurs with significantly lower cyclic stresses in deep compared to superficial dentin [92]. To avoid catastrophic, unrepairable tooth fractures, restorative procedures should be performed in a manner to protect and preserve the remaining tooth structure, especially after endodontic treatment.

2.7 The Periodontium

The periodontium includes the gingiva, the periodontal ligament (PDL), the alveolar bone, and the dental cementum (Figure 2.34). Its main function is to provide an attachment for the teeth to the alveolar bone. Periodontium is actually a fibrous joint of the gomphosis type, where a conical process (root) is inserted into a socket via a fiber ligament (i.e. PDL). In humans, periodontium is the only gomphosis-type joint and allows minor adjustments in the position of the teeth. Thus it is a resilient suspensory apparatus that provides optimal conditions for masticatory functions. The apical periodontium, including the PDL, cementum and the alveolar bone, is of prime importance for endodontology and these parts of the periodontium will be described in some detail.

2.8 The Periodontal Ligament (PDL)

The PDL is a dense connective tissue with islands of interstitial loose connective tissue interspersed between the dense bundles of collagen fibers (Figure 2.2, Figure 2.34). The principal fibers of the PDL extend from the cementum to the alveolar bone. However, each fiber does not reach the entire distance between cementum and bone, and collectively they constitute an intricate branching and reuniting pattern of fibers. They are embedded deep into the two mineralized tissues as Sharpey's fibers (Figures 2.34 and 2.35). The fibers inserting into cementum are smaller and more numerous than those entering into alveolar bone.

Four groups of principal fibers are present in the PDL. The alveolar crest fibers pass downward from the cementum in the cervical region of the tooth to the alveolar crest; the horizontal fibers comprises the cervical third of the PDL; the oblique fibers run from the alveolar bone somewhat apically to the cementum; and the apical fibers radiate from

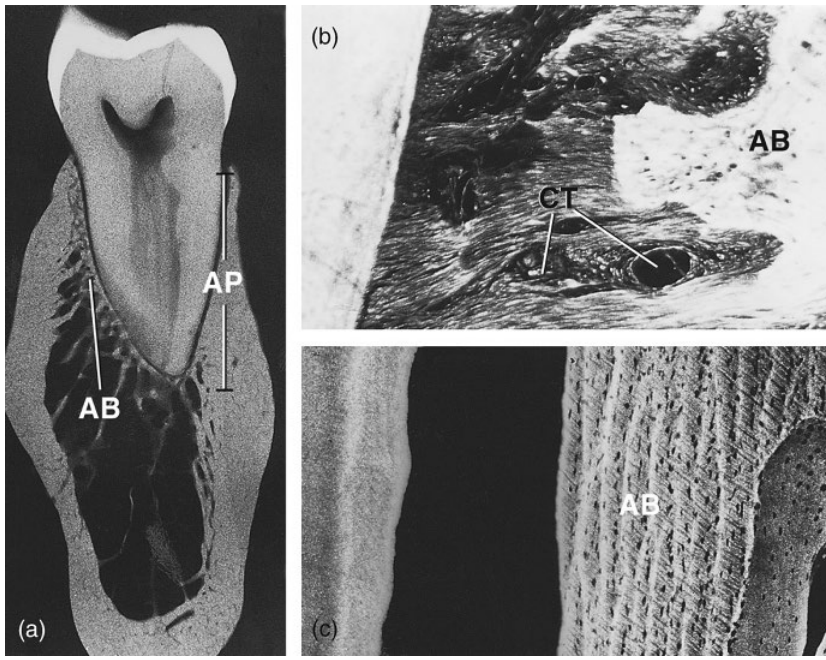


Figure 2.34 Radiograph (a) and microradiographs (b, c) of alveolar bone (AB) in undermineralized (a, c) and demineralized sections (b). Note islands of blood vessels and loose connective tissue (CT) among the fibers of the periodontal ligament and radiolucent Sharpey's fibers that attach to alveolar bone (AP, alveolar process of the mandible).

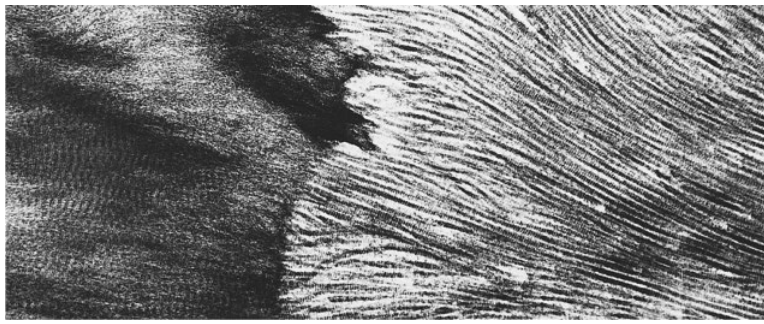


Figure 2.35 Electron micrograph showing fine collagen fibers inserting into a cellular cementum as Sharpey's fibers. (Reproduced from [58] with permission from *Acta Odontologica Scandinavica*.)

the cementum towards the alveolar bone in all directions.

The principal fibers of the PDL have a functional arrangement in that groups of fibers act against different types of forces, including resistance to rotation of the teeth. Although collagen is inelastic, slight movement of the teeth is possible due to the wavy course of the fibers, allowing them to stretch during stress. Changes in the blood flow and blood pressure of the PDL induce minor movement of the teeth [1]. The blood and tissue fluid also act as a hydrodynamic system that absorbs occlusal forces.

2.8.1 Cells of the PDL

Fibroblasts are the prevailing cells of the PDL. Those that are located between the principal fibers are long, slender cells, but

those in the interstitial tissue are irregular or stellate-shaped. The function of the fibroblasts is to maintain the collagen fibers and the glycosaminoglycans and glycoproteins that constitute the ground substance during the normal turnover processes and during repair. Macrophages and mast cells, including associated cytokines, are also found in the normal PDL and they increase markedly during inflammation. Cementoblasts locate on the cementum side and osteoblasts on the alveolar bone side of the PDL. Osteoclasts and odontoclasts allow bone and tooth resorption. They play important roles in the turnover of the PDL, during periodontal, including apical, disease and during orthodontic movement of teeth. They also play an essential role in the development of apical periodontitis. Odontoclasts and osteoclasts also play a central role in shedding of primary teeth and during eruption of the permanent teeth.

Sympathectomy increases osteoclast-mediated bone resorption [113] and root dentin resorption [78], indicating that sympathetic nerves have an inhibitory effect on osteo- and odontoclasts. Sympathetic nerves in the PDL are also important for the recruitment of granulocytes as demonstrated by experimental tooth movement [78]. In experimentally induced periapical lesions, sympathetic nerves have an inhibitory effect on the size of the lesion, the number of osteoclasts lining the lesion and the amount of IL-1α within the lesion [79].

2.8.2 Epithelial Cell Remnants

Both the developing and mature teeth are surrounded by a continuous, net-like pattern of epithelial cells originating from the Hertwig's root sheath. These so-called Malassez's epithelial rests (Figure 2.2) are located close to the root cementum in its entire length, and persist within the periodontal membrane throughout the life of the tooth [225]. The reason why the epithelial rests of Malassez persist in the PDL is unknown, but it has been proposed that these cells are important to prevent ankylosis and hinder bone ingrowth [225]. Stimulation of these epithelial cells may induce cell proliferation [66] that may form cyst linings at the periphery of lesions. These epithelial cells undergo apoptosis, which, together with proliferation, may play a role in the decrease and/or turnover of the epithelial cells of Malassez's rests in the periodontium [32].

Malassez's cells may function as targets for developing periodontal nerves. Ruffini-like receptors and free nerve endings relate close to these cells (Figure 2.36) [81]. Furthermore, immunohistochemical studies have shown that Malassez's cells contain neuropeptides such as CGRP and SP and may thus have functional endocrine roles [81]. Malassez's epithelial rests may also play a role in the formation of cementicles by undergoing mineralization. They may be attached or free and may contain cell rests much like pulp stones which may be found in the apical area of the teeth (Figure 2.37).

2.8.3 Turnover

It is important to note that bone is a more dynamic tissue than cementum. The normal turnover of bone also affects the alveolar processes. Cementum is usually covered by a thin, unmineralized precementum, and slow cementogenesis compensates for wear of the teeth. Bone, on the other hand, is covered by osteoid only during bone formation. Unmineralized matrix tends to resist odontoclastic activity [190]. Thus, osteoclastic activity seems to predominate over odontoclastic or cementoclastic activity in the PDL and Howship's lacunae in bone defects develop more commonly than tooth resorption. Both resorptive processes may take place simultaneously. Reparative cementum formation by cellular cementum may fill in resorption defects.

The difference in resorptive activity between bone and cementum in the PDL is the basis for orthodontic tooth movement. Moderate forces applied to a tooth will result in bone resorption on the pressure side and

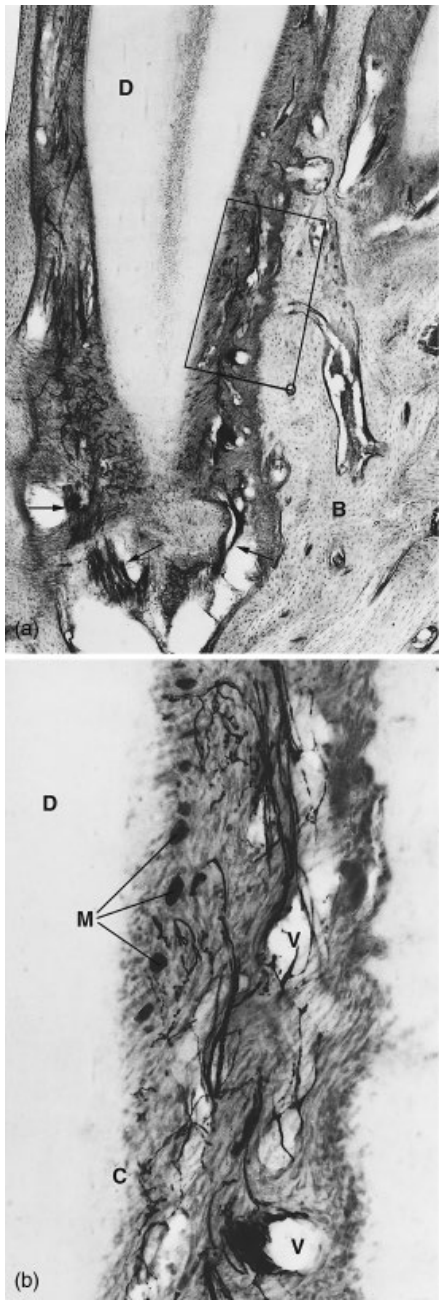


Figure 2.36 (a) Apical periodontal ligament of cat incisor richly supplied with protein gene product (PGP) immunoreactive nerves (arrows) from apical bone (B). (b) Enlargement of framed area showing nerve fibers supplying the blood vessels (V) and extensive branching of nerves towards the root cementum (C) and Malassez's epithelial cells (M) (D, dentin). (Reproduced from [81] with permission from *Acta Odontologica Scandinavica*.)

bone formation on the tension side without resorption of the cementum. Thus, the tooth will move in the direction of the force applied. The use of excessive force may also result in resorption of the cementum and may reach clinically significant proportions.

2.8.4 Circulation in the PDL

The blood supply to the PDL is complex. Although tendinous, the PDL is highly vascularized. The total vascular volume has been calculated to be approximately 20% of the tissue, compared to only 3–4% in most other tissues. PDL receives its blood via vessels from the alveolar bone, periosteum, gingiva, and pulp. The main vessel supply originates from the intraosseous arteries. As the arterial supply arises from different sources and the venules and veins drain both into the bone marrow and gingiva, the vascular bed of the PDL should not be regarded as an isolated functional unit. This implies that inflammatory and pathological changes in blood flow, tissue fluid pressure, or blood pressure in the surrounding adjacent tissues, will also influence the periodontal circulation. Inflammatory vasodilation in parallel-coupled vessels in the alveolar bone or gingiva may cause reduced blood flow in the PDL due to a fall in pressure in the arterioles feeding the PDL.

The main vessels of the ligament run parallel to the long axis of the tooth in compartments of loose connective tissue between the fibers. The arterioles branch to form capillaries arranged in a flat, basketlike network that surrounds the root surface. The capillary network is located closer to the alveolar bone than to the cementum, and the vessels perforating the alveolar walls are most abundant in the apical third.

Blood flow in the PDL seems to be controlled mainly by sympathetic fibers causing vasoconstriction and by sensory fibers for vasodilation [104]. However, due to the lack of suitable methods to measure blood flow in a tissue with such a tiny volume and a position secluded between bone and tooth as the

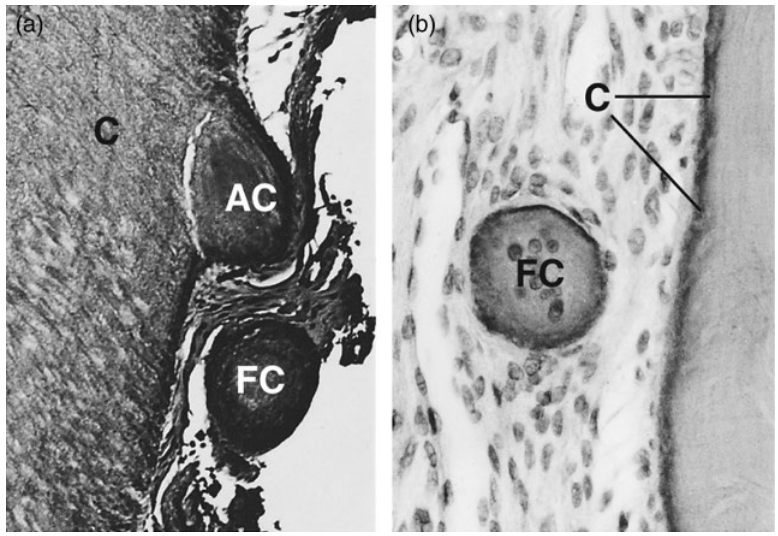


Figure 2.37 (a) Attached (AC) and (b) free cementicles (FC) in the periodontal ligament (C, cementum).

PDL, reliable quantitative measurements of blood flow in the PDL are lacking.

Qualitative measurements of PDL blood have shown that sympathetic vasoconstrictor fibers take part in regulation of PDL blood flow. The constrictor effect is greatly reduced, but never abolished, by alpha-adrenoceptor antagonists, indicating that some of the sympathetic fibers innervating the PDL contain NPY, which has been confirmed by immunohistochemical studies [81, 157] (Figure 2.36). Sympathetic induced vasodilations, due to activation of beta receptors that are most probably located in postcapillary resistance vessels, have also been found in the PDL [1].

Changes in PDL blood flow affect the tooth position, and external forces applied to the tooth crown may greatly influence PDL blood flow [29, 161]. Such changes in the normal conditions may possibly be related to changes in PDL tissue fluid pressure [111]. This pressure has been recorded as relatively high compared to most other tissues [111, 161]. It is claimed to affect tooth position, blood flow, the eruptive force of teeth, and probably pain sensation. In common with the pulp, also the PDL is enclosed in a rigid low-compliance environment between alveolar bone and tooth. Changes in blood volume induced by venous stasis or cardiac arrest are thus rapidly transmitted to the PDL tissue fluid pressure [111].

Unlike the pulp, where the main sensation is pain, the PDL also recognizes touch, pressure, movements, and position of teeth, in addition to pain. A variety of Ruffini-like mechanoreceptor structures are found in the PDL [22]. Some of the fully encapsulated mechanoreceptor structures locate in the interstitial loose connective tissue next to blood vessels. Thus, increased PDL blood flow causing increased tissue pressure would most probably cause excitation of mechanoreceptors. An increased blood volume in the PDL raises the tissue pressure and causes tooth extrusion, whereas decreased blood volume causes tooth intrusion and reduces the tissue pressure [1, 111]. Another aspect of the low compliance in the PDL is that changes in tissue pressure most likely will affect pain sensation, i.e., increased tissue fluid pressure causes increased activity in sensory A-delta and C fibers, much the same way as in the pulp.

2.8.5 Innervation of PDL

Although less innervated than the dental pulp, the PDL is richly supplied with nerves

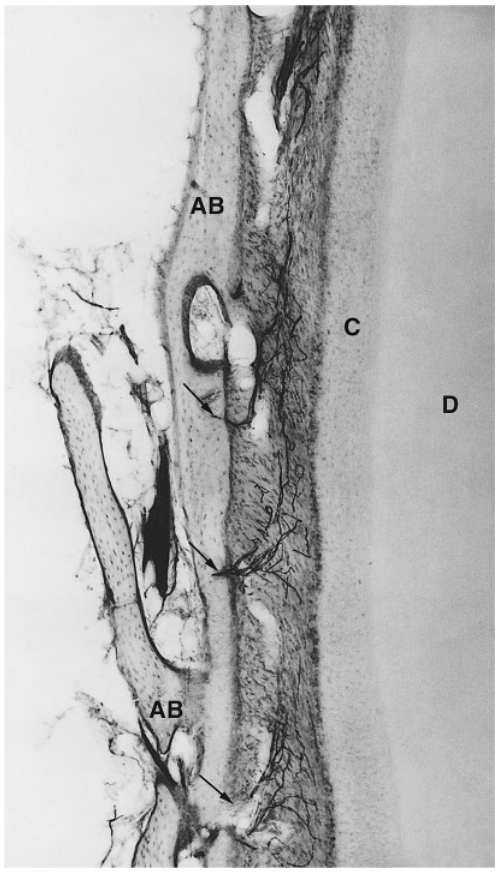


Figure 2.38 Section through the periodontal ligament from apical third of cat canine showing numerous nerve fibers (arrows) approaching the ligament from alveolar bone (AB) (D, dentin; C, cementum). (Reproduced from [81] with permission from *Acta Odontologica Scandinavica*.)

entering both from the apical and lateral alveolar bone (Figures 2.36 and 2.38). The apical part of the ligament is most heavily innervated and the major part seems to be of sensory origin, whereas sympathetic, NPY-carrying fibers are rarely found. However, some larger vessels in the mandibular canal are densely innervated by NPY fibers in their walls [81]. Sprouting of sympathetic NPY-containing nerves may be observed in the inflamed PDL after pulp exposure [79]. The significance of this sprouting is not clearly understood, but it may affect blood flow and immunomodulation. Since peripheral endogenous sympathetic neurotransmitters are important regulators of vascular growth factors [30], it might suggest that sympathetic nerves play a role in revascularization during repair and healing processes in the inflamed PDL.

The periodontal ligament contains myelinated and unmyelinated nerve fibers. CGRP fibers appear more frequently than SP fibers at all levels in the PDL [81]. Most nerves in the PDL localize in the apical third, closely associated with blood vessels. They are frequently observed on the exact border between PDL and cellular cementum, where some form round, coiled, nerve-like endings. Others are closely associated with periodontal epithelial rests of Malassez's cells (Figure 2.36) [81, 122]. In the apical part of cat PDL, immunoreactive cells forming a net-like pattern are regularly displayed in Malassez's epithelial rests surrounded by numerous nerve fibers. Some of the cells located in Malassez's rests contain CGRP and SP. Thus, in common with specialized epithelial cells from other locations [148], the Malassez's epithelium may comprise cells that could be classified as endocrine cells due to their content of neuropeptides. Periapical inflammation and orthodontic tooth movement induce a transient periapical sprouting of sensory axons [25, 108, 214]. Larger axons often form specialized terminals, predominantly in the apical area, described as Ruffini-like endings. Thin axons usually terminate as free nerve endings. Both electrophysiological and histological data suggest that the Ruffini-like terminals as well as free nerve endings may function as mechanoreceptors [122]. Cementum appears not to be innervated [81], but extensive branching of sensory nerves is often found adjacent to the apical cellular cementum, where few blood vessels are located (Figure 2.36).

2.9 Cementum

Cementum is avascular, mineralized connective tissue that covers the root of the tooth. The main function of cementum is to attach

the principal fibers of PDL to the tooth. Generally, the coronal half of the cementum is acellular while the apical part is cellular and has cementocytes embedded in its matrix. The acellular cementum is thin, about 50–200 μm thick [227]. The cellular cementum is thicker and usually multilayered, with individual layers of 10–100 μm thickness [227]. Acellular cementum is important for tooth support while cellular cementum has a role in adjustment of tooth position after eruption.

Cementum-dentin junction (CDJ), a region where cementum attaches to dentin, is a 100–200 μm thick interspace with a 10–50 μm hygroscopic proteoglycan-rich layer [83, 227]. The tight attachment of cementum to dentin at CDJ is mediated by bridges of continuous collagen fibers [83, 208] (Figure 2.39). The hygroscopic CDJ has also been suggested to be a gomphosis, a fibrous joint between cementum and root dentin that is capable of accommodating functional loads in a way similar to that between cementum and alveolar bone [83, 84].

Age-related changes occur in physicochemical properties of cementum, suggesting cementum as adaptive in nature [96]. Cementum hardness increases with age, but the width of the CDJ decreases, as measured from the cementum-enamel junction (CEJ) to the tooth apex [96]. The thickness of the cellular cementum increases with age. Compensatory cementum deposition occurs in the apical area to counterbalance occlusal attrition. Occasionally cementum formation may exceed this physiologic limit and result in hypercementosis, which may affect a single tooth or all the teeth. Local abnormal thickening of the cementum may be found in connection with chronic periapical inflammation. A more generalized hypercementosis may be associated with certain systemic disorders. Cementomas, cement-producing tumors, have also been described. If a root fractures, cementum may form between the root fragments and at the peripheral site of the fracture. If root resorption occurs, repair of the defects by cellular cementum formation may take place.

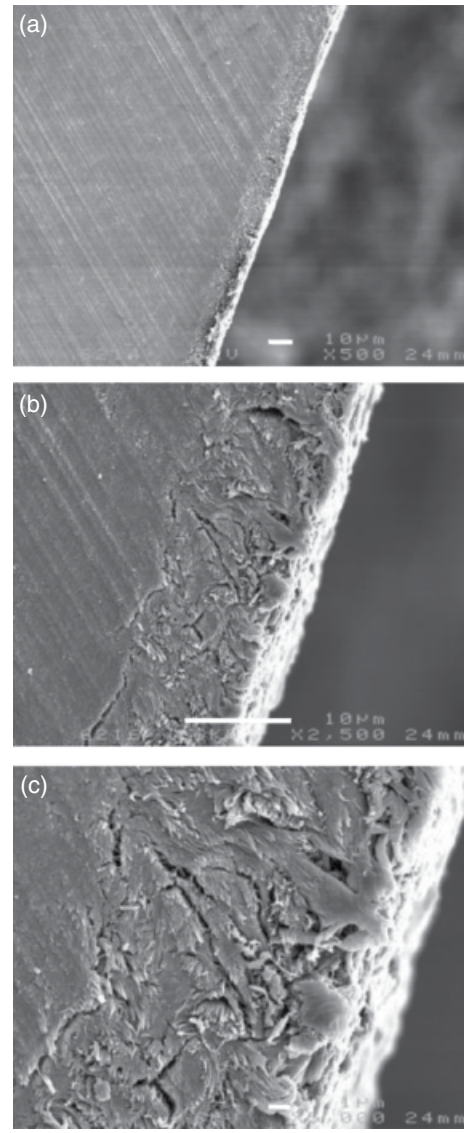


Figure 2.39 FE-SEM images of human cement–dentinal junction (CDJ). (a) 10 to 15 μm cementum layer in intimate contact with dentin. Magnification = 500×; bar = 10 μm. (b,c) Higher magnification demonstrates the mineralized collagen fiber continuity from cementum to underlying dentin. Magnifications = 2,500× (b) and 5,000× (c); Bars = 10 μm (b) and 1 μm (c). (Reproduced from [208] with permission from *Endodontic Topics*.)

Cementicles are mineralized structures that may be found freely residing in the PDL or attached to the root surface (Figure 2.37). They may be formed by mineralization of

degenerating epithelial rests or from thrombosed vessels. Cementicles contain bone sialoprotein (BSP) and osteopontin, two noncollagenous matrix proteins typically found in bone and cementum [17]. When present, cementicles are often found on most teeth.

2.9.1 Cement Structure and Formation

Collagen fibers are major organic components of cementum. Two types of collagen fibers are present in cementum, Sharpey's fibers and matrix fibers. Fine Sharpey's fibers (or extrinsic fibers) represent the termination of the principal fibers of the PDL and penetrate the cementum (Figure 2.35). The matrix fibers are oriented parallel to the root surface and they are interwoven with the Sharpey's fibers. The Sharpey's fibers are formed by the fibroblasts in the PDL while the matrix fibers are formed by the cementoblasts. When the position of the tooth is altered, e.g., during tooth eruption or during orthodontic tooth movement, new attachment of periodontal fibers will take place and the fibers in the cementum orient at different angles to the surface. In cellular cementum, the layers of cellular intrinsic fiber cementum form an alternating lamellar pattern of two types of lamellae, transversely and longitudinally arranged, and this arrangement may be controlled by cementoblasts [227].

The cellular components of cementum include cementoblasts and cementocytes. The cementoblasts, which line the root surface, have all the ultrastructural characteristics of cells capable of synthesizing collagen and protein-polysaccharide complexes. During the formation of cellular cementum at the apical portion of the root, some cementoblasts progress to become cementocytes. The cementocytes are harbored in lacunae, which are embedded in the matrix of the cellular cementum. The functions of cementocytes are not known, but they have recently been compared to osteocytes; cementocytes and osteocytes harbor several commonalities in transition, communication, and specific markers, suggesting that cementocyte may have similar dynamic functions to osteocyte [234].

The ground substance of interfibrillar matrix contains proteoglycans and glycoproteins similarly with other periodontal tissues. Two major constituents are BSP and osteopontin. Collagens have been characterized better than other components in cementum, but there are new developments in the composition of extracellular matrix. Recent mice experiments revealed a novel structural component, Fibulin-4, expressed in dental cementum [180]. Proteomic analysis from mouse dental cementum identified a protein-protein interaction network that includes indicators of metabolic function, possibly reflecting the activity of cementocytes [178].

The precementum is a thin unmineralized layer, which covers the cementum. It prevents, up to a certain point, the root from resorption. If it mineralizes, root resorption may occur.

2.10 Alveolar Bone

The alveolar bone is that part of the alveolar processes of the mandible and maxilla that lines the alveoli for the teeth (Figure 2.40). It has all the features of cortical bone but is further characterized by harboring the Sharpey's fibers from the PDL. Alveolar bone has numerous channels for blood and lymph vessels (Volksmann's canals) from the cancellous bone to the PDL and is therefore often referred to as the cribriform plate. Despite all the channels passing, it appears as a radiopaque line on clinical radiographs (Figure 2.41a), which gives it the name lamina dura. It is an important diagnostic landmark. A breach in its continuity on radiographs may be a sign of resorption, often associated with pulp infection or periodontal disease. However, the degree of mineralization of the alveolar bone is no different from that of the rest of the cortical bone in the

alveolar process (Figure 2.41b). The tangential superimposition of the alveolar bone on radiographs gives the lamina dura its characteristic radiodensity.

The buccal and lingual laminae of the alveolar processes of the mandible and maxilla vary depending on the location within the jaw. The buccal (vestibular) lamina is usually thinner than the lingual (palatal) lamina, except in the mandibular molar region, where the lingual lamina is thinner. This relationship is important to keep in mind when teeth are extracted and for draining abscesses from periapical areas since they tend to follow the path of least resistance. The close relationship between the alveoli and the maxillary sinus (Figure 2.42a) is also of importance during surgical extractions, implant placement, and endodontic procedures. (Figure 2.42b)

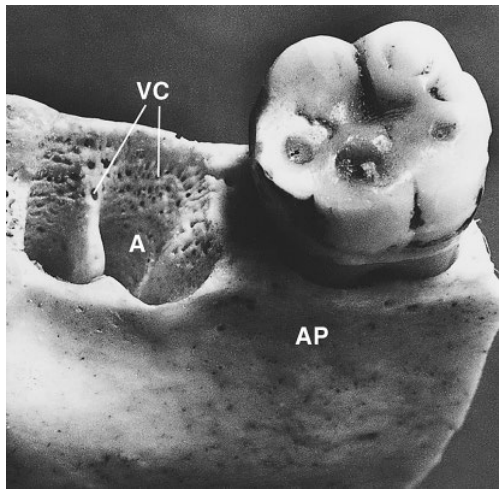


Figure 2.40 Alveolus with Volkmann's canals (VC) perforating the alveolar bone (AP, alveolar process of the mandible). (Reproduced from [140] with permission from Munksgaard.)

2.10.1 Alveolar Bone Structure

The alveolar bone has all the basic characteristics of bone tissue, including osteoblasts, osteocytes, and osteoclasts (Figure 2.43). Its development is closely associated with the presence of teeth. If teeth are lost, the alveolar bone undergoes resorption. If teeth do not erupt, alveolar bone will not develop.

Osteoblasts are matrix-producing cells, with a well-developed Golgi apparatus, granular endoplasmic reticulum, and mitochondria. Osteoblasts that become embedded

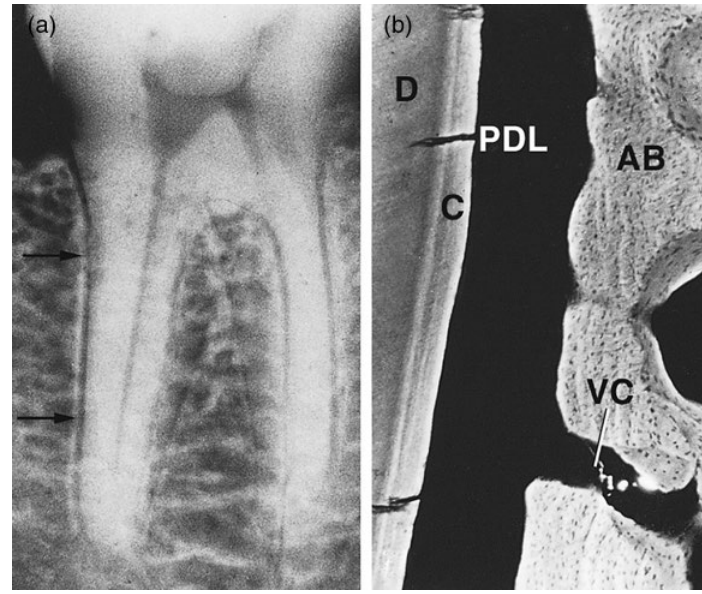


Figure 2.41 (a) Radiograph of a molar tooth showing the radio-dense alveolar bone (arrows) which gives it the name lamina dura. (b) Microradiograph of a ground section showing dentin (D), cementum (C), periodontal ligament space (PDL) and alveolar bone (AB) (VC, Volkmann's canal).

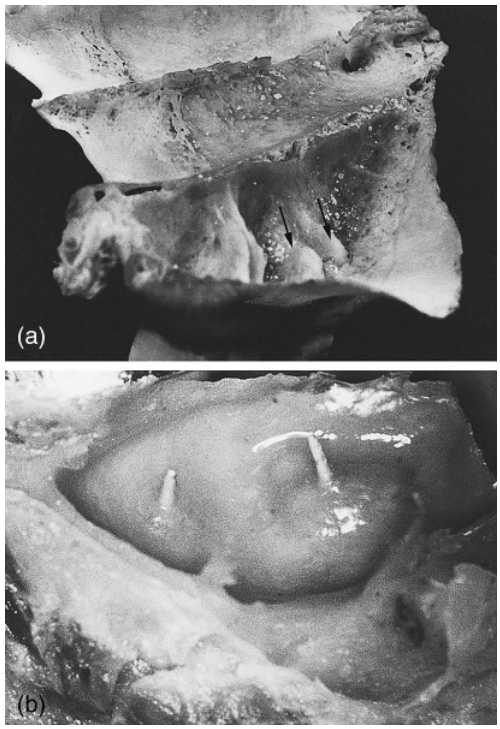


Figure 2.42 (a) A split maxilla at the level of the hard palate showing apices of the roots of the first permanent molar (arrows) extending into the maxillary sinus. (Reproduced from [140] with permission from Munksgaard.) (b) A dissected specimen from a series of experimental endodontic procedures on monkeys showing perforation into the maxillary sinus by gutta-percha points. (Courtesy of Drs D. Ørstavik and I.A. Mjör.)

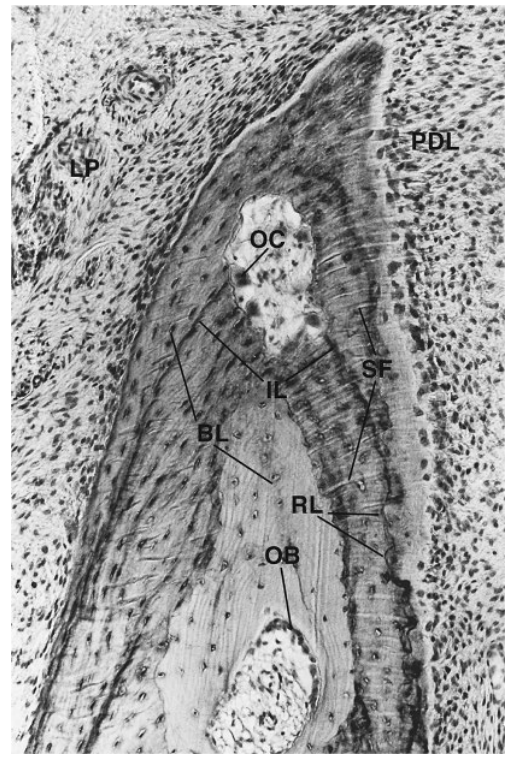


Figure 2.43 Demineralized section showing human alveolar crest and adjacent tissues (PDL, periodontal ligament; OB, osteoblasts; SF, Sharpey's fibers; LP, lamina propria of attached gingiva; BL, bone lacunae with osteocytes; OC osteoclast; IL incremental lines; RL, reversal lines). (Courtesy of Dr K. Reitan.)

in the bone matrix are referred to as osteocytes. They lose many of their organelles, but they maintain cytoplasmic contact with neighboring osteocytes. Osteocytes are significant in controlling responses to mechanical forces and therefore may be central to tooth eruption, physiological and pathological tooth movement, and in orthodontics [21]. Collagen formation may take place in the periosteocytic space between the osteocytes and the wall of the lacunae. Mineralization of the lacunae may occur, resulting in "plugged lacunae." Osteolysis may also occur in the lacunae and it represents a part of the mineral metabolism of bone tissue [224]. However, the main part of the turnover associated with bone remodeling involves osteoclastic activity (Figure 2.43) and new bone formation by osteoblasts.

The normal turnover rate results in bone tissue of different ages in the alveolar processes. Osteons with different degrees of mineralization are present at any given time. Bone lamella, which are the incremental growth lines of bone tissue, are discernible. Osteoid is present anywhere bone formation takes place, but it does not cover fully formed bone. The unmineralized core of the relatively thick Sharpey's fibers inserting into the alveolar bone gives it a striated appearance in microradiographs (Figure 2.34c).

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3

Etiology and Pathogenesis of Pulpitis and Apical Periodontitis

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3.1 Introduction

This chapter will address the etiological factors and the pathological processes involved in the development of pulpitis and apical periodontitis. The sources of microbial irritation of the dental pulp are diverse, and may arise from the coronal (most common), lateral or apical routes. Microbial irritation of the dental pulp has a profound effect on this tissue, often giving rise to severe symptoms, but frequently leading to the asymptomatic degeneration of the tissue. Likewise, apical periodontitis may be asymptomatic, may be associated with severe symptoms, and may be associated with spreading, occasionally life-threatening infections. The exact reasons for the variation of symptoms among cases with seemingly similar pathological changes, and the lack of correlation between symptoms and histological appearance of the dental pulp, are not fully understood. While many neuropeptides and other inflammatory mediators, which are associated with pain, are found in large concentrations in painful pulpitis and apical periodontitis, it is not clear why they increase in some cases. Frequently, the pulp becomes necrotic without a notable association with symptoms.

Diseases of the pulp and periapical tissues result from an inflammation of the respective tissues, ultimately leading to degeneration and necrosis of the pulp, periapical bone resorption, the development of an inflammatory periapical lesion, possibly cystic formation, and possibly severe infections and/or osteomyelitis. In some situations, root resorption of various types may occur, either directly related to a traumatic injury or orthodontic treatment, or insidiously for known or unknown reasons. It is generally accepted that root resorption follows a similar pathogenesis as bone resorption, after the disruption of the cementum that protects the root surface or the predentine internally. All these pathological entities are variants of inflammatory reactions, and are mediated by a wide variety of cytokines, chemokines, neuropeptides, proteases, and other inflammation-associated molecules. The development of neoplastic changes is not known within the pulp, even in response to metastatic affection of the jawbones. In these cases, the periapical bone may be resorbed, but the pulp generally maintains its vitality. This fact is of importance in the clinical differentiation of pulpal disease from other disease that may mimic endodontic pathosis, but does not originate from the dental pulp. It is interesting why the pulp appears to be immune from developing neoplasms, which if present, would have resulted in cases of spontaneous pulp necrosis or neoplastic disease of pulpal origin.

Animal studies have shown that once the pulp is exposed to oral bacteria, pulp necrosis

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and the development of apical periodontitis are rapid, and generally occur within a few weeks in different mammalian models. The bacterial population within the pulp space continues to evolve following necrosis, depending on environmental, compositional and nutritional factors. It is important to note that the pathogenesis of disease and response to treatment may depend on the duration of microbial irritation. Prolonged microbial irritation, in primary infections or following incomplete treatment, affects the spatial distribution of the microbial biofilms and their potential expansion into the complex root canal environment, the dentinal tubules, and potentially the periapical region. The effectiveness of endodontic disinfection decreases in these locations, and therefore, the response to treatment may be slower and less robust, than if the tooth were to be extracted.

3.2 Etiology of Pulpitis and Apical Periodontitis

Microbial infections from carious lesions are the most common etiology for pulpitis and apical periodontitis. In normal conditions, the crowns of erupted teeth are covered by biofilms composed of symbiotic microbial communities. In sugar-rich environments, specific bacterial taxa from these communities release acids that demineralize dentin, resulting in the formation of carious lesions. While *Streptococcus mutans* was considered the singular pathogen in caries for a long time, recent studies suggest that caries is caused by a complex microbiota [39, 128, 149]. Carious lesions limited to the enamel induce subtle changes in the pulp such as accumulation of MHC Class II antigen-expressing cells. Once the caries biofilm destroys enamel and reaches dentin, it induces further inflammatory changes in the pulp [90, 91, 177, 232–234].

The pulp may also be directly exposed to microorganisms and their byproducts by routes other than carious lesions, for example in cases of traumatic injuries resulting in complicated tooth fractures or in situations of iatrogenic pulp exposures. The role of periodontal disease in causing pulpal inflammation and necrosis remains controversial [81, 152]. Some studies suggest that periodontal disease induces the influx of inflammatory cells and pulpal calcifications [115, 184]. However, total necrosis of the pulp was only noted in teeth where all the main apical foramina were infected with bacterial plaque. A preclinical study in which marginal periodontitis was experimentally induced reported mild pathological pulpal changes in 57% of teeth with periodontitis [13]. Pulpal necrosis was noted in only one tooth out of the 92 teeth in the experimental group. Other studies suggest that periodontal disease does not induce changes in the pulp [137].

One of the seminal studies on the role of bacterial infection in pulpal and periapical disease compared the outcomes of pulpal exposures between germ-free and normal rats [98]. In the gnotobiotic rats, the pulps remained vital and the apical tissues remained normal during the study period. In this group of rats, formation of complete dentinal bridges was noted by 28 days after the pulpal exposure. In contrast, the normal rats developed pulpal necrosis early in the study period, which was followed by the development of periapical abscesses and granulomas. Another seminal study on infections of the root canal system included one that examined the association between different taxa. In this primate study, the root canal systems of teeth were infected with different combinations of bacterial species. This was the first study to show that taxa form multispecies communities in periapical tissues which elicit stronger inflammatory responses when combined [50]. These findings that pulpal and periapical diseases are caused by polymicrobial infections that form biofilms are supported by several other studies [12, 50, 165, 208].

The development of sophisticated molecular tools have further improved our understanding of the polymicrobial nature of pulpal and periapical infections [59]. For example, a clinical observational study examined samples collected from the deepest layer of

the dentinal caries lesions associated with pulpal exposures in teeth with symptomatic irreversible pulpitis (N=10) [170]. Half of the advanced caries lesions were highly dominated by lactobacilli. In the remainder, the most dominant genera were *Pseudoramibacter*, *Olsenella*, *Streptococcus* and *Stenotrophomonas*. Conversely, other studies have reported *Prevotella* as the dominant genus in deep dentinal caries [29, 106, 133, 182]. These differences may be due to methodological issues or may be related to geographical differences in the composition of the oral microbiota [11].

The integration of data sets from culture and molecular studies show that over 460 unique bacterial taxa belonging to 100 genera and nine phyla have been identified in endodontic infections. The predominant phyla include Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria [191]. These studies also show that the microbial diversity in endodontic infections is not limited to bacteria; instead, it also includes fungi, archaea [219, 220], and viruses [28, 47, 82, 120, 158].

While microbial infection is the predominant etiology for pulpal inflammation, procedures such as cavity preparation and restorations can also adversely affect the pulp. Enamel and dentin are among the hardest tissues in the body and the heat generated during cavity preparation can potentially induce inflammation of the pulp [116, 183, 197]. Most current restorative material do not adversely affect the pulp as long as the tissue is not directly exposed to them. However, microleakage around restorations can induce pulpal inflammation [73].

3.3 Inflammation Versus Infection of the Pulp and Periapical Tissues

3.3.1 Bacterial Irritation and Invasion of the Dental Pulp

Coronal sources of pulpal irritation such as caries, coronal cracks and fractures, attrition, abrasion erosion, or congenital defects in the crown lead to pulp inflammation. Even early pit and fissure caries is associated with inflammation that corresponds with the pulpal extent of the affected dentinal tubules [20]. Likewise, exposed root surface that does not have cementum coverage allows bacterial invasion of the external surface of dentinal tubules and result in mild inflammation in the corresponding area of the pulp [114]. In these cases, the bacteria may not be physically present in the pulp, but secrete enzymes, toxins or cell wall molecules that travel through the tubules and cause pulpal irritation. As the bacterial irritation approaches the pulp, the inflammation increases in intensity in the zone closest to the irritants, and eventually progresses to distant areas of the pulp. The degree of pulpal inflammation correlates with the amount of bacterial irritants and their proximity to the pulp [140, 165]. Eventually, bacterial infection reaches the pulp and progresses through the vital pulp, leading to pulpal degeneration and initiating periapical inflammation. The same process of inflammation followed by potential infection eventually occurs in the periapical tissues, particularly after complete pulp necrosis. The apical infection may result in an acute or chronic abscess formation, particularly when virulent bacteria reach the periapical region in sufficient numbers. Therefore, both the pulp and the periapical tissues undergo a stage of inflammatory changes, before becoming physically infected by bacteria. One of the main aim of the diagnostic process clinically is to determine whether the diseased pulp and periapex are inflamed or infected, because this would impact anesthetic considerations, emergency and definitive treatment, as well as the determination of prognosis.

Studies have shown that when exposed to oral bacteria, pulpal inflammation and eventual degeneration are generally mediated by innate immunity. The evidence for this comes from murine models, where it was shown that the rate of pulp necrosis is similar in animals deficient in adaptive immune cells, such as T- and B-cells, and normal animals

[55, 222]. However, adaptive immunity, particularly B-cells and immunoglobulins, are protective against severe infections caused by large numbers of pathogenic bacteria [212].

Potent bacterial molecules such as bacterial peptidoglycan, lipopolysaccharide (LPS) (also known as endotoxin) from Gram-negative bacteria, and lipoteichoic acid (LTA) from Gram-positive bacteria are capable of inducing significant pulpal and periapical inflammation. For example, animal studies have shown that LPS placement in otherwise sterile root canals induces periapical lesions [34, 45]. LPS is a potent mediator of bone resorption [23], that acts via activation of the arachidonic acid pathway, cytokine production, and complement activation [56]. Moreover, LPS has been shown to activate TLR4 receptors on trigeminal nociceptors [221], and is clinically associated with pain when present in carious lesions [104] and infected pulp [84, 92, 134]. However, LPS is by no means the only bacterial irritant involved in the pathogenesis of pulpitis and apical periodontitis. Studies that examined the progression of pulpal and apical disease in LPS hyporesponsive models showed comparable overall progression of the disease to normal animals, indicating the LPS, peptidoglycan, bacterial proteinases, toxins, and other virulence factors may contribute to the process [60].

Clinically, periapical bone loss is usually visible radiographically when the pulp is necrotic. This is a surprising finding, as periapical inflammation is seen histologically in animal studies during the period of pulp inflammation. Indeed, it has been shown that the clinical phenomenon is related to the low sensitivity of the periapical radiograph in detecting early apical lesions, and that more sensitive techniques, such as cone beam computed tomography (CBCT) is significantly more sensitive than periapical radiography in detecting apical periodontitis in cases with pulpitis [1]. Therefore, the pulp does not have to be totally necrotic for periapical bone resorption, and the formation of a periapical lesion to develop.

3.4 The Dental Pulp

3.4.1 The Defense System in the Dental Pulp

The dental pulp is well equipped to detect invading pathogens and to mount an immune response to them. As with all other tissues in the body, the defense system of the pulp can be classified into the innate and the adaptive immune responses. The innate responses are not antigen-specific and include the outward flow of dentinal fluid and the deposition of intratubular immunoglobulins [130, 136, 155]. It also includes the resident cells (such as odontoblasts and fibroblasts) and innate immune cells (such as dendritic cells, natural killer cells and T cells). Odontoblasts are found at the interface of the pulp-dentin junction and have processes which extent into dentinal tubules. While the primary function of odontoblasts is to produce the predentin matrix during tooth formation and to control its mineralization, they also play an important role in the immune response of the pulp. It has been hypothesized that they represent the first biologically active line of defense for the pulp and, that their role is similar to that of the skin and mucosal epithelial cells. Odontoblasts express Toll-like receptors (TLRs), a class of pattern recognition receptors. Specifically, they express TLRs-1, 2, 4, 6 and 9 [44, 95, 217]. Stimulation of these TLRs activates the MyD88 dependent pathway which triggers NF-ḱB and MAPK activation, resulting in the expression of pro-inflammatory and chemokine genes such as CCL2, CCL5, CCL7, CXCL8, and CXCL10 [199]. Odontoblasts also express nucleotide-binding-domain leucine-rich repeat (NLR)-family proteins such as NOD2, which recognize peptidoglycan components common to both gram-positive and gram-negative bacteria [198]. Activated odontoblasts release molecules such as nitric oxide (NO), beta defensins (BD), and liposaccharide binding protein (LBP), IL-6, CXCL1, CXCL 2, CXCL 8, CCL2 and IL-10 [41, 42, 44, 111, 154, 217]. Some of these diffuse through the dentin towards the carious lesion to

destroy the pathogens (such as NO and BD) or to reduce their pathogenicity (such as LBP). Others diffuse into the pulp where they activate and mobilize immune cells [44] (Figure 3.1).

Fibroblasts also play a role in the immune defense of the pulp, which is in addition to their role in the synthesis and turnover of the extracellular matrix. They express several pattern recognition receptors such as TLRs-2, 4, 5, and NOD1,2 [83, 103, 126, 199]. Once activated they release mediators such as TNFα, CXCL8, and others [102]. An interesting study compared the responses of fibroblasts and odontoblast-like cells to various TLR agonists [199]. Lipoteichoic acid (LTA), a TLR2 specific agonist, induced upregulation of CXCL-2 and -10 in odontoblast-like cells but not in fibroblasts. While LPS, a TLR4 specific agonist, induced expression of CCL7, CCL26 and CXCL11 in fibroblasts but not in odontoblast-like cells, thus suggesting that odontoblasts and fibroblasts mount specific immune responses by differentially influencing the various immune cells in the pulp.

Resident immune cells in the pulp include leucocytes, mononuclear phagocytes dendritic cells, and natural killer (NK) cells [71]. These cells are continuously sampling their environment to detect invading pathogens. Once a pathogen is detected the number of immune cells increases dramatically. Neutrophils are among the earliest cells, which are recruited followed by monocytes, which differentiate into macrophages [32, 80, 90, 131]. Dendritic cells and NK cells also accumulate at the site of infection where they capture bacterial antigens or destroy the invading pathogen [44, 96, 101, 129, 233]. Interactions between dendritic cells and NK cells result in reciprocal activation and increased cytokine production by both cell types. These innate immune cells also activate the adaptive immune response [107, 224]. Dendritic cells migrate to regional lymph nodes where they present antigens to and activate CD4+ T cells (aka Th0 cells) [17].

In addition to the innate immune responses described above, the nerves innervating the pulp also detect and respond to pathogens. An elegant study using human and rat trigeminal neurons as well as rat dental pulps demonstrated the expression of CD14 and TLR4 in nerves innervating the pulp [221]. Subsequent studies demonstrated that LPS sensitizes the ion channel TRPV1 via activation of TLR4 in trigeminal sensory neurons [40, 52]. These studies along with earlier ones on the neuronal responses to pulpal exposures provide further support of the role of neurogenic inflammation in pulpitis [105, 108, 211].

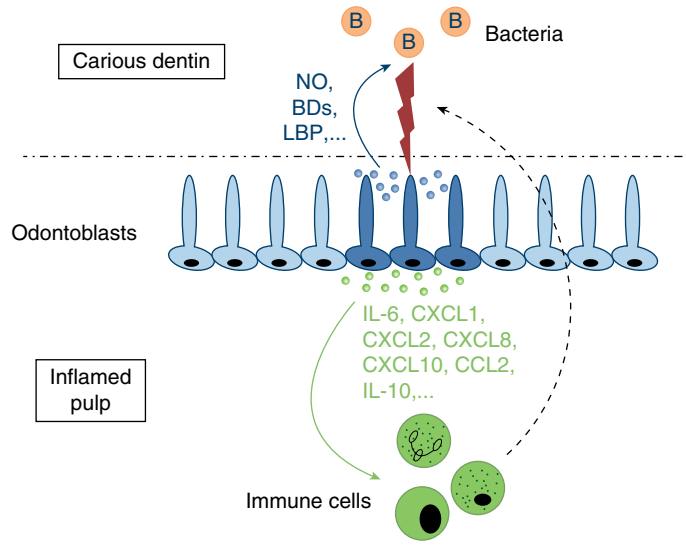

Figure 3.1 Illustration of the two key aspects of the defense mechanism mounted by odontoblasts. In response to bacteria (B) in carious dentin, odontoblasts (blue) release mediators such as nitric oxide (NO), beta defensins (BDs), liposaccharide binding protein (LBP), IL-6, CXCL1, CXCL 2, CXCL 8, CCL2 and Il-10. Some of these mediators diffuse through the dentin towards the carious lesion to destroy the pathogens or to reduce their pathogenicity. Others diffuse into the pulp where they activate and mobilize immune cells. (Reproduced with permission from [51].)

3.4.1.1 Adaptive Immunity

As mentioned earlier, dendritic cells activate CD4+ T cells in lymph nodes. The latter then differentiate into effector CD+4 helper cells or induced regulatory T cells (Figure 3.2). As the carious lesion progresses toward the pulp, there is an increase in the accumulation of T cells [50, 79, 91]. Healthy pulps contain a small number of B-cells, which increase in number with caries progression and inflammation [71, 79]. The predominant B-cells derived immunoglobulins in inflamed pulps are IgG1 followed by IgA and IgE [30, 80].

3.4.2 Classification of Pulpitis

The most commonly accepted diagnostic classifications are based on our current understanding on treatment prognosis. Diagnosis is based on the patients' symptoms (presence, duration, severity, and type), the presence of caries or restorations, the response to pulp sensibility tests, and the clinical and radiographic exams. A normal pulp is one in which the tooth is asymptomatic, responds to all clinical tests (palpation, percussion, cold, or electrical stimuli) within normal limits and has a normal radiographic appearance. Normal pulps may contain calcifications.

Inflammation of the pulp is commonly categorized into reversible and irreversible pulpitis. The former refers to a state of mild inflammation that can be "reversed". In other words, the pulp is capable of healing if the appropriate therapy, such as removal of an irritant, is performed. The etiologies for reversible pulpitis include caries, trauma and a recent or defective restoration. Clinical exams reveal absence of palpation and percussion sensitivity, a brief, non-lingering response to thermal stimuli, and occasionally pain on biting. The radiographic appearance of teeth with reversible pulpitis is normal.

When pulpal inflammation is more severe, and the prognosis is that it is unlikely to revert to a normal pulp, the pulpal state is classified as "irreversible pulpitis". These teeth are often symptomatic and are associated with spontaneous pain and/ or pain that lingers after the removal of a thermal stimulus. The teeth may be sensitive to percussion and often have a normal radiographic appearance.

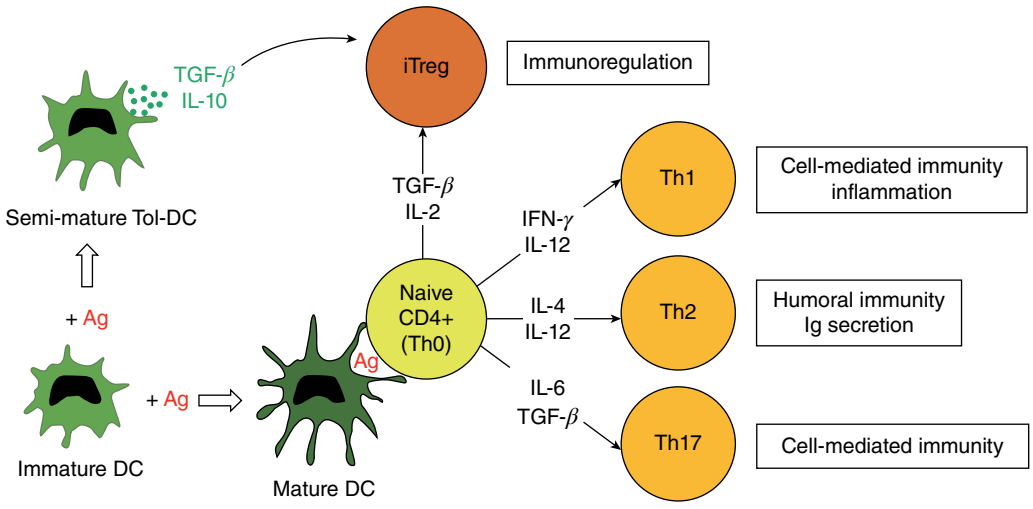

Figure 3.2 Illustration of the role of dendritic cells (DCs) in activating the adaptive immune response. Immature DCs usually become mature DCs after encountering antigens (Ag). Mature DCs present antigens to naïve CD4+ cells which then clonally expand and differentiate into effector T-helper cells (Th1, Th2 or Th7) or into induced regulatory T cells (iT-reg). Alternatively, immature DC mature partially to form Tolerogenic-DCs (Tol-DC) which in turn induce iT-reg cell differentiation. TGF – Transforming Growth Factor; IL – interleukin; IFN – interferon, Ig – immunoglobulin. (Reproduced with permission from [51].)

Etiological factors include deep caries, deep restorations, and cracks. These pulps are subclassified as "symptomatic irreversible pulpitis". In contrast, some teeth with carious exposures or trauma present with no symptoms. Based on our current understanding, these inflamed pulps have a poor prognosis and are expected to become necrotic. These pulps are subclassified as "asymptomatic irreversible pulpitis".

At this point there is inadequate evidence to support that irreversible pulpitis is truly irreversible. Based purely on patients' pain history and the diagnostic tools currently available, it is not possible to determine whether the degenerative inflammatory process involves the entire pulp or whether it is limited to only a part of the pulp. As described later in this chapter, the histological evaluation of human pulps clinically diagnosed as "irreversibly" inflamed noted that in some teeth the pulp tissue in one, but not all, pulp horns was inflamed [167]. With the development of new pulp-capping materials, it now appears that partial or full pulpotomy is a viable treatment option and that extirpation of the entire pulp may not be necessary in teeth with irreversible pulpitis [8, 48].

3.4.3 Histological and Molecular Evaluation of Pulpitis

As mentioned earlier, multiple cells in the pulp express pathogen recognition receptors and respond rapidly to microbial infection. A recent study examined the histological and molecular response to pulpal injury and infection by exposing rat pulps to PBS or LPS [166]. The tissues were examined at 3 hours, 9 hours, and 3 days after the exposure. Pulps in intact teeth were used as controls. Infiltration by inflammatory cells was seen as early as 9 hours after exposure and an osteodentine matrix was noted 3 days after the exposure. As compared to pulps exposed to PBS, the area of the pulp infiltrated by inflammatory cells and the osteodentine deposition was larger in pulps exposed to LPS (Figure 3.3). Similar changes were noted with the expression of dentin sialophosphoprotein. Flow cytometry analysis revealed an increase of leukocytes and dendritic cells, while the percentage of T cells and NK cells remained unchanged. The same study also examined the expression of inflammatory genes. Treatment with LPS increased expression of IL-6, IL-1 β, IL-10, TNFα, iNOS, CCL2, CXCL1, and CXCL2 at the 3-hour time point. MMP3 was upregulated at 9 hours and 3 days after exposure to LPS.

A clinical observational study examined the histologic status of pulps clinically diagnosed as being irreversibly inflamed (n=32), reversibly inflamed (n=59) and normal (n=4) [167]. A majority (84.4%) of pulps diagnosed as being irreversibly inflamed displayed areas of coagulation or liquefaction necrosis with bacterial colonization and severe infiltration by leucocytes in parts of the coronal pulp. The inflammatory/immune reactions were much less severe in what remained of the coronal pulp and in some teeth; normal uninflamed tissues were noted in the contralateral pulp horns (Figure 3.4).

The remainder of the pulps diagnosed as irreversibly inflamed (15.6%) displayed localized accumulation of inflammatory cells with no tissue necrosis and no bacterial infections – a histological diagnosis consistent with reversible pulpitis. In almost all (96.6%) the pulps diagnosed with reversible pulpitis, the histological diagnosis was consistent with the clinical diagnosis. A mild to moderate accumulation of chronic inflammatory cells and formation of tertiary dentin was noted in these pulps.

Another clinical observational study examined gene expression in pulpitis patients presenting with mild to severe pain as well as in normal teeth [67]. Genes involved in immune response, cytokine-cytokine receptor interaction and signaling, integrin cell surface interactions, and others were expressed at relatively higher levels in the pulpitis group as compared to normal pulps (Figures 3.5 and 3.6).

Moreover, several genes known to modulate pain and inflammation showed differential

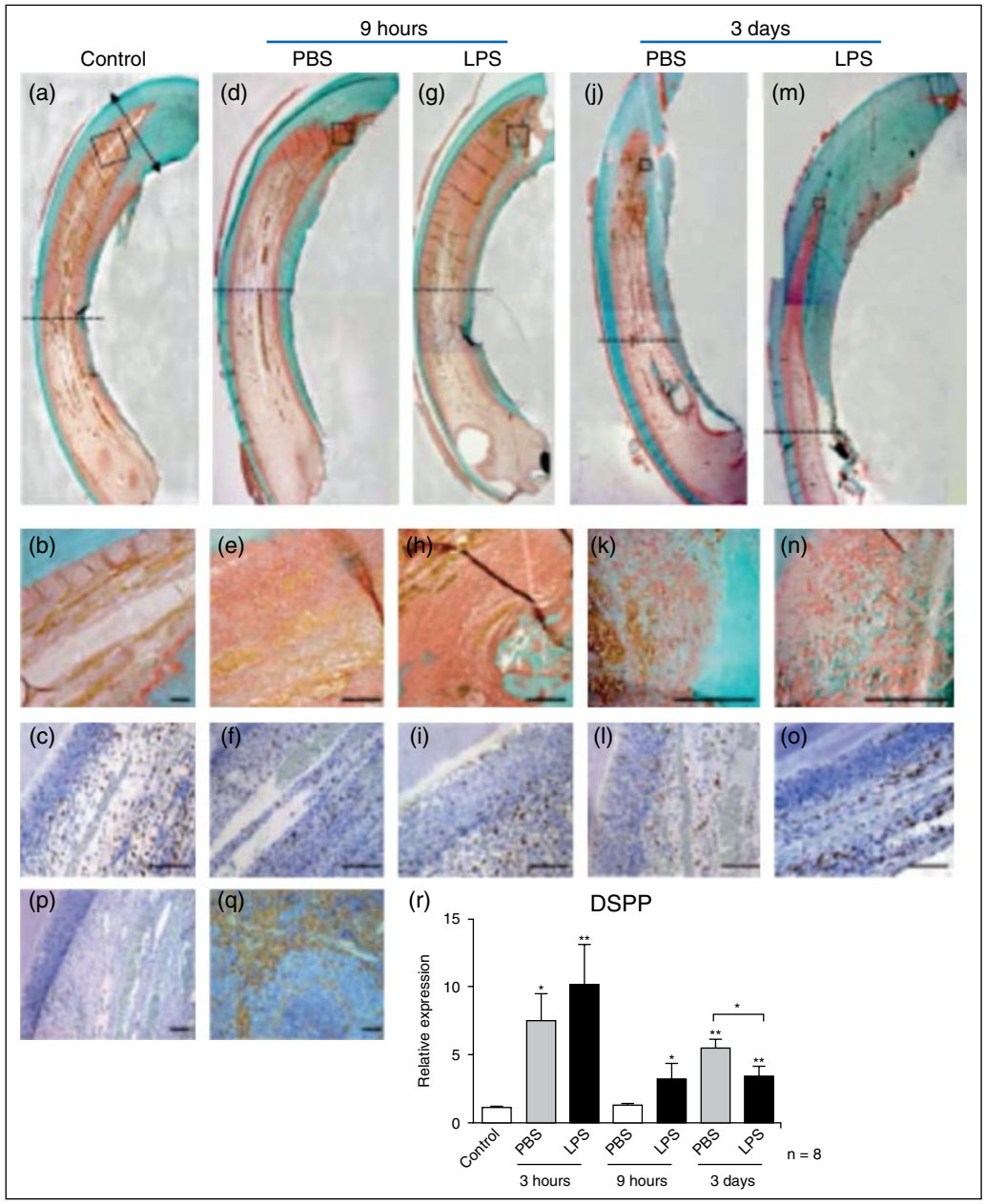

Figure 3.3 Masson trichrome staining and immunohistochemical analysis of rat pulps exposed to LPS or PBS. Unexposed pulps were used as controls. The arrow in panel (a) show the site at which the tooth was amputated, and pulp exposed. As compared to PBS (d: low magnification; e: higher magnification), inflammatory exudate was greater after exposure to LPS (g: low magnification; h: higher magnification). Wound healing with deposition of a collagenous matrix (j: low magnification; k higher magnification) was noted by day 3 in the PBS group. Expression of dentin sialophosphoprotein (DSPP) increased at the 3-hour and 9-hour time points (r). (Reproduced with permission from [166].)

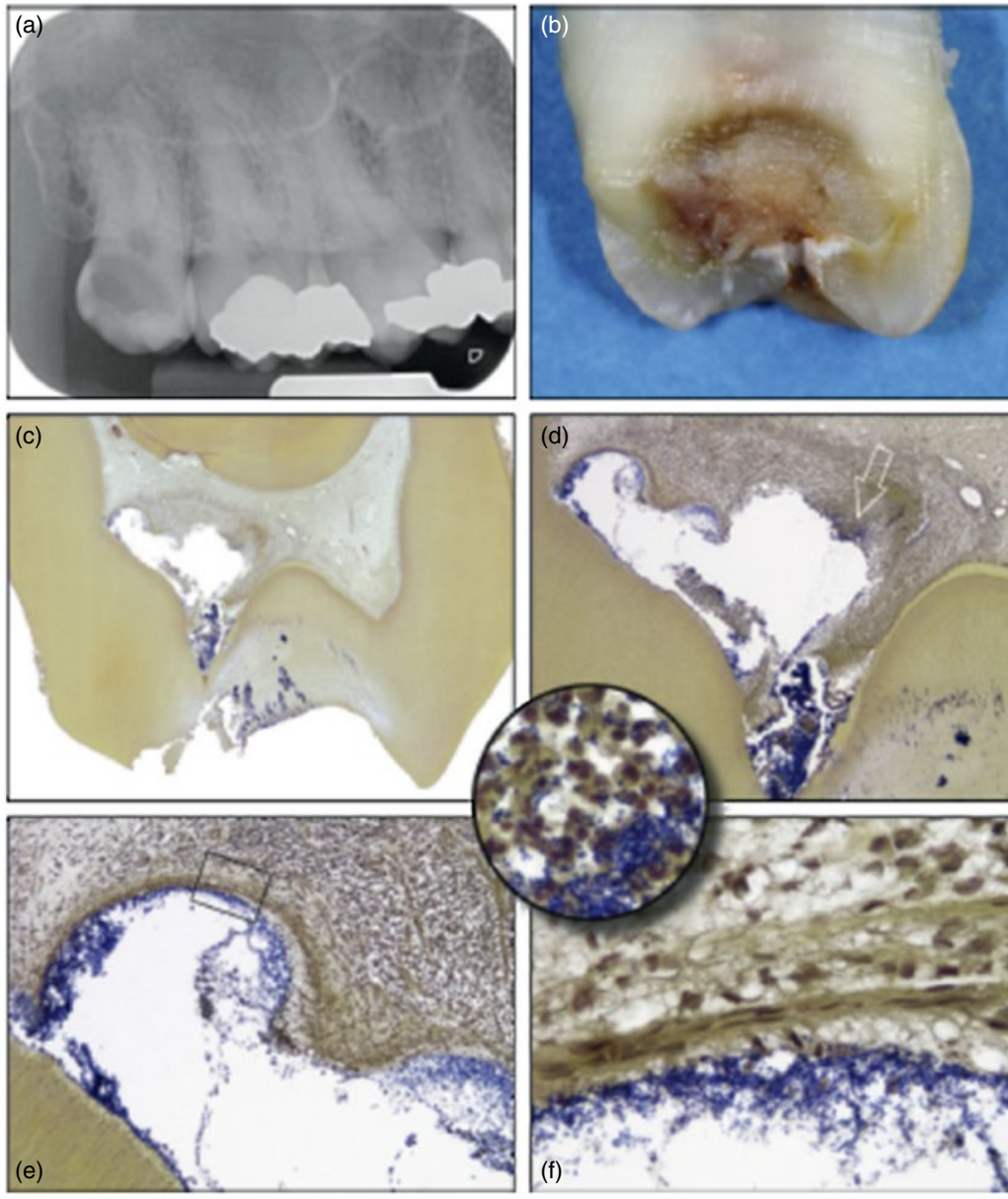

Figure 3.4 Histological analysis of a tooth clinically diagnosed with irreversible pulpitis. The patient, a 30-year-old man presented with severe pain. (a) Periapical radiograph of the tooth. (b) Preparation of a bucco-lingual plane for sectioning. (c) An overview of the pulp chamber showing the abscess. Note that the contralateral pulp horn appears normal. (d) Magnified view of the abscess. Note necrotic debris (original magnification 16×). (e) Partial view of the abscess (original magnification 40×). (f) Higher magnification of the rectangular area in E (original magnification 400×). Inset A high power view (original magnification 400×) of the abscess indicated by the arrow in D. Note accumulation of PMNs and bacteria. (Reproduced with permission from [167].)

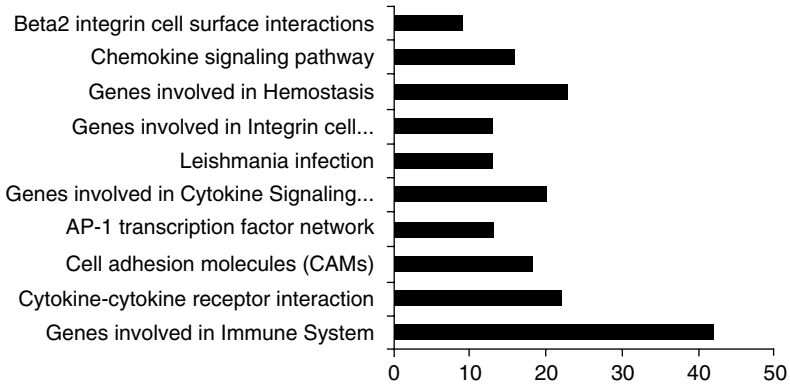

Figure 3.5 Gene Set Enrichment Analysis results between pulpitis and normal samples. Inflamed pulps were collected from patients diagnosed with irreversible pulpitis (n=20). Normal pulps from teeth extracted for various reasons served as controls (n=20). Genome-wide microarray analysis was performed using Affymetrix GeneTitan Multichannel Instrument. Each bar represents the functional categories and the number of significantly regulated genes between pulpitis and normal groups (q<0.05). (Reproduced with permission from [67].)

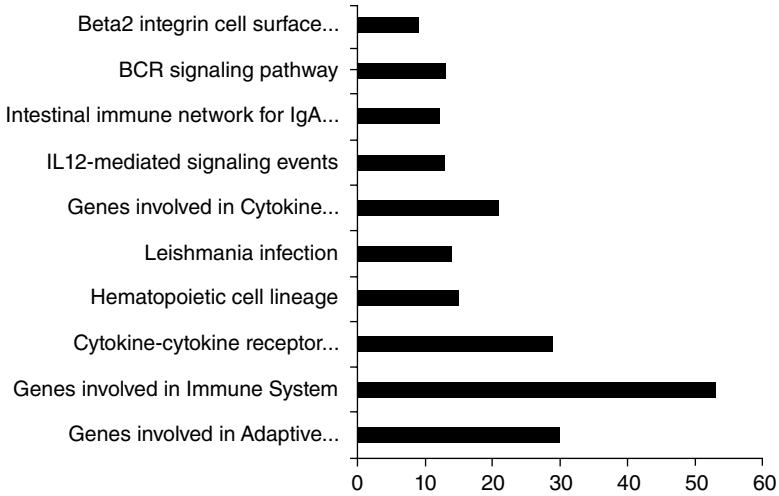

Figure 3.6 Gene Set Enrichment Analysis results between groups that reported none to mild pain (≥30mm on VAS) and those that reported moderate to severe pain on VAS associated with pulpitis. Samples were collected and analyzed as described in Figure 3.5. Each bar represents the functional categories and the number of significantly regulated genes between none to mild and moderate to severe pain groups (q<0.05). (Reproduced with permission from [67].)

expression in asymptomatic and mild pain patients (≥30mm on the Visual Analog Scale) compared to those with moderate to severe pain (Table 3.1).

A limited number of clinical studies have examined molecular markers in pulpal blood at the protein levels. The immunoglobulins IgG, IgA, Ig M as well as elastase and PGE_2 are elevated in inflamed pulp as compared to healthy pulps [2]. Expression of the cytokine IL-8 is increased in inflamed symptomatic pulps as compared to normal pulps and asymptomatic pulps with caries exposure [49]. The content of the dentinal fluid may also be reflective of molecular changes in the pulp. An interesting clinical study compared the expression of MMP9 in dentinal fluids collected from teeth diagnosed with irreversible

Table 3.1 Selected up-regulated or down-regulated genes in pulpitis patients. Black – down-regulated; Red – upregulated; Green – no difference.

Gene symbol	Pulpitis vs normal	None to mild vs moderate to severe pain	Gene function (genecards.org)
AMELX			Enamel biomineralization
CALCRL			Bone metabolism
CCL20			Chemotaxis
CD14			Innate immune response
CD163			Acute-phase receptor
CD79A			B-cell receptor function
COL10A1			Collagen formation
COL11A2			Collagen formation
COL12A1			Collagen formation
COL14A1			Collagen formation
COL15A1			Collagen formation
COL15A1			Collagen formation
COL18A1			Collagen formation
COL1A1			Collagen formation
COL1A2			Collagen formation
COL21A1			Collagen formation
COL4A1			Collagen formation
COL4A2			Collagen formation
CXCL3			Chemotaxis
DEFA1B/1A			Antimicrobial activity
DEFA3			Antimicrobial activity
DSPP			Dentin formation
IL10RA			IL-10 signaling
IL1A			Inflammatory response
IL1B			Inflammatory response
IL6			Inflammation, B-cell maturation
IL8			Chemotaxis
LBP			Innate immune response
MMP13			Collagen degradation
MMP20			Amelogenin degradation
MMP9			Collagen degradation
NOD2			Innate immune response
NR5A2			Antiviral activity
PTGS2			Prostaglandin formation
SCN8A			Sodium ion permeability
TLR1			Innate immune response
TLR2			Innate immune response
TLR3			Innate immune response
TLR4			Innate immune response
TLR6			Innate immune response
TLR8			Innate immune response
TLR9			Innate immune response
TNFA			Inflammatory response

pulpitis (n=19) as compared to normal controls (n=12) [235]. MMP9 is a proteolytic enzyme secreted by neutrophils and may be a marker of tissue destruction. The data show a clear increase in levels of MMP9 in the symptomatic teeth as compared to controls.

The role of epigenetic modifications in pulpitis is only just beginning to be explored. Epigenetic modifications include DNA methylation, modifications of histones and regulation of non-coding RNAs. DNA methylation in pulpal inflammation has only been examined in two studies to date. One of these was on the methylation pattern of the IFN γ and noted partial methylation or unmethylation in samples of inflamed human pulps [25]. Another study reported no difference in the methylation of CD14 and TLR2 in normal and inflamed pulps [24]. Recent studies suggest that histone methylation on H3K27 plays a role in pulpal inflammation and reparative processes [89, 230].

MicroRNAs (miRs) are small (18–22 nucleotides), single-stranded, non-coding RNA oligonucleotides. Our lab was the first to report on miR expression in inflamed pulps [236]. Inflamed pulps were from carious teeth diagnosed with symptomatic or asymptomatic irreversible pulpitis (n=18). Normal pulps were extirpated from healthy third molars or teeth extracted for orthodontic purpose (n=12). As compared to normal pulps, 36 human miRs were dysregulated in inflamed pulps. +In a separate *in vitro* study, we demonstrated that one of the microRNAs differentially expressed in pulpitis- miR181a modulates expression of the cytokine IL-8 [68], thus demonstrating the role of microRNA in modulating the immune response to microbial infections of the pulp.

3.5 The Periapical Tissues

3.5.1 The Defense System in the Periapical Tissues

As noted before, periapical bone resorption and the formation of a periapical lesion start before the pulp is totally necrotic. As the irritants progress within the pulp, it is important that apical bone resorbs and is replaced by a soft tissue, which can mount a formidable immune response. The essential function of the periapical lesion is to defend the body against advancing bacteria from the degenerating pulp. In so doing, the periapical lesion defends against two serious diseases: acute spreading infections and osteomyelitis (Figure 3.7).

While these conditions may occur with some frequency, their incidence and severity are certainly modulated by the apical immune response in most cases. The development of an apical lesion that can be observed radiographically in patients takes a few weeks to months. In animal models the size of the

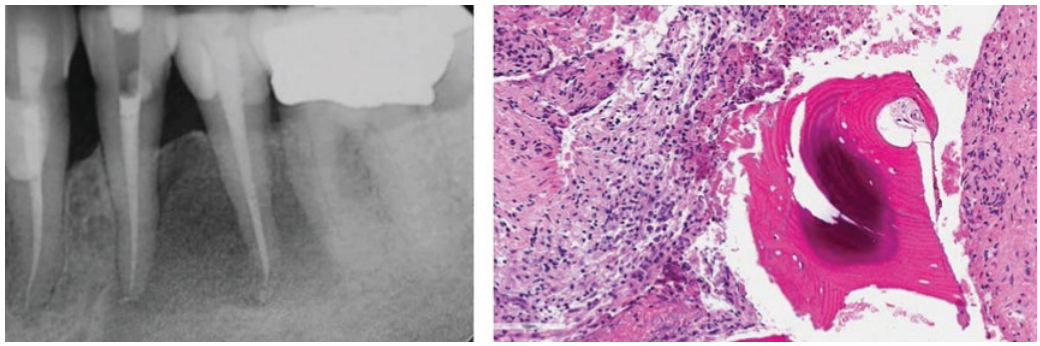

Figure 3.7 Radiograph is of a patient who had non-surgical endodontic treatments on the mandibular left canine and first and second premolars. The molar was normally responsive. The patient started experiencing numbness in the area, and root-end surgery was performed on the premolars. The biopsy report showed intense inflammatory response and multiple bone fragments with empty lacunae. The diagnosis was osteomyelitis.

lesion increases steadily and then plateaus as the bacterial irritation and the defense mechanisms reach a stable stage [64]. In some cases, periapical lesions can reach very large sizes (Figure 3.8). It is thought that the size of the lesion may be related to the types and numbers of bacterial irritants, the development of an apical cyst, and the types and concentrations of inflammatory mediators within the lesion. The available evidence suggests that the ultimate size of the lesion is a regulated mechanism that is related to a large number of microbial and host factors [200].

3.5.1.1 Acute Infections

Acute endodontic infections represent about 56% of all non-traumatic dental emergencies [163]. In the U.S., endodontic infections result in over 400,000 emergency room outpatient visits per year [148]. Endodontic abscesses result in about 8,000 hospitalizations per year [5], was the cause of death for 66 hospitalized patients over a period of 8 years, according to one study [185]. Given that in the U.S., 27–41% of the adult population have at least one tooth with apical periodontitis [22], it is evident that despite the large numbers, severe, life-threatening endodontic infections affect a small number of cases. The more common clinical presentation for symptomatic cases, are ones where there is symptomatic apical periodontitis associated with symptomatic irreversible pulpitis or with necrotic pulp, or acute apical abscesses with localized swelling, lymphadenopathy and minimal constitutional symptoms. According to one study, 57% of all cases of symptomatic irreversible pulpitis are associated with symptomatic apical periodontitis [151]. Conversely, it was shown that about 40% of cases with pulp necrosis and chronic apical periodontitis develop with minimal pain [139].

It is not clear what specific factors are involved in the development of symptomatic apical periodontitis or severe endodontic infections. Numerous studies have explored the possibility that specific virulent endodontic pathogens may be associated with endodontic infections. For example, several studies have shown the association of *Fusobacterium nucleatum* and *Parvimonas micra* [27, 61, 178], and black-pigmented bacteria belonging to the

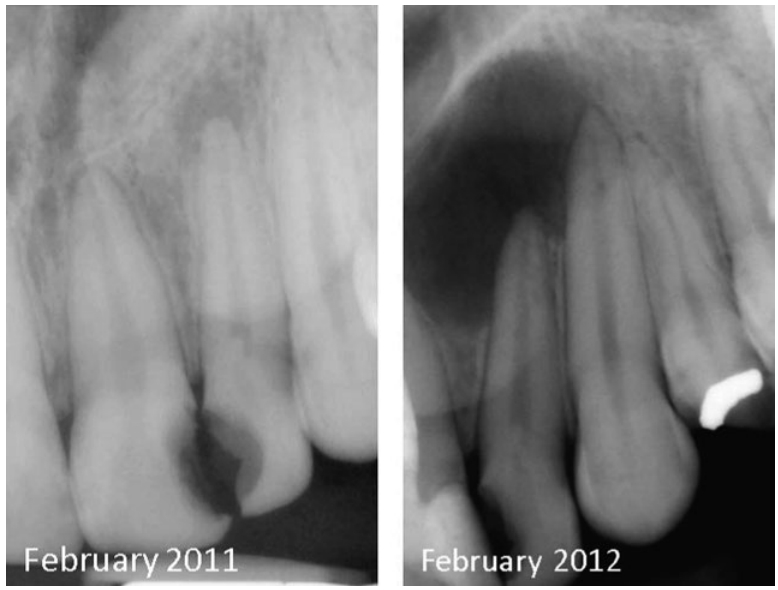

Figure 3.8 Teenage patient diagnosed with pulp necrosis and asymptomatic apical periodontitis on the maxillary left lateral incisor. The patient did not return for treatment until a year later. Note the substantial increase in lesion size within that time. (Reproduced with permission from [57].)

genera *Prevotella* and *Porphyromonas* [75, 77, 208, 209] with pain. Molecular studies, which employ more sensitive techniques, have shown that spirochetes that are members of the *Treponema* spp. (mainly *T. denticola*) are significantly associated with the presence of pain and apical acute infections, particularly in primary infections [117, 176, 189]. The association between this microorganism and acute endodontic infections is an example of the accuracy and sensitivity of molecular microbiology, because members of the *Treponema* genus, as are many other spirochetes, are very fastidious and thus difficult to identify by culturing [58]. Molecular studies have also identified other genera of microorganisms that are associated with pain, such as *Dialister*, *Filifactor*, *Olsenella*, *Granulicatella*, and *Synergistes* [171, 192]. However, contemporary deep sequencing of bacterial samples from endodontic infections has clearly revealed that the bacterial community harbors hundreds or thousands of bacterial taxa [87, 121, 190]. Moreover, these studies have shown that the relative abundance of the most prevalent individual taxa is very low, and there are no dominant microorganisms present. Therefore, it is not clear how the microbial community profile influences the development of symptoms. It is also likely that these complex microbial communities interact differently with the host response in different patients, making the exact identification or true culprits rather challenging.

Viral infections may exacerbate or potentiate symptoms in endodontic infections. Earlier studies showed that infection with human cytomegalovirus or Epstein-Barr virus was associated with larger and symptomatic periapical lesions [174, 175, 195]. However, a recent systematic review and meta-analysis revealed no significant associations between these viruses and symptoms [93]. It is noteworthy that *varicella zoster* infection is frequently followed by a post-herpetic pain that could present clinically as dental pain. This viral infection has been associated with the pathogenesis of pulpal and apical pathosis in several case reports, case series, and a recent systematic review [76, 94, 156, 187].

3.5.1.2 Chronic Infections

Apical periodontitis appears to be a very prevalent disease among dental patient and non-patient populations (Table 3.2).

As noted before, this is further evidence that most endodontic pathosis is asymptomatic, and represents a homeostatic relationship between the microbial irritants and the host response. However, chronic or asymptomatic disease may exacerbate, resulting in acute infections. It is thought that this occurs due

Table 3.2 Prevalence of apical lesions related to at least one tooth in the adult population in selected representative studies. The asterisk denotes dental clinic populations.

Bergström et al. 1987 [15]	(Sweden)	48%
Odesjo et al. 1990 [150]	(Sweden)	43%
De Cleen et al. 1993 [35]	(Netherlands)	45%*
Saunders et al. 1997 [181]	(Scotland)	41%*
Kirkevang et al. 2001 [109]	(Denmark)	42%
Boucher et al. 2002 [19]	(France)	63%*
Kabak and Abbott 2005 [97]	(Belarus)	80%*
Palmqvist 1986 [153]	(Sweden) (>65y/o)	72%
Ainamo et al. 1994 [4]	(Finland) (>75y/o)	41%
Caplan et al., 2006 [22]	(USA) (<45y/o)	27%
Caplan et al., 2006 [22]	(USA) (>45y/o)	41%

to changes in the composition of the microbial community, or in bacterial load. It may also be related to changes in the host response, such as if the immune system becomes stressed or compromised. Very little objective data is available in this area from clinical studies, because of the ethical difficulty of studying lesion progression without treatment on patients.

A large volume of data is available from animal models or the role of various immunological factors on the size of chronic periapical lesions. Most of these animal models are of chronic periapical lesions, which are the typical lesions that develop upon pulp exposure and extirpation. Animals may develop acute infections in rare situations such as uncontrolled diabetes mellitus [62] or in severe combined immunodeficiency when they are also inoculated with endodontic pathogens [212]. This tendency for a chronic lesion development under normal conditions illustrates the success of the immune responses in preventing severe spreading infections or osteomyelitis due to periapical infections. However, lesions vary in size and rate of progression in these models, depending on a variety of microbial and host response factors.

Animals with deficiency in TLR-4, which recognizes LPS, have smaller periapical lesions at some time points but not others [60, 85]. Interestingly, animals deficient in TLR-2, which recognized lipoteichoic acid of Gram-positive bacteria, have larger, not smaller, induced periapical lesions than controls [33]. It was recently reported that TLR-2 deficiency may enhance CD-14 and TLR-4 signaling, which may explain the increased periapical lesion size in these cases [169]. Furthermore, endodontic pathogens, like *F. nucleatum*, may cause cytokine production, which is mediated by the p38 mitogen activated protein kinase (MAPK) signaling that is independent of TLRs, nucleotide-binding oligomerization domain-1 (NOD-1), NOD-2 and nuclear factor kappa B (NF-kB) signaling [162]. Taken together, these studies show that periapical lesions result from many diverse bacteria, for which different patterns of recognition are at play.

In the pathogenesis of chronic periapical lesions, in response to normal oral microorganisms, the absence of T- and B-cells [55, 210], complement factor C5 [86], interleukin-4 (IL-4) [38, 180], Interferon (IFN) gamma, IL-12, or IL-18 [179] do not seem to affect the pathogenesis of periapical lesions.

However, periapical lesions are larger in size if there is deficiency of adhesion molecules, like intercellular adhesion molecule-1 (ICAM-1) [38] or P/E selectins [100], IL-6 [9, 88], IL-10 [38, 180] or chemokines like CCR2 [70] and CCR5 [38] which are critical for migration of circulating inflammatory cells to the site of inflammation.

Estrogen appears to be protective against the expansion of periapical lesions [72, 228]. This effect can be modulated by antiestrogen agents, like raloxifene [74]. By contrast, medications like bisphosphonates, for example alendronate [186, 228, 229] and metformin (a hypoglycemic agent) [127], reduce bone resorption in periapical lesions. There are many other medications and host conditions that may influence the expansion rate, ultimate size symptoms in apical periodontitis (and potentially the healing following treatment), and therefore, this is the subject of active research.

3.5.2 Classification of Apical Periodontitis

3.5.2.1 Histopathological Classification of Apical Periodontitis

The histopathological classification of apical periodontitis has historically been of great importance, as it was thought to influence the prognosis of non-surgical root canal treatment. In this regard, apical periodontitis has been classified into three main histopathological conditions: apical granuloma, apical cyst and apical abscess. Other less common apical entities include foreign body reaction (such as to food particles, dental materials or sealers), apical scar (which is formed of fibrotic tissue and may result following surgical

treatment of large apical lesions), or cholesteatoma (which is a lesion filled with clefts that result from cholesterol crystals together with giant cells) may be present.

3.5.2.1.1 Apical Granuloma

Apical granuloma assumed this designation due to the presence of inflammatory cells such as neutrophils, lymphocytes, plasma cells, macrophages, and mast cells, which may surround larger multinucleated giant cells or foam cells, giving the appearance of granules. Other granulomatous diseases include tuberculosis, leprosy, and sarcoidosis. Apical granulomas may also include epithelial cell rests of Mallassez. These are the remnants of the epithelial root sheath of Hertwig, which is involved in embryological root development.

Apical granulomas are the primary lesions involved in generating the apical immune response. Even in other histopathological conditions such as cysts or abscesses, a part of the lesion, usually in the periphery, is apical granuloma and is thought to have given rise to the cyst or abscess. The apical granuloma contains the cellular and molecular factors involved in the immune response mentioned above. The differential numbers of inflammatory cells present may depend on the technique used for examination, whether the lesion is from primary or persistent apical periodontitis and whether it is of human or animal model origin. Human studies have shown that macrophages [202, 203] or lymphocytes [14] may dominate the periapical granuloma. Animal studies show that T-helper cells are prevalent in initial phases of the lesion production, and T-suppressor cells dominate later phases of the lesion [201, 225]. Recently, it was shown that T-regulatory (T-reg) cells play an important role in the modulation or control of periapical lesion expansion. T-reg inhibition resulted in a significant increase in periapical lesion severity, which was associated with upregulation of pro-inflammatory, T-helper 1 and T-helper 17 [66].

Periapical granuloma is thought to contain little or no bacteria directly within it. Animal studies show no bacteria in apical granuloma [64, 223], and human lesions (which presumably have a longer induction period by the time they are sampled than in most animal studies) have a low prevalence of bacteria detected histologically [143, 168]. Persistent lesions may have a higher prevalence of bacteria within the lesion [205–207]. When present, bacteria are typically surrounded by intense inflammatory cells, mostly neutrophils and macrophages [64]. Occasionally an area of necrosis is seen within the granuloma that is surrounded by intense inflammatory cellular response, and the lesion is termed an apical abscess. One study showed that 35% of apical lesions that are extracted with the tooth are apical abscesses [146].

3.5.2.1.2 Apical Cyst

Apical cyst results from the proliferation of epithelial rests of Mallassez within a granuloma (Figure 3.9). The epithelium initially forms strands that eventually coalesce. The enlargement of the mass of epithelial cells may create nutritional deficiency in the center of the mass, causing necrosis of tissue and liquefaction to form the cyst with cyst fluid. Alternatively, the epithelial strands may surround a portion of the granulomatous tissue causing the cyst formation [213]. Cyst expansion is thought to be due to pressure from the fluid within the cyst, especially that epithelia cells may secrete bone-resorptive cytokines [123, 214]. Cysts may reach very large sizes clinically, and may cause movement of roots of neighboring teeth.

The diagnosis of an apical cyst is variable. Some pathologists determine that the lesion is a cyst whenever they see evidence of epithelial proliferation within the submitted tissue. Others would only diagnose a cyst when they see intact epithelial lining surrounding a fluid-filled cavity, which may contain cholesterol clefts. This variability in determining the diagnosis has led to wide differences among studies that examined the prevalence of granulomas and cysts (Table 3.3).

A further classification of apical cysts calls for the differentiation of "Bay" [188] or

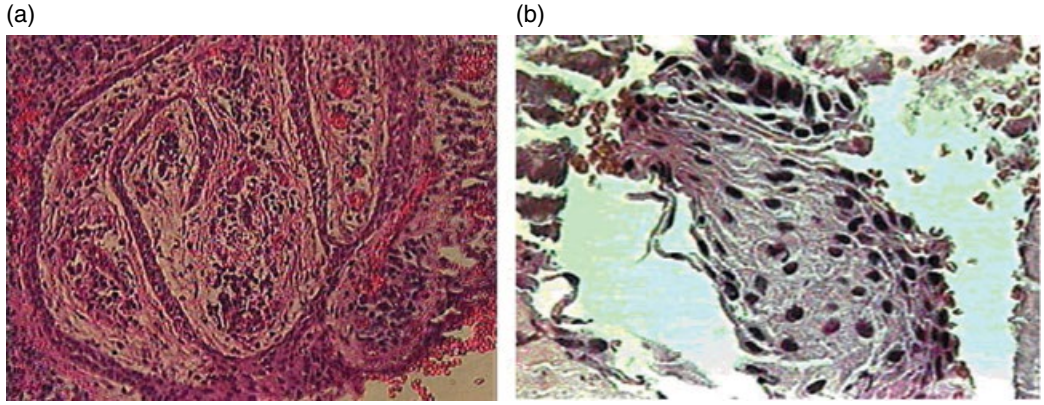

Figure 3.9 Biopsy specimens of tissues recovered during apical surgery on a case with non-healing apical periodontitis and diagnosed by a pathologist as periapical cyst. (a) Epithelial proliferation into strands within the granulomatous tissue (original magnification 100×). (b) Epithelial proliferation (original magnification 200×).

Table 3.3 Prevalence of cysts and granulomas in several frequently cited studies.

	Sample Size	Cysts %	Granulomas %
Priebe et al. 1954 [161]	101	54	46
Patterson et al. 1964 [157]	510	14	20
Bhaskar 1966 [16]	2308	42	48
Lalonde and Luebke 1968 [113]	800	44	45
Mortensen et al. 1970 [142]	396	41	59
Block et al. 1976 [18]	230	7	93
Winstock 1980 [227]	9804	8	83
Stockdale and Chandler 1988 [204]	1108	17	77
Spatafore et al. 1990 [196]	1659	42	52
Nair et al. 1996 [164]	256	15	50
Koivisto et al. 2012 [110]	9723	33	40

"Pocket" [164] cysts from true apical cysts. In Bay or Pocket cysts, the root apex projects into the cyst, and the root canal appears to open directly into the lumen of the cyst. In a true apical cyst, the cyst's epithelial lining and connective tissue appear to be continuous, and separated from the root apex.

The diagnosis of apical cysts versus granuloma can only be made histologically of submitted biopsy material, or with an extracted tooth. Attempts to use radiography [226], even cone beam computed tomography (CBCT) [172], to make this distinction have not been successful. More recent attempts to use specific indices and algorithms for CBCT evaluations may be useful in this respect [78]. There are some general radiographic features of an apical radiolucency that suggest that it is a cyst, such as the large size, being surrounded by a well-corticated border, and appearing to push the roots of neighboring teeth. Give the difficulty of clinical diagnosis of a cyst versus a granuloma, it has been difficult to prove or disprove the effectiveness of non-surgical root canal treatment in the treatment of apical cysts. In the 1950s and

1960s, it was thought that surgical enucleation was necessary for the treatment of apical cysts, as is the case with other types of cysts. However, arguments were later made that since cysts were quite prevalent in apical periodontitis diagnosis, the fact that non-surgical root canal treatment of cases with apical periodontitis had higher success than the average prevalence of apical granulomas meant that there must be a reasonable number of cysts that healed with non-surgical treatment. More recently, the case was made that the prevalence of true apical cysts (as opposed to pocket cysts) is small and that these would be resistant to non-surgical treatment [144, 145]. These hypotheses remain to be proven, especially that the diagnosis of a true apical cyst would be impossible to do preoperatively at this time.

The other important issue regarding apical cysts as a pathological entity is that biologically, several studies done to distinguish granulomas and cysts with respect to cellular and molecular content have failed to show notable differences [21, 36, 37, 132, 218]. One recent study examined the literature on gene expression in apical granulomas and radicular cysts more critically using contemporary bioinformatics tools [160]. In this study, it was revealed that in cysts the main genes expressed were TP53 (tumor protein p53) and EP300 (E1A binding protein p300), whereas periapical granulomas were associated with IL2R, CCL2, CCL4, CCL5, CCR1, CCR3, and CCR5 genes [160].

3.5.2.2 Clinical Classification of Apical Periodontitis

The clinical classification of apical periodontitis has changed over the years and in different regions of the world. Fundamentally, a few clinical signs and symptoms warrant the diagnosis of apical periodontitis. In the absence of histopathological diagnosis, the clinical diagnosis is used for determining the treatment plan, the prognosis, the need for further exploration and testing, and the response to treatment.

Patients presenting with apical periodontitis may have no symptoms, may complain of mild to moderate pain with mastication, and may present with severe pain, with or without swelling in relation to the offending tooth. Radiographic features include an area of hypodensity related to the apex (or lateral border) of the offending tooth, widening or expansion of the periodontal ligament space and absence of lamina dura in the area of the radiolucency. The area of radiographic hypodensity may be diffuse in its extent, or well defined and surrounded by corticated border. Occasionally there is an area of increased radiographic density at the border of the radiolucency. Other signs of disease include a sinus tract that leads to the area of apical radiolucency.

The clinical diagnosis of apical periodontitis utilizes these signs and symptoms in a manner that is recognizable and reproducible by the clinician. The exact terminology is further discussed in other chapters. However, with respect to the relationship of pathogenesis and diagnosis of apical periodontitis some known findings have emerged from decades of study. Clinical symptoms may be associated with the presence of certain bacterial species or viruses, as noted before (see also Chapter 4 for more discussion of the microbiology of endodontic infections). Symptoms may also be associated with the presence of bacterial cell wall components like LPS or endotoxin in the root canal [84, 92]. Periapical pain is likely also associated with several inflammatory mediators that reduce the pain threshold such as neuropeptides, bradykinin, leukotrienes, and eicosanoids [26, 138, 215]. Periapical lesion size may be associated with the levels of bone resorptive cytokines like IL-1beta [112].

3.5.3 Modulation of the Immune Response and Bone Resorption in Apical Periodontitis

Periapical lesions represent a dynamic process of bone resorption and deposition, coupled with the development of the soft tissue lesion that contains a number of immune and structural cells (Figure 3.10). Immune cells include

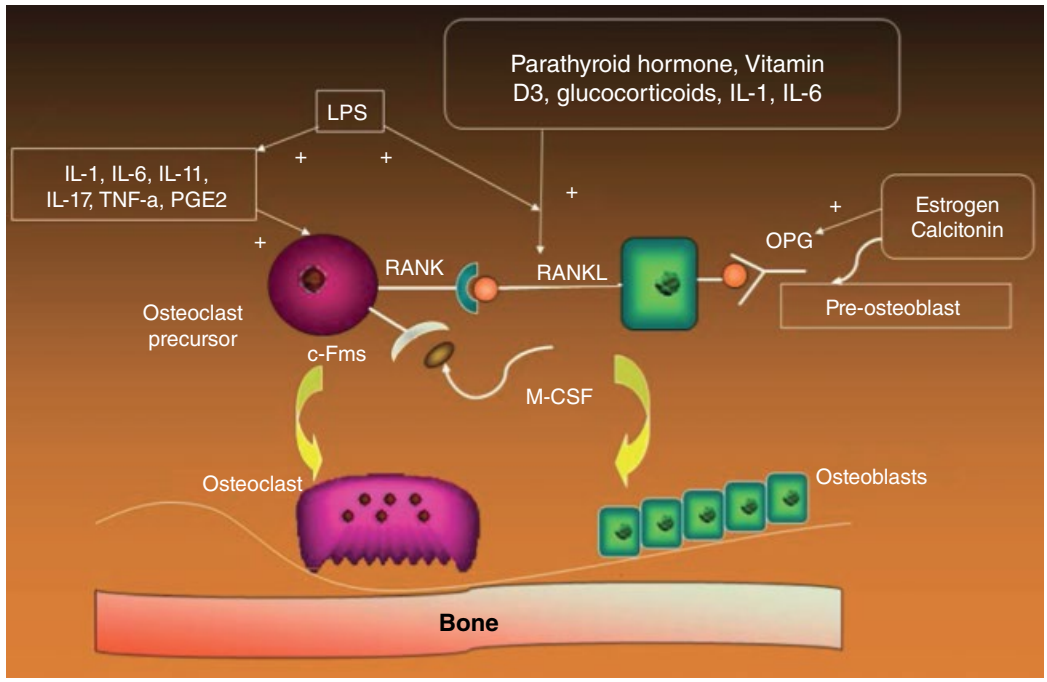

Figure 3.10 Interactions between osteoblastic cells and osteoclast precursors during bone remodeling (reproduced with permission from [59]).

lymphocytes, macrophages, neutrophils, plasma cells, and mast cells. Structural cells include fibroblasts and osteoblasts. In addition, endothelial cells and osteoclasts play an important role in the progression of the inflammatory process. Occasionally, epithelial cells are present, and may give rise to cystic formation as noted previously.

Innate immunological cells and factors appear to play a critical role in initial development of periapical lesions. Animal models of lesion induction show the presence of neutrophils at the site of the apical foramen. They appear to play a significant role in the control of microbial progress into the lesion (Figure 3.11).

Studies have shown that suppression of neutrophils causes significant disruption of lesion formation in animal models [147, 231]. Neutropenic patients may have spontaneous necrosis and abscess formation in relation to mild caries [159]. The role of adaptive immunity becomes evident when the host is exposed to significant bacterial load of virulent oral bacteria. Thus studies in which animals deficient in T- and B-cells (*scid* mice) were exposed to 10^{10} cells/mL of *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros* (now *Parvimonas micra)*, and *Streptococcus intermedius* [212] or *Treponema denticola* [54], the compromised animals developed severe swellings and experience disseminated infections.

However, in most cases, the bacterial irritants are not as severe, and the immunological response is capable of controlling it without significant morbidity. A balance between the various immunological factors and the advancing microbial irritants determines the exact size of the periapical lesion. In most animal studies, the size of the lesion reaches a specific radiographic or histologic level within 4–6 weeks that is then stable for the rest of the experiment [10, 55, 60]. This balance is maintained by pro-inflammatory and anti-inflammatory cells and molecular mediators, which act in concert to titrate the resulting response. This is as critical in the

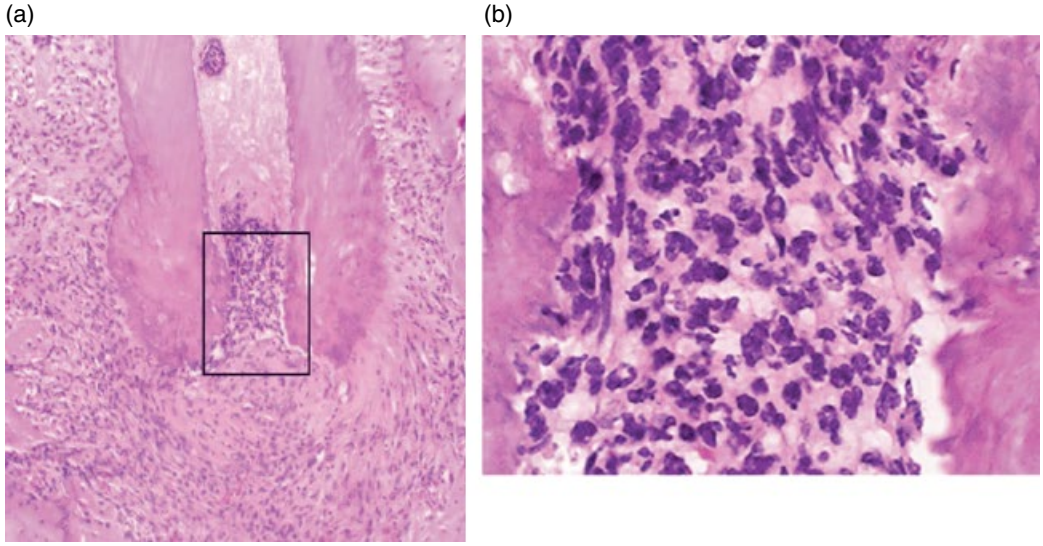

Figure 3.11 Induced periapical lesion in a mouse model. (a) Pulp necrosis and intense inflammatory response at the apical foramen and sporadically within the lesion (original magnification 100×). (b) Higher magnification of the box in (a) showing that most inflammatory cells are neutrophils (original magnification 400×).

case of apical periodontitis as it is in pulpitis and other types of inflammation, limiting the side effects of inflammation. This process assures the protective function of inflammation and limits its destructive effects.

As noted before, active bone resorption is associated with developing lesions. Bone resorption involves the kinetics related to nuclear factor kappa B (NF-kB) which is ubiquitously expressed in periapical lesions [173]. Specifically, the receptor activator of NF-kB (RANK) on osteoclasts and their precursors interacts with the RANK ligand (RANKL) on osteoblasts to effect bone resorption. RANKL is so critical for bone modulation that an inactivator of this molecule causes osteonecrosis in an animal model [3]. At the same time, osteoprotegrin (OPG) acts as a decoy to stop the RANK/RANKL reaction and maintain bone structure (Figure 3.10). This occurs when a balance is reached between the advancing irritants and the host response, by limiting bone resorption, resulting in stable lesion size. The cessation of further lesion development, beyond the needed extent, may be orchestrated by T-reg cells [66] and a variety of cytokines, hormones, and other factors (Figure 3.10).

It follows from this argument that one could potentially assay a periapical lesion (through the root canal of a necrotic pulp or directly) for factors that may indicate whether a lesion is actively developing, stable or healing. Preliminary work in this area has been reported. In one study, active versus inactive lesions were arbitrarily based on a RANKL/OPG ratio of five-fold or greater [69]. This was correlated with gene expression from an array of 84 wound healing genes for 83 granulomas and 25 control periodontal ligament specimens. It was shown that active lesions were associated with upregulation of TNF (a pro-inflammatory cytokine) and CXCL11 (a chemokine), whereas inactive lesions had upregulated SERPINE1 (a remodeling enzyme), TIMP1 (a remodeling enzyme), COL1A1 (extracellular matrix component), TGF-beta1 (a growth factor), and ITGA4 (a cellular adhesion molecule) [69]. In another study, the designation of active versus inactive lesions was based on the diagnosis of chronic apical abscess for active lesions or asymptomatic (chronic) apical periodontitis

for inactive lesions [119]. The lesions were assayed for matrix metallo-proteinases or their inactivators. Active lesions were found to have upregulated MMP-2, MMP-7, and MMP-9, whereas inactive lesions had upregulated TIMP-1 [119].

3.5.4 Healing of Apical Periodontitis

Once the microbial irritants are eliminated, by tooth extraction, or are substantially reduced, by non-surgical or surgical endodontic treatment, periapical healing ensues. Healing occurs by increased vascularity, formation of collagen, development of osseous islands that eventually coalesce, and deposition of cellular cementum (Figure 3.12) [65]. The periapical lesion contains many of the growth factors needed for wound healing and mineralization. These include transforming growth factors (TGF-alpha and TGF-beta) [69, 216], epidermal growth factor [124], vascular endothelial growth factors [53, 118], and bone morphogenetic protein-2 (BMP-2) [135].

Multiple host-related factors may influence the rate of healing of periapical lesions. For example, diabetes mellitus [7, 63] and smoking [43] were shown to be associated with reduced periapical healing in cohort studies. The study of the role of systemic factors on the healing of periapical lesions is confounded by the fact that many of the patients involved take medications that may alter the bone kinetics within the lesion. For example, it was shown that metformin, which commonly used for the treatment of type 2 diabetes [127], bisphosphonates, which are used for osteoporosis among other conditions [99] and statins [122, 125], which are used by many elderly patients, reduce the size of induced periapical lesions in animal models.

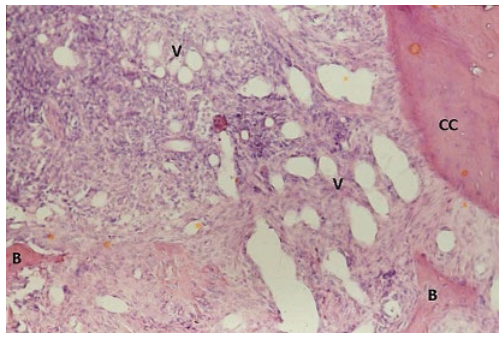

Figure 3.12 Healing four months following non-surgical endodontic treatment of a canine with induced periapical lesion in a ferret. Elements of healing include bone (B), vascular proliferation (V) and cellular cementum (CC). (Reproduced with permission from [65].)

Genetic polymorphism in the IL-1beta allele2 [141] or some of the Fc-gamma receptors [193, 194] may be associated with reduced healing of apical periodontitis. Fc-gamma receptors are located on many inflammatory cells, particularly macrophages, and bind the Fc portion of immunoglobulin to facilitate phagocytosis of the antigens attached to the Fab portion of the antibody. However, it is premature at this stage to blame genetic polymorphism for reduced healing, as some of the findings are not supported by other studies [6].

3.6 Concluding Remarks

It is now thought that a low-grade inflammation of the pulp enables tissue regeneration i.e., tertiary dentinogenesis while a more intense and severe inflammation results in necrosis [31, 91]. In this regard, the mechanisms involved in excessive pulp calcification such as following trauma and in heavily restored dentition are not fully understood. Therapies designed to reduce the pulpal inflammatory response may help maintain the vitality of the pulp, an effective immunocompetent tissue. In a canine model of pulpitis, treatment with MMP-3 reduced the expression of the pro-inflammatory cytokine IL-6 and decreased the number of macrophages and antigen-presenting cells [46]. Thus, reversing the induced pulpal inflammation. Improved understanding to the molecular networks which contribute to tissue regeneration and the maintenance of heathy vital pulps have the potential to significantly benefit dental patients and improve oral health.

Healing of apical periodontitis by nonsurgical and surgical endodontic treatment is generally slower than following the extraction of the tooth. It is thought that this may be related to a more effective elimination of microbial factors with extraction. However, this delay in healing with endodontic procedures affect the practitioner's ability to follow up on treatment and render additional treatment when it is indicated. In future, it may become feasible to enhance periapical healing following endodontic treatment by the application of commercially-available growth factors, like Emdogain, or other proteins so that the prognosis can be determined after a shorter period of time. Furthermore, assessment of the level of activity in periapical lesions, as noted previously, may assist with treatment planning and prediction of the development of symptoms. The ultimate understanding of the pathogenesis and healing of the pulp and periapical tissues will involve a better understanding of the endodontic microbiome, as well as the patient's genomic, epigenetic, and medical considerations.

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4

Microbiology of Apical Periodontitis

José F. Siqueira Jr and Isabela N. Rôças

4.1 Introduction

Infection of the dental root canal system is the major cause of apical periodontitis (Figure 4.1). Although chemical and physical factors can induce inflammation in the periradicular tissues, a large body of scientific evidence indicates that microorganisms are essential to the progression and perpetuation of apical periodontitis [99, 137, 291]. Fungi, archaea, and viruses have been found in association with apical periodontitis, but bacteria are the major microorganisms implicated in the etiology of this disease.

Root canal infection only develops in teeth with partial or total pulp necrosis or in canals that had the pulps removed during previous treatment. Pulp necrosis is a sequel to caries, trauma, periodontal disease, or iatrogenic operative procedures. These conditions create pathways by which oral bacteria can gain access to the root canal system. A necrotic pulp lacks active circulation and can therefore no longer mobilize inflammation and protect itself against invasion and colonization by oral bacteria. After an endodontic infection is established, bacteria contact the periradicular tissues via apical and lateral foramina. As a consequence, inflammatory changes take place at the periradicular tissues and give rise to the diverse forms of apical periodontitis.

Because apical periodontitis is regarded as an infectious disorder caused by bacterial infection of the root canal system, successful treatment of this disease is contingent upon complete elimination or effective control of the infecting microbiota. A thorough understanding of the disease's etiology is essential for successful treatment. In this context, knowledge of the microbiological aspects of apical periodontitis is of utmost importance for endodontic practice of high quality and founded on solid scientific basis.

This chapter discusses several aspects related to the microbiology of endodontic infections and pathogenesis of apical periodontitis. For a better understanding of several terms used, Table 4.1 depicts definitions for many of them.

4.2 Microbial Causation of Apical Periodontitis

The first recorded observation of bacteria in the root canal dates back to the 17th century, when the Dutch amateur microscope builder Antony van Leeuwenhoek [1632–1723] wrote: "The crown of this tooth was nearly all decayed, while its roots consisted of two branches, so that the very roots were uncommonly hollow and the holes in them were stuffed with a soft matter. I took this stuff out of the hollows in the roots and mixed it with clean rain water, and set it before the magnifying glass so as to see if there were as many living creatures in it as I had aforetime

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Companion website: www.wiley.com/go/orstavik/essentialendodontology

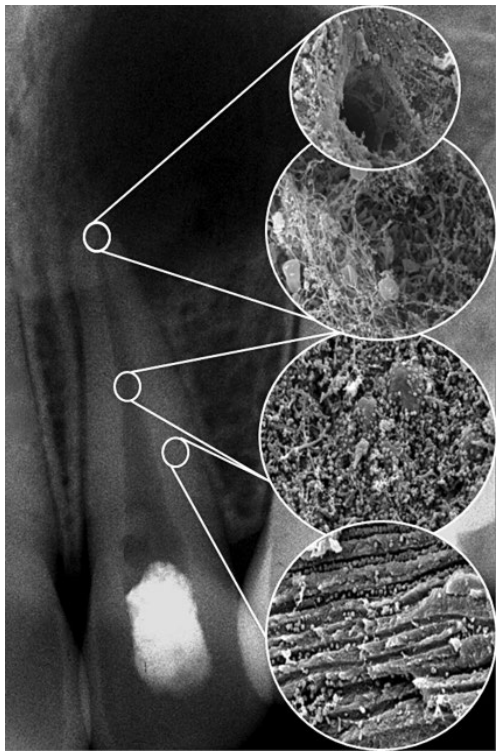

Figure 4.1 Bacteria colonizing the root canal system are the major causative agents of apical periodontitis lesions.

discovered; and I must confess that the whole stuff seemed to me to be alive" [38]. However, at that time, the role of Leeuwenhoek's animalcules as infectious agents was unknown. It took over 200 years before his observations were confirmed and a cause-and-effect relationship between bacteria and apical periodontitis was suggested.

In 1894, Willoughby Dayton Miller, an American dentist who developed his seminal experiments in oral microbiology inspired by Robert Koch, in Berlin, Germany, published a milestone study reporting on the association of bacteria with apical periodontitis, after the analysis of material collected from root canals [131]. By means of bacterioscopy of the canal samples, Miller found the three basic bacterial morphotypes, i.e., cocci, bacilli and spirilla (Figure 4.2). He wrote: "We assume, in a general way, that bacteria must in some manner be connected with these processes (pulp diseases)… There are, then, as I have already pointed out, different species of bacteria in the diseased pulp that have not yet been cultivated on artificial media, and of whose pathogenesis we know nothing definite. Their great numbers in some pulps, and especially the repeated occurrence of spirochaetes, justify the supposition that, under certain circumstances, they may play an important role in suppurative processes."

Miller raised the hypothesis that bacteria were the causative factors of apical periodontitis and that the species composition of the microbiota in the coronal, middle and apical parts of the root canal was clearly different. Some bacteria from root canal samples that were seen under light microscopy could not be cultivated using the methods available at that time. Most of those bacteria were conceivably anaerobic bacteria. Nonetheless, in spite of the considerable technologic advances in the last century regarding bacterial cultivation, it is widely recognized that a large number of microbial species still remain uncultivated [3, 179].

Miller's findings, although pioneering, were only suggestive of a causal relationship between bacteria and apical periodontitis. Two events occurring simultaneously do not necessarily imply a cause-and-effect relationship. It was not until approximately 70 years after Miller's classic findings that the causal relationship between bacteria and apical periodontitis was unequivocally demonstrated by an elegant study in germ-free rats from Kakehashi et al. [99]. These authors exposed the dental pulps of conventional and germ-free rats to the oral cavity and observed the pulp and periradicular responses. Histologic evaluation performed at intervals ranging from 1 to 42 days postoperatively revealed that in conventional animals, without exception, all older specimens showed complete pulp necrosis with periapical inflammatory lesions. On the other hand, the dental pulps of germ-free animals repaired themselves by dentinal bridge formation, which was already evident at 14 days; by 21 and 28 days, the

Table 4.1 Definitions of terms used in this chapter.

Term	Definition
16S ribosomal RNA (rRNA)	A component of the smaller (30S) prokaryotic ribosomal subunit. The gene that encodes this structure possesses hypervariable regions containing species-specific signature sequences, which are useful for bacterial and archaeal identification at the species level. It also contains conserved universal regions that permit amplification of part or the entire 16S rRNA gene of virtually all bacteria or archaea
Amensalism	A relationship between two species of organisms in which one is inhibited and the other is unaffected
Archaea	A domain containing prokaryotic microorganisms different from bacteria, which compose another domain (Bacteria). Eukarya (containing organisms with nucleated cells) is the third domain of life.
Asaccharolytic	A microorganism that cannot metabolize carbohydrates and consequently needs other carbon sources, such as peptides, for obtaining energy
Biofilm	A sessile multicellular microbial community characterized by cells firmly attached to a surface and emmeshed in a matrix of extracellular polymeric substances produced by themselves
Community	A group of interacting populations
Community profile	Species richness and abundance in a community
Community structure	Species richness and abundance in a community
Density	Number of cells (individuals)
Diversity	Measure of the richness and relative abundance of species in a community
Ecology	Study of the interrelationships between organisms and their environments
Endogenous infection	Infection caused by members of the normal human microbiota
Exogenous infection	Infection caused by microorganisms that do not belong to the normal microbiota and that were introduced in the host
Food chain	A linear succession of organisms in a community that are linked to each other through the transfer of nutrients and energy
Food web	A network of interconnected food chains. It shows how different species are connected by different metabolic paths
Horizontal (or lateral) gene transfer	The exchange of genetic material between contemporaneous organisms as opposed to vertical transfer, which represents inheritance of genetic material from an ancestor. Horizontal transfer permits that one species transfers its genetic material to another species.
Infection	Invasion and proliferation of microorganisms in a place where they are not expected to be present. Infection does not necessarily result in disease
Infectious disease	Development of signs and symptoms after microbial infection and damage to host tissues
Inflammophilic bacteria	Bacterial species that flourish in contact with inflamed tissues, deriving nutrients from inflammatory exudates and tissue breakdown products
Load	Number of cells (individuals)
Metaproteomics	The study of all proteins recovered directly from environmental or clinical samples
Microbiota	A collective term for microorganisms forming a complex and diverse community in a given anatomical site

(*Continued*)

Table 4.1 (Continued)

Term	Definition
Modulin	Bacterial structural components or released products with the ability to modulate the host immune response, especially by stimulating synthesis and release of cytokines
Niche	Functional role (metabolic functions) of an individual in the community
Opportunistic pathogen	A microorganism that causes disease only when host defenses are impaired
Pathogen	A microorganism that causes disease
Pathogenicity	Ability to cause disease
Phylotype	As-yet-uncultivated species that are known only by a 16S rRNA gene sequence rather than by phenotypic characteristics
Polymicrobial infection	An infection caused by several different species
Population	A group of growing cells (microcolony)
Primary or true pathogen	A microorganism that often causes disease within a given host
Putative or candidate pathogen	A microorganism that has been found in association with disease in cross-sectional studies, but evidence from longitudinal observations is lacking
Relative abundance	Proportion of each species within a community
Richness	Number of different species in a community (composition)
Synergism	Interaction between two or more species or factors that results in an effect greater than the sum of their individual effects
Virulence	Degree of pathogenicity
Virulence factor	Microbial products, structural components or strategies that contribute to pathogenicity

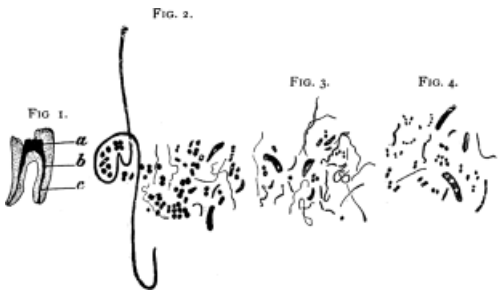

Figure 4.2 Drawings from Miller's classic article [131] showing different bacterial forms in a root canal sample observed by microscopy.

newly formed hard tissue was completely sealing the previously exposed area, regardless of the angle or severity of exposure. In every instance, the pulp tissue remained vital beneath the dentinal bridge.

In 1976, Sundqvist used anaerobic cultivation methods to evaluate the bacteriology of pulps of human intact teeth that had become devitalized after trauma [291]. His findings revealed that whereas the necrotic pulps of teeth without apical periodontitis lesions were bacteria-free, those showing apical periodontitis lesions were virtually always infected. Anaerobic bacteria predominated, comprising more than 90% of the isolates. It could also be inferred from his findings that the necrotic pulp tissue itself and stagnated tissue fluid in the root canal are not able to induce and maintain apical periodontitis.

Strong evidence about the causal relationship between bacteria and apical periodontitis was also provided by Möller et al. [137]. In a study in monkey's teeth, these authors demonstrated that only devitalized pulps that were infected caused apical periodontitis, whereas devitalized but uninfected pulps showed an absence of pathological changes in the periradicular tissues. In addition to corroborating

the importance of bacteria for the development of apical periodontitis, this study also showed that the necrotic pulp tissue, in the absence of infection, is not able to induce and perpetuate periradicular inflammation.

Bacteria colonizing the root canal system in association with apical periodontitis are primarily organized in biofilm structures. Several morphologic studies have reported on bacterial organizations resembling biofilms in root canal infections [138, 148, 233, 276], but it was not until the study of Ricucci and Siqueira [183] that the strong association of bacterial biofilms present in the apical portion of the canal and primary and post-treatment apical periodontitis was demonstrated. Given the cross-sectional nature of most endodontic microbiology studies, it is still not clear whether bacterial organization in biofilms is essential for apical periodontitis to develop. However, from a clinical standpoint, it is important to recognize that bacterial biofilms are highly prevalent in the apical canal of untreated and treated teeth with apical periodontitis and may represent a challenge for proper antimicrobial treatment.

4.3 Endodontic Biofilms and the Community-as-Pathogen Concept

Biofilm can be defined as a sessile multicellular microbial community characterized by cells firmly attached to a surface and emmeshed in a matrix of extracellular polymeric substances (EPS) [28, 39] (Figure 4.3). The vast majority of microbial species in nature live in metabolically integrated biofilm communities, and the human body is no exception [28, 126].

While most acute medical infections that require rapid diagnosis and intervention to avoid dramatic damage or death are caused by planktonic bacteria, chronic infections associated with sustained inflammation and gradual tissue damage are usually related to biofilms [168, 273, 330]. Biofilm infections

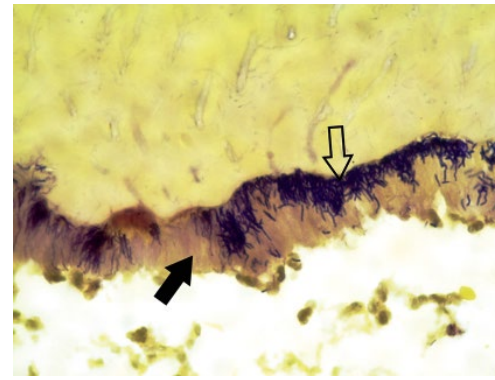

Figure 4.3 Bacterial biofilm in a tooth with primary apical periodontitis. Notice the bacterial cells adhering to dentin (blank arrow) and embedded in a thick extracellular matrix (black arrow) (Brown and Brenn staining, original magnification 400×).

are suggested to be responsible for 65–80% of the human infectious diseases, including endocarditis, middle ear infections, osteomyelitis, prostatitis, and orthopedic device-related infections [27, 30, 168]. In the oral cavity, caries, gingivitis, and marginal periodontitis are typical examples of diseases caused by bacterial biofilms [125]. Apical periodontitis has also been recently introduced in the set of biofilm-induced oral diseases [183].

4.3.1 Structure

Cells in bacterial biofilms are commonly aggregated in microcolonies (populations) embedded and non-randomly distributed throughout the EPS matrix [29, 39, 283, 286]. Distribution of the populations along the biofilm structure is dictated by diverse factors, including arrival time, positive metabolic interactions among the community members, and proximity to convenient nutrient sources. Populations are separated by water channels that travel throughout the biofilm structure, carrying water and nutrients and draining waste products. The largest accumulations of cells commonly occur at the bottom of the biofilm structure, close to the surface to which the biofilm is adhered.

The EPS matrix is produced by the community members themselves and account for

the largest portion of the biofilm dry mass (>90%); cells constitute <10% of the biofilm mass [58]. The EPS matrix is mostly composed by polysaccharides, but proteins, nucleic acids, and lipids can also be present [28]. It serves numerous essential functions to the biofilm community, as it mediates adhesion to surfaces, provides mechanical stability, allows for accumulation of extracellular enzymes involved with nutrient acquisition and defense against antimicrobials, favors positive intercellular interactions by keeping cells in close proximity, serves as a reserve of nutrients, retains water, and protects the community against antimicrobial agents and host defenses [58, 239]. Bacteria in biofilms exhibit increased resistance against phagocytosis by neutrophils [94] and macrophages [297].

Biofilm cells exhibit a different phenotype in comparison with their counterparts occurring in the planktonic state. This is largely due to differential gene expression between the two states [16, 154, 228]. The changed gene expression and low growth rate of the biofilm phenotype also generally confers increased resistance to antimicrobial agents, environmental stress, and host defenses [120].

4.3.2 Bacterial Interactions and Intercommunication in Biofilms

In multispecies biofilms, cells are in close contact with one another, which favors interactions and communication between them. Interactions affect the overall function and physiology of the biofilm community, as well as its resistance to external threats and virulence [20]. Interactions between biofilm bacteria may enable them to behave collectively as a group.

Communication between bacteria is often referred to as quorum sensing and may occur with both Gram-positive and Gram-negative species [44, 159, 329]. Quorum sensing involves the production, release and subsequent detection of chemical signaling molecules called autoinducers. As a bacterial population producing and releasing autoinducers multiplies, the extracellular concentration of autoinducers also increases. When autoinducer concentration reaches a crucial threshold level, the group responds by changing gene expression [159]. This gives bacteria a means to perform specific behaviors and functions only when living in groups. Communication signals are known to be much more relevant among members of a given population than between distinct populations, but the possibility exists that the signals released by cells present in separate populations may also affect each other [20]. Quorum-sensing systems are important for regulation of virulence, resistance to starvation and host defenses, production of secondary metabolites, and biofilm formation. For instance, some opportunistic pathogens express virulence factors in response to sensing their own cell density. Several candidate endodontic pathogens have been demonstrated to produce quorum-sensing signal molecules [61, 167, 324, 334].

Communication among bacteria in multispecies biofilms can lead to reciprocal transcriptomic and proteomic responses with consequent regulation of nutrient acquisition, metabolic processes and expression of virulence factors [81, 144]. Based on the numerous possible interactions between community members, it is possible to infer that the higher the community diversity, the higher its complexity.

The high cellular density and the stable conditions for cell–cell contact in biofilms also favor horizontal gene transfer between community members [20]. Therefore, genes responsible for antimicrobial resistance or related to virulence properties may be transferred to other members of the community.

Occurrence of syntrophy between community members, in which one species depends on the products released by another, helps shape the organization of populations throughout the biofilm structure. In this sense, cooperative interactions for breakdown of complex substrates also assumes relevance (see section “Microbial ecology and the root canal ecosystem”).

4.3.3 Community-as-Pathogen Concept

Biofilms associated with most endogenous infections are usually composed of several different species. Consequently, a polymicrobial etiology for these diseases has been proposed. With the biofilm concept, the current trend is to interpret the bacterial community as a whole as the unit of pathogenicity for these diseases [93, 109, 273]. Thus, rather than the specific factors released by one or a few of the colonizing microorganisms, it is the total species composition and relative abundance of each species in the biofilm community as well as the myriad of bacterial interactions that modulate virulence.

It seems that the pathogenesis of apical periodontitis requires the concerted action of bacteria in a multispecies community [273]. The types and levels of the community members as well as the interactions between them result in the accumulation of a multitude of virulence factors in the biofilm matrix [269]. As the biofilm reaches the apical region of the root canal system, diffusion of the virulence factors from its biomass evokes and maintains inflammation in the periradicular tissues [258].

Biofilm infections generally represent a persistent source of aggression to the tissues. In root canal infections, persistence is aggravated by the inaccessibility of host defenses to the anatomic site of infection. The juxtaposition of bacterial biofilms to the periradicular tissues triggers destructive inflammatory responses.

Biofilm collective pathogenicity is influenced by synergistic interactions among community members, and the resulting outcome can be more severe than expected for the individual components. As noted earlier, synergistic interactions enhance protection against host defenses and antimicrobial agents and increases virulence of the multispecies community. Furthermore, survival and persistence of the biofilm in an inflamed environment may actually be favored in some circumstances: In fact, it has been suggested that oral bacterial communities associated with long-standing inflammatory processes can be regarded as "inflammophilic" [80]. This means that the community members not only have the ability to survive the attack of the inflammatory response, but also to take advantage of this condition.

Inflammation is an essential process to combat infection, but in biofilm infections it usually does not succeed in eradicating the infection, resulting in tissue damage without elimination of its source. Inflammation can represent an important source of nutrients to some of the bacteria occurring in biofilms in the apical root canal. Nutrients are in the form of glycoproteins from the exudate and tissue breakdown products, such as degraded collagen and heme-containing compounds (haptoglobin, hemopexin and hemoglobin) [80, 258]. It has been shown that the bacterial load in periodontal biofilms is higher with increasing inflammation [1], and the periodontitis-associated community exhibits increased expression of genes related to iron acquisition and proteolytic activity [45].

After the development of apical periodontitis, inflammation-derived products become the main source of nutrients to intracanal bacteria. As a consequence, asaccharolytic and proteolytic bacteria that obtain iron and use these products (peptides and amino acids) as their main source of energy dominate the microbiota in the apical root canal system [258].

Therefore, inflammation acts as a double-edged sword. On the one hand, persistent biofilm intraradicular infection simultaneously activates both innate and adaptive immune responses that play an important role in preventing the root canal infection from reaching bone and spreading to other body areas. On the other hand, neither of these responses can eliminate the intraradicular biofilm and the result is tissue damage (see Mechanisms of bacterial pathogenicity below). In addition, persistent inflammation introduces a new and sustainable source of substrate for growth and survival of inflammophilic bacteria in the apical canal.

Based on the community-as-pathogen concept, the pathogenic endodontic bacterial

community becomes enriched in virulence factors, which accumulate in the biofilm and are gradually released to stimulate and sustain periradicular inflammation. The community also is adapted to survive in the presence of inflammation, from which it can derive an important source of nutrients for its maintenance.

4.3.4 Biofilms in Teeth with Apical Periodontitis

Several morphological studies have described bacterial organizations resembling sessile biofilm structures in the root canals of teeth with apical periodontitis [22, 138, 148, 186, 229, 276]. While many bacterial cells are suspended in fluid or enmeshed in the pulp necrotic tissue in the main root canal lumen (planktonic state)(Figure 4.4), dense bacterial biofilm communities have been observed adhering to the root canal walls to a varying degree [148, 276] (Figure 4.5). Planktonic bacterial cells in the main canal may be newcomers or they may be cells detached from the biofilm structures adhering to the canal walls. Biofilms are found not only in the main canal,

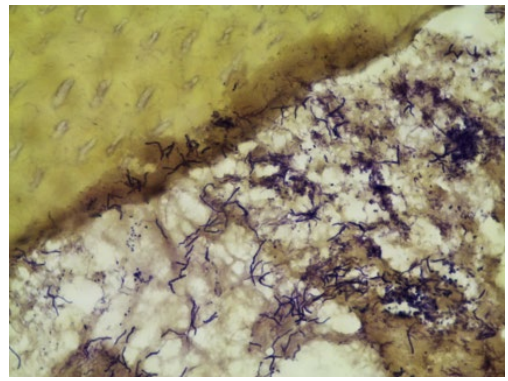

Figure 4.4 Planktonic bacteria occurring in the canal lumen. Cells are usually suspended in fluid and enmeshed with the necrotic tissue (Brown and Brenn staining, original magnification 400×).

but they can also propagate to apical ramifications, lateral canals, isthmi, and recesses of the system [22, 147, 182, 184]. Occasionally, they can also reach the apical foramen/ina and extend to the outer root surface, forming an extraradicular biofilm [54, 185, 298].

The high prevalence of biofilms in the apical root canal and their strong association with apical periodontitis were demonstrated in a histobacteriological study by Ricucci and

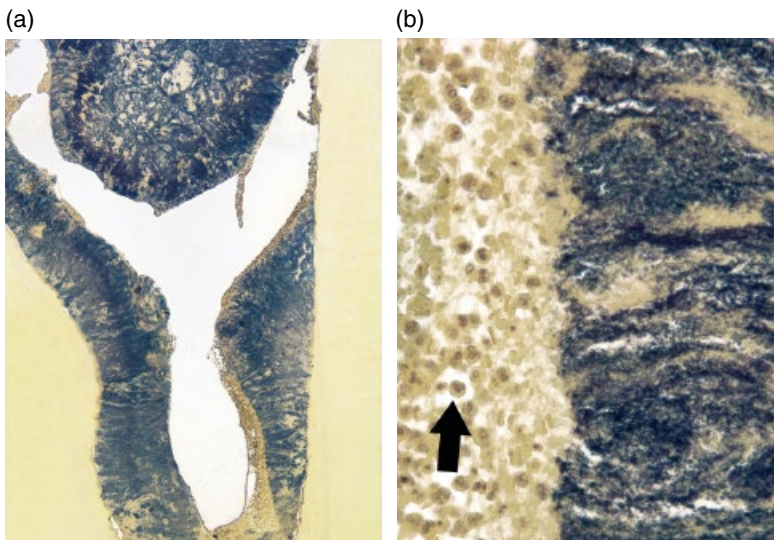

Figure 4.5 (a) Thick bacterial biofilm in a tooth with extensive coronal destruction and apical periodontitis, which was open to the oral cavity and had a history of several exacerbations before extraction (Taylor-modified Brown and Brenn staining, original magnification 50×). (b) Higher magnification showing dense population of bacterial cells faced by accumulation of inflammatory cells, especially polymorphonuclear neutrophils (arrow) (400×). Courtesy of Dr Domenico Ricucci.

Siqueira [183]. Intraradicular biofilms were found in the apical canal of 80% of teeth with primary apical periodontitis and 74% of teeth with post-treatment apical periodontitis. Endodontic biofilms were usually thick and multilayered, and the relative matrix-cell proportions were highly variable (Figure 4.5). Whereas most of the walls were heavily colonized, there were areas in which colonization was slight or even absent.

Biofilms have been more frequently found in teeth with large lesions and those histopathologically diagnosed as cysts [183]. This is possibly related to the fact the large lesions and cysts represent long-standing pathological processes. Consequently, the intraradicular infection, which is the cause of the lesion, is even "older" in these cases. Bacteria involved in these processes are expected to have had sufficient time and conditions to organize themselves in a mature biofilm community. The observation that the treatment outcome is influenced by the size of apical periodontitis [287] can be justified by the difficulties to control an infection that is complex and composed by large numbers of cells and species [196, 291], usually organized in biofilms [183].

A particular finding from the Ricucci and Siqueira's study [183] was the observation of bacterial flocs in some clinical specimens. Flocs are large bacterial colonies surrounded by EPS matrix and regarded as "planktonic biofilms". These structures may originate from growth of aggregates/coaggregates of planktonic cells or they may have detached from biofilms adhered to a surface [82].

Bacterial invasion of dentinal tubules is commonly seen underneath biofilm structures [183]. The diameter of dentinal tubules is large enough to permit penetration of most oral bacteria. Dentinal tubule infection can occur in about 50–80% of teeth with apical periodontitis [79, 130]. A shallow penetration is more common, but bacterial cells can sometimes be observed as deep as 300 m in dentin [276]. A study in monkeys showed that infection may even reach the entire length of the dentinal tubules and cause changes in the periodontal ligament, especially when cementum is lost by resorption [302]. While some tubules can be heavily infected, adjacent tubules may be free of infection (Figure 4.6). Motility does not appear to be a necessary bacterial attribute for dentinal invasion, since most bacteria so

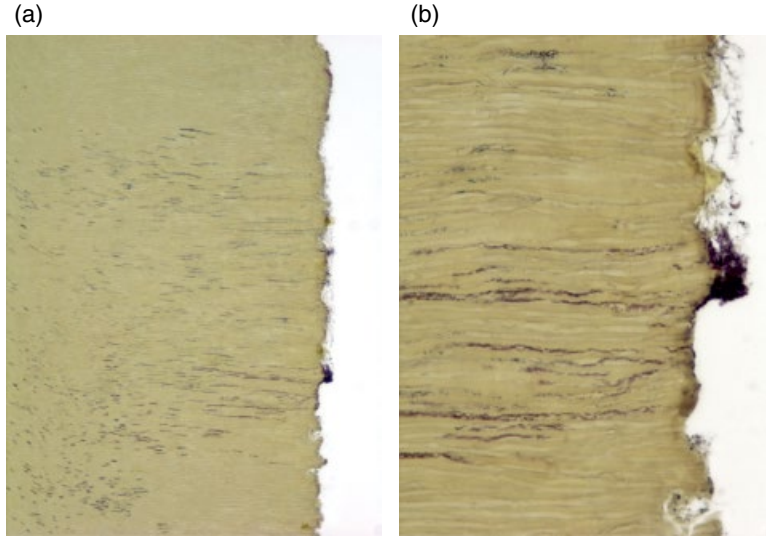

Figure 4.6 (a) Bacteria invading dentinal tubules in a tooth with apical periodontitis (Taylor-modified Brown and Brenn staining, original magnification 100×). (b) Higher magnification (400×). Courtesy of Dr Domenico Ricucci.

far identified in tubules are non-motile species [130, 165]. Dividing cells are frequently observed within tubules in *in situ* investigations [276] (Figure 4.7), indicating that bacteria can derive nutrients within tubules, probably from degrading odontoblastic processes, denatured collagen, bacterial cells that die during the course of infection, and intracanal fluids that enter the tubules by capillarity. Actually, several candidate endodontic pathogens have been shown to be capable of penetrating dentinal tubules in *in vitro* [116, 163, 260] and *in vivo* studies [130, 165].

The occurrence of biofilms attached to the outer root surfaces around apical or lateral foramina is rather uncommon. However, when present, extraradicular biofilms are usually associated with symptoms or sinus tract (see further discussion on "Extraradicular infection").

4.3.5 Dynamics of Endodontic Biofilm Formation – a Theory

The mechanisms of biofilm formation on different surfaces in nature are similar to dental plaque. Basically, the process starts by colonization of a solid surface by planktonic bacterial cells floating in a fluid that bathes that surface. Initially, a conditioning film forms on the tooth surface as a result of adsorption of macromolecules (proteins and glycoproteins) from saliva to the surface. Bacteria occurring in the planktonic state approach and adhere to the surface by non-specific and specific means. EPS matrix is produced and permits stronger adhesion. Finally, these pioneer species grow and co-aggregate to others, including newcomers, to form populations that organize themselves in a multispecies community – biofilm.

These mechanisms may contribute to biofilm formation in cases that a pulpless canal is open to the oral cavity and saliva seeps into it. However, in most circumstances, the process of biofilm formation in root canals is expected to follow a difference course of events. Pulp inflammation, necrosis and infection is usually a sequel to caries – a disease also caused by biofilms [127]. The biofilm associated with caries gradually advances towards to the pulp as the process destroys dentin. When the pulp tissue is exposed to the caries biofilm, severity of inflammation intensifies and may result in localized areas of tissue necrosis (Figure 4.8). Then, the biofilm advances to occupy these areas, moving in apical direction as the bacterial cells adhere and grow along the dentinal canal walls. Cells detaching from the biofilms and late-coming planktonic cells arriving from the oral cavity will also occur in the canal lumen with necrotic tissue. The events of bacterial aggression, inflammation, necrosis and infection occur by compartments of pulp tissue and gradually move towards to the apical portion of the canal. Consequently, the process of biofilm formation in necrotic root canals occurs gradually as the biofilm in the frontline of the advancing infection moves apically.

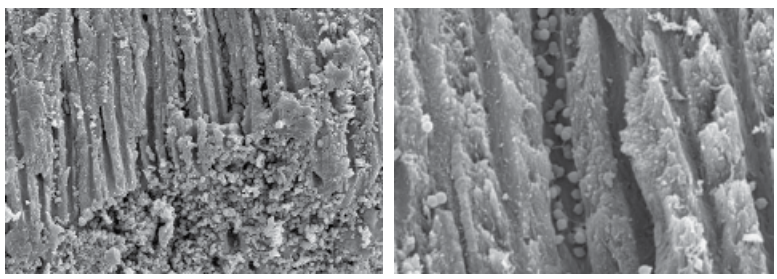

Figure 4.7 Bacterial infection of the root canal in a tooth with apical periodontitis. Scanning electron micrographs showing (a) dense bacterial aggregates colonizing the root canal walls and some cells invading the dentinal tubules (original magnification 1800×), and (b) dividing bacterial cells within tubules (5500×).

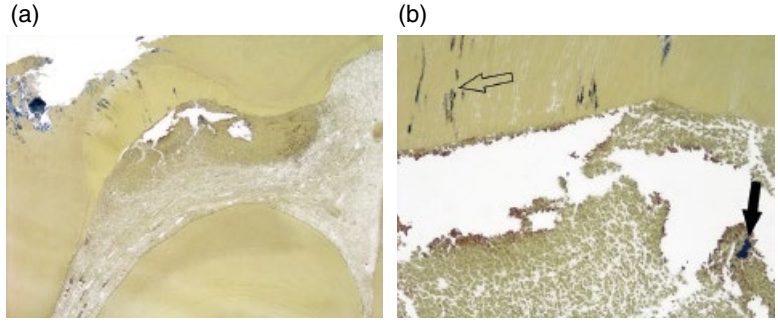

Figure 4.8 (a) Caries biofilm reaching the pulp and causing extensive inflammation (Taylor-modified Brown and Brenn staining, original magnification 16×). (b) Higher magnification showing bacterial invasion of the tertiary dentin (blank arrow). Bacteria are also seen in the pulp (black arrow), which is severely inflamed (100×). Courtesy of Dr Domenico Ricucci.

4.3.6 Apical Periodontitis is a Disease Caused by Biofilms

Apical periodontitis fulfils the criteria commonly used to establish a causal link between an infectious disease and biofilms [82, 160, 183]. They are as follows.

1) The infecting bacteria are adhered to or associated with a surface.
   Findings for apical periodontitis: As discussed above, bacteria infecting the root canal are often organized in communities attached to dentinal walls of the main canal and other areas of the system [22, 138, 148, 183, 186, 229, 276].
2) Direct examination of infected tissue shows bacteria forming populations encased in an extracellular matrix.
   Findings for apical periodontitis: Histobacteriological studies have shown that bacterial communities adhering to the canal walls are enmeshed in an amorphous extracellular matrix with varying thickness [183, 186, 239], a structure similar to biofilms reported in other human sites, including dental plaque.
3) The infection is generally confined to a particular site and although dissemination may occur, it is a secondary event.
   Findings for apical periodontitis: In endodontic infections, biofilms are frequently restricted to the root canal system (intraradicular biofilms) [183]. In rare conditions, the biofilm is found extending to the external root surface, but dissemination beyond this area or the apical periodontitis lesion is rarely, if ever, reported in chronic cases.
4) The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible microorganisms are susceptible to killing in the planktonic cell state.
   Findings for apical periodontitis: Endodontic infections cannot be successfully treated by systemic antibiotics. Antibiotics are prescribed usually in conditions where there are signs of dissemination of the infecting bacteria. The reason for this inefficacy is that the bacteria are located in an avascular necrotic space, in which antibiotics cannot penetrate in adequate concentration to be effective. In addition, bacterial cells living in biofilms are reported to be 100 to 1000 times more resistant to antibiotics than their planktonic counterparts [153].
5) Ineffective host clearance, which may be evidenced by the location of bacterial colonies in areas of the host tissue associated with inflammatory cells.
   Findings for apical periodontitis: accumulations of inflammatory cells, mostly polymorphonuclear neutrophils, are often seen facing endodontic biofilms [183].
6) Elimination or significant disruption of the biofilm structure and ecology leads to remission of the disease process.

Findings for apical periodontitis: if the endodontic treatment succeeds in rendering the root canal free of detectable bacteria at the time of filling, the success rate is significantly increased [50]. Moreover, the frequent observation of biofilms in root canal-treated teeth with apical periodontitis, and a lack thereof in teeth with healthy periapical areas [183, 187], indicates that there is a potential for fulfilment of this criterion.

4.3.7 Requirements for an Endodontic Pathogenic Community

The biofilm community should fulfil the following requisites to initiate and maintain disease:

1) The community density must be high enough to reach a pathogenic load.
2) The community must exhibit an array of antigens and virulence factors, which should be expressed during root canal infection, accumulated in the community EPS matrix, and released to the environment.
3) The community must be spatially located in the root canal system in such way that cells and/or virulence factors and antigens can gain access to the periradicular tissues.
4) The community must contain pathogenic species that are well integrated and organized in a synergic relationship with the other biofilm species.
5) The host must mount a defense strategy at the periradicular tissues, which inhibits the spread of the infection to the bone and beyond, but which also results in tissue damage.

4.4 Mechanisms of Bacterial Pathogenicity

The ability of microorganisms to cause disease is regarded as *pathogenicity*. *Virulence* denotes the degree of pathogenicity of a microorganism, and *virulence factors* are the microbial products, structural components or strategies that contribute to pathogenicity.

The collective pathogenicity of the bacterial community will depend on the overall population density, species composition, and the synergistic interactions between them. Bacterial organization in biofilms permits the accumulation of virulence factors and their gradual release from the EPS matrix to affect the adjacent host tissues. Different virulence factors usually act in combination at various stages of infection, and a single factor may have several functions in different stages. Virulence factors may be involved in attachment to host surfaces, tissue and host cell invasion, spread through the host tissues, direct and indirect tissue damage, and survival strategies, including evasion of host defense responses.

Bacteria exert their pathogenicity by destroying host tissues through direct and/or indirect mechanisms. Direct harmful effects caused by bacteria usually involve secreted products, including enzymes (proteases, peptidases, hyaluronidase, etc), exotoxins, and metabolites (butyrate, propionate, ammonium, polyamines, indole, volatile sulfured compounds, etc) [245]. In apical periodontitis, the indirect mechanisms dominate in the development of tissue damage. Thus, it is the host immune response to the bacterial challenge that ultimately causes the tissue destruction typical of apical periodontitis. Stimulation and activation of the host immune responses is caused by bacterial antigens and structural components (modulins), including lipopolysaccharide (LPS or endotoxin), peptidoglycan, lipoteichoic acids, fimbriae, flagella, outer membrane proteins, and exopolysaccharides [86, 303].

Bone resorption illustrates this. Inflammatory and non-inflammatory host cells are stimulated by bacterial components to release chemical mediators such as cytokines and prostaglandins, which are involved in the induction of bone resorption characteristically observed in chronic apical periodontitis lesions [226]. Bacterial DNA also activates macrophages and dendritic

cells and triggers the release of pro-inflammatory cytokines [108]. Pro-inflammatory cytokines stimulate osteoclastic bone resorption either by enhancing the proliferation and differentiation of osteoclast precursors or by promoting activation of mature osteoclasts, or both [213].

Another example of indirect damage caused by bacteria is pus formation in acute apical abscesses. Although some bacterial products may cause tissue damage and play a direct role in the generation of pus, the main cause of connective tissue destruction and liquefaction is products released by polymorphonuclear neutrophils (PMNs) in the response to the infection. Hyperactive, supernumerary, or dysregulated PMNs cause tissue damage through the release of toxic substances such as oxygen-derived free radicals or tissue-degrading lysosomal enzymes.

The sheltered location of the root canal microbiota implies that for bacteria to exert their pathogenicity, they must either invade the periradicular tissues or their products and/or structural components must penetrate the tissues and evoke a response by the host tissues. Bacteria invade tissues either by means of their motility or by growth. Motile bacteria, such as treponemes, can escape phagocytes by a rapid movement. However, most endodontic bacteria are non-motile and tissue invasion by growth requires that the rate of reproduction overcomes the host defense mechanisms. Frank invasion of the periradicular tissues is rather uncommon and, when it occurs, bacteria are usually rapidly eliminated. However, in some instances, massive invasion of the periradicular tissues by bacteria results in severe inflammation and abscess formation. The presence of virulent species or strains, and a highly virulent multispecies community predisposes to abscess formation (see "Symptomatic infections").

It is assumed that the oral microbiota contains only a few truly pathogenic species, and most of them exhibit low virulence. This is consistent with the chronic progressive nature of the most common forms of apical periodontitis. Therefore, because bacterial infection of the periradicular tissues rarely occurs (except for abscesses), direct or indirect damage to the tissues is caused by bacterial secreted products or structural components that diffuse out from the intraradicular biofilm or are released by bacterial cells that reach the periradicular tissues, but which are quickly destroyed therein.

Nearly all virulence factors are tightly regulated, and their expression is linked to environmental signals or cues. Biochemical and physical parameters that affect virulence factor regulation include starvation, populational density, pH, temperature, iron availability, oxygen tension, and redox potential. Therefore, upon receiving the appropriate environmental signals, different sets of virulence genes can be turned on or turned off. This affords bacteria the ability to adapt themselves to different and varying environmental conditions.

Stressful conditions, such as starvation, may stimulate the virulence apparatus in certain pathogens [110, 129]. Periods of starvation are commonly experienced by living bacteria in their natural environments. Once starvation genes are expressed, bacteria shift their behavior in order to survive in conditions of nutrient depletion. The major induced mechanisms include control of the energy generation during starvation and enhancement of the scavenging ability of the scarce nutrient. These mechanisms may allow bacteria to survive in root canal-treated teeth and induce post-treatment apical periodontitis lesions.

4.4.1 Bacterial Virulence Factors Released in the Canal Milieu

The pathogenic ability of multispecies biofilm communities is related to the accumulation and gradual release of virulence factors and antigens from the different component species in the EPS matrix. Evaluation of the bacterial substances released during the course of a multispecies infection thus provides valuable information about the physiologic and pathogenic behavior of the community.

LPS is one of the dominating constituents of the outer membrane of most Gram-negative bacteria and is composed by a hydrophilic polysaccharide and a hydrophobic glycolipid component (lipid A) [193]. Most of its biological effects are related to the lipid A portion, which is exposed after cell death or during multiplication. Interaction of LPS with host cells cause numerous biological effects, including: activation of macrophages/monocytes with consequent synthesis and release of pro-inflammatory cytokines, prostaglandins, nitric oxide, and oxygen-derived free radicals [86, 303, 328]; activation of the complement system [96, 332]; stimulation of osteoclast differentiation and bone resorption [345]; and activation of pattern recognition receptors expressed on trigeminal afferent neurons, triggering intracellular signaling cascades that lead to peripheral release of neuropeptides and central nociceptive neurotransmission [316]. The content of LPS in infected root canals is higher in teeth with primary infections, symptomatic apical periodontitis, large periradicular bone destruction, and in cases with persistent exudation [31, 69, 91, 92, 230, 231]. LPS from endodontic pathogens has been suggested to play an important role in tissue damage associated with abscesses [142] and bone resorption [32].

Although LPS has been widely studied and regarded as an important virulence factor from Gram-negative bacteria with regard to the pathogenesis of apical periodontitis, it seems too simplistic to attribute to this substance all the biological effects of endodontic infections related to causation of apical periodontitis. In a multispecies community like endodontic biofilms, many other virulence factors are expected to be produced and participate in disease pathogenesis. Indeed, many other potential virulence factors are released in the root canal milieu during the course of infection.

Lipoteichoic acid (LTA) is an anionic polymer that is a major component of the Gram-positive cell wall. Given its biological effects, LTA may be considered as the counterpart of LPS for Gram-positive bacteria. LTA stimulates macrophages/monocytes to release of pro-inflammatory cytokines [303, 321] and activate the complement system [66]. A study quantified LTA in teeth with post-treatment apical periodontitis and found this molecule in all cases examined [9].

Several end-products of the bacterial metabolism are released to the extracellular environment and may be toxic to host cells, cause degradation of constituents of the extracellular matrix of the connective tissue, and interfere with host defense processes [46, 73, 304]. Among them, short-chain fatty acids (SCFAs) are well known metabolic end-products of anaerobic bacteria that have been regarded as potential virulence factors [150, 175]. Provenzano et al. [172] evaluated the occurrence of SCFAs in primarily infected root canals before and after treatment and revealed that butyric and propionic acids were the most frequently found. Both SCFAs were also found after chemomechanical procedures. Lactic acid was not present in detectable levels before treatment, but was very frequent after calcium hydroxide medication.

Polyamines are produced by bacteria following enzymatic decarboxylation of amino acids. Examples of polyamines include putrescine, spermidine, spermine, and cadaverine. Polyamines can dysregulate apoptosis of PMNs and lead to premature cell death [122]. A study found greater amounts of polyamines in root canals of teeth with spontaneous pain and swelling when compared with asymptomatic teeth [121].

Community profiling studies have revealed a high inter-individual variability in the bacterial diversity of endodontic infections [225, 238], with innumerable bacterial combinations leading to the same disease outcome. In these cases, despite the differences in species composition between individuals, the bacterial communities are likely to exhibit a similar physiologic and pathogenic behavior. Disease-associated biofilm communities have been shown to exhibit conserved metabolic gene expression profiles, despite the high subject-to-subject variability in species

composition [97]. Thus, even though communities associated with a given disease vary in their species composition, they may behave similarly towards host tissues. This suggests that there is some redundancy in terms of bacterial physiology and function in disease-associated communities, in which the overall diversity of bacterial products released is lower than the species diversity. In this context of functional redundancy, a given product that is essential to the physiology or pathogenicity of a community may be provided by different species in different individuals. This helps explain the high variability in community species composition from different individuals with the same disease (e.g., asymptomatic apical periodontitis, acute apical abscess.

Metaproteomics technologies have been used for large-scale characterization of the entire protein complement of microbial communities at a given point in time [327]. Therefore, the products of gene expression (proteins) are identified directly in samples. Some studies have used metaproteomics to evaluate endodontic infections. Nandakumar et al. [149] identified bacterial proteins in cases of primary or persistent infections and found proteases, virulence factors, autolysins, and proteins involved with adhesion, conjugation, and antibiotic resistance. Provenzano et al. [171] evaluated the metaproteome of primary infections associated with acute apical abscesses and asymptomatic apical periodontitis and reported an overall greater number of proteins in the former. The large majority of microbial proteins were related to metabolic and housekeeping processes, including protein synthesis, energy metabolism and DNA processes, indicating the occurrence of viable and active cells. Several proteins related to pathogenicity and resistance/survival were identified, including proteins involved with adhesion, biofilm formation and antibiotic resistance, stress proteins, exotoxins, invasins, proteases and endopeptidases (mostly in abscesses), and an archaeal protein linked to methane production.

In another study, Provenzano et al. [173] evaluated the bacterial metaproteome in root apices and their associated inflammatory lesions from teeth with post-treatment apical periodontitis. Proteins from viable and metabolically active bacterial cells were detected both in the apices and in the associated lesions. Several bacterial proteins related to pathogenicity and resistance/survival were identified in both apices and lesions, including proteins involved with antibiotic resistance, proteolytic function, stress-response, adhesion, and virulence.

4.5 Microbial Ecology and the Root Canal Ecosystem

A root canal containing necrotic pulp tissue provides a space for colonization and affords bacteria a moist, warm, nutritious and anaerobic environment, which is by and large protected from the host defenses because of lack of active blood circulation in the necrotic tissue. Also, the root canal walls are non-shedding surfaces, being conducive to persistent colonization and formation of complex sessile biofilm communities.

Intuitively, the root canal system can be considered a rather lush environment for bacterial growth. Consequently, one might assume that colonization should be easy for virtually all oral bacterial species. Over 700 different bacterial species have been reported to occur in the oral cavity and about 100 to 300 species can make up one individual's oral microbiota [37, 106]. Theoretically, these species share similar opportunities to invade and colonize root canals. Nonetheless, the relative dominance of 10 to 30 species found in primarily infected root canals argues otherwise. Even without the significant presence of host defense factors, the necrotic root canal provides a rather selective environment for bacteria to adapt and colonize. Environmental pressures must occur in the root canal that favor the establishment of some species and inhibit others.

4.5.1 Ecological Determinants

The ecological factors that influence the establishment of a colonizing microbiota in most environments include: oxygen tension and redox potential (Eh); available nutrients; microbial interactions; host defense factors; temperature; pH; and receptors for bacterial adhesions.

4.5.1.1 Oxygen Tension and Redox Potential

The root canal infection is a dynamic process and different bacterial species dominate the community at different phases of the infectious process. In the earliest stages of root canal infection, the number of bacterial species and cells colonizing the root canal system are low. Pulp infection is frequently a sequel to caries. The bacterial species in the frontline of the caries biofilm are the first ones to reach the pulp. Except for lactobacilli, most species frequently detected in advanced caries lesions associated with pulp exposure and irreversible pulpitis are also found in endodontic infections [209, 210] (Figure 4.9). Early colonizers or pioneer species set the stage for further colonization by other species.

Shifts in the composition of the microbiota are observed over time. They are largely due to changes in environmental conditions, including oxygen tension and redox potential. A study in monkeys revealed that facultative bacteria predominate in the very initial phases of the pulp infectious process [49]. After a few days or weeks, oxygen is depleted within the root canal as a result of pulp necrosis and consumption by facultative bacteria. Further oxygen supply is interrupted because of loss of blood circulation in the necrotic pulp tissue. An anaerobic milieu with consequent low redox potential develops, which is highly conducive to the survival and growth of obligate anaerobic bacteria. With the passage of time, anaerobic conditions become even more pronounced, particularly in the apical segment of the root canal. Several anaerobic bacterial species have been identified in the apical segment of the root canals of teeth with primary apical periodontitis [12, 207].

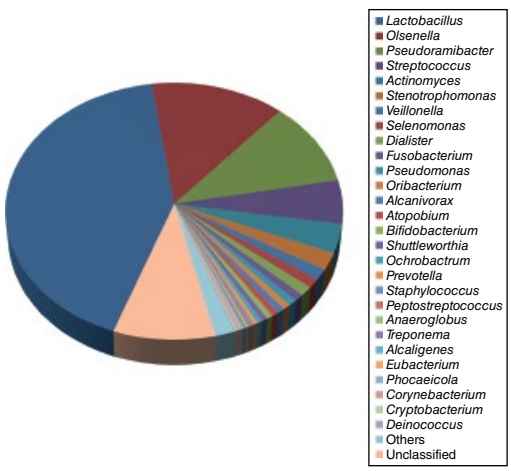

Figure 4.9 Average relative abundance of bacterial genera in deep dentinal caries lesions in teeth with symptomatic irreversible pulpitis. Data according to Rôças et al. [210].

4.5.1.2 Available Nutrients

The selective physical environment of the root canal system is characterized by an apparent excellent access to nutrients, which however may be limited depending on the spatial location of a given bacterial population and the stage of infection. The large majority of nutrients available for endodontic bacteria are derived from the host. However, certain essential nutrients to some species are not provided by the host and must be delivered by other species in the infected site (discussed below in "Microbial Interactions"). Because the root canal system may not be rich in nutrients, there will be competition for the amounts available. Oral bacterial species have different nutritional demands and competence in acquiring or scavenging for nutrients. As a consequence, bacterial species that can best utilize and compete for nutrients in the root canal system will best succeed in colonization.

In the root canal system, bacteria can utilize the following nutrient sources: 1) necrotic pulp tissue, containing remnants of dead pulp cells and other degenerated constituents of the pulp connective tissue; 2) components

(usually proteins and glycoproteins) of tissue fluids and exudate that seep into the root canal system via apical and lateral foramina; 3) saliva that may coronally penetrate into the canal; and 4) products of the metabolism of other bacterial species (discussed below). Because the largest amount of nutrients is available in the main canal lumen, which is the most voluminous part of the root canal system, most of the infecting microbiota, particularly fastidious anaerobic species, is expected to be located in this region.

The dynamics of nutrient utilization by microbial species can also induce shifts in the infecting microbiota of the root canal system, with saccharolytic species dominating the very early stages of the infectious process but being soon outnumbered by asaccharolytic species, which will dominate later stages. For better understanding, an analogy can be made based on studies of nutrient utilization in serum [295]. In the initial phases of the infectious process, the low amount of carbohydrates available in serum is rapidly consumed by saccharolytic bacteria. In an intermediary phase, carbohydrates are split off from glycoproteins and their content is completely consumed. Proteins are hydrolyzed and some amino acid fermentation takes place. This indicates a shift from a saccharolytic community to a proteolytic one. Species from the genera *Prevotella*, *Porphyromonas*, and *Fusobacterium* predominate in this phase. In a final phase, progressive protein degradation and extensive amino acid fermentation is observed, with *Parvimonas*, *Prevotella*, *Porphyromonas*, and *Fusobacterium nucleatum* prevailing over other species [295].

The necrotic pulp tissue represents a finite source of substrate for bacterial growth, given the small volume of tissue occupying the root canal system. However, development of periradicular inflammation ensures a sustainable source of nutrients in the form of proteins, glycoproteins, degraded collagen and iron-containing compounds carried into the canal by seepage of the inflammatory exudate via apical and lateral foramina. At this stage, bacteria that have a proteolytic capacity or establish a cooperative interaction with those that can utilize this substrate in the metabolism start dominating the community. As the infectious process reaches the stage of induction of periradicular inflammation, proteins become the principal source of nitrogen and carbon, particularly in the apical portion of the canal, favoring the establishment of anaerobic species that utilize peptides and/or amino acids in their metabolism. Thus, the root canal environment in untreated necrotic teeth affords bacteria a shifting pattern of nutrient availability and type over time, which will impact on the composition of the microbiota.

4.5.1.3 Bacterial Interactions

The structure of the endodontic microbiota can also be shaped by ecological relationships between the species that invade the root canal system. Because endodontic infections are characterized by multispecies biofilm communities, different bacterial species are in close proximity with one another and a multitude of interactions become inevitable. These interactions can be positive or negative.

Positive interactions enhance the survival capacity of the interacting species. Sometimes different species coexist in habitats where neither could exist alone. Positive bacterial interactions in multispecies communities include interbacterial nutritional interactions (food chains/webs, and concerted action to break down complex substrates); local environmental modification; collective protection against external threats; cell–cell signaling (quorum-sensing systems); and horizontal gene transfer.

Interbacterial nutritional interactions are mainly represented by food chains/webs that include utilization of metabolic end-products from one species by another in a relationship of mutualism or commensalism (Figure 4.10). Mutualism between two species occurs when both benefit from the relationship, as for instance the bi-directional use of metabolites. Commensalism is a unidirectional relationship between bacteria, in

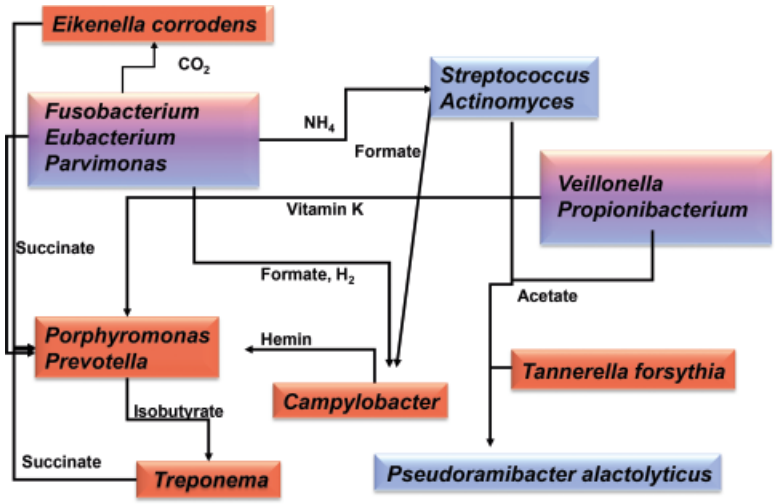

Figure 4.10 Nutritional relationships that may occur between bacterial species in an endodontic multispecies community.

which one species benefits and the other is unaffected.

Some species can modify the environment and thereby provide growth conditions favorable to other species. For instance, by reducing the oxygen tension in the environment, pioneer facultative bacteria can favor the establishment of anaerobes. Also, by releasing proteases or antibiotic-inactivating enzymes in the environment, some species can provide protection to the entire community from host defenses (e.g., antibodies and complement) and antibiotics, respectively.

Negative interactions act as feedback mechanisms that limit population densities. Examples that may occur in the endodontic milieu include competition and amensalism. Competition occurs when two species are striving for the same resource and focuses on available nutrients and space for colonization. Amensalism (antagonism) occurs when one species produces a substance (bacteriocin or metabolic end-product) that inhibits another species. Pioneer species colonizing a habitat may inhibit the establishment of competitive latecomers. The simplest way to avoid inhibition factors released by one bacterial species is to find sites that are not colonized by antagonistic species. Another method is to counterattack the antagonistic species by producing inhibitory or killing factors against it.

4.5.1.4 Other Ecological Determinants

Host defense factors. In the initial process of pulp infection, invading bacteria face the attack from the host immune defenses in the inflamed pulp. Only those that endure will successfully colonize the canal. When the whole pulp has become necrotic, the root canal microbiota is relatively protected from the host defenses because of the lack of an active circulation. However, in the apical part of the canal, the microbiota may still be influenced by specific host defense components entering the canal with the inflammatory exudate. Only the species capable to overcome these defenses can survive. The ability to form biofilms is one important survival strategy for bacteria in this regard.

Temperature. The temperature levels within the root canal likely range from 35–38°C, which is conducive to colonization by virtually all oral and many environmental bacteria.

pH. The pH in the necrotic pulp ranges from 6.4 to 7.0 [299], but it can rise slightly as a result of the metabolism of proteins by

some bacterial species. Some putative endodontic pathogens grow better at slightly alkaline pH values and present marked alterations in the enzyme profile under such conditions. For instance, trypsin-like activity in *Porphyromonas gingivalis* is maximal at pH 8.0 [128]. Bacteria vary in regard to their pH tolerance, with most species best growing within a range of 6 to 9 pH [7]. Fungi generally exhibit a slightly wider pH range, growing within a range of 5 to 9 pH [7].

Receptors for bacterial adhesins. Adhesins are bacterial molecules involved with specific host tissue recognition and adhesion as well as cell–cell specific binding between bacteria. They are usually chemical components of fimbriae, pili, cell walls, and capsules and play an important role in the strong adhesion of bacteria to the surfaces for initial biofilm formation. Cell–cell binding also favors the attachment of latecomers with the consequent establishment of complex multispecies bacterial communities. Because adhesins bind to specific complementary receptors on host surfaces, the types of host molecules expressed influence the species that will adhere and colonize the surface. While this factor is an important determinant of the bacterial species that colonize the tooth surfaces for dental plaque formation, there is only scarce information on the types of receptors for bacterial adhesins involved with intracanal biofilm formation. Plasminogen, a major plasma protein that can coat the dentinal walls following tissue fluid seepage in the canal, and collagen type I from predentin and dentin may serve as receptors for bacterial adhesion in necrotic canals [107, 117, 139]. In cases where saliva leaks into the root canal, a conditioning film composed of salivary proteins can form on the canal walls and favor bacterial adhesion and biofilm formation [65].

4.5.2 Ecology of the Endodontic Biofilm Community

As noted earlier, a dynamic bacterial biofilm community establishes itself in the necrotic root canal system and is shaped over time by gradual species succession [49, 294]. The pioneer species influence the pattern of bacterial succession within the root canal. With the passage of time, the number of species gradually changes and increases and the community becomes more complex. Community members may be joined or replaced by other species.

The spatial organization of the bacterial populations within the endodontic biofilm is unlikely to be at random. Species that are metabolic interdependent will likely be located in close proximity to each other.

In the late stages of the pulp infectious process, certain groups of anaerobic bacteria start dominating the microbiota. Eventually, a stable situation and a high level of community organization may be reached, with populations co-existing in harmony and balance with their environment. This means that the community reach a climax in its maturity and organization [124, 284]. A multitude of niches (metabolic functions) may take place in the biofilm consortium. Consequently, physiologically different species can coexist indefinitely provided they are functionally compatible.

Environmental conditions within the root canal system are not uniform and differences will influence the composition of the infecting community and the establishment of different ecological niches. Organization of populations in the endodontic community are conceivably dictated by the ecological determinants occurring in different parts of the root canal system. Environmental conditions may vary along the entire extent of the root canal system, with differences being more pronounced at the two ends (coronal and apical). Oxygen and nutrient gradients arguably set up in root canals exposed to the oral environment [285]. In the coronal region of the canal, given the proximity with the oral cavity, the oxygen tension is higher than in other areas of the canal. As a consequence, facultatives and aerotolerant anaerobes are expected to prevail in this area, whereas the anaerobic conditions in the apical segment

are highly conducive to the establishment of a microbiota dominated almost exclusively by obligately anaerobic bacteria [49]. Similarly, bacteria located in the most coronal aspects of the canal can utilize carbohydrates from the host diet and saliva, which can seep into the canal via coronal exposure. Bacteria in the most apical area of the root canal system utilize protein- and glycoprotein-rich tissue fluids and exudate which penetrate in the canals via apical and lateral foramina. Therefore, the dominance of different bacterial groups along the biofilm structure occurring in different parts of the root canal system can be predicted based mostly on oxygen tolerance and nutrient requirements.

4.6 Types of Endodontic Infections

Endodontic infections can be classified according to their anatomical location (intraradicular or extraradicular infection) and the time participating bacteria gained entry into the root canal (primary, secondary, or persistent infection) [245]. The composition of the microbiota may vary depending on the different types of infection and different forms of apical periodontitis.

4.6.1 Intraradicular Infection

As the name implies, this is caused by bacteria colonizing the root canal system. it can be subdivided into three categories.

4.6.1.1 Primary Intraradicular Infection

Primary intraradicular infection is caused by bacteria that invaded and colonized the necrotic pulp tissue. It has also been referred to as initial infection and is the cause of primary apical periodontitis. Primary infections are characterized by a mixed consortium composed of 10 to 30 bacterial species per canal [140, 180, 196, 267, 272, 277]. The number of bacterial cells in an individual infected canal may vary from 10^3 to 10^8 [18, 222, 250, 291, 311]. Teeth with sinus tracts and/or large apical periodontitis lesions have been shown to harbor a microbiota that is more complex in terms of number of species (richness) and cells (density) [196, 251, 291]. The microbiota of primary infections is conspicuously dominated by anaerobic bacteria, particularly Gram-negative species belonging to the genera *Fusobacterium*, *Treponema*, *Tannerella*, *Dialister*, *Porphyromonas*, *Prevotella*, and *Campylobacter*. Gram-positive anaerobes from the genera *Parvimonas*, *Filifactor*, *Actinomyces*, *Olsenella*, and *Pseudoramibacter*, as well as facultative or microaerophilic streptococci are also commonly found in primary intraradicular infections.

4.6.1.2 Secondary Intraradicular Infection

Secondary intraradicular infections are caused by microorganisms that were not present in the primary infection, but were introduced in the root canal system at some time during or after professional intervention. In any circumstance, if penetrating microorganisms succeed in surviving and colonizing the root canal, a secondary infection is established.

The main sources of secondary infections *during treatment* include: remnants of dental plaque, calculus or caries on the tooth crown; leaking rubber dam; contamination of endodontic instruments; and contamination of irrigant solutions.

Microorganisms can also enter the root canal system *between appointments*. This may occur following leakage along the temporary restorative material; breakdown, fracture or loss of the temporary restoration; fracture of tooth structure; and exposure to the oral environment in teeth left open for drainage.

Finally, microorganisms can penetrate the root canal system *after placement of the root canal filling* in the following situations: leakage through the temporary or permanent restorative material; breakdown, fracture, or loss of the temporary/permanent restoration; fracture of the tooth structure; recurrent decay exposing the root canal filling material; or loss of seal provided by the temporary

material as a consequence of delay in placement of the permanent restoration.

The species causing secondary infections will depend on the source of contamination. Nonoral species may be found, including *Pseudomonas aeruginosa, Staphylococcus* species, enteric rods, *Candida* species, and *Enterococcus faecalis* [77, 176, 177, 262, 279, 318]. If the cause of secondary infection is coronal leakage or any other condition that exposes the root canal to saliva, then the species involved will come from the subject's own oral cavity.

4.6.1.3 Persistent Intraradicular Infection

Persistent intraradicular infections are caused by bacteria that in some way resisted intracanal antimicrobial procedures and endured periods of nutrient deprivation in an infected canal during treatment. The cultivable microbiota associated with persistent infections is usually composed of fewer species than primary infections, and Gram-positive facultative bacteria are the most prevalent [135, 169, 266, 271, 272, 292]. Fungi can be found in teeth with post-treatment disease in frequencies significantly higher when compared with primary infections [278].

Both persistent and secondary infections are for the most part clinically indistinguishable and can be responsible for several clinical problems, including persistent exudation, persistent symptoms, inter-appointment exacerbations, and post-treatment apical periodontitis (Figure 4.11).

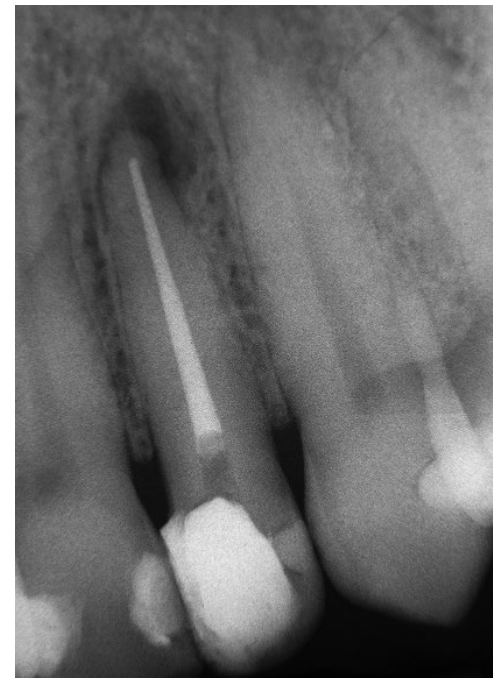

Figure 4.11 Tooth with post-treatment apical periodontitis. Persistent or secondary intraradicular infections are the main causative agents of this form of the disease.

4.6.2 Extraradicular Infection

Extraradicular infection is characterized by bacterial invasion of the inflamed periradicular tissues. While it is almost invariably a sequel to the intraradicular infection, an established extraradicular infection may be dependent on or independent of the intraradicular infection. The most common form of extraradicular infection, which is dependent on the intradicular infection, is the acute apical abscess. It has been suggested that apical actinomycosis, caused by *Actinomyces* species or *Propionibacterium propionicum*, may be a form of extraradicular infection independent of the intraradicular infection [145]. This condition accounts for about 2–4% of the apical periodontitis lesions, but its independent nature remains unproven [257]. The question as to whether the extraradicular infection is dependent on or independent of the intraradicular infection assumes special relevance from a therapeutic standpoint, since the former can be successfully managed by root canal therapy while the latter may have to be handled by periradicular surgery.

4.7 Identification of Endodontic Bacteria

Although the final pathogenicity is strongly determined by the biofilm as a community, identification of the species associated with a given infectious disease is still necessary for the understanding of the etiology and

pathogenesis of the disease as well as for the development of better strategies to treat and prevent the disease. Microbiological studies for identification of the species participating in endodontic infections can be chronologically divided into five generations on the basis of the different strategic approaches used and their contribution to knowledge [272, 275]:

- *First generation:* This is represented by studies of the endodontic microbiota using open-ended culture methods to detect virtually all the cultivable species present in the root canal. The great contribution of this generation relates to the disclosure of many cultivable species strongly associated with apical periodontitis, including *Fusobacterium nucleatum*, *Prevotella* species, *Porphyromonas* species, *Parvimonas micra*, and streptococci.
- *Second generation:* This encompasses studies that employ closed-ended (species- or group-specific) DNA-based molecular microbiology methods, such as polymerase chain reaction (PCR) and the conventional checkerboard hybridization approach, to target cultivable bacterial species. Results from these studies generally showed higher prevalences for many cultivable bacterial species and contributed to strengthen their association with apical periodontitis. In addition, some difficult-to-grow species from the genera *Tannerella*, *Dialister*, *Filifactor*, and *Treponema* were included in the set of candidate endodontic pathogens.
- *Third generation:* This involves open-ended DNA-based molecular studies, using broad-range PCR followed by cloning and Sanger sequencing, terminal-restriction fragment length polymorphism (T-RFLP) or denaturing gradient gel electrophoresis (DGGE), for detecting cultivable species and as-yet-uncultivated phylotypes in endodontic infections. These molecular methods are generally laborious, time-consuming, and expensive, resulting in the analysis of only a few samples per study. However, they have permitted cataloging the cultivable and as-yet-uncultivated/uncharacterized portions of the endodontic microbiota, refining the knowledge of bacterial diversity associated with apical periodontitis.
- *Fourth generation:* These studies use closed-ended molecular methods, such as species- or group-specific PCR and the reverse-capture checkerboard assay, for large-scale investigations of the prevalence and levels of cultivable and as-yet-uncultivated bacteria in endodontic infections. Findings from this generation confirmed the association of several cultivable bacteria with apical periodontitis and included some as-yet-uncultivated phylotypes in the set of candidate endodontic pathogens.
- *Fifth generation:* High-throughput (formerly "next-generation") sequencing (HTS) approaches have been introduced which have permitted DNA sequencing from samples to a far deeper coverage and higher throughput in comparison with the traditional Sanger sequencing approach [88, 315]. Consequently, bacterial diversity in samples have been explored to reveal even low-abundance components of the community. These methods, especially the pyrosequencing and Illumina approaches, have been used for open-ended analysis of endodontic samples, and substantially expanded the knowledge of the bacterial diversity associated with apical periodontitis.

4.7.1 Taxonomy of Endodontic Infections

Current evidence reveals that over 500 different bacterial species have been identified in endodontic infections; these species fall into 9 of the 13 phyla that have oral representatives, namely Bacteroidetes, Firmicutes, Spirochaetes, Fusobacteria, Actinobacteria, Proteobacteria, Synergistetes, "Candidatus Saccharibacteria" (formerly TM7), and SR1 [272] (Figure 4.12). However, there may be representatives of at least 10 other phyla in endodontic infections as revealed by HTS methods [90, 114, 156, 225, 255, 259, 301, 308, 339]. Named species that have been

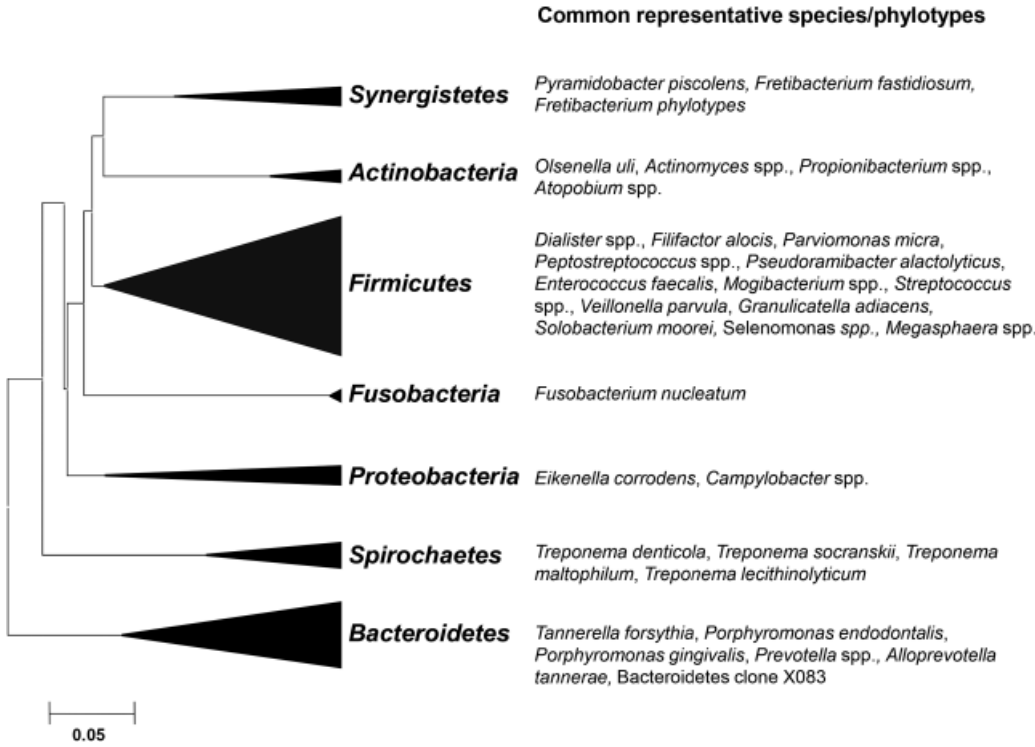

Figure 4.12 Bacterial phyla and their main representatives in endodontic infections.

regarded as candidate endodontic pathogens include Gram-negative bacteria from the genera *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Dialister*, *Treponema*, *Tannerella*, *Pyramidobacter* and *Campylobacter*, and Gram-positive bacteria from the genera *Parvimonas*, *Pseudoramibacter*, *Streptococcus*, *Enterococcus*, *Olsenella*, *Filifactor*, *Actinomyces*, and *Propionibacterium* [12, 64, 68, 104, 140, 180, 196, 221, 224, 268, 272, 291] (Table 4.2).

4.7.2 As-yet-Uncultivated Phylotypes

Phylotype is a term used for those as-yet-uncultivated species that are known only by a 16S rRNA gene sequence identified by molecular microbiology methods from the third, fourth and fifth generations. Third-generation studies of the endodontic bacterial diversity have shown that 40% to 60% of the species-level taxa (richness) still remain to be cultivated and validly named [140, 180, 221, 222, 313]. As-yet-uncultivated phylotypes may represent about 30–40% of the endodontic bacterial community in terms of relative abundance [221]. Uncultivated phylotypes detected in endodontic infections have been classified into several known genera, including *Dialister*, *Treponema*, *Prevotella*, *Solobacterium*, *Olsenella*, *Fusobacterium*, *Megasphaera*, *Veillonella*, and *Selenomonas* [140, 195, 212, 219, 221, 224, 248, 268]. One of the most prevalent as-yet-uncultivated phylotypes encountered in endodontic infections in fourth-generation studies is *Bacteroidaceae* sp. HOT-272 (synonym, Bacteroidetes oral clone X083) [196, 197]. Several members of the Synergistetes phylum have been identified in infected root canals; most of them still remain uncultivated [195, 208, 268, 270]. Some, however, have been recently cultivated using special strategies, which permitted them to be characterized, and formally named as *Pyramidobacter piscolens* [41] and *Fretibacterium fastidiosum* [307].

Table 4.2 Bacterial genera and respective common representative species occurring in endodontic infections.

Genus	Common representatives	Primary infections (asymptomatic apical periodontitis)	Primary infections (acute apical abscess)	Persistent/ secondary infections (post-treatment apical periodontitis)
Gram-negative				
Anaerobic rods				
Dialister	*D. invisus, D. pneumosintes,* uncultivated phylotypes[a]	+++	+++	+
Porphyromonas	*P. endodontalis, P. gingivalis*	+++	+++	+
Tannerella	*T. forsythia*	+++	+++	+
Prevotella	*P. intermedia, P. nigrescens, P. multissacharivorax, P. baroniae, P. denticola,* uncultivated phylotypes[a]	+++	+++	+
Alloprevotella	*A. tannerae*	++	++	-
Fusobacterium	*F. nucleatum,* uncultivated phylotypes[a]	+++	+++	+
Campylobacter	*C. rectus, C. gracilis, C. showae*	++	+	+
Fretibacterium	*F. fastidiosum,* uncultivated phylotypes[a]	++	++	-
Pyramidobacter	*P. piscolens*	++	++	+
Selenomonas	*S. sputigena, S. noxia,* uncultivated phylotypes[a]	++	+	-
Anaerobic cocci				
Veillonella	*V. parvula,* uncultivated phylotypes[a]	++	+	-
Megasphaera	uncultivated phylotypes[a]	+	+	-
Anaerobic spirilla				
Treponema	*T. denticola, T. socranskii, T. parvum, T. maltophilum, T. lecithinolyticum*	+++	+++	-
Facultative rods				
Capnocytophaga	*C. gingivalis, C. ochracea*	+	-	-
Eikenella	*E. corrodens*	++	++	-
Gram-positive				
Anaerobic rods				
Actinomyces	*A. israelii, A. gerencseriae, A. meyeri, A. odontolyticus*	++	+	++
Pseudoramibacter	*P. alactolyticus*	+++	+	++
Filifactor	*F. alocis*	++	+	+
Peptostreptococcaceae (Eubacterium)	*P. infirmum, P. saphenum, P. nodatum, P. brachy, P. sulci*	+	+	+

Table 4.2 (Continued)

Genus	Common representatives	Primary infections (asymptomatic apical periodontitis)	Primary infections (acute apical abscess)	Persistent/ secondary infections (post-treatment apical periodontitis)
Mogibacterium	*M. timidum, M. pumilum, M. neglectum, M. vescum*	+	+	+
Propionibacterium	*P. acnes, P. propionicum*	++	+	++
Eggerthella	*E. lenta*	+	+	-
Olsenella	*O. uli, O. profusa*	++	+	+
Atopobium	*A. parvulum, A. minutum, A. rimae*	+	-	+
Solobacterium	*S. moorei,* uncultivated phylotypes[c]	+	-	+
Anaerobic cocci				
Parvimonas	*P. micra*	+++	+++	++
Peptostreptococcus	*P. stomatis, P. anaerobius,* uncultivated phylotypes[a]	+	+	-
Anaerococcus	*A. prevotii*	+	-	-
Streptococcus	*S. anginosus, S. constellatus S. intermedius*	+++	+++	+++
Gemella	*G. morbillorum*	+	+	+
Facultative rods				
Actinomyces	*A. naeslundii*	+	-	-
Facultative cocci				
Streptococcus	*S. mitis, S. sanguinis, S. gordonii, S. oralis*	++	+	+++
Enterococcus	*E. faecalis*	+	-	+++
Granulicatella	*G. adiacens*	+	-	-

[a] for uncultivated phylotypes, Gram-staining patterns, cell morphology, and relationship to oxygen are estimates based on the general features of the genus.
+++, very frequently found in many studies
++, frequently found in many studies
+, found in many studies
-, rarely found, if ever

4.8 Endodontic Biofilm Community Profiles

Bacterial community profiles are determined by species richness and abundance. DNA-based molecular methods have been used to profile the biofilm communities associated with different types of endodontic infections and manifestations of apical periodontitis. These studies have demonstrated that the endodontic bacterial communities associated with primary [26, 119, 277] and post-treatment apical periodontitis [18, 26, 114, 194, 205, 223, 266] are mixed, composed of several different species, including as-yet-uncultivated bacteria [118, 248]. Different profiles have been reported for endodontic bacterial communities associated with different

clinical conditions, including asymptomatic apical periodontitis, acute apical abscesses, and post-treatment apical periodontitis (see below) [205, 221, 277, 301].

No two endodontic infecting communities are the same in terms of richness and abundance, resulting in a high inter-individual variability [26, 114, 151, 225, 277]. Even two infected teeth in the same individual show different profiles [2]. This indicates that apical periodontitis has a heterogeneous etiology, and multiple species combinations can lead to similar disease outcomes. In spite of the inter-individual variability, samples from individuals living in the same geographical location are more similar among them when compared to individuals living in distant locations [64, 119, 205, 252, 277]. This geography-related pattern in community profiles raises questions about the effectiveness of specific antimicrobial treatment (antibiotics) protocols for worldwide use.

4.9 Microbiota in the Apical Root Canal

The apical segment of the root canal system is a critical territory for the host, the infecting microbiota, and the clinician. Because the apical foramina are the main portals of exit of bacteria and their virulence factors, the host defenses must be mounted and concentrated near those areas in order to prevent infection from gaining access to the bone and disseminating to other body areas. In the apical canal, bacteria are located in a strategic position to obtain nutrients from and cause damage to the periradicular tissues. The clinician in turn must control the infection especially in this area and create conditions to prevent reinfection in order to succeed with root canal treatment.

The apical canal offers different ecological conditions in terms of oxygen tension and type of nutrients available, favoring the establishment of a microbiota that significantly differs from the one occurring in the most coronal parts of the canal system [2, 12, 49, 131, 296]. Bacterial counts in the apical canal may range from 10^4 to 10^6 cells [12].

The bacterial species identified in this region are highly likely to be the most important ones involved in the etiology of apical periodontitis, because they are in close contact with the affected tissues. While most studies have evaluated the microbiota of the entire root canal system, a few studies have looked at the bacterial species occurring exclusively in the apical root canal. Such studies can only be performed in extracted teeth or in teeth subjected to root-end resection. Several candidate endodontic pathogens have been identified in the apical canal segment, including *Prevotella* species, *Porphyromonas* species, *Pseudoramibacter alactolyticus*, *Streptococcus* species, *Olsenella uli*, *F. nucleatum*, *P. micra*, *Tannerella forsythia*, and *Treponema* species [12, 40, 207, 247, 253].

A molecular study found a mean number of 28 species in both apical and middle/coronal samples; however, the mean shared species was only 54%, ranging from values as low as 2% to 79% [2]. Therefore, while harboring similar number of species, the composition of the bacterial community in the apical canal was different from the coronal parts. There is also a high variability between subjects in terms of apical microbiota composition [2, 259].

Some species are more frequently found in the apical portion of the canal than in its matched coronal regions, including *Prevotella baroniae*, *T. forsythia*, and *F. nucleatum*, while others, such as *Streptococcus* species, are more prevalent in middle/coronal samples [207]. The complexity of the apical microbiota has been confirmed by studies using HTS methods [156, 259].

Bacteria in the apical canal of treated teeth with persistent disease are highly likely to be the cause of treatment failure. Molecular analysis of the apical root canal system of adequately treated teeth with persistent apical periodontitis have disclosed highly complex bacterial communities [255] (Figure 4.13). The community composition varied significantly from individual to individual. The mean

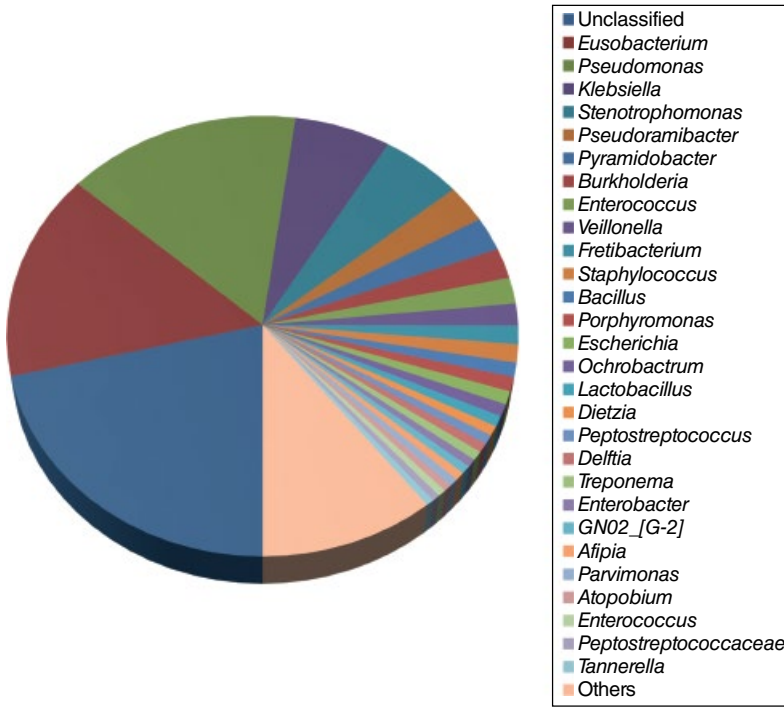

Figure 4.13 Average relative abundance of bacterial genera in root apex samples from teeth with post-treatment apical periodontitis. Data according to Siqueira et al. [255].

bacterial load in cryopulverized apical canal samples of adequately treated teeth with apical periodontitis was about 10^4 cells [5].

4.10 Symptomatic Infections

4.10.1 Factors Influencing the Development of Symptoms

In some cases, bacterial infection of the root canal can give rise to acute forms of apical periodontitis, including symptomatic apical periodontitis and acute apical abscess (Figure 4.14). Endodontic microbiology studies have long looked for association between specific bacterial species and symptomatic disease. Although it has been suggested that some Gram-negative anaerobic bacteria can be associated with symptoms [71, 74, 204, 274, 291, 305, 335], several studies have found similar prevalences of the same species in asymptomatic cases [15, 60, 78, 98, 196, 242, 243].

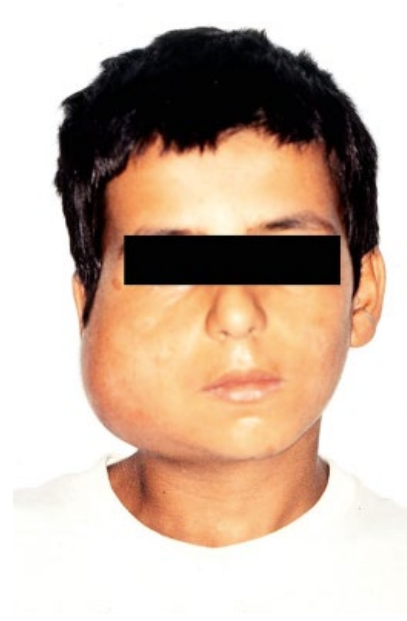

Figure 4.14 Acute apical abscess with extensive swelling. In cases like this, an extraradicular infection is established.

This suggests that factors other than the mere presence of a given pathogenic species can influence the development of symptoms. Actually, symptomatic manifestations of apical periodontitis are a result of interplay of diverse factors as proposed elsewhere [245, 275]. They are as follows.

4.10.1.1 Difference in Virulence Ability among Clonal Types of the Same Species

Clonal types of a given pathogenic bacterial species can significantly diverge in their virulence ability [8, 56, 75, 143, 155]. A disease ascribed to a pathogen is in fact caused by specific virulent clonal types of that species [143]. Therefore, the presence of virulent clonal types of candidate endodontic pathogens in the root canal may be a predisposing factor for pain. This helps explain why the same species are detected in both symptomatic and asymptomatic cases.

4.10.1.2 Cell Numbers or Infectious Load

Other important factors contributing to the development of symptomatic infections are the overall and specific bacterial loads. Overall bacterial load refers to the total cell numbers in the community, whereas the specific load relates to the counts and relative abundance of certain pathogenic species. The total number of bacterial cells in the community results in a heavy infectious bioburden for the host to cope with, and is characterized by a massive accumulation of virulence factors. Total bacterial counts in acute apical abscesses can vary from 10^4 to 10^9 cells per sample [104, 112, 325]. The levels of some specific virulent species are also of importance for the community pathogenicity. The possibility exists that the number of cells of a given species reported to occur in both symptomatic and asymptomatic infections is larger in the former. A molecular study detected *T. forsythia* in significantly higher density in symptomatic than in asymptomatic endodontic infections [227]. Another molecular study revealed that while the mere presence of the target species/phylotypes was not associated with symptoms (abscessed teeth), the counts of some taxa (*Porphyromonas endodontalis*, *Prevotella baroniae*, *Treponema denticola*, and *Streptococcus* species) were significantly higher in abscesses than in asymptomatic cases [201] Thus, presence of a potentially virulent pathogen in high counts may increase the virulence of the whole community and give rise to symptoms.

4.10.1.3 Bacterial Interactions and Collective Pathogenicity

Most endodontic pathogens are only capable to cause disease when in association with other species [13, 55, 101, 241, 293, 333]. This is because of synergism occurring among different species in a community. Interactions may influence virulence and play a role in symptom causation. Studies comparing symptomatic and asymptomatic endodontic infections showed different dominant species in the communities, with a significantly higher species richness in symptomatic teeth [220, 221, 225, 277, 337]. The increased diversity in teeth with symptomatic infections is expected to influence the collective pathogenicity of the biofilm, resulting in incalculable synergistic interactions among the community members. Every component of a polymicrobial infection, even species regarded as avirulent and/or in low numbers in the community, have been suggested to affect virulence of the whole biofilm community [42, 43, 164, 236]. Communication between members of a bacterial community can change the expression of virulence factors by certain pathogenic species and affect the collective pathogenicity [42]. Some pairs of species have been found in association with endodontic symptoms [70, 174]. Actually, some endodontic pathogens form different partnerships and associations in symptomatic infections in comparison with asymptomatic ones [202], leading to communities that can be more virulent and capable of causing symptoms.

4.10.1.4 Environmental Cues for Expression of Virulence Factors

A pathogenic species does not always express its virulence factors throughout its lifetime. The environment plays an important role in inducing the turning on or the turning off of virulence genes [10, 57, 105, 329]. Bacteria can sense environmental changes and respond accordingly by genetic expression of products that favor adaptation and survival. Several endodontic pathogens, such as treponemes, *F. nucleatum*, *P. gingivalis*, and *Prevotella intermedia*, have been shown to exhibit gene expression and virulence influenced by environmental cues [61, 102, 103, 336, 343]. The possibility exists that symptoms may also result from root canal environmental conditions that are conducive to the expression of bacterial virulence genes.

4.10.1.5 Host Resistance and Disease Modifiers

Individuals significantly differ in their ability to cope with infections, and differences may even become evident during each individual's lifetime [132]. Those individuals with reduced resistance to infections may be more prone to develop clinical symptoms. In addition, disease modifiers, such as genetic polymorphisms and diabetes, may influence the severity of the disease and predispose to symptoms [4, 36, 59].

4.10.1.6 Concomitant Herpesvirus Infection

Herpesvirus infection may be a disease modifier and is usually associated with diminished host resistance. Human cytomegalovirus (HCMV) and/or Epstein–Barr virus (EBV) have been detected in specimens of apical periodontitis lesions in association with symptoms [215, 217]. Other herpesviruses, such as human herpesvirus (HHV)-8, *varicella zoster* virus (VZV) and HHV-6 have also been identified in acute apical abscess samples [52, 53]. However, it still remains to be determined whether herpesvirus participate in the pathogenesis of acute infections or they are only bystanders attracted to the area as a consequence of the bacterially-induced inflammation (see further discussion on "Other microorganisms in endodontic infections").

4.10.2 Shift in the Microbiota Before Symptom Appearance

Symptomatic endodontic infections, including the acute apical abscess, may develop in teeth with or without periradicular radiolucency. In the latter, the initial infection of the canal is assumed to reach significant virulence to cause a rapidly evolving acute periradicular inflammation, before bone resorption develops. This is because bone resorption is usually a slow chronic process associated with long-standing root canal infection. However, when an abscess develops in a tooth with radiographically visible periradicular lesion, this indicates that it resulted from exacerbation of a previously existing chronic process. In these cases, a change in the host-pathogen relationship predisposed to exacerbation. It may be a temporary or definitive decrease in the host immune resistance, as for instance caused by stress or viral infection (e.g., flu, cold, herpesvirus); however, it may also be precipitated by a shift in the structure of the endodontic microbiota.

Cross-sectional studies have shown that the structure (richness and relative abundance) of the endodontic bacterial communities in symptomatic teeth is significantly different from that of asymptomatic teeth [225, 277]. Differences are evident in the type of dominant species, the total number of species (richness) and the bacterial load. This may indicate that ecological rearrangements in the bacterial community structure precede the appearance of symptoms. The ecological succession from asymptomatic to symptomatic condition may be related to the emergence of newly dominant community members, caused either by environmental changes that predispose to the growth of some specific members or even by arrival of novel species. Differences in the type and load of dominant species and the resulting

bacterial interactions may be responsible for differences in the pathogenicity degree of the whole biofilm community and lead to symptoms.

4.11 Persistent/Secondary Endodontic Infections

Given the essential role played by bacteria in the etiology of apical periodontitis, endodontic treatment should focus on both elimination of bacterial cells colonizing the root canal system and prevention of reinfection. Sterilization is the ideal goal of the treatment, but the realistic goal with techniques and substances currently available is disinfection. Thus, bacteria must be eliminated to levels that are compatible with periradicular tissue healing [271]. If bacteria are allowed to remain in the root canal at the time of filling, there is an increased risk of adverse treatment outcome [50, 281, 317].

Secondary infections due to canal contamination during treatment or coronal leakage after treatment can be the cause of post-treatment apical periodontitis, but bacterial persistence (persistent infections) is the most common cause of treatment failure [271]. It is salient to point out that even when antimicrobial endodontic treatment does not completely eradicate the intraradicular infection, substantial levels of bacteria are eliminated and the root canal environment is markedly disturbed. For bacteria to survive they need to resist or escape intracanal disinfection procedures and rapidly adapt to the drastically altered environment.

Most intracanal bacteria are sensitive to standard treatment procedures. Nevertheless, some bacteria may endure treatment procedures, especially if they are located in areas not reached by instruments and the antimicrobial substances used. When treatment is performed below acceptable standards, the risks of bacterial persistence are obviously high. However, areas such as isthmi, dentinal tubules, recesses, lateral canals and apical ramifications are difficult to reach with instruments and irrigants, and bacteria established therein can remain unaffected by treatment [6, 22, 147, 181, 186, 309, 314].

Bacteria persisting in the root canals after treatment procedures do not always maintain an infectious process and cause post-treatment apical periodontitis [50, 281]. The possible reasons for this are shown in Table 4.3. Actually, for residual bacteria to cause persistent infections and influence the treatment outcome they need to fulfil certain requirements depicted in Table 4.4.

Virtually all root canal-treated teeth with post-treatment apical periodontitis have

Table 4.3 Situations in which residual bacteria may not influence treatment outcome.

Residual bacteria do not cause post-treatment disease when:
1) they die after placement of the root canal obturation
2) they remain in quantities and virulence that are subcritical to cause or sustain periradicular inflammation
3) they are located in areas with access denied to the periradicular tissues

Table 4.4 Requisites for residual bacteria to influence treatment outcome.

For residual bacteria to maintain or cause disease they need:
1) to withstand periods of nutrient deprivation, scavenging for low traces of nutrients and/or assuming a state of low metabolic activity
2) to find a steady source of nutrients in order to survive and flourish
3) to resist treatment-induced disturbances in the ecology of bacterial community, including disruption of quorum-sensing systems, food webs/chains and genetic exchanges
4) to reach numbers sufficient to cause damage to the host
5) to have unrestrained access to the periradicular tissues through apical/lateral foramina or lateral perforations to cause damage
6) express virulence factors that reach critical concentrations in the modified root canal environment

been demonstrated to harbor an intraradicular infection [115, 186, 200, 266]. This indicates that residual bacteria can in some way acquire nutrients within filled root canals. Because no obturation technique or filling material promote a predictable antibacterial and fluid-tight coronal, lateral, and apical seal of the root canal system [76], persisting bacteria can derive nutrients from saliva (coronally seeping into the canal) or from the periradicular inflammatory exudate (apically or laterally seeping into the canal) [256]. Even though most necrotic pulp tissue is removed during chemomechanical procedures, residual bacteria can utilize some necrotic tissue remnants as nutrient source. These tissue remnants may be localized in isthmi, recesses, dentinal tubules, and lateral canals, which very often remain unaffected by instruments and irrigants [35, 240, 320, 346]. In addition, even in the main root canal lumen, some dentinal walls may remain untouched after instrumentation [240, 331], with different instrumentation techniques leaving up to 50% of the root canal surface area untouched [123, 158, 166, 254].

If residual bacteria are located in the very apical part of the root canal or in ramifications, they have unrestricted access to nutrients in the form of protein- and glycoprotein-rich tissue fluids and exudate. Actually, histobacteriological analyses of teeth with post-treatment apical periodontitis have frequently shown persistent infections in areas of apical ramifications [6, 146, 182, 186] and lateral canals [181, 186].

Unlike primary infections, a more restricted group of species has been found in root canal-treated teeth associated with post-treatment apical periodontitis.

E. faecalis has been one of the most frequently identified species in samples taken from root canal-treated teeth with apical periodontitis [48, 83, 87, 135, 141, 161, 169, 170, 203, 206, 232, 266, 292, 326, 344] (Figure 4.15). Fourth generation studies using

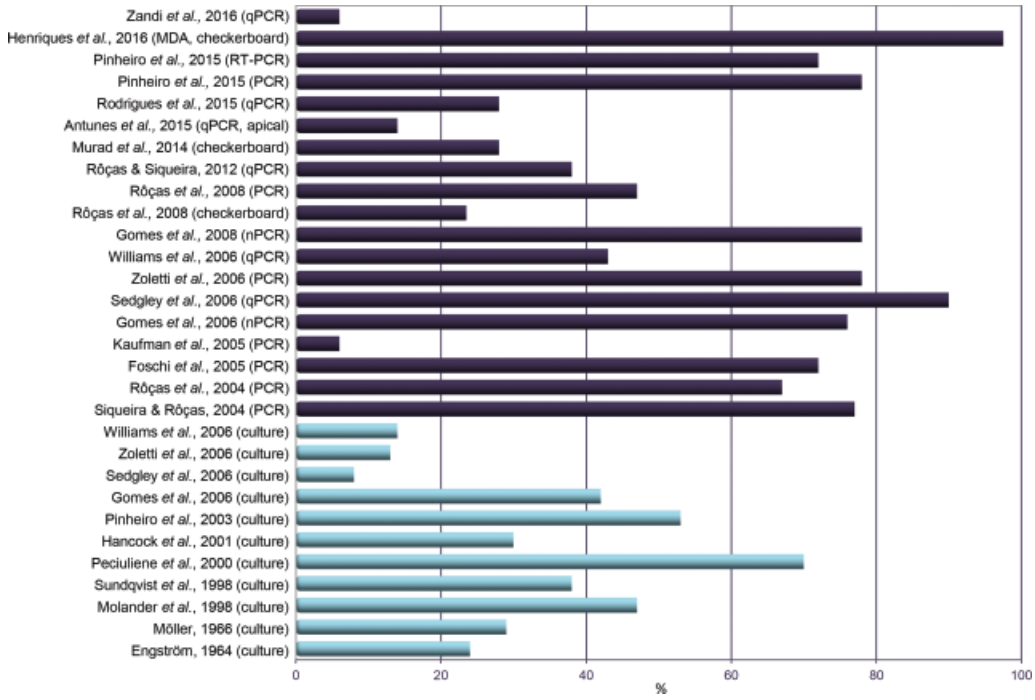

Figure 4.15 Prevalence of Enterococcus faecalis in association with post-treatment apical periodontitis according to different studies using either culture or molecular methods for identification.

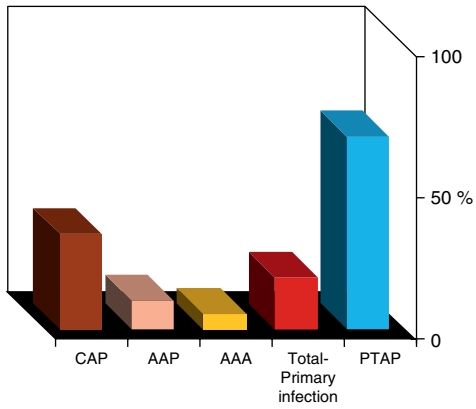

Figure 4.16 Prevalence of Enterococcus faecalis in endodontic infections associated with different forms of apical periodontitis. CAP, chronic apical periodontitis; AAP, acute apical periodontitis; AAA, acute apical abscess; PTAP, post-treatment apical periodontitis. Data according to Rôças et al. [203].

quantitative real-time PCR analysis reported that this species constitute a median of some 1% (range 0.1% to 100%) of the overall bacterial load in treated canals [200, 232].

E. faecalis is not commonly encountered in primary infections [17, 48, 203, 244, 342], and root canal-treated teeth are about nine times more likely to harbor *E. faecalis* than untreated teeth [203] (Figure 4.16).

This species is rarely, if ever, found as a persister in studies evaluating the antimicrobial effects of treatment and the microbiological conditions of the canal at the time of filling [21, 211, 222, 261, 264, 265, 281, 312, 338]. Enterococci have been reported to occur frequently in cases treated in multiple visits and/or in teeth left open for drainage [279]. They have also been found in high prevalence in root canal-treated teeth with leaking coronal restorations [170]. Because enterococci can be food-born colonizers and have been found in a variety of cheeses [63], their occurrence in the oral cavity has been suggested to be related to food ingestion [178, 340].

Based on many cross-sectional studies, as shown in Figure 4.15, *E. faecalis* was regarded as possibly the main causative agent of post-treatment apical periodontitis. Recently, however, its status has been questioned by the following findings:

1) *E. faecalis* is not detected in all studies evaluating the microbiota of root canal-treated teeth with post-treatment disease [25, 212].
2) When present, *E. faecalis* is rarely the most dominant species in the community [87, 194, 205, 223, 255].
3) *E. faecalis* has been found in similar prevalence values in root canal-treated teeth with or without apical periodontitis lesions [100, 344].

Streptococcus species have also been commonly found in the canals of teeth with post-treatment apical periodontitis [5, 23, 169, 206, 266]. Their prevalence and relative abundance in treated canals can be even higher than *E. faecalis*, as demonstrated by fourth and fifth generation studies [5, 200, 338, 339]. Other bacteria found in teeth with post-treatment disease include fastidious anaerobic bacterial species, such as *P. alactolyticus*, *Propionibacterium* species, *Filifactor alocis*, *Dialister* species, *Fusobacterium* species, *P. micra*, *P. piscolens*, and *Prevotella* species [72, 135, 194, 200, 205, 223, 266, 268, 339].

As-yet-uncultivated bacteria have also been identified in teeth with post-treatment apical periodontitis. In terms of both richness and relative abundance, uncultivated phylotypes may correspond to about one half of the bacterial community [223]. Some uncultivated phylotypes, such as *Bacteroidaceae* sp. HOT-272 (*Bacteroidetes* oral clone X083), are one of the most prevalent uncultivated taxa found in treated canals [223]. The fact that as-yet-uncultivated bacteria can be dominant community members helps explain why some culture studies failed to detect bacteria in treated canals.

Like primary infections, the bacterial community profiles in treated teeth vary from individual to individual, indicating that distinct bacterial combinations play a role in post-treatment disease [205, 223, 255]. Infections associated with treatment failure

are characterized by a mixed community, which is however less diverse than primary infections.

Secondary infections with *Pseudomonas aeruginosa*, enteric rods, and staphylococci leading to prolonged endodontic therapy have been reported [77, 177, 262]. These bacteria are most likely secondary invaders that may gain entry into the root canal due to a breach in the aseptic chain during intracanal intervention.

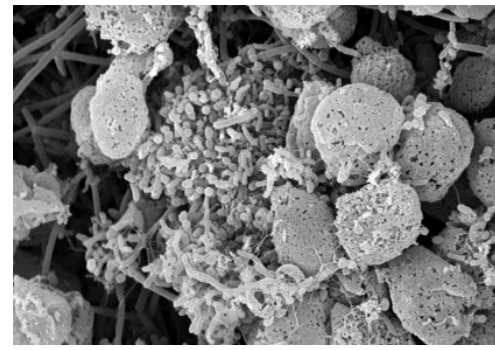

Figure 4.17 Large bacterial colony being attacked by phagocytes within the lumen of a pocket (bay) cyst (original magnification 3300×). Reproduced from Siqueira [256].

4.12 Extraradicular Infections

Apical periodontitis is an inflammatory disease that develops in response to intraradicular bacterial infection and usually represents an effective barrier against spread of the infection to the alveolar bone and other body sites. However, bacteria may occasionally succeed in overcoming this defense barrier and cause extraradicular infection. The acute apical abscess is the main example of this condition. There are other forms of extraradicular infection which, unlike apical abscesses, may be characterized by mild or even absence of symptoms. Such infections have been suggested as possible causes of post-treatment apical periodontitis, either by forming a biofilm adhering to the outer apical root surface [152, 189, 190, 298] or by mounting cohesive actinomycotic colonies within the body of the inflammatory lesion [85].

The extraradicular infection may have the following possible origins:

1) It may be an extension of the intraradicular infectious process, caused by bacteria that directly invaded the periradicular tissues and overcame the local host defenses. Most oral bacteria found in endodontic infections are opportunistic pathogens and as such lack a virulence apparatus that allows them to invade the periradicular tissues, subvert the host defenses, and survive within a hostile inflamed environment. However, some candidate oral pathogens, such as *Treponema* species, *Porphyromonas endodontalis*, *P. gingivalis*, *T. forsythia*, *Prevotella* species, and *F. nucleatum*, have been shown to possess such virulence traits [19, 51, 89, 95, 306, 341].
2) Bacteria may reach the periradicular tissues by penetrating into the lumen of "bay" cysts, which is in direct communication with the apical foramen/ina (Figure 4.17).
3) Bacteria may persist in the extraradicular space following remission of an acute apical abscess. These persisters would then maintain an extraradicular infection associated with chronic inflammation and an actively draining sinus tract – a chronic apical abscess.
4) Apical extrusion of infected debris during chemomechanical preparation is another way by which bacteria may reach the periradicular tissues. Once therein, bacteria embedded in dentinal chips can be physically protected from the host defense mechanisms and persist in the inflamed tissues.
5) Bacterial colonies or biofilms located in the very apical part of the root canal system may assume an extraradicular location following resorption of the apical root segment.
6) Bacteria occurring in intraradicular biofilms may reach the external apical root surface through dentinal tubules in an area where root surface cementum was resorbed, and then form an extraradicular biofilm.

Presumably, the extraradicular infection can be dependent on or independent of the intraradicular infection [257]. The dependent infection is fostered by the intracanal bacterial community. Thus, if the clinician succeeds in controlling the latter, the host can manage to control the extraradicular infectious component. Independent extraradicular infections in turn are those that are not sustained by an intraradicular infection and consequently do not respond to nonsurgical endodontic treatment. Apical actinomycosis, caused by *Actinomyces* species and *Propionibacterium propionicum*, has been suggested as another form of independent extraradicular infection (Figure 4.18) [85, 280]. However, its occurrence as a self-sustained pathologic entity involved as exclusive cause of treatment failure remains to be proven by studies using modern sensitive methods to simultaneously evaluate the bacteriological conditions of the apical root canal system.

Extraradicular biofilms have been found to occur in approximately 6% of the teeth with apical periodontitis [183] (Figure 4.19). Sometimes, these biofilms may exhibit foci of calcification, resembling dental calculus [185, 190]. In the large majority of cases, the extradicular biofilm is associated with an intraradicular biofilm, suggesting a dependent infection [183, 288]. However, the extraradicular biofilm may be occasionally independent of the intraradicular infection and cause treatment failure [189]. Extraradicular biofilms are virtually always associated with symptoms in untreated teeth [183] and may be the cause of persistent symptoms [189] or exudation [190] in teeth undergoing root canal treatment.

A histobacteriologic study evaluating the distribution of infection in teeth with chronic apical abscesses and sinus tracts revealed the extraradicular occurrence of bacteria in 83% of the cases. In 71% of the examined teeth, bacteria were forming a biofilm adhering to the outer root surface. Most of these structures showed some mineralization indicative of calculus formation [191].

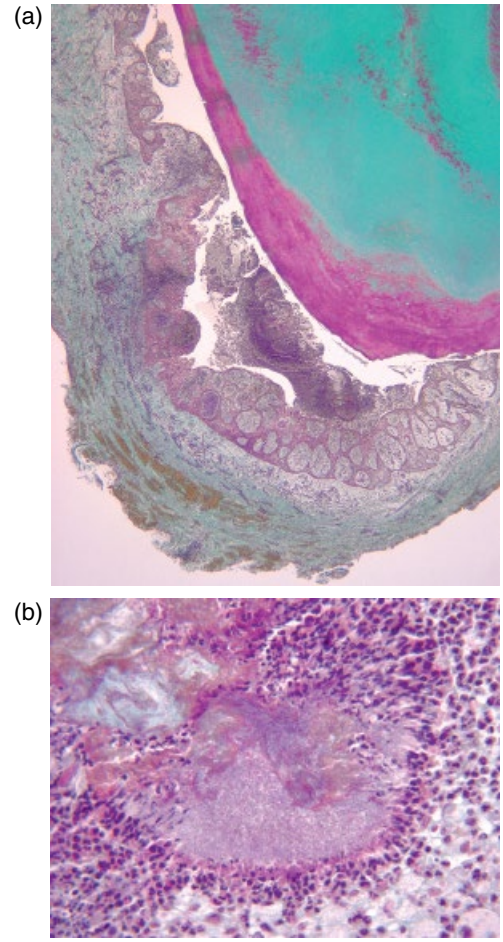

Figure 4.18 Apical actinomycosis. (a) Bacterial aggregate in an epithelialized apical periodontitis lesion, suggestive of actinomycosis (Masson trichrome, original magnification 25×). (b) Higher magnification of the actinomycotic aggregate, which is surrounded by inflammatory cells (400×). Courtesy of Dr Domenico Ricucci.

Except for cases with sinus tracts, it is still controversial whether asymptomatic, chronic apical periodontitis lesions can harbor bacteria for very long beyond initial tissue invasion or without being maintained by concomitant root canal infection [11, 182]. Studies have reported the extraradicular occurrence of a mixed infection by anaerobic bacteria in post-treatment lesions [62, 84, 214, 237, 288–290, 300, 323, 337]. As with apical actinomycosis and apart from the discussion as to whether contamination can be

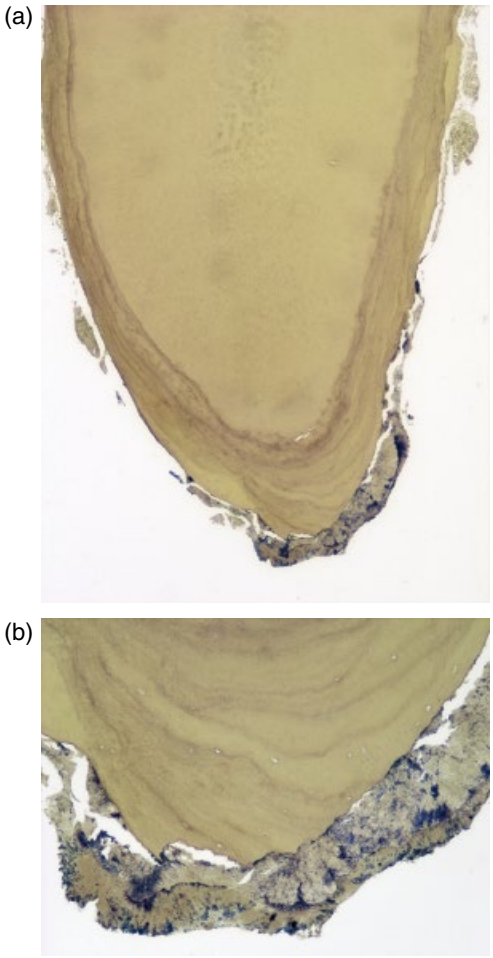

Figure 4.19 (a) Extraradicular biofilm (Taylor-modified Brown and Brenn staining, original magnification 25×). (b) Higher magnification showing dense population of bacterial cells adhering to cementum (100×). Courtesy of Dr Domenico Ricucci.

effectively prevented during surgical sampling of apical periodontitis, there is no clear evidence showing that these cases of extraradicular infections were independent of an intracanal infection.

The prevalence of extraradicular infections in untreated teeth with asymptomatic apical periodontitis is low [148, 183, 263], which is coherent with the high success rate of non-surgical endodontic treatment [33, 188]. In teeth with post-treatment disease, for which extraradicular bacteria have been suggested as a possible cause of failure, a histobacteriologic study showed no case suggestive of independent extraradicular infection [186]. Actually, there are only a few cases of post-treatment apical periodontitis reported that may have been caused by an extraradicular infection not associated with infection in the apical canal system [189, 192]. In addition, the high healing rate of apical periodontitis following retreatment [34, 188] indicates that the major cause of post-treatment disease is located intraradicularly and accessible to non-surgical therapeutic procedures. This is confirmed by microbiological studies showing that virtually all teeth with post-treatment apical periodontitis are associated with intraradicular bacterial infection [115, 186, 200, 205, 266].

4.13 Other Microorganisms in Endodontic Infections

Bacteria are the main microorganisms found in endodontic infections. However, other microorganisms have also been sporadically found. They include fungi, archaea, and viruses.

4.13.1 Fungi

Fungi are eukaryotic microorganisms that occur in two basic forms: molds (multicellular filamentous fungi consisting of branching cylindric tubules) and yeasts (unicellular fungi with spherical or oval-shaped cells). Fungi have only occasionally been found in primary root canal infections [272]. However, some studies have detected *Candida* species in about 20% of the samples from primary root canal infections [14, 133]. Reasons for discrepancies between studies are not evident, but may include differences in the identification methods used and in the general health conditions of the patients.

Fungi have been more frequently detected in studies evaluating the microbiological conditions of root canal-treated teeth.

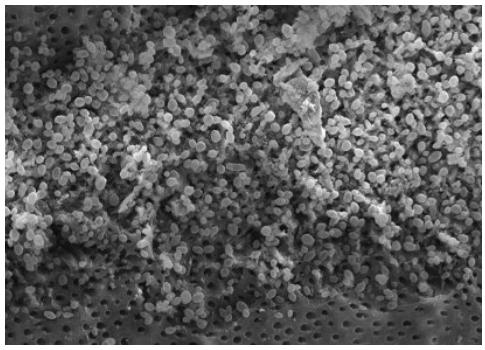

Figure 4.20 Colonization of the dentinal root canal walls by Candida albicans (scanning electron microscopy, original magnification 600×).

Detection frequencies for *Candida* species in persistent/secondary infections range from 3% to 18% [25, 47, 135, 136, 162, 169, 194, 266, 292]. Fungi can gain access to root canals via contamination during endodontic therapy or can overgrow after inefficient intracanal antimicrobial procedures that caused an imbalance in the microbiota [278]. *Candida albicans* is by far the fungal species most commonly detected in teeth with post-treatment disease. This species has been considered as a dentinophilic microorganism due to its ability to colonize and invade dentin [234, 235, 246] (Figure 4.20). In addition to the invading ability, *Candida albicans* has also been demonstrated to be resistant to some intracanal medicaments, such as calcium hydroxide [319]. Whether or not fungi participate in the pathogenesis of apical periodontitis still remains to be clarified.

4.13.2 Archaea

Archaea represents one of the three primary evolutionary domains of life, along with Bacteria and Eukarya. This domain comprises a highly diverse group of prokaryotes, distinct from bacteria. To date, no member of the Archaea domain has been described as a human pathogen. Methanogenic archaea have been detected in samples from subgingival plaque associated with periodontal disease [111]. Although many studies have failed to detect archaea in primary endodontic infections [198, 199, 249], others have detected methanogenic archaea in low prevalence [156, 157, 313], and one notable exception is a study that detected archaea in 25% of the canals with primary infections [310]. In that study, archaeal diversity was limited to a *Methanobrevibacter oralis*-like phylotype and the relative abundance of archaeal population accounted for up to 2.5% of the total prokaryotic community (i.e., bacteria plus archaea) [310]. Given its overall low prevalence as reported by most studies, it is unlikely that archaea plays a significant pathogenic role in apical periodontitis.

4.13.3 Viruses

Viruses are not cells but particles structurally composed of a nucleic acid molecule (DNA or RNA) and a protein coat. They are inert in the extracellular environment; as obligate intracellular parasites, viruses are totally dependent on living cells to perform life functions. When they infect living cells, the viral nucleic acid molecule directs the replication of the complete virus and assumes control of the metabolic activities of the host cell. Because viruses require viable host cells to infect and use the cellular machinery to replicate the viral genome, they cannot thrive in the root canal containing necrotic pulp tissue. Viruses have been reported to occur in root canals only in teeth with vital pulps. For instance, the human immunodeficiency virus (HIV) has been detected in vital pulps of HIV-seropositive patients [67] and some herpesviruses have been identified in both non-inflamed and inflamed vital pulps [113]. On the other hand, different herpesviruses have been detected in apical periodontitis lesions [52, 53, 215–217], where living cells abound.

It has been suggested that herpesviruses, especially human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV), may be implicated in the pathogenesis of apical periodontitis [282]. This would be either a direct result of virus infection and replication, or a

result of virally induced impairment of local host defenses, which might give rise to overgrowth of pathogenic bacteria in the apical canal segment. A hypothesis for herpesviruses participation in the pathogenesis of apical periodontitis has been proposed [282]. Bacterial infection of the root canal would cause an influx of herpesvirus-infected cells into the periradicular tissues. Reactivation of these herpesviruses by tissue injury caused by bacteria might lead to local impairment of host immune defenses in the periradicular microenvironment, affecting the response against infection. In addition, herpesvirus-infected inflammatory cells are stimulated to release pro-inflammatory cytokines and contribute to increased inflammation [134, 282, 322].

Herpesviruses have been identified in samples from symptomatic apical periodontitis lesions [215, 217], acute apical abscesses [24, 52], large lesions [216, 217], and lesions from HIV-positive patients [218]. However, the mere occurrence of herpesviruses in samples of apical periodontitis does not necessarily imply a role in disease causation. Herpesviruses infecting inflammatory cells may occur chronically throughout the human body. As these virus-infected inflammatory cells are attracted to and accumulate in the inflamed periradicular tissues, these viruses will also be present and detected. Herpesvirus participation in disease etiology could be inferred if high viral titers and/or viral RNA transcripts or proteins were detected in the lesion samples, or the clinical condition improves and the lesion heals after antiviral treatment. Pending such documentation, the role of herpesviruses in the pathogenesis of apical periodontitis remains unknown.

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5

Epidemiology, Treatment Outcome, and Risk Factors for Apical Periodontitis

Lise-Lotte Kirkevang and Michael Vaeth

5.1 Introduction

Apical periodontitis is a common disease; in some populations, about half of the adult population have at least one tooth with apical periodontitis. If untreated, apical periodontitis may cause pain, reduce chewing function, and eventually lead to tooth loss. Prevention and treatment of apical periodontitis have become a central part of a dentist's work and an essential aspect of dental health care planning. Identification of suitable preventive measures and optimal treatment strategies therefore become important.

A patient seeking dental treatment is mainly concerned with elimination of pain and discomfort. The patient expects that treatment decisions are based on best available evidence and wish to be informed about the benefits and risks associated with different treatment alternatives. The dentist must be able to share evidence-based information with the patient so that the patient can select a treatment based on their individual values and preferences. Browsing the internet, patients can easily obtain further information on available treatments, and use it to challenge the clinician's recommendations. The dentist must therefore have up-to-date knowledge about the supporting evidence of the treatment procedures that are presented to the patient.

To select the best available treatment to a patient the prognosis of the different treatment alternatives needs to be known. What is the probability of a successful outcome? The information needed to answer this question can only be provided by clinical and epidemiologic studies [51]. Due to the usually asymptomatic, chronic nature of periapical inflammation, evaluation of endodontic treatment is primarily based on radiographic recording of periapical structures, while clinical signs and symptoms may contribute additional information regarding clinical functioning and patient satisfaction. Information from clinical research represents a best-case scenario of what can be achieved with present knowledge and modern treatment principles, while epidemiologic studies may elucidate to what extent the dental profession is succeeding in controlling and eliminating apical periodontitis.

Moreover, to identify the best treatment, the meaning of "best" must be established. What is the relevant outcome and how do we define a successful outcome? Endodontic outcome studies have usually focused on apical periodontitis, and defined success as the absence or reduction of a radiographic periapical lesion. However, for patients, absence of pain, return of chewing function, or preservation of a tooth may be the most important aspects of a successful treatment. Moreover, additional complications arise when comparing the relative success of endodontic and implant treatment. Comparable outcome measures are needed but rarely used.

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

Companion website: www.wiley.com/go/orstavik/essentialendodontology

Table 5.1 Common epidemiological terms.

Population	A group of people in a defined setting
Sample	A selected part of a population
Selection bias	Systematic over- or underestimation of results due to distortion of a sample
Length bias	Distortion of sample due to overrepresentation of long disease duration
Information bias	Systematic over- or underestimation of results due to inappropriate data collection or registration
Confounding	Systematic over- or underestimation of results caused by a variable that is associated with both outcome and the study factor in question
Confounding by indication	Confounding resulting from the treatment procedure not being chosen at random, but being guided by, either the nature of the disease, or by a standard procedure at an investigating clinic.
Exposure	A study factor thought to cause disease
Outcome	A measure of present disease
Prevalence	The number/proportion of cases of a disease at a given point in time
Incidence	The number/proportion of new cases developing over a time period
Risk	The probability associated with the outcome in a specified period

Thus, a "successful treatment" has different meanings, and results from different studies may be difficult to compare.

In this chapter, the current knowledge about the prognosis of apical periodontitis is described. This knowledge derives from studies using clinical and epidemiological research approaches. Experimental research focuses primarily on diseased mechanisms or evaluation of technical equipment under strictly controlled laboratory conditions, and these results are seldom directly applicable to a real-life situation. Traditionally, epidemiologic research investigates how diseases occur and persist in different groups of people or in populations, while clinical research concerns the individual patient and the treatment of their health problems. But, epidemiological methods can also be used to analyze disease patterns and identify prognostic factors. Epidemiological information is therefore important for planning and evaluation of preventive health care and to guide management of patients in whom disease has already developed. Moreover, epidemiologic research methodology is used increasingly in a clinical setting to make predictions about health outcomes based on results from studies of groups of similar patients. Clinical epidemiology plays a central role in evidence-based medicine; a basic understanding of epidemiologic research methodology is therefore required to appreciate the strengths and weaknesses of the different studies. The basic epidemiological terminology is explained in Table 5.1.

5.2 General Aspects of Epidemiology

WHO defines epidemiology as "…the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems" [184]. Epidemiologic investigations are traditionally divided in descriptive epidemiology and analytic epidemiology according to the purpose of the research; see Box 5.1 for a fuller explanation.

Traditionally, dentistry focuses on treatment of teeth and dentists may consider results from epidemiological studies less

Box 5.1

WHO defines epidemiology as follows: "Epidemiology is the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems. Various methods can be used to carry out epidemiological investigations: surveillance and descriptive studies can be used to study distribution; analytical studies are used to study determinants" [184].

Traditionally, epidemiology is divided in descriptive epidemiology ad analytic epidemiology: Descriptive epidemiology concerns *the distribution of health-related states or events*, the main aspects being the person (*who?*), the place (*where?*), and the time (*when?*), whereas analytic epidemiology focus on *determinants*, which are causes and other conditions influencing the occurrence of *the health-related states and events* (*why*, *how?*). In both cases *health-related states or events* must be determined (*what?*) and properly defined and these events are used to identify the *outcome* of the study.

relevant to their daily dental practice. However, proper answers to many important questions depend on epidemiologic data in addition to information from clinical research. Different designs are used in epidemiological studies reflecting the type of question being considered. Box 5.2 gives a brief description of the most common designs used in of epidemiology.

5.2.1 Prevalence

Questions like "What is the frequency of apical periodontitis and how does it depend on age, gender and other person-specific characteristics" and "Which tooth-specific factors and conditions are associated with presence of apical periodontitis?" relate to the prevalence of apical periodontitis in a population and the factors associated with presence of disease. These questions are relevant for health care planning and resource allocation and they are usually addressed by *cross-sectional studies* that include a sample of individuals who participate in a single examination. This gives a snapshot of the distribution of the disease in the population. The outcome is typically absence or presence

Box 5.2 Epidemiological designs

Randomized controlled trial (RCT): A trial, typically conducted to compare the effect of two treatments, in which the patients are allocated at random to one of the treatment groups.

Cohort study: A group of persons/patients are followed forward in time from a specific time point. The starting point may be defined as a given date, or by some event, e.g. a treatment. In *population-based cohort study*, participants are selected from the general population. In a *patient-based cohort study*, participants are patients from one, or several, clinics. In a *historical cohort study*, sometimes denoted a retrospective cohort study, participants are identified in the past and followed to the present date.

Case-control study: The past exposure history of a group of cases, i.e. diseased persons or patients with an unfavorable outcome, is compared with the exposure history of a control group selected from the source population (the population from which the cases are identified). Many different types of case-control designs have been developed. Case-control studies are sometimes called retrospective studies; they rarely used in endodontic research.

Cross-sectional study: In a cross-sectional study, information about a group of individuals is collected at a given point in time. This design allows estimation of disease prevalence and of the distribution of a condition in the population from which the sample is selected. Causal relations cannot be assessed in a cross-sectional study.

of apical periodontitis, defined, either by a binary variable or by a scoring system with several ordered categories reflecting an increasing severity of the disease.

Indices often used to classify sound and diseased periapical bone have their origin in a study from 1956 by Strindberg [169], who conducted a comprehensive longitudinal study on factors related to the results of pulp therapy. The width of the periodontal ligament, the integrity of the lamina dura and the presence of periapical radiolucency were proposed as the main indicators of periapical disease. Brynolf in 1967 investigated to what extent histologic changes were reflected in radiographs by comparing histologic and radiographic appearances of periapical changes in human autopsy material [21]. The study very thoroughly described the characteristics defining the different levels of apical inflammation. The Brynolf study formed the basis for development of a five grade index for registration of periapical inflammation, the Periapical Index (PAI) [128].

To produce valid information the distribution of age, gender, and other relevant factors in the study sample should mirror the distribution in the population. A random sample or a stratified random sample is usually the best way to meet this requirement. Other sampling strategies may result in study data that are subject to selection bias and the results of the study would then be of limited use to society or health care planners. A comparison of two studies from Denmark in 1997–98 highlights the problem [87, 88]; see Box 5.3 for details.

The endodontic literature includes a large number of reports from cross-sectional studies, many of which are based on samples of patients from dental schools or specialist clinic. Table 5.2 gives summary information about cross-sectional studies in endodontics. Secular trends in proportion of individuals with apical periodontitis, in proportion of teeth with apical periodontitis, and in proportion of root-filled teeth are shown in Figure 5.1.

Box 5.3

Two cross-sectional studies with participants living in the municipality of Aarhus, Denmark were conducted in 1997–98. One study was based on a random sample of persons born between 1935 and 1975. In this sample the average age was 42.3 years, 42% had at least one tooth with apical periodontitis, and 52% had at least one root filling. The other study was based on a sample of patients enrolled at the Dental School at Aarhus University in 1997–98; the study reported on the endodontic status of the patients prior to the treatment they received at the Dental School. The patient sample was slightly older, the average age being 45.8 years, but here 90% had at least one tooth with apical periodontitis and 99% had at least one root filling. Patients seeking treatment at the dental school obviously differ from the general population with respect to endodontic status and treatment needs [87, 88].

5.2.2 Incidence

The question "Which factors and conditions influence the incidence of apical periodontitis?" concerns the development of disease in healthy teeth. A cross-sectional study cannot answer this question because it does not include a time dimension and clinical research focuses on treatment rather than prevention. However, development of appropriate preventive measures hinges on an understanding of the factors and conditions that influence the incidence of apical periodontitis. This knowledge is only available from *population-based cohort studies*. Population-based cohort studies are observational studies that allow an investigation of the natural development of the disease and its treatment in the general population. A well-defined group of persons and/or teeth is followed forward in time with periodically examinations of the disease status. Both healthy and diseased persons/teeth are included and followed, and an analysis of the

Table 5.2 Summary information about cross-sectional studies with presence of apical periodontitis as outcome.

Author	Country	Year	Population	Age	Method	Number of persons	Persons with AP (%)	Number of teeth	Average number of teeth	Teeth with AP (%)	Teeth with RF (%)	RF teeth with AP (%)
Bergenholtz et al. (1973) [15]	Sweden	1973	DSP	20–70+	F+P	240	57	5472	22.8	6	12.5	31
Kerekes and Bervell (1976) [83]	Norway	1976	DSP	19–81	F	200	34.5	4832	24.2	2.8	5.7	25.4
Allard and Palmqvist (1986) [5]	Sweden	1986	GP	>65	F	183	72	2567	14	9.8	17.6	27
Petersson et al. (1986) [141]	Sweden	1986	GP	—	I	861	—	4985	—	6.6	13.3	33.8
Bergström et al. (1987) [16]	Sweden	1987	GP[a]	21–60	F	250	46.8	6600	26.4	3.5	6.5	28.8
Eckerbom et al. (1987) [36]	Sweden	1987	PP	20–60+	F	200	63	4889	24.4	4.6	13	26.4
Eriksen et al. (1988) [43]	Norway	1988	GP	35	P+I	141	29.8	3917	27.8	1.4	3.4	25.6
Petersson et al. (1989) [140]	Sweden	1989	PP	—	F	567	76.5	11497	20.3	8.7	22.2	26.5
Ödesjö et al. (1990) [123]	Sweden	1990	GP	20–80+	F	967	33.2	17430	18.2	2.9	8.6	24.5
Eriksen and Bjertness (1991) [42]	Norway	1991	GP	50	P+I	119	—	2940	24.7	3.5	6	36.6
Imfeld (1991) [75]	Switzerland	1991	GP	66	P+I	143	—	2004	14	8	20.3	31
De Cleen et al. (1993) [32]	Netherlands	1993	PP	20–59+	P	184	44.6	4196	—	6	2.3	39.2
Buckley and Spångberg (1995) [22]	USA	1995	DSP	—	F	208	—	5272	25.3	4.1	5.5	31.3
Eriksen et al. (1995) [41]	Norway	1995	GP	35	P	118	14.4	3282	27.8	0.5	1.3	38.1
Soikkonen(1995) [168]	Finland	1995	GP	76–86	P+I	169	41.4	2355	13.9	6.6	21.5	16.8
Saunders et al. (1997) [154]	England	1997	DSP	20–59+	F	340	67.7	8420	—	4.6	5.6	58.1
Weiger et al. (1997) [182]	Germany	1997	PP	12–89	P+I	323	—	7897	24.4	3.1	2.7	60.9
Marques et al. (1998) [107]	Portugal	1998	GP	30–39	P+I	179	26	4446	—	2	1.6	21.7
Sidaravicius et al. (1999) [161]	Lithuania	1999	GP	25–44	P+I	147	70	3892	26.5	7.2	15.1	35
De Moor et al. (2000) [33]	Belgium	2000	DSP	18–59+	P	206	63.1	4617	—	6.6	6.8	40.4

(*Continued*)

Table 5.2 (Continued)

Author	Country	Year	Population	Age	Method	Number of persons	Persons with AP (%)	Number of teeth	Average number of teeth	Teeth with AP (%)	Teeth with RF (%)	RF teeth with AP (%)
Kirkevang et al. (2001) [88]	Denmark	2001	GP	20–60+	F	614	42.3	15984	26	3.4	4.8	52.3
Boucher et al. (2002) [20]	France	2002	DSP	18–70+	F	208	—	5373	—	7.4	19.1	29.6
Lupi-Pegurier et al. (2002) [105]	France	2002	DSP	>20	P	344	—	7561	22	7.3	18.9	31.5
Ridell et al. (2006) [148]	Sweden	2006	GP	19	—	—	—	—	—	—	—	—
Dugas et al. (2003) [35]	Canada	2003	DSP	25–40	F+P+I	610	—	16148	26.5	3.1	2.5	45.4
Jiménez-Pinzón et al. (2004) [78]	Spain	2004	DSP	—	F	180	61.1	4453	—	4.2	2.1	64.5
Georgopoulou et al. (2005) [56]	Greece	2005	PP	16–77	F	320	85.9	7664	24	13.6	8.9	60
Kabak and Abbott (2005) [80]	Belarus	2005	DSP	15–65+	P	1423	80	31212	—	11.7	20.3	45.2
Loftus et al. (2005) [103]	Ireland	2005	DSP	16–75+	P	302	33.1	7424	24.6	2	2	25
Tsuneishi et al. (2005) [177]	Japan	2005	DSP	20–89	F	672	69.8	16232	—	9.4	20.5	40
Peciuliene et al. (2006) [133]	Lithuania	2006	DSP	—	F	83	—	2186	26.3	—	12.9	43.1
Skudutyte-Rysstad et al. (2006) [165]	Norway	2006	GP	35	P	146	16	3971	27.2	1.1	1.5	42.6
Chen et al. (2007) [25]	USA	2007	GP	—	P	244	—	3533	14.5	5.1	4.8	35.5
Sunay et al. (2007) [171]	Turkey	2007	DSP	—	P	375	—	8863	—	4.2	5.3	51.1
Touré et al. (2008) [175]	Senegal	2008	DSP	—	F	208	—	6234	30	4.7	2.6	—
Gulsahi et al. (2008) [60]	Turkey	2008	DSP	—	P	1000	23.8	24344	—	1.4	3.3	18.2
Hollanda et al. (2008) [68]	Brazil	2008	PP	48[b]	P	1401	—	29467	—	—	21.4	—
Da Silva et al. (2009) [30]	Australia	2009	DSP	—	P	243	—	5647	23.24	—	8.8	21.6
Al-Omari et al. (2011) [3]	Jordan	2011	DSP	16–59	P	294	83.7	7390	—	11.6	5.7	71.9
Matijevic et al. (2011) [109]	Croatia	2011	PP	—	P	1462	76.9	38440	—	8.5	8.5	54

Peters et al. (2011) [134]	Netherlands	2011	PP	18–59+	P	178	36.5	4594	25.8	2.6	4.9	24.1
Gumru et al. (2011) [61]	Turkey	2011	DSP	19	P	1077	—	28974	26.9	2.2	—	42
Lopez-Lopez et al. (2012) [104]	Spain	2012	DSP	19–70	P	397	34	9390	—	2.8	6.4	23.8
Kalender et al. (2012) [81]	Brazil	2012	DSP	18–50	P+I	1006	68	24730	—	7	8.9	62
Huumonen et al. (2012) [73]	Finland	2012	GP	30–95	P	5244	—	120250	22.9	—	7	—
Jersa and Kundzina (2013) [77]	Latvia	2013	PP	35–44	P	312	72	7065	—	7	18	30.5
Hebling et al. (2014) [67]	Brazil	2014	GP	60–94	F	98	42.9	942	11.6	12.1	13.4	65.1
Berlinck et al. (2015) [18]	Brazil	2015	DSP	0–60+	F	1126	—	25292	—	7.9	6.9	16.7
Oginni et al. (2015) [124]	Nigeria	2015	DSP	—	F	756	67.2	21468	—	—	12.2	41
Huumonen et al. (2017) [72]	Finland	2017	GP	30–95	P	5335	27	120635	—	—	—	39
Kielbassa et al. (2017) [85]	Austria	2017	PP	18–70	P	1000	60.5	22586	—	6.4	11	42.6

Abbreviations: AP: Apical periodontitis; RF: Root filling; DSP: Dental school patients; GP: General population; PP: Patient population; P: Panoramic images; I: Intraoral radiographs; F: Full mouth survey

Footnotes:

[a] Patients from general practice

[b] Mean age

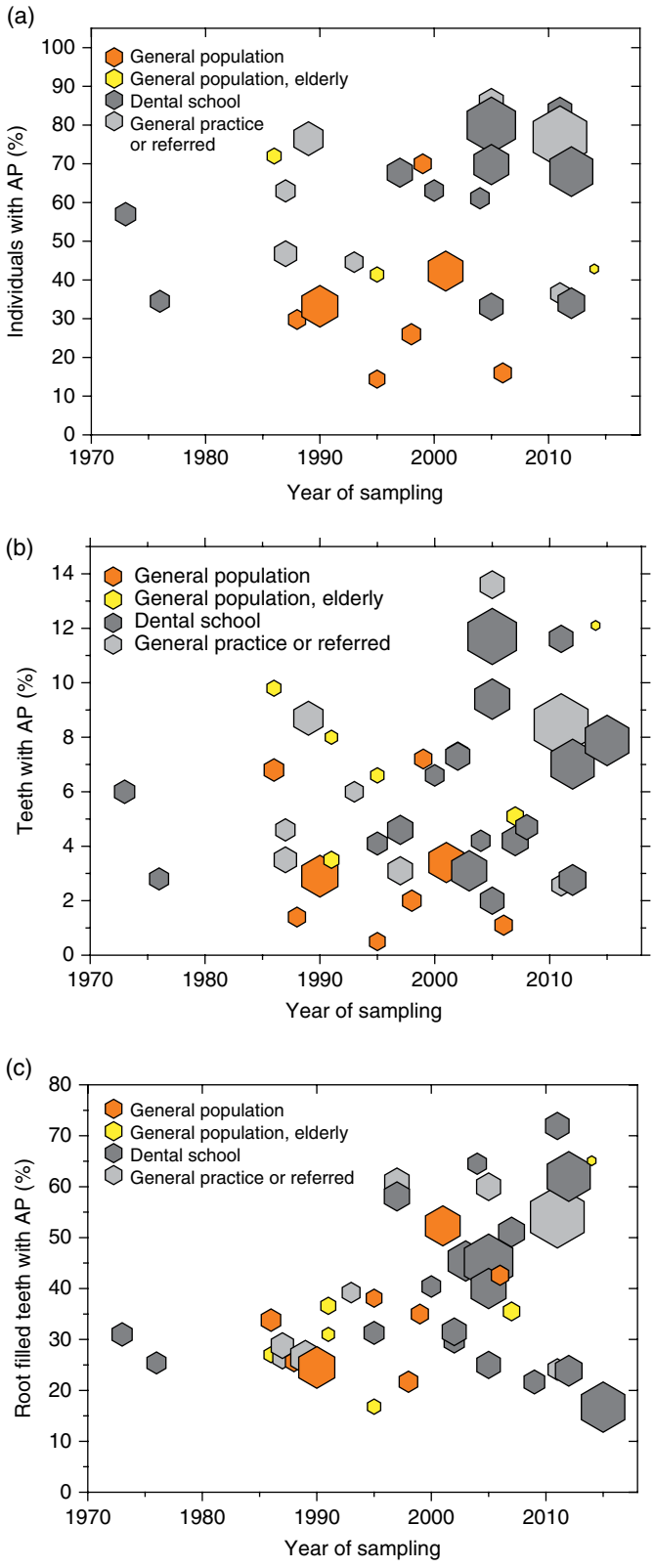


Figure 5.1 (a) Proportion of individuals with one or more teeth with apical periodontitis. (b) Proportion of teeth with apical periodontitis. (c) Proportion of root-filled teeth with apical periodontitis. The size of the bubbles reflects the size of the study.

data may identify factors associated with changes in disease status from one examination to the next. The times of examination may not coincide with times of treatment, therefore the exact preoperative status of the tooth will not be known to the researchers, but only be estimated from the information available from a prior examination. Furthermore, information on intra-operative factors, e.g. type of irrigant from clinical records, is seldom available. The population-based cohort studies usually rely on radiographic assessment of disease status and the quality of the root filling.

The population-based cohort studies require extended follow-up and are often costly and time-consuming. Moreover, the success of cohort study depends on the cohort members' willingness to continue participation in the examinations. Relatively few population-based cohort studies can be found in the endodontic literature (Table 5.3).

5.2.3 Prognosis

Other important questions relate to the prognosis of the treatment, e.g. "What is the probability that the disease will respond to a specific treatment?" or "Which factors and conditions influence the prognosis of the treatment?". Here, our knowledge derives primarily from clinical studies. These are typically series of cases, all of which have received the same treatment. Some studies compare different treatments, with or without random allocation to the treatment groups.

The predominant clinical study in endodontics describe treatment of patients and/or teeth who are followed from treatment and forward in time. The outcome is observed after a pre-specified period or at a common closing date, and such a clinical study may therefore be viewed as a *patient-based cohort study*. The patient-based cohort study is often conducted by specialists at a single clinic according to a detailed protocol, therefore the results may not translate directly to other settings or to general practice. In other words, the external validity may be low.

The clinical setting ensures that patient records are available from which relevant clinical information can be accessed, but patient-based cohort studies are also costly and time-consuming. Sometimes a patient-based cohort study relies on information about previously performed treatments retrieved from patient files. A new examination may then provide outcome information and the study can be conducted quickly, because there is no waiting time for follow-up information. Information from historic records may, however, be hampered by lack of standardization and frequent occurrence of missing data, which may affect the quality of the study. These studies are usually denoted retrospective cohort studies or perhaps more precisely *historically prospective cohort studies*; such studies are frequent in the endodontic literature. Table 5.4 gives a summary of the findings from patient-based cohort studies.

5.2.4 Prevalence Versus Incidence

The description above shows that the choice of study design and study setting depends on the questions being addressed. Moreover, the distinction between prevalence and incidence must be recognized. Prevalence describes the status of the disease in the members of a population at a given time and gives therefore a static picture of the distribution of the disease. Incidence, on the other hand, is related to the dynamics of the disease; in the present context, it describes the occurrence of apical periodontitis in the population over time and is usually quantified as a rate of occurrence or as a proportion of new cases during a specific time interval. Healing and extractions describe other aspects of the disease dynamics. It is also important to distinguish between prevalence and incidence when discussing conditions associated with the apical periodontitis. A high disease prevalence in a certain group could reflect an increased incidence, but delayed healing or reluctance to seek treatment could also be possible explanations.

Table 5.3 Summary information about population-based cohort studies.

Study	Exam year	Country	Number of persons	Follow-up months	Persons with AP	Persons with AP(%)	Persons with RF	Persons with RF(%)	Number of teeth	Teeth per person	Teeth with AP	Teeth with AP(%)	Teeth with RF	Teeth with RF(%)	RF teeth with AP	RF teeth with AP(%)
Petersson et al.	1974	Sweden	351	—	—	25	—	46	2100	6	107	5.1	258	12.3	82	31.8
1991/1993[a] [137, 139]	1985	Sweden	345	120	—	29	—	52	1962	5.7	121	5.9	323	16.5	94	29.1
Frisk et al. 2005 [53]	1968	Sweden	1220	—	—	41.9	—	18.3	24156	19.8	—	0.7[b]	—	3.4	—	—
	1980	Sweden	1023	144	—	41.9	—	22.1	20255	19.8	—	0.6[b]	—	4	—	—
	1992	Sweden	867	288	—	31.1++	—	21.9	17253	19.9	—	0.5[b]	—	3.8	—	—
Eckerbom et al. 2007 [38][c]	1975	Sweden	200	—	126	63	—	83.5	4889	24.4	255	5.2	636	13	168	26.4
	1980	Sweden	(200) 115	60	44	61.7	98	85.2	2825	24.6	97	3.3	393	13.9	68	17.3
	2002	Sweden	115	240	42	63.2	100	87	2461	21.4	168	6.8	598	24.3	93	21.4
Kirkevang et al. 2012 [92]	1997	Denmark	616	—	259	42	319	51.8	16016	26.0	534	3.3	776	4.8	402	51.8
	2003	Denmark	473	60	236	49.9	279	58.4	12345	26.1	461	3.7	705	5.7	311	44.4
	2008	Denmark	360	120	189	52.5	214	59.4	9360	26.0	395	4.2	543	5.8	233	42.9

Abbreviations: AP: Apical Periodontitis; RF: Root-filled
Footnotes:
[a] Molars and premolars
[b] Mean
[c] Initially patients from a radiographic clinic

Table 5.4 Summary information about patient-based cohort studies with healing of apical periodontitis as outcome.

									Healed(%)		
Study	**Year**	**Country**	**Design**	**No of patients**	**No of teeth**	**No of roots**	**Follow-up months**	**AP(%)**	**No AP**	**AP**	**Overall**
Strindberg [169]	1956	Sweden	ClinCoh	254	529	—	6–120	42	93	88	90
Seltzer et al. [158]	1963	USA	ClinCoh	2784	2921	—	6	—	94	76	84
Engström [40]	1964		ClinCoh	—	306	—	48–60	53	88	76	82
Harty et al. [65]	1970	UK	HistProsp	—	1139	1139	24+/6–24	—	—	—	90
Jokinen et al. [79]	1978	Finland	HistProsp	1199	1782	2459	24–84	33	61	38	53
Kerekes et al. [82]	1979	Norway	HistProsp	—	—	501	36–60	34	92	89	90
Barbakow et al. [10]	1980	S. Africa	HistProsp	—	332	—	12+	—	—	—	87
Oliet [125]	1983	USA	ClinCoh	—	153	—	18	—	—	—	89[a]
Swartz et al. [172]	1983	USA	HistProsp	—	1007	1770	12+	—	—	—	88[a]
Byström et al. [24]	1987	Sweden	HistProsp	—	—	79	24–60	100	—	85	84
Ørstavik et al. [129]	1987	Norway	ClinCoh	—	—	546	12	29	—	—	82
Eriksen et al. [44]	1988	Norway	ClinCoh	—	—	121	36	100	—	82	90
Sjögren et al. [164]	1990	Sweden	HistProsp	356	—	849	96–120	24	96	86	53
Smith et al. [167]	1993	UK	HistProsp	—	821	—	60+	—	—	—	90
Ørstavik [126]	1996	Norway	HistProsp	—	—	599	48	—	94	75	87
Sjögren et al. [163]	1997	Sweden	ClinCoh	53	—	—	60+	100	—	—	89[a]
Trope et al. [176]	1999	USA	RCT	102	—	—	12	—	—	—	88[a]
Weiger et al. [183]	2000	Germany	RCT	67	—	—	12–60	100	—	—	74
Benenati et al. [13]	2002	USA	HistProsp	—	894	—	6–72	—	—	—	62 (91[a])
Hoskinson et al. [69]	2002	UK	HistProsp	167	200	489	48–60	70	88	74	77
Peters et al. [135]	2002	Netherlands	RCT	38	—	—	12–54	100	—	—	76
Huumonen et al. [70]	2003	Finland	RCT	156	—	—	12	100	—	—	76

(*Continued*)

Table 5.4 (Continued)

Study	Year	Country	Design	No of patients	No of teeth	No of roots	Follow-up months	AP(%)	Healed(%)		
									No AP	AP	Overall
Peters et al. [136]	2004	Switzerland	HistProsp	179	233	—	12–36	44	95	76	87
Marending et al. [106]	2005	Switzerland	ClinCoh	66	—	—	30	52	—	—	88
Negishi et al. [115]	2005	Japan	HistProsp	57	114	—	12+	—	—	—	85
Marquis et al. [108]	2006	Canada	HistProsp	325	373	—	48–72	57	93	80	85
Doyle et al. [34]	2007	USA	HistProsp	—	196	—	12+	65	87	75	82
Gilbert et al. [58]	2010	USA	PracBased	—	115	—	14–343	49	81	60	71
Riccucci et al. [147]	2011	Italy	ClinCoh	470	816	1369	60	53	92	83	89
Ng et al.	2011	UK	ClinCoh	534	702	1170	24–48	66	—	—	87
Ng et al. [118]	2011	UK	ClinCoh	—	1617	—	24–48	—	—	—	95[b]
Setzer et al. [159]	2011	USA	HistProsp	42	50	—	48+	36	—	—	52 (96[b])
Bernstein et al. [19]	2012	USA	ClinCoh	1312	—	—	36–60	—	—	—	89[b]
Arya et al. [7]	2017	—	ClinCoh	46	200	—	12	100	—	—	62
Barborka et al. [11]	2017	USA	HistProsp	—	100	—	60–72	—	—	—	72
Pirani et al. [143]	2017	Canada	HistProsp	94	193(213)	—	60	—	—	—	85 (88[b])
He et al. [66]	2017	USA	ClinCoh	54	54	—	12	83	—	—	69

Abbreviations: ClinCoh: Clinical cohort; HistProsp: Historical prospective; RCT: Randomized controlled trial; PracBased: Practice-based; AP: Apical Periodontitis
Footnotes:
[a] Healing;
[b] Survival

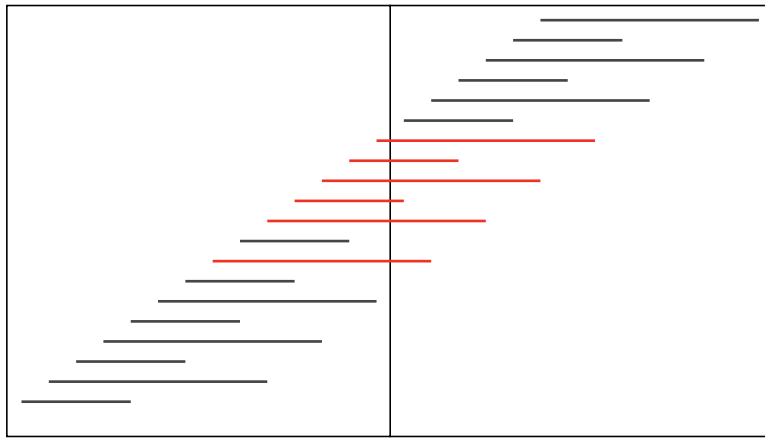


Figure 5.2 Length bias in cross-sectional samples. The horizontal lines represent time periods from treatment to healing for a number of teeth. Two equally frequent types of recovery are shown: one group of teeth has a slow recovery, the other heals quickly. The vertical line represents the sampling time of a cross-sectional study. The horizontal lines for teeth included in the cross-sectional sample are red. Here, teeth with slow recovery dominate.

Conditions associated with disease prevalence may therefore not necessarily be potential targets for preventive efforts focused on reducing the disease incidence.

Furthermore, it is well known that cross-sectional sampling results in a length-biased sample, since individuals with protracted disease durations will be over-represented with this sampling strategy. Figure 5.2 illustrates this phenomenon. The horizontal lines represent the time period from treatment to healing for a number of teeth. Two types of recovery are shown, one group of teeth has a slow recovery; the other heals quickly. The two types have identical incidence, but in a cross-sectional sample, in the figure represented by the vertical line, teeth with slow recovery dominate. Extrusion of root filling material in the periapical tissues may prolong the healing period, so this group of teeth will be over-represented in a cross-sectional sample of root-filled teeth with apical periodontitis, and the effect of a root filling surplus would be overestimated.

Systematic reviews and meta-analyses are often used to gain more information on a specific research question, e.g. the influence of age on root canal treatment outcome [160]. If the studies included in such review and/or meta-analyses are homogeneous, the accumulation of cases may improve the certainty of an estimate. However, often the studies differ so much that valid conclusions are not easily drawn [119]. Figure 5.3 gives an overview of how different study and evidence types compare overall as regards the strength of evidence they provide.

5.3 Elements of an Epidemiologic Study

Endodontic studies of patients/teeth usually focus on treatment or prevention of apical periodontitis, and a typical outcome therefore describes the absence, reduction or elimination of a periapical lesion as observed on a periapical radiograph. Index systems or scales with ordered categories have developed to describe the extent and severity of the disease [113, 128, 149], but in published research the treatment outcome is often reported as success or failure defined by a suitable cut-point on the outcome scale. A valid comparison of success probabilities from different studies requires that the same

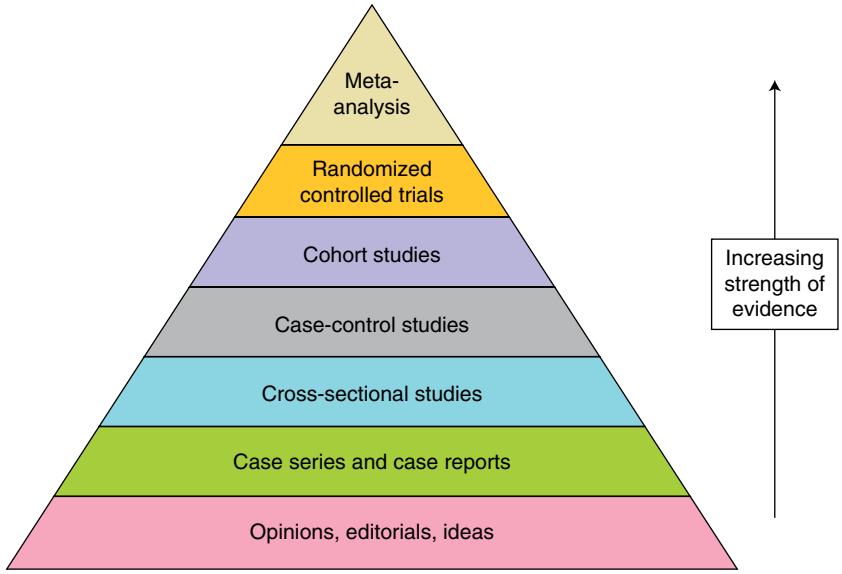


Figure 5.3 Evidence pyramid showing the relative strength of evidence for different study designs and sources of information.

outcome scale with the same cut-point is used to define a success and that the outcome is assessed from the same type of radiographs. Moreover, the timing of the treatment outcome is important. The length of the follow-up period should reflect the chosen outcome; for healing from one to several years might be appropriate, for tooth survival a longer period may be relevant, perhaps 5–10 years. Further confusion arises when the success-failure terminology is used when reporting results from a cross-sectional study. To conclude that the treatment is successful an improvement of the disease status must be observed and repeated follow-up measurements of the case, patient or tooth are therefore required.

Even when the outcome is assessed on a well-defined scale, by trained and calibrated observers, misclassifications or measurement errors will occur (Figure 5.4). The reliability and validity of the scoring system is therefore also important. Reliability concerns the reproducibility of measurement: Will a trained observer always give the same score, if an image is assessed at several occasions? How often will trained observers disagree?

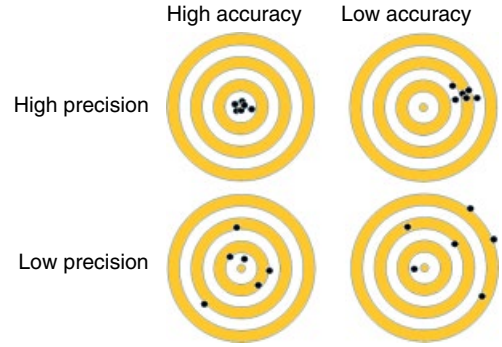


Figure 5.4 Accuracy (validity) and precision (reliability).

Validity measures the trueness of the measurement. In a clinical setting, the true value is unknown, but in vitro experiments may allow histological evaluation of the results from the different imaging techniques and thereby provide information on the validity of the method of measurement. Unfortunately, the terminology used to describe properties of a measurement method is not consistent; several names are in use for the same property and a given name may have different definitions. Recently, efforts to establish an international consensus on terminology and definitions of measurement properties have

Table 5.5 Common terminology for measurement properties.

Measurement error and misclassification	
Validity	The COSMIN panel [110] defines *validity* as "the degree to which an instrument truly measures the construct(s) it purports to measure". *Accuracy* is another commonly used name for this property and in statistics the name *unbiasedness* is used.
Reliability	The COSMIN panel [110] defines *reliability* as "the degree to which the measurement is free from measurement error". Other commonly used names are *repeatability* or *reproducibility*. In statistics reliability is often denoted *precision*.
Terminology related to measurement of disease status as present or absent	
Sensitivity	The probability of correctly classifying a diseased individual
Specificity	The probability of correctly classifying a healthy individual
Positive predictive value	The probability of an individual classified as diseased is classified correctly.
Negative predictive value	The probability of an individual classified as healthy is classified correctly.
ROC curve	The receiver operating characteristic (ROC) curve is a plot of the sensitivity against 1 – specificity as the diagnostic threshold varies.

led to the COSMIN checklist [110, 111] Table 5.5 reviews this proposal and other commonly used terms.

When the relevant outcome is identified, the next issue becomes how to obtain or measure the outcome and the determinants that you want to include in the study. Again, the answer depends on the aims of the study. If the aim of the study is to give an overall description of the dental health in a large population, panoramic images may be convenient and provide sufficient information to register an outcome such as number of root-filled teeth. However, periapical radiographs are necessary, if the study focuses on the healing of apical periodontitis using the periapical index as the outcome and with quality of the root filling as a possible determinant. Moreover, if the patient's symptoms or satisfaction is used as treatment outcome, radiographic information may not be needed at all.

5.4 Evaluation of Epidemiologic Data

5.4.1 Data Structure

Statistical evaluation is essential in epidemiology. Standard statistical methodology assumes independence between different observations, but in dental research so-called multi-level data are frequent and special considerations are therefore often needed. The term multi-level reflects that information describing the patient, e.g. age, gender, and smoking habits, is shared by all the patient's teeth; whereas information related to the tooth, e.g. tooth type, presence of coronal restoration, may differ among the patient's teeth.

In endodontic research information is collected from persons, teeth, roots, and occasionally also from root canals, and in the data analysis, the person or the tooth is usually chosen as the unit of analysis. When outcome is measured at the level of individual persons, an assumption of independence is usually appropriate. When the tooth is the unit of analysis, a positive association between outcomes is usually expected for outcomes obtained from teeth from the same person; whereas teeth from different persons are expected to be independent. Part of a dependence may be explained by known factors and conditions and if data are available, the statistical analysis can adjust for their effect. If a positive dependence between outcomes is ignored in the statistical analysis, the uncertainty in the data will be underestimated,

confidence intervals become too narrow, and statistical tests will give too many rejections. In analyses where the tooth is the unit of analysis, between-persons comparisons, e.g. smokers versus non-smokers, are more vulnerable to such bias, whereas within-person comparisons, e.g. teeth with coronal restorations versus teeth without coronal restorations are less problematic. Strindberg (1956) [169] in a statistical appendix examines the interdependence between treatment outcomes for two roots of the same tooth and between two teeth in the same mouth. The analysis of his data indicates that both associations are present, but none of them is particularly strong. The impact of the association between treatment outcomes for teeth in the same mouth is illustrated in the example shown in Box 5.4

Data on the determinants, the potential risk factors or prognostic factors, are also multilevelled. When the unit of analysis is the tooth and the outcome therefore is measured on tooth level, information on root canal and root level is usually aggregated to the tooth level; for multi-rooted teeth, the quality of a root filling is not presented as a score for each root, but aggregated to a single score. Similarly, when the unit of analysis is the person, tooth level risk factors are usually aggregated to person level variables, e.g. as the number of root-filled teeth or the number of de crown.

Box 5.4

In a Danish population-based cohort study, the incidence of apical periodontitis over a five-year period among smokers and non-smokers was compared. A logistic regression with tooth as the unit of analysis gave an odds ratio of 1.77 for smokers relative to non-smokers. Ignoring the association between outcomes in teeth from the same person, the 95% confidence limits for the odds ratio were 1.40 and 2.23, whereas an analysis that accounted for this association gave a slightly wider confidence interval with 1.32 and 2.35 as confidence limits [91].

5.4.2 Adjustment for Confounding in Observational Studies

An investigator planning an experiment may ensure that the experiment is balanced in the sense that the experimental units allocated to the different treatment groups are similar. In epidemiology, most studies are observational; the investigator is an observer and not able to influence the allocation of treatments or interventions.

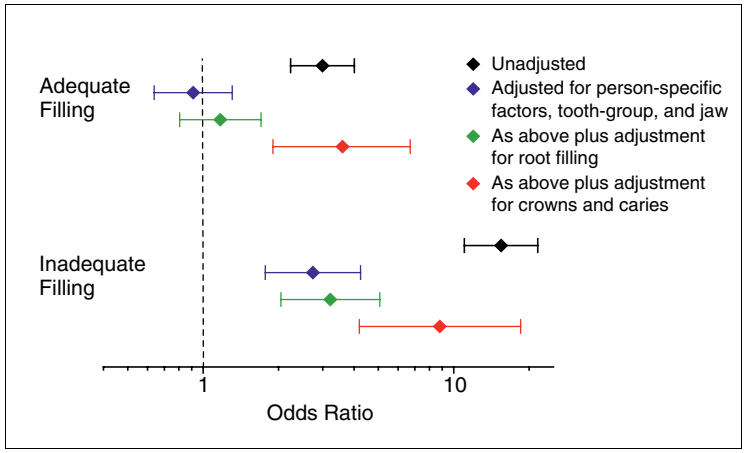
Also epidemiologic studies in endodontics are usually observational studies; to estimate the influence on the outcome of a specific factor it is therefore necessary to adjust for the effects of confounding factors, i.e. factors related to both the outcome and the factor in question. Separate analyses of each factor with no adjustment or analyses with incomplete adjustment may result in biased estimates of the association between the risk factor and the outcome. To identify the influence of a specific factor it is therefore important to correct for all known confounding factors in the analysis. Moreover, valid comparisons of estimates from studies with different degree of adjustment may not be possible. An example that illustrates the importance of these points is presented in Box 5.5

5.4.3 Regression Models

Adjustment for confounding factors is usually obtained by some sort of regression analysis. The choice of regression model depends on the outcome selected; see Box 5.6

Modern statistical software packages include modules that will analyze data with any of these regression models (SAS, Stata, R etc.). Note, however, that regression analyses with adjustment for confounding factors and prediction models with many predictors may require large data sets to produce reliable results. A rule of thumb, derived from computer simulations, indicates that for a logistic regression the number of records should be at least 15 events per parameter fitted in the model. Similar rules of thumb exist for other

Box 5.5



Comparison of different adjustments for confounding factors.

The figure shows the effect of adjustment for confounding factors on the association between the quality of a coronal filling and incidence of apical periodontitis. The association is described by an odds ratio and estimated in a logistic regression using the data on incidence of apical periodontitis in a five-year period. The diamonds represents odds ratio estimates for apical periodontitis for teeth with an adequate coronal filling (top) and teeth with an inadequate coronal filling (bottom) relative to teeth with no coronal filling; 95% confidence intervals are shown as bars together with the estimates.

Results are shown from four different analyses with varying degree of adjustment for other risk factors. The first analysis is a simple unadjusted analysis, and the second analysis includes adjustment for person-specific factors (gender, age, smoking habit), tooth group (front, premolar, molar), and jaw. The estimates are here reduced considerably and the odds ratio for adequate coronal fillings is no longer significantly different from 1. The estimates becomes slightly larger in the third analysis, which also includes adjustment for presence of a root filling. In the final analysis adjustment for presence of crowns and of carious lesions are also included and this has a large impact on the size of the estimate. This change reflects that presence of a crown increases the risk of developing apical periodontitis. In the previous analyses, these teeth were categorized as teeth without a coronal filling leading to an underestimation of the risk associated with coronal fillings. This example illustrates the importance of including corrections for all relevant factors in the analysis and that useful comparisons of estimates from studies with different degree of adjustment may not be possible. Data from [91].

Box 5.6 Types of outcome and the corresponding regression models

Outcome	Regression method
Two categories (dichotomy)	Logistic regression
Several categories	Multinomial logistic regression
Several ordered categories	Ordinal logistic regression
Counts	Poisson regression, Negative binomial regression
Quantitative, normal error	Multiple regression

regression models, see Harrell (2015) [64], chapter 4 for a fuller discussion of issues related regression analysis strategies.

Many statistical software packages include options that will allow the user to correct for correlation between outcomes from the same person. Different approaches have been developed, but a detailed description is beyond the scope of the present chapter. Kirkwood and Sterne (2003) [95] chapter 31 gives a brief overview of the available methodology.

In a cohort study of incidence of apical periodontitis with outcome for each tooth registered as absence or present of apical periodontitis a simpler approach may be useful. If interest primarily focuses on the role of tooth-specific risk factors, the impact of person-specific risk factors are disturbing the evaluation and corrections for their influence are therefore needed. An analysis based completely on comparisons of teeth within a person will automatically correct for all person-specific factors, both measured and unmeasured, and may therefore be a convenient choice. This approach is implemented in a conditional logistic regression analysis.

Information on treatment outcome in terms of overall success rates is useful as it provides information on what is achieved in different settings and different populations. However, to be able to estimate the prognosis for a specific treatment, the dentist also needs to take factors related to the patient, the tooth, and the treatment, into account. Usually, these factors are described as person or tooth-specific preoperative, intra-operative and postoperative factors.

5.5 Factors and Conditions Associated with Treatment Outcome

When presented with a tooth with apical periodontitis the dentist must decide if the tooth should be treated and if so, which treatment for perform. The prognosis of the different treatment choices depends on conditions of the specific tooth, and to some extent on factors related to the patient. Considering different treatment scenarios, the dentist must therefore assess how the different aspects of the particular treatment session affects the chance of a successful treatment. The current available prognostic information derives mainly from patient-based cohort studies in which the association between tooth- and patient-specific conditions and endodontic disease has been assessed. The different factors are interrelated, so the apparent association between a condition and treatment outcome may actually rather reflect the confounding effects of other factors. Single-factor analyses may therefore not give valid prognostic information. Factors and conditions associated with the prognosis are often denoted prognostic factors or, if focus is on treatment failure, risk factors. The following is a review of prognostic value of a number of person-specific and tooth-specific factors related to prevention and treatment of apical periodontitis. Focus is on the outcome *absence of apical periodontitis*, but information related to *tooth survival* and other patient-related outcomes is also important.

5.5.1 Treatment Outcome

5.5.1.1 Periapical Disease

The focus of most studies in endodontology has been on presence of apical periodontitis assessed by conventional radiographs, sometimes supplemented with information about clinical symptoms. Fewer studies have reported on outcomes related to tooth survival and patient satisfaction.

In reviews the calculated success rate of primary root canal treatment varies from around 70% to more than 90% [12, 98, 119, 181]. The main source of information derives from historically prospective cohort studies, as in the review by Ng. et al. (2007) [119]. Reviews are usually considered to generate results at the one of highest levels of evidence, but the validity of a review obviously depend on the quality of the included studies and the

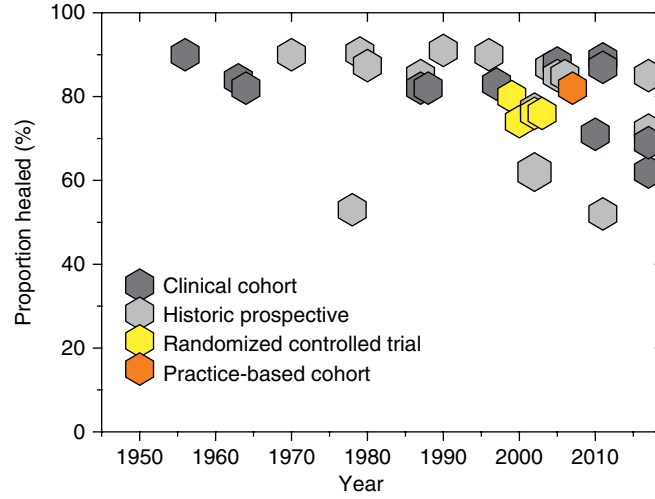


Figure 5.5 Endodontic treatments results from patient-based cohort studies. Proportion of successful treatments plotted against the year the study was conducted and stratified according to the type of the study.

validity of results from retrospective studies may be questionable.

In patient-based cohort studies, the success rate of primary root canal treatment has been found to be from 70 to more than 90% (Figure 5.5). The findings in these studies relate typically to treatment of teeth in highly controlled environments, from specialist clinics, dental schools done by dental students, post-graduate students or faculty teachers and represent the results that can be achieved when selected patients are treated under ideal conditions.

Most of the published outcome studies report on primary root canal treatment, or do not distinguish between primary and secondary treatments. In a review of secondary root canal treatments a success rate of 78–82% was found [118].

In population-based cohort studies the estimated success rates vary extensively depending on the population studied. In the private sector in USA a success rate as high as 89% [185] has been reported. However, the frequency of apical periodontitis before treatment was only 13% in this population. In a Danish study the estimated success rate was 59%, but here the frequency of apical periodontitis at baseline much higher (42%) [92]. In a Swedish study 32% of the teeth had apical periodontitis at baseline. The success rate for the teeth with apical periodontitis was 33%, whereas 83% of teeth that initially had no apical periodontitis were also without apical periodontitis 10 years afterwards [137]. A recent Swedish study from a public county clinic reported a baseline frequency of apical periodontitis of 61% and the success rate after 3 years of 58% [97]. Few population-based cohort studies allow estimation of retreatment outcomes. In a Danish study, 26% had healed within a 5-year period, but after 10 years, almost 50% had healed [92].

The findings from population-based cohort studies suggest that endodontic treatments performed in the general clinic have lower success rates than those reported in patient-based cohort studies of patients in specialized clinics and institutions. Cross-sectional studies do not allow estimation of success rates, but the prevalence of apical periodontitis found in these studies reflects the overall treatment outcome and treatment strategy in the population (Table 5.2, Figures 5.1a–c)

5.5.1.2 Patient-Related Outcome

Reviews have reported prevalence of pain one-week postoperatively to be about 14% [132], and this has been confirmed in a recent study where 90% had no or little pain 7 days after endodontic treatment [114], with follow-up periods of at least 6 months the estimate of pain decrease to about 5% [122]. These estimates are supported by recent

patient-based cohort studies, which also suggest that less than 5% of patients experience persistent pain 3–5 years after endodontic treatment [19, 179].

5.5.1.3 Retention of Root-filled Teeth

Teeth are being root canal treated to eradicate endodontic disease, and for patients to retain their teeth functional and without pain, and even though root-filled teeth are lost more often than non-root-filled teeth (Table 5.6), the retention rate of root-filled teeth in different populations is high.

A review of survival of root-filled teeth found survival rates of 86–93% in patient-based cohort studies, with observation periods ranging from 2–10 years [117]. This has been confirmed in recent patient-based cohort studies where 10-year survival rates of 86% [23] and 81.5% [100] was reported, while insurance data show that around 90% of endodontically treated teeth were retained in the oral cavity after 5–10 years [52, 150, 151].

In population-based observational studies of general populations almost 90% of root-filled teeth were retained 10 years after endodontic treatment [93, 139], and in a study covering 20 years approximately 70% root-filled teeth were retained [38]. Tooth loss also depends on the study population, and in another Swedish population receiving high-cost dental care fewer root-filled teeth (65%) were retained after 20 years [138]. Overall, it seems evident that a high proportion of root-filled teeth are retained over periods of 10–20 years.

5.5.2 Person-specific Risk Factors

5.5.2.1 Gender

Most studies have found that the patient's gender has little or no influence the outcome of root canal treatment. Some demonstrate a slightly higher success rates for females – others the opposite effect [69, 91, 116, 118, 120, 147].

Table 5.6 Loss of non-root filled and root filled teeth after 5(A) and 10(B) years. Percentages and relative risk [92].

Panel A					
Root filling status		**Lost in 2003**			
in 1997*	**Yes**	**No**	**Total**	**Percent**	**Relative risk**
No	65	11754	11819	0.50%	
Yes	42	579	621	6.80%	12.3
Total	107	12333	12440	0.90%	
* 473 persons					
Panel B					
Root filling status		**Lost in 2008**			
in 1997**	**Yes**	**No**	**Total**	**Percent**	**Relative risk**
No	122	8952	9074	1.30%	
Yes	57	398	455	12.50%	9.3
Total	179	9350	9529	1.90%	
** 360 persons					

It seems prudent to conclude that endodontic outcomes are independent of the patient's gender.

5.5.2.2 Age

Cross-sectional studies show that the prevalence of root canal treated teeth as well as the prevalence of AP among elderly individuals is high. Furthermore elders have a high frequency of root canal treated teeth [63].

However, in population-based cohort studies the occurrence of apical periodontitis in root-filled teeth does not seem to be associated either with age or with study population [53, 91, 137]. The proportion of persons who have at least one tooth with a root filling has however been shown to increase with age in some studies [92, 137]. This may affect the overall prevalence of apical periodontitis, since root-filled teeth more often have apical periodontitis than non-root-filled teeth. If teeth are not extracted the prevalence of apical periodontitis will accumulate, mimicking an age dependence. Other studies demonstrate no effect of age or even a slight decrease in persons with apical periodontitis with age [37, 53]. A recent review on patient-based cohorts concludes that the patient's age is not a prognostic factor for non-surgical root canal treatment [160].

In conclusion, older people may be more likely than younger people to have teeth with apical periodontitis, in particular if few extractions are performed, but patient age should not be regarded as a prognostic factor for the outcome of root canal treatment.

5.5.2.3 Smoking

The impact of smoking on apical has previously been investigated in cross-sectional studies and in cohort studies with conflicting findings. Five out of six cross-sectional studies found a significant positive association between presence of apical periodontitis and smoking; one population-based cohort study indicated an increased risk of receiving a root canal treatment, whereas one population-based and one patient-based cohort study found no statistically significant effect of smoking on development of apical periodontitis [180].

Smoking may delay healing, and smoking habits may be related to unfavorable lifestyle choices, but smoking should not be considered a risk factor when deciding whether to perform root canal treatment or not.

5.5.2.4 Socio-economic Status

The influence of socio-economic status has not been investigated in relation to treatment outcome in patient-based cohort studies. In cross-sectional studies have shown that after adjustment for dental status the socio-economic status of the individual did not provide additional information on the periapical status [4, 54, 94].

In conclusion, the socio-economic status of a patient is not associated with presence of apical periodontitis and is not likely to influence the prognosis of a root canal treatment.

5.5.2.5 General Health

Most patient-based cohort studies exclude patients with general diseases. A robust assessment of the effect of a patient's general health on treatment outcome is therefore difficult to obtain. In the study by Strindberg (1956) [169] no effect of the patient's general health was demonstrated. A historic prospective patient-based cohort study from 2005 found that a compromised immune system was associated with poorer prognosis [106].

Studies have investigated the effect of diabetes mellitus on AP, but even though diabetes in some studies has been shown to be associated with a less favorable outcome after endodontic treatment the current evidence is inconclusive and insufficient to claim an association [6, 84].

The association between endodontic lesions and cardiovascular disease has been investigated and inconsistent results have been found. However, only four studies were cohort studies. Three of these studies reported that the presence of AP could affect the cardiovascular system [17].

The findings in cross-sectional studies regarding the association between general

health and presence of apical periodontitis are inconsistent [4, 54].

It seems prudent to conclude that relevant information is lacking on the association between general health and endodontic treatment outcome.

5.5.2.6 General Dental Health

Poor dental health and extensive dental treatment experience have been associated with higher risk of a less favorable endodontic treatment outcome in both patient-based and population-based cohort studies [54, 91, 137, 169].

Findings from cross-sectional studies support this finding. Both the extent and quality of previous dental work and signs of present or previous dental disease has been found to be associated with presence of apical periodontitis [59, 131].

In conclusion, the extent and quality of previous dental treatments as well as signs of present or previous dental disease may predict the success of future treatment. This may reflect both to patient habits and quality of dental work.

Overall, person-specific factors seem to be less important in predicting endodontic treatment outcome when adjusted for tooth-specific variables more closely related to treatment [91, 118].

5.5.3 Tooth-Specific Risk Factors

5.5.3.1 Preoperative Risk Factors

Preoperative Diagnosis

The preoperative diagnosis (pulpitis, necrosis pulpae, apical periodontitis) is one of the preoperative factors that has been studied most frequently. The presence of a preoperative periapical lesion has been shown to be associated with unfavorable treatment outcome in patient-based cohort studies [19, 118, 147, 169], population-based cohort studies [91, 93, 139], and reviews.

In reviews it has been suggested that presence of a periapical lesion may reduce the chance of a successful outcome with 10–20% (Table 5.2) [12] compared to teeth without a preoperative periapical lesion. Moreover, some studies have demonstrated that the size of a periapical lesion size may influence the outcome with larger lesions having a negative effect on outcome [27, 118, 147, 169]. This may reflect that larger lesions may take longer to heal and therefore require longer follow-up time compared to smaller lesions. The importance of the length of follow-up was highlighted in a study by Weiger et al. 1998 [181] that concluded that calculation of success rates without taking the individual observation time into account would result in misleading outcome information. Still, the patient-based cohort study by Ng et al. 2011 [118] reported that larger lesions had a reduced success rate – even after adjusting the results for follow-up time after treatment. Recently, it was demonstrated that different grades of periapical inflammation, as described using the PAI scores, influenced the prognosis of the tooth significantly, the more severe the inflammation was the poorer was the tooth's prognosis [71, 89, 90].

The preoperative diagnosis of a tooth does affect the outcome. Teeth presenting with a preoperative periapical lesion have a poorer prognosis compared to teeth without a preoperative periapical lesion.

Tooth Type

Most clinical and population-based cohort studies have found that molars have a higher risk of failure [10, 26, 55, 82, 91, 172]. In a review it was demonstrated that mandibular premolars had the lowest risk of failure [120].

Comparisons of results are complicated by differences in reporting; some studies report outcome for teeth other consider separate outcomes for each root, and this may at least in parts explain the differences seen between the findings. If tooth is the unit, multi-rooted teeth have a higher risk of apical periodontitis, let alone because of the number of roots. Assuming independence between treatment outcomes in roots from the same tooth, it follows that if one root canal has a probability of 80% success, then a tooth with three canals will have a probability of success (no apical

periodontitis in any canal) of approximately 50%. Similarly, if the root-level probability is 70%, a tooth with three canal will have 30% probability of success. This crude estimation of course does not take root morphology and other relevant factors into account.

Endodontic treatment of multi-rooted teeth may have a lower success probability compared to single-rooted teeth.

Sinus Tract

Some studies have suggested that a sinus tract may facilitate bacteria from the oral cavity to colonize the periapex, and increase the risk for an extraradicular infection, but findings have been inconclusive. Two patient-based cohort studies found that a preoperative presence of a sinus tract did not affect the outcome [27, 147] whereas one study found that presence of sinus tract had a negative effect on the treatment outcome [118].

Little is known about the influence of a sinus tract on the outcome of endodontic treatment, but the studies available suggest that an association, if any, is negative.

Preoperative Pain

Prolonged preoperative pain may be a predictor for pain postoperatively together with negative patient expectations. It has been reported that 5–10% of patients receiving primary root canal treatment may experience postoperative pain even after 6 month [121, 122, 179]. Nevertheless, presence of preoperative pain has not been shown to be associated with an unfavorable treatment outcome of a root canal treatment. [24, 47, 127, 130, 147, 164].

Marginal Bone Level

Several studies have found an association between marginal periodontitis and presence of apical periodontitis, with periodontal involvement having a negative effect on treatment outcome [19, 84, 90, 145, 166]. A compromised periodontal support may be associated with reduced success probability of an endodontic treatment.

Root Filling

Reviews based on patient-based cohort studies have reported success rates for secondary endodontic treatment of more than 70% [116, 178], and when stratified according to length of follow-up period 70% (2–4 years) and 83% (4–6 years) [174].

Very few population-based cohort studies have reported results related to outcome of retreatment. In a Danish study 26% of the re-treated teeth had healed within a 5-year period, and after 10 years almost 50% had healed [92], and Petersson et al. (1991) [139] found that 66% had healed after 10 years.

The presence of a previous root filling may not affect treatment outcome if the dentist is able to gain access to the periapical area and effectively clean the root canal. The findings from population-based studies may suggest that is not always possible in general practice and that a previous root filling therefore may be associated with increased risk of treatment failure.

5.5.3.2 Intra-operative Risk Factors

Treatment procedures and treatment decisions are actions, interventions, and choices taken by the dentist as the treatment progresses, often in response to events that occur during cleaning, preparation, and obturation of the root canal. In case series and patient-based cohort studies it may be difficult, or even impossible, to separate the influence of the selected response from the influence of the triggering event on the outcome of the endodontic treatment. In epidemiology, the term *confounding by indication* is used to describe this situation (Table 5.1). A valid assessment of the treatment procedure requires the conduct of a randomized controlled trial, but these trials are unfortunately not common in endodontic research. The available evidence from the observational studies may occasionally lead to diverging interpretations.

Aseptic Procedures

Infection with microorganisms is the primary cause of endodontic disease, so minimizing

or eliminating contamination of the working area when performing endodontic treatment is essential. Rubber dam has been available to the dental profession for more than a hundred years, and during this time, universities and endodontic specialists have taught and recommended the use of it. Failure to use rubber dam has been shown to be associated with the use of less potent root canal irrigants, and moreover places the patient at risk of swallowing or aspirating materials and instruments [2, 45]. In a historic prospective population-based cohort study the use of rubber dam was shown to increase the survival rate of teeth receiving primary root canal treatment compared to teeth where rubber dam had not been used [102]. Furthermore, studies have shown that patients who had experienced the use of rubber dam during endodontic treatment would prefer rubber dam for eventual future endodontic treatments [2].

Aseptic procedures improve the chance of a successful outcome.

Choice of Instrumentation

The purpose of root canal treatments is to clean and prepare the root canal system as completely and efficiently as possible, without ledging, zipping, stripping, or straightening the canal. The mechanical preparation of the root canal is considered one of the most important stages in a root canal treatment, and the last decade has witnessed the introduction of many new instruments and techniques. Numerous *in vitro* studies have demonstrated that NiTi instruments better respect the original root canal anatomy and facilitate root canal preparation compared to the use of traditional stainless steel instruments, especially in curved root canals [74].

So far, relatively few studies have evaluated the effect of different instruments or techniques on treatment outcome in patients. An *in vivo* study showed that inexperienced operators have fewer procedural errors when using more contemporary techniques [142]. However, the available evidence does not permit any firm conclusions regarding differences in outcome of root canal treatment related to use different file materials and systems [8, 74, 86, 155].

A few community trials report that adaptation to newer instrumentation produces result of higher technical quality, assessed in radiographs, than traditional instrumentation procedures and that the improvement was maintained over time [31, 96, 112]. In one community trial, Kock et al. (2013) [96], the effect on outcome was assessed – the proportion of technical adequate root fillings increased, unfortunately without a concomitant effect on the periapical status.

Little is known about the overall effect of different instrumentation techniques on treatment outcome. Root canal preparation with NiTi instruments is easier to perform, and result in better maintenance of the original canal shape and a more predictable preparation of the root canal, but it has not yet been demonstrated that the use of these instruments has an important impact on the outcome of root canal therapy.

Size of Apical Preparation

Bacteria in the root canal are found in biofilm, on the root canal walls and they may have penetrated into the dentinal tubules where they are difficult to reach with disinfective agents. To eradicate these bacteria it has been advocated that the infected layers of dentine in the apical part of the root canal should be mechanically removed. Several patient-based cohort studies have investigated the effect of the size of the apical preparation on endodontic treatment outcome, but the results are inconclusive. Most studies find no effect of increasing apical enlargement [69, 82, 118, 130, 135] and even a negative effect of excessive preparation of the apical dentine has been described [169].

The findings were all from patient-based cohort studies and confounding by indication cannot be excluded. Additional studies are needed to establish the optimal apical preparation size to be able to eradicate bacteria at the same time as saving root dentine.

Patency

The concept of retaining patency by letting a thin file bypass the apical foramen repeatedly during root canal preparation has been debated for decades. In some studies apical disturbance has been shown to affect the outcome negatively [1, 12, 14], whereas in other studies have suggested a positive effect of patency [65, 118]. In 1956 Strindberg [169] found more failures if the root canal was blocked and the apical part of the root canal inaccessible for instrumentation.

Also, the effect of patency has only been investigated in patient-based cohort studies without randomization and therefore the conflicting findings may reflect differences in treatment philosophy.

Complications During Treatment

Different complications may emerge during treatment related to either obstruction of the root canal or perforation of dentine. *Obstruction* of the root canal may be a consequence of insufficient irrigation from packing of dentine chips in the apical part of the root canal, or caused by a fractured instrument. Both complications may result in impaired cleaning of part of the root canal and this may be problematic, especially in infected root canals. Patient-based cohort studies generally agree that canal obstruction is problematic [29, 115, 164, 169]; in particular, instrument breakage may affect the outcome negatively [76, 169]. *Perforation* through dentine during endodontic treatment has a negative effect on the treatment outcome [29, 76, 118, 164], in particular if a perforation is situated close to the osseous crest [48, 118, 144]. Moreover, a preoperative lesion at the site of the perforation may affect heling negatively [99, 144].

Treatment complications have a negative effect on treatment outcome.

Irrigation

Thorough irrigation during root canal preparation is considered essential, and many laboratory-based studies have compared different types and concentrations of irrigation solutions [74]. The SBU report (2010) [8] found insufficient scientific evidence to support the use of a specific endodontic irrigant to ensure a favorable treatment outcome. A Cochrane review concluded that, even though there was little evidence, sodium hypochlorite and chlorhexidine appeared to be more effective at reducing bacterial cultures compared to saline [49]. The two irrigants should, however, not be used together since a combination of chlorhexidine and sodium hypoclorite result in an precipitate containing para-chloro-aniline, which, besides being difficult to remove, is suspected of being cytotoxic and carcinogenic.

Based on clinical microbiological and ex vivo microbiological data the ESE guidelines for canal irrigation recommend a solution possessing both disinfectant and tissue-dissolving properties (ESE 2006) [45].

Antiseptic Procedures; Additional Disinfection/Medication

To further improve the cleansing and disinfection of infected root canals different protocols using a variety of disinfective agents has been studied. These include antibiotic or steroid containing substances (e.g. MTAD, Ledermix®), 2% chlorhexidine or iodine potassium iodide, sonic or ultrasonic activation of the irrigant used and photodynamic disinfection using light or laser. It was found that teeth dressed with steroid had lower success rate than those dressed with antibiotics or antiseptics [120]. Some of these approaches have showed promising results in *in vitro* and *ex vivo* studies [162].

Scientific evidence is lacking to determine if additional antiseptic measures, medication or other, approaches have any effect on the outcome of root canal treatment [8, 162].

Single-visit – Multiple Visits

Randomized controlled trials have established that treatment outcome does not differ between single-visit and multi-visit treatments. Evidence is, however, lacking regarding differences in the prevalence of postoperative pain or flare-up following either single- or

multiple-visit root canal treatment [9, 12, 50, 152, 153, 170].

Root Filling Quality

Root canal treatment should include a complete obturation of the root canal space, as voids or gaps invites treatment failure due to infection from remaining or intruding bacteria.

Patient-based cohort studies and reviews have shown that the length of a root filling may affect the treatment outcome. An optimal length (0–2mm) is better than a to short or overextended root filling [12, 98, 118, 120, 147].

A possible interaction between root filling quality and infected/non-infected root canals has also been investigated. The results indicate that the length of the root filling was less important, if no preoperative lesion was detected [46, 57, 167].

If a preoperative lesion was present a sufficient length of a root filling resulted in significantly higher success rate compared to teeth where the root filling was either too long or too short. [156, 157, 164, 167].

When a root filling extends beyond the apical foramen, the extruded materials may have an irritating effect on the periapical tissues, this may result in longer healing time, suggesting that longer observation times may be necessary for a trustworthy assessment of treatment outcome [62, 120, 181]. Over-filling is often preceded by an over-instrumentation and pulp remnants and microorganisms may have been pushed beyond the apex, and this may also influence the treatment outcome [12].

The homogeneity of root fillings has been also been investigated, and the results indicate that root fillings without visible voids are associated with a better prognosis [101, 118, 120, 169]. Most of these studies are, however, retrospective patient-based cohort studies so findings should be interpreted with caution. The SBU 2010 [8] found lack of scientific support to determine the effect on root filling quality on outcome.

In cross-sectional studies absence of apical periodontitis is more frequent in teeth with adequate root canal treatment (length/lateral seal) and adequate restorative treatment [59]. In population-based cohort studies, however, the effect of root filling quality on incidence of apical periodontitis, is reduced when adjusted for baseline information of apical periodontitis suggesting the inadequate quality of a root filling could be associated with healing rather than development of apical periodontitis [90, 91].

An inferior quality of root fillings influence the outcome of root canal treatment negatively, in particular in teeth with preoperative infection.

5.5.3.3 Postoperative Risk Factors

Coronal Restoration

The coronal restoration of a root-filled tooth serves as a barrier against reinfection of the root canal system and restores the functional status of the tooth.

Most studies have found that both the quality of the root filling and of the coronal restoration may affect the periapical status [59, 90, 91, 118, 120, 146, 173]. Chugal et al. 2007 [28], however, found no such effect and argued that lack of stratification on key confounding factors has resulted in an overestimation of the contribution of an adequate coronal restoration to the success of endodontic treatment.

The quality of the coronal restoration has been found to influence the treatment outcome, with inferior quality being linked to poorer outcome.

5.5.4 Concluding Remarks

A treatment should be based on best available evidence, also when few good-quality studies supporting our diagnostic performance and clinical procedures are available. Additional high-quality studies are urgently needed, and until such studies have been conducted, analyzed, and published we must rely on a cautiously interpretation of the findings in the studies currently available.

Many different factors have been suggested to influence the treatments outcome, but it is

difficult to single out which factors are the most important for a successful treatment. We know that bacteria cause apical periodontitis, therefore it is not surprising to find the best success rates of root canal treatments in teeth with no pretreatment apical periodontitis, and when the treatment was performed with the highest clinical standard, *lege artis*.

Still, it is evident that apical periodontitis is a prevalent disease in most populations. In general populations around 30–40% of individuals have at least one tooth with apical periodontitis and 25–50% of root-filled teeth have apical periodontitis. These findings may suggest that endodontic treatments performed in general practice do not always follow the international guidelines for good endodontic treatment [39, 45]. On the other hand, population-based cohort studies have found that few patients experience pain 3–5 years after treatment, and furthermore that retention of teeth 10 years after endodontic treatment is high.

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6

Radiology of Apical Periodontitis

Shanon Patel and Conor Durack

6.1 Introduction

Apical periodontitis (AP) is an inflammatory condition that occurs around the root of a tooth. Infection of the root canal system is the etiology of AP, and usually results in destruction of the periapical bone around the root [61, 73, 108].

AP is a common disease, which increases in prevalence with age. Epidemiological studies have reported a prevalence of 7% of teeth, and 70% of individuals with AP [39]. While a provisional diagnosis of *acute* AP may be confidently diagnosed from its clinical presentation, the diagnosis of *chronic* AP is usually dependent on radiological signs.

The aim of endodontic treatment is to prevent, or in some cases cure, AP. Whether it is maintenance of pulp vitality, endodontic (re-) treatment or surgical endodontic treatment from diagnosis, management, and ultimately assessment of the outcome of therapy, endodontic treatment is reliant on radiographs [40, 49, 125].

The two core imaging modalities for the radiographic detection and monitoring of apical periodontitis are periapical radiographs and Cone Beam Computed Tomography (CBCT).

Periapical radiographs are currently the primary tool as the first line for radiographic assessment of the periapical tissues. It is a quick and simple technique to use, and images are relatively easy to interpret. In addition, it has good specificity and high image resolution. The limitations of periapical radiography are the superimposition of overlying anatomy; the 2-dimensional nature of the image being produced; and the geometric distortion (Figure 6.1). These factors result in less than ideal sensitivity [93].

The radiation dose generated from a high-resolution, small field-of-view CBCT scan is higher than from a conventional radiograph. However, the continual improvements in CBCT software and hardware result in a gradual reduction in radiation exposure [7]. CBCT gives a more accurate interpretation of the dentoalveolar anatomy as it overcomes the limitations of periapical radiographs. However, the hardware costs (and therefore scans) are significantly higher than conventional radiographs. Moreover, using the scanner and interpretation of the resulting scans requires specific training [19].

It is probable that CBCT will become an integral part of the radiographic assessment and management of AP in the near future. This is reflected in the recently published CBCT position statement by the European Society of Endodontology [ESE] [91], and the joint statement by the American Association of Endodontists & American Academy of Oral & Maxillofacial Radiology [AAE/AAOMR] [3]. With this in mind, it seems prudent to describe both the periapical radiographic and CBCT features of AP in this chapter.

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

Companion website: www.wiley.com/go/orstavik/essentialendodontology

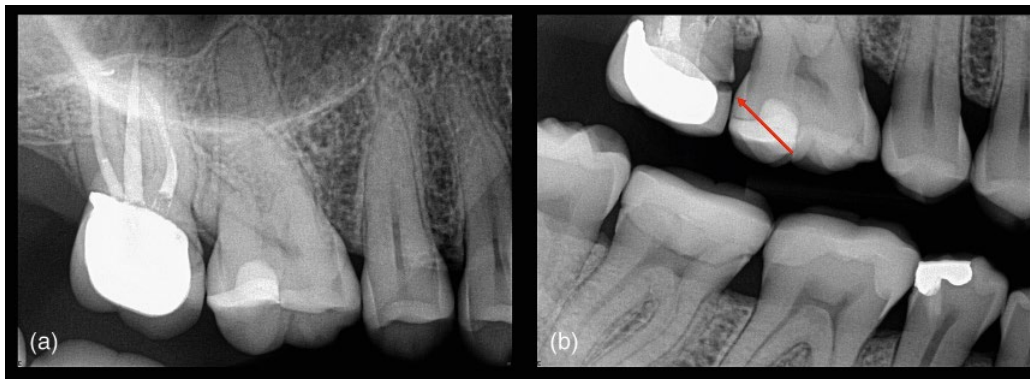

Figure 6.1 Geometric distortion. (a) Periapical radiograph of a root treated upper right molar tooth does not reveal anything untoward, (b) however, the change in the bitewing radiograph reveals a deficient crown margin (red arrow).

The application of CBCT into endodontics has resulted in a steady increase in research and publications on the radiological interpretation of AP [62, 88, 90, 116].

In this chapter we describe the radiological presentation of healthy and diseased periapical tissues at diagnosis and follow-up of treatment. Differential diagnostics and sources of error are also discussed, as will some aspects of. alternative imaging techniques applied for assessing AP.

6.2 Normal Apical Periodontium

The periodontium comprises the root cementum, the periodontal ligament, and the alveolar bone. The apical periodontium specifically refers to the relationship of these structures to each other at the apex of the root of a tooth. There are a range of radiological features of the root apex, the periodontal ligament and the lamina dura of the alveolar bone, which are considered typical of a normal apical periodontium. These so-called "typical" radiological features are used as a reference standard when assessing the presence, or absence of AP. However, root morphology varies significantly between tooth groups and to a lesser degree between teeth of the same group. Furthermore, the interpretation of radiographic images of the apical periodontium is complicated by "noise" created by the superimposition of anatomical structures over the area of interest or area under observation. This makes the definition of the normal radiological features of the apical periodontium difficult when using conventional radiography, resulting in a corresponding difficulty in diagnosing minor signs of disease.

The structures of the apical periodontium and those anatomical structures in close proximity to the apical periodontium must be identified and evaluated radiographically for abnormal variations when assessing teeth for AP (Figure 6.2).

6.2.1 Root Apex and Pulp Canal Foramen

Apical periodontitis will typically develop and be evident radiographically at the site from which microbial toxins and mediators of inflammation egress into the periodontal ligament space and alveolar bone from an infected pulp. The apical foramen and, to a lesser extent, the ramifications of the pulp are the most common portals of exit of these toxins and inflammatory mediators. The normal radiographic appearance of the tooth apex should therefore be appreciated prior to assessing radiographic images for the presence of disease.

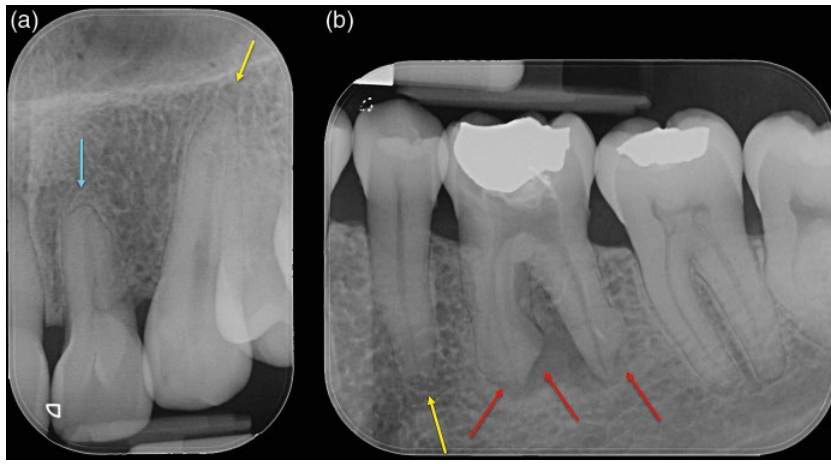

Figure 6.2 Normal apical periodontium. (a) Shortening of the root and blunting of the root end (blue arrow) of tooth 22 following resorption caused orthodontic fixed appliance therapy. The root end of the adjacent tooth 23 (yellow arrow) has a well-rounded, well-defined radiographic appearance typical of a root apex unaffected by disease processes or pressure related resorption. (b) External inflammatory resorption on the apices of the roots of tooth 36 due to an infected necrotic root canal system and apical periodontitis of the tooth. The root apices of tooth 36 have become flattened and uneven in comparison to the root end of tooth 35 (yellow arrow), which is unaffected by apical periodontitis.

The tip of the root apex often deviates at an angle from the long axis of the root [72]. This deviation can be in any direction and will vary, depending in part on the tooth type. In situations where the root tip and the long axis of the root are in alignment, the apical foramen will generally be located more coronal to the radiographic apex. These features have a significant bearing on the radiographic interpretation of the position of the apical foramen.

Conventional radiography: As the apical foramen is not a structure that can typically be seen radiographically, the root apex should be considered the defining landmark in assessing the most likely position of the apical foramen [127].

The apex of a healthy tooth is normally represented radiographically as a rounded, well-defined structure. However, for the reasons outlined above, the radiographic apex rarely coincides with the site of the apical foramen, the latter typically being situated more coronal to the former [72].

Resorption of the root apex as a result of orthodontic treatment, trauma or due to chronic AP can alter the shape and therefore the appearance of the root apex [112]. Blunting of the root end is a typical feature of teeth that have undergone orthodontic tooth movement, while those affected by chronic AP may become flattened or adapt a ragged, uneven appearance. The root ends of teeth that have suffered traumatic injuries may develop any of the changes outlined above. Repair of root tips damaged by resorption can undergo repair with new cementum deposition, but features of the previous tooth destruction generally persist (Figure 6.3).

CBCT: The use of CBCT allows an exact location of the root apex. The course of the root can be followed, and deviations of the apical third of the root in all planes can be traced. The apical foramen can often be identified and related to the apex of the root, especially in cases where the canal is wide. In sclerosed teeth or teeth with narrow canals, the apical foramen may not be identifiable (Figure 6.4). Resorptive processes and their effect on the shape of the root apex can often be readily appreciated [8].

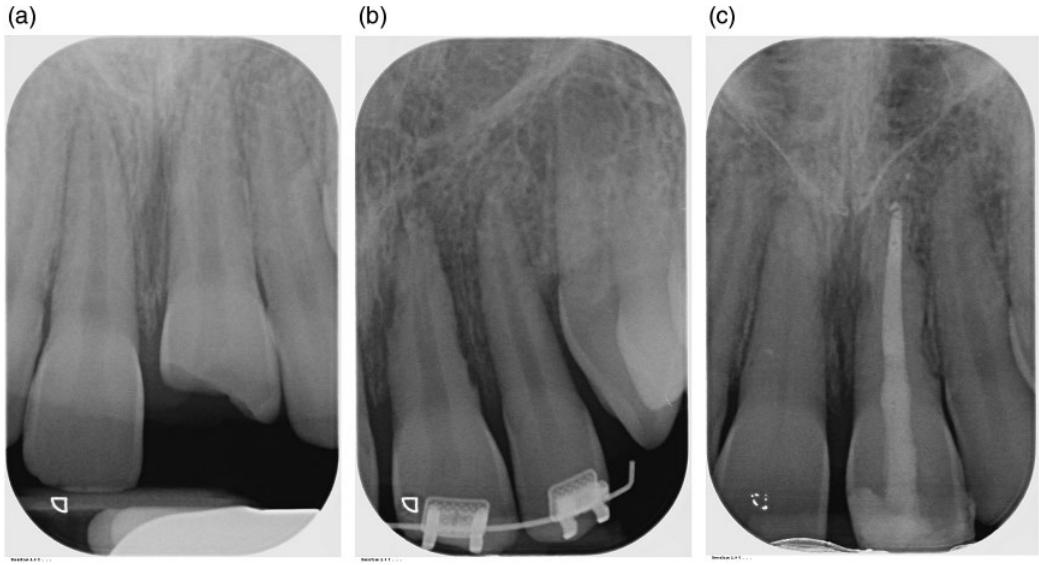

Figure 6.3 Dental trauma with external inflammatory resorption. (a) Periapical radiograph of maxillary central incisor teeth following a traumatic dental injury in which tooth 21 was severely intruded. (b) Within one week tooth 21 had undergone external inflammatory resorption with the development of excavations on the root surface of the tooth and associated radiolucencies in the adjacent bone. The entire root length, including the apical third, was involved. (c) Periapical radiograph of tooth 22 one year after the injury demonstrates resolution of the apical periodontitis and cessation of the resorptive process, although the shape of the root surface has been permanently altered.

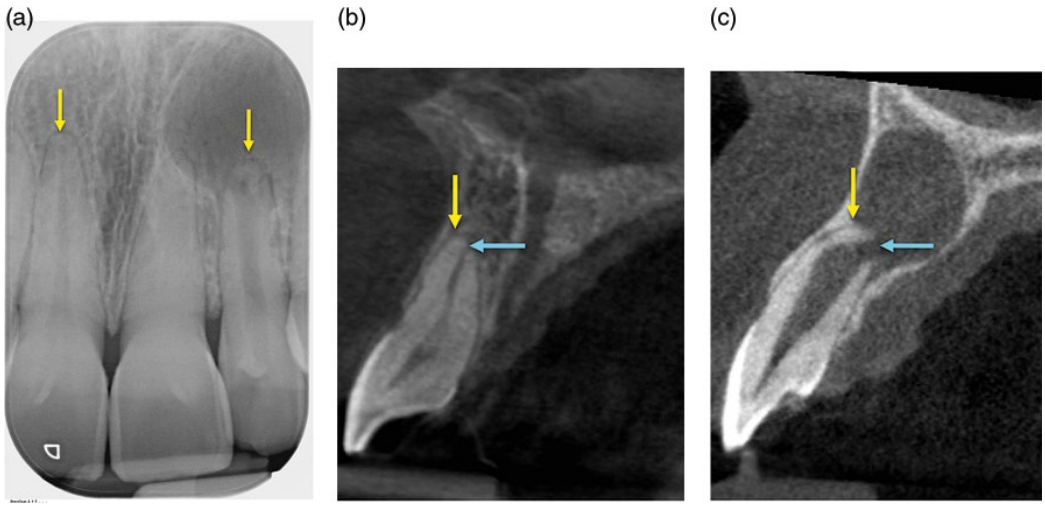

Figure 6.4 Apical periodontium. (a) Periapical radiograph of teeth 11, 21 and 22. The yellow arrows indicate the position of the radiographic apices of teeth 11 and 22, note the large periapical radiolucency associated with the 22. (b, c) Sagittal CBCT views of (b) tooth 11 and (c) tooth 22. The yellow arrows indicate the position of the most apical point of the roots of the teeth, which would correspond with the radiographic apices on the conventional radiographs. The position of the apical foramina of the teeth (blue arrows) are located at a more coronal position.

6.2.2 Cementum

Deposition of cementum on the apex of a root occurs throughout life. Further compensatory deposition of cementum on the root apex takes place in response to occlusal wear and attrition. Excessive formation of cementum (hypercementosis) on the root apex sometimes occurs as a result of occlusal stress or in response to pulpal insults.

Conventional radiography: The radiographic features of increased cementum deposition are variable and dependent on the site of the deposition. They can range from an apparent elongation of the root as a result of the deposition of cementum on the root apex, to the formation of a very bulbous root end as a result of the deposition of cementum on the apical third of the root in some or all planes (hypercementosis). The deposited cementum is often more radio-opaque than the adjoining dentine, allowing the differentiation between the two tissues in conventional radiographs. However, this is not always the case and there may be very little evidence of demarcation between the two tissues.

CBCT: CBCT evaluation will permit a more accurate appreciation of the location and distribution of increased cementum deposition or hypercementosis on the affected root or roots.

6.2.3 Periodontal Ligament Space

Conventional radiography: The periodontal ligament space is the soft tissue connecting the dental cementum and the lamina dura of the surrounding bone. The fact that the periodontal ligament is radiolucent and that the bordering structures of the periodontal ligament space are mineralized and relatively radio-dense means that the periodontal ligament space is represented radiographically as a thin, well-defined radiolucency surrounding the root of the tooth. The periodontal ligament effectively supports the tooth within the alveolar bone and permits physiological mobility. The width of the periodontal ligament space may increase with increased tooth mobility as might occur in advanced marginal periodontitis or in traumatic occlusion (Figure 6.5).

CBCT: The appearance of the periodontal ligament space is similar when assessed using conventional radiography and CBCT. However, CBCT allows the periodontal ligament space to be observed in all plains and without interference caused by adjacent anatomical structures (Figures 6.6 and 6.7). Indeed there is in vitro and in vivo evidence to suggest that CBCT permits better visibility of both simulated periodontal ligament and natural periodontal ligament spaces when compared to conventional radiography [58, 98].

6.2.4 Lamina Dura

The lamina dura is a term used to describe the bone immediately adjacent to periodontal ligament space. It is a continuation of the cortex of the jaw bone, but has multiple openings through which nerves and vessels pass.

Conventional radiography: The lamina dura is generally more radiopaque than the adjacent medullary bone but this may vary. In the absence of significant anatomical noise the lamina dura may appear as a continuous radiopaque border surrounding the root of the tooth. But the perforations for vascular and neural vessels may be so large as to be visible in the radiograph.

CBCT: The appearance of the lamina dura on CBCT is similar to its appearance on conventional radiographs. However, due to the 3-dimensional nature of the system, more details are visible on CBCT [98].

6.2.5 Cortical Bone

The maxilla and the mandible are covered by a layer of compact bone known as the cortical plate. The thickness and integrity of the cortical plate of the maxilla and mandible vary regionally. In the maxilla the facial cortical plate is thin as it runs posteriorly from the midline to the disto-buccal root of the first molar, at which point it widens, encasing the

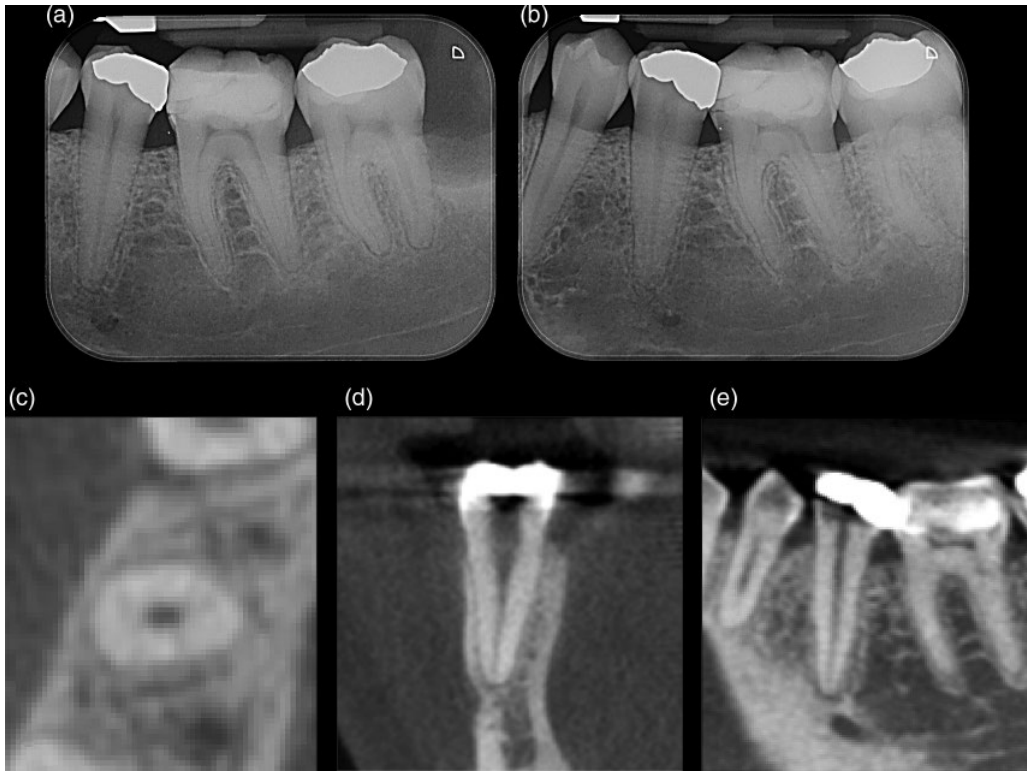

Figure 6.5 Periodontal ligament space. (a and b) Periapical radiographs of mandibular posterior teeth. The PDL space and lamina dura associated with tooth 45 can be evaluated in the mesio-distal plane. (c–e) CBCT images of the same tooth in the (c) axial, (d) coronal and (e) sagittal planes permit a more objective appreciation of the PDL space and lamina dura associated with the tooth.

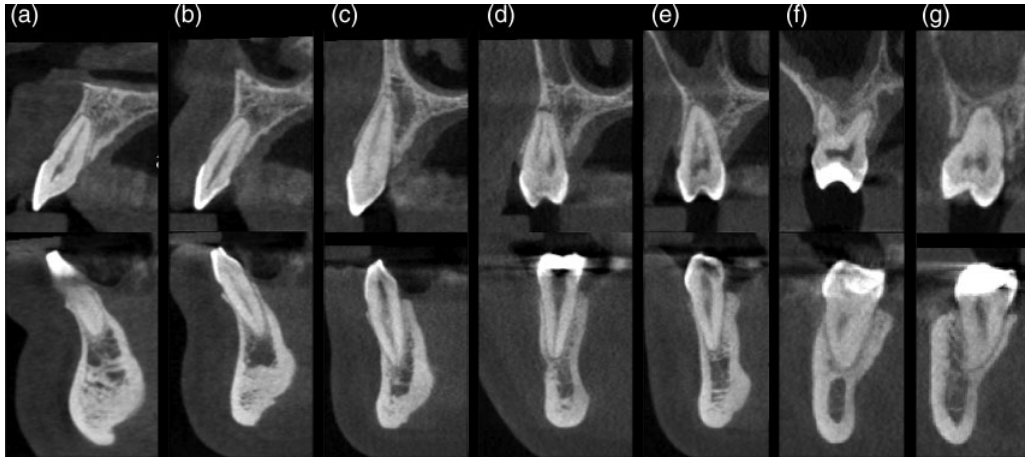

Figure 6.6 Periodontal ligament space. (a-e) Sagittal and coronal CBCT slices of the maxillary (top row) and mandibular (bottom row) anterior (a-c) and posterior (d-g) teeth. The slices accurately demonstrate the relationships of the roots of the teeth to the cortical plates as well as the orientation of the teeth within the jaws. Regional variations in jaw thickness and shape can also be readily appreciated.

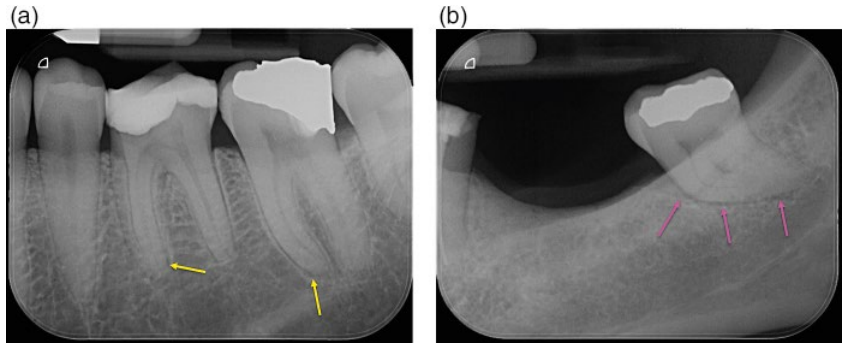

Figure 6.7 Periodontal ligament space. (a) Periapical radiograph of tooth 36 and 37, demonstrating widening of the PDL space on the mesial roots each tooth (yellow arrows). Both had symptoms of irreversible pulpitis. (b) Periapical radiograph of tooth 37, which is a lone standing tooth and the terminal tooth in the arch. Widening of the PDL space on the mesial aspect of the tooth (pink arrows) is related to traumatic occlusion.

buccal aspect of the first and second molars in a thicker cortex. The palatal cortical plate of the maxilla is generally thicker than the facial. However, the palatal cortical plate is thinner in the regions of the molars, particularly the first molar, due to the palatally tipping, palatal roots of these teeth.

The facial surfaces of the roots of the maxillary teeth are generally in intimate contact with the facial cortical plate, with the notable exception of the lateral incisor, the root of which is usually more palatally inclined. Indeed, apart from lateral incisor, the apices of the maxillary teeth generally touch the facial cortical plate. Furthermore, the integrity of the facial cortical plate is sometimes broken over the roots of some maxillary teeth, resulting in a bony dehiscence, often over the apex of an affected tooth. Less frequently, such a dehiscence may extend from the apex to the marginal periodontium, or anywhere between those points.

The facial cortical plate of the mandible is thin over the incisor teeth. However, the facial cortical plate thickens as it runs posteriorly from the canine to the third molar. The buccal cortical plate is particularly thick over the second and third molars where it forms the external oblique ridge.

The alveolar process of the mandible containing the incisor teeth is very narrow. The alveolar process widens as it runs posteriorly and the alveolar process containing the molar teeth is much wider, despite the presence of the submandibular fossa (depression in the mandible which harbors the submandibular gland).

The lingual cortical plate of the mandible is generally thicker than the facial plate in the incisor, canine and premolar regions. However, in the molar regions the lingual cortical plate is thinner than that on the buccal aspect of the molars.

The facial surfaces of the roots of the mandibular anterior teeth are usually in close contact with the facial cortical plate. The apices of mandibular incisor teeth are sometimes exposed by breaks in the integrity of the very thin labial plate. Mandibular premolar teeth generally sit closely against the buccal cortical plate, but the relationship is less intimate than between the anterior teeth and the labial cortical plate. Indeed, on occasion, mandibular premolars will be surrounded by medullary bone and will not contact the buccal cortical plate at all. The roots of mandibular molar teeth are more lingually inclined, with the lingual root surfaces and root apices often contacting the lingual cortical plate.

Conventional radiography: An intimate knowledge of the anatomy of the facial skeleton is required to interpret the presentation of anatomical structures on conventional radiographs, as the true form of the structures is generally confused by the superimposition and compression of adjacent or

overlying structures on the radiograph. The relationship of the cortical plate to the roots of teeth cannot be accurately appreciated nor can the thickness of the cortical plate overlying the root apices. The presence of dehiscences in the cortical plate also cannot be identified using conventional radiography.

CBCT: The selection of appropriate CBCT slices allows the thickness of the cortical plate to be measured with precision. In the same way, the cortical plate can be related to the roots of adjacent teeth and the integrity of the cortical plate can be inspected and the presence of dehiscences identified.

6.2.6 Cancellous Bone

The dense, outer cortical bones of the jaws and facial skeleton form a protective layer and encase the enclosed cancellous bone. Cancellous bone is a light porous bone, arranged into a matrix of interspersing bony projections from the cortex of bone spicules called trabeculae (Figure 6.8). The spaces between trabeculae contain vasculature and bone marrow. The arrangement of the trabeculae gives rise to the typical granular appearance of the bone of the alveolar process surrounding the roots of teeth on radiographs. The trabeculae tend to be thicker and horizontally striated in the mandible and the marrow spaces tend to be wider. In the maxilla the trabeculae tend to be thinner and more compact than in the mandible, resulting in typically smaller marrow spaces. However, there is significant inter-individual variation and the organization of the trabeculae, and therefore the radiographic appearance of the trabeculae and marrow cavities at any given site is related to functional stress on the teeth supported by the bone at that site [15, 47, 50].

Conventional radiography: The true definition of the trabeculae and marrow spaces of cancellous bone on periapical radiographs is obscured by the anatomical noise created by the overlying cortical plate, as well as by the compression of adjacent anatomical structures on to the 2-dimensional radiograph. As such, the subtle changes in the organization of the cancellous bone often associated with the development of AP are difficult to identify. On occasion, to the inexperienced clinician, a well-defined, larger marrow space superimposed over a root apex may be misinterpreted as a AP.

CBCT: By choosing the appropriate CBCT slice, the structure of the cancellous bone can be examined free from anatomical noise and related to the roots of the tooth in the area of interest.

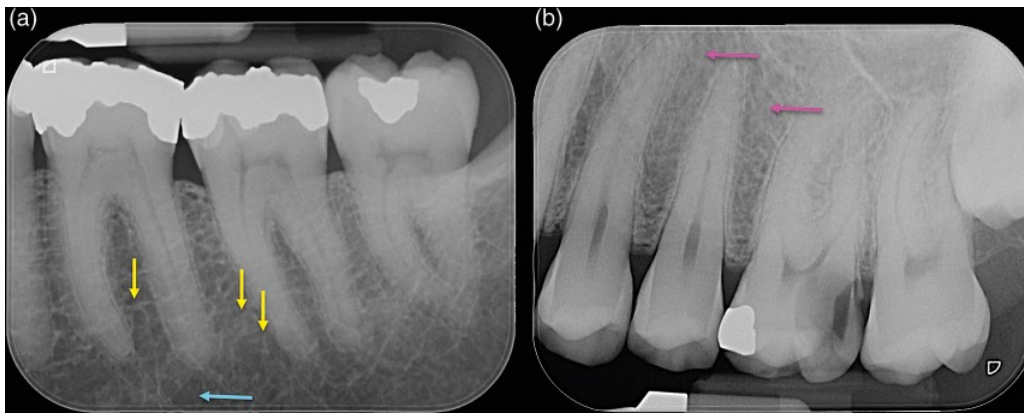

Figure 6.8 Periodontal ligament space. (a) Periapical radiograph of the mandibular right first, second and third molars. The predominantly horizontally oriented boney trabeculae (yellow arrows). Wider marrow spaces (blue arrow) may be mistaken for periapical radiolucencies. (b) The trabeculae in the maxilla (pink arrows) are thinner and more compact compared to those in the mandible.

6.2.7 Neighboring Anatomical Structures

An array of normal anatomical structures in the maxilla and mandible may be captured on a radiograph during the radiographic assessment of a particular tooth. The superimposition of these structures over the area of interest may complicate the interpretation of a conventional radiographic image, when assessing for AP [55]. The following is a list of anatomical structures of the jaws, the conventional radiographic features of which may be misinterpreted as features of AP.

Incisive canal and foramen. The incisive canal is a channel in the bone extending from the floor of the nasal cavity and opening on to the anterior hard palate, in the midline just posterior to the maxillary central incisors. The canal is a conduit for the ascending greater palatine artery and the descending naso-palatine nerve (Figure 6.9). The incisive canal and foramen is represented radiographically as a radiolucency, which may be heart-shaped, oval, round, or a thin wedge. Superimposition of the incisive canal and/or foramen over the apices of the maxillary incisor teeth may mimic a radiolucency associated with AP in conventional radiographs.

Nasal cavity. The nasal cavity is situated immediately superior to the palatine process of the maxilla (hard palate). The most anterior and inferior part of the floor of the nasal cavity is immediately superior to the alveolar process of the maxilla that contains the maxillary incisor teeth. The walls of the nasal cavity are comprised of cortical bone, and the floor of the nasal cavity will manifest radiographically as a radiopaque line in PAs. When

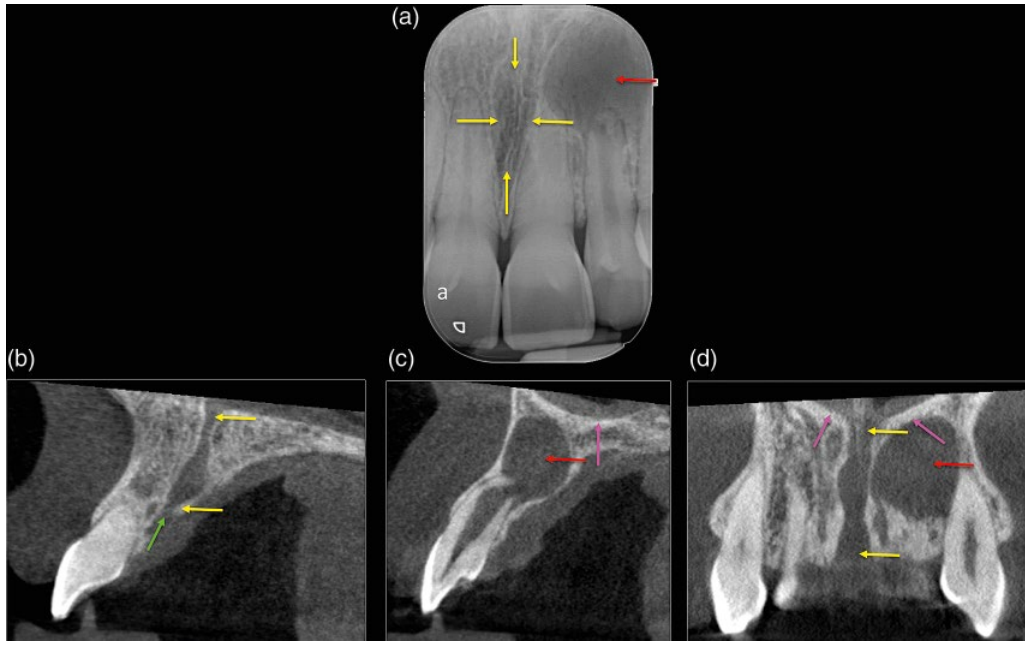

Figure 6.9 Incisive foramen. (a) Periapical radiograph of teeth 11, 21 and 22. The incisive foramen/canal (yellow arrows) is represented on the radiograph as an elongated radiolucency between teeth 11 and 21. There is a large periapical radiolucency associated with tooth 22 (red arrow). (b) Sagittal CBCT view demonstrating the incisive canal (between yellow arrows) and the incisive foramen (green arrow). (c) Sagittal CBCT view demonstrating the periapical radiolucency associated with tooth 22 (red arrow) and its relationship with the nasal cavity (pink arrow). (d) Coronal CBCT view demonstrating the relationship of the incisive canal (between yellow arrows) to the nasal cavity (pink arrows) and the periapical radiolucency associated with tooth 22 (red arrow).

projected over the apices of the maxillary anterior teeth, the floor of the nasal cavity may appear as a corticated radiolucency on periapical radiographs.

Tip of the nose. Periapical radiographs of the maxillary anterior teeth may include the tip of the nose, which appears a diffuse radiopacity over the root apices.

Canine fossa. The canine fossa is a depression on the anterior surface of the maxilla, lateral to the canine eminence. It can manifest radiographically as a relative radiolucency superimposed over the apices of the maxillary lateral incisor tooth, and may mimic AP.

Maxillary sinus. The maxillary sinus is contained within the body of the maxilla. As an air-filled cavity the sinus is seen radiographically as a radiolucency with its caudal periphery coming in intimate contact with the roots of the molars and frequently the premolar teeth. However, as it is enclosed in cortical bone the border of the sinus appears as a thin radiopaque line or multiple thin radiopaque lines. Folds of cortical bone projecting into the lumen from the sinus borders produce the appearance of multiple radiopaque lines.

The border of the maxillary sinus may appear as a unilocular or bilocular projection over the roots of the maxillary molar and premolar teeth. The corticated rim of the sinus border may be mistaken for the lamina dura associated with adjacent teeth and the body of the sinus may be mistaken for lesions of AP. Furthermore, the presence of a true periapical radiolucency associated with AP, may be masked by the radiolucency of the sinus (Figure 6.10).

Maxillary torus. Maxillary tori are benign bony protuberances, comprised of dense cortical bone and occurring on the alveolar and palatine processes of the maxilla. As they are highly mineralized they stand out as densely radiopaque when captured on conventional radiographs (Figure 6.11). Due to their relative radiodensity, their radiographic manifestation may obscure normal and pathological radiographic features of the periapical area.

Mental foramen. The mental nerve passes through the mental foramen to supply sensory innervation to the lower lip and chin. The mental foramen is generally situated in close proximity to the apical area of the mandibular second premolar and is often located between the first and second premolar teeth and just inferior to the root apices of these teeth (Figure 6.12). However, the exact location is variable. As a bony opening of varying

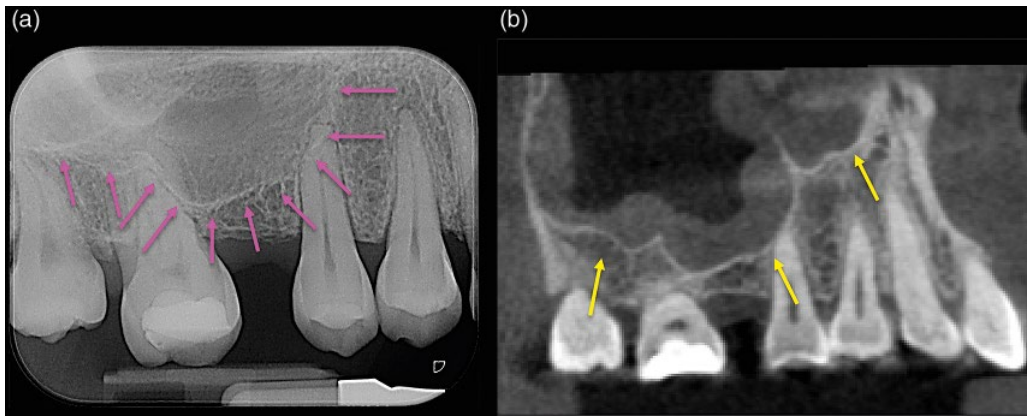

Figure 6.10 Maxillary sinus. (a) Periapical radiograph of the maxillary right posterior teeth demonstrating the inferior border of the right maxillary sinus represented by a thin, poorly defined radiopaque line (pink arrows). (b) Sagittal CBCT view accurately demonstrating the true dimensions of the maxillary air sinus and its multiple lobes (yellow arrows).

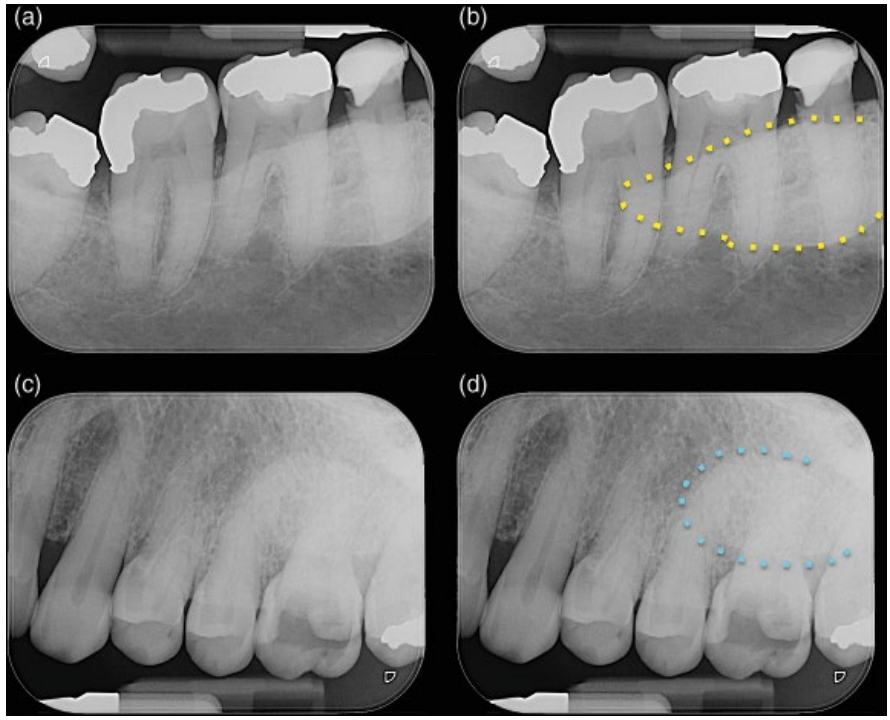

Figure 6.11 Tori. (a, b) Periapical radiographs of the mandibular right posterior teeth. A large mandibular torus can be seen as a well-defined radiopacity projecting over the roots of the 45 and 46 teeth (broken yellow line). (c–d) Periapical radiographs of the maxillary left posterior teeth. A large maxillary torus can be seen as less well defined radiopacity projecting over the roots of the 25 and 26 teeth (broken blue line).

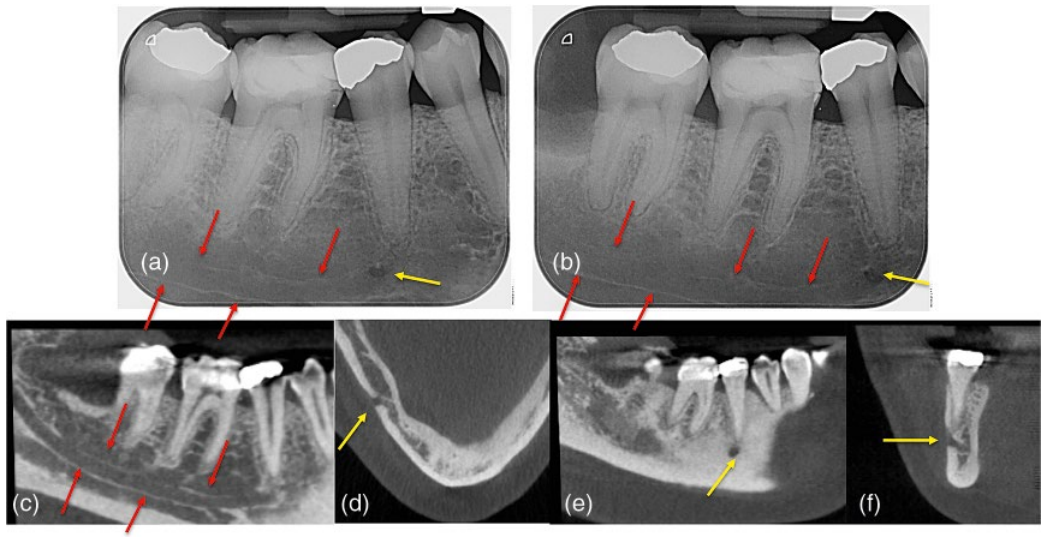

Figure 6.12 Mandibular anatomy. (a–b) Periapical radiographs of mandibular right molar and premolar teeth. The inferior dental nerve canal is represented by parallel, thin, radiopaque lines (red arrows) running in a mesio-distal direction, terminating mesially at the mental foramen, which appears as a well-defined, oval radiolucency (yellow arrow). (c) Sagittal CBCT view of the ID nerve cana. The true course and relationship of the canal can be appreciated and related to apices of the teeth. (d–f) Axial (d), Sagittal (e) and coronal (f) CBCT views demonstrating the position of the mental foramen (yellow arrows) in all planes.

size and shape, the foramen appears radiographically as a radiolucency of varying definition. Depending on the exact site and the projection geometry of the radiograph the mental foramen may be superimposed on the root of a premolar (or less frequently a molar) tooth on a periapical radiograph, mimicking AP. Sometimes the mental foramen is too inferiorly located to be evident on a periapical radiograph.

Mandibular canal. The inferior alveolar nerve and inferior alveolar artery run through the body of the mandible in the mandibular canal. The mandibular canal runs from the mandibular foramen, on the medial aspect of the ramus, to the mental foramen where the mental nerve and vessel branches exit. It appears radiographically as a radiolucent channel, sometimes with a contrasting rim. Depending on the vertical (superior-inferior) position of the canal, it may or may not be evident on periapical radiographs of the mandibular posterior teeth. However, when captured on periapical radiographs it will pass close to the apices of the teeth, potentially obscuring the appearance of the periapical structures (Figure 6.12).

Mandibular torus. Bony protuberances of the mandible will present radiographically in a similar fashion to maxillary tori and will have a similar effect on the interpretation of conventional radiographs (Figure 6.11).

6.3 Radiographic Appearance of Apical Periodontitis

6.3.1 Conventional Radiographic Appearance

Established periapical bone destruction associated with apical periodontitis is generally readily identifiable on conventional radiographs. However, incipient apical periodontitis is often much more difficult to detect using this imaging modality due to overlying anatomical noise [11, 62]. Changes in the structure and form of the apical periodontium, particularly the periodontal ligament space, the lamina dura and the trabeculae of the cancellous bone are often early signs of the development of apical periodontitis [52]. A familiarity with the normal radiographic appearance of these structures is therefore fundamental to potentially identifying early changes at the onset of the disease process. While the apical foramen remains the primary portal of exit of infection from the root canal system to the periodontal ligament and alveolar bone, accessory lateral and furcal canals will often provide a conduit for the egress of infection such that periodontitis develops at these sites. Furcal accessory canals are reported to be present in 76% of molars [22].

6.3.2 Incipient Apical Periodontitis on Conventional Radiographs

Subtle alterations in the normal trabecular pattern of the cancellous bone are among the earliest indicators of the development of AP evident on conventional radiographs. The trabeculae begin to show evidence of disruption and disorganization (outside of normal functional modifications) around the apex (or other portal of exit) of the affected tooth (Figure 6.13). The area of trabecular disruption may be diffuse and difficult to demarcate from surrounding healthy tissue or, alternatively, it may be well-defined and easily differentiated from the adjoining bone. [20].

Widening of the periodontal ligament space of an affected tooth is often a feature of incipient AP. However, a widened periodontal ligament is not exclusively associated with infections of endodontic origin and other causes include, but are not restricted to, occlusal trauma, orthodontic trauma, marginal periodontitis, and neurogenic inflammation [23, 97]. Even in healthy teeth unaffected by trauma, a widened periodontal ligament space may be seen on conventional radiographs when the vertical or horizontal angle of exposure is increased i.e. when the paralleling technique is not utilized [13].

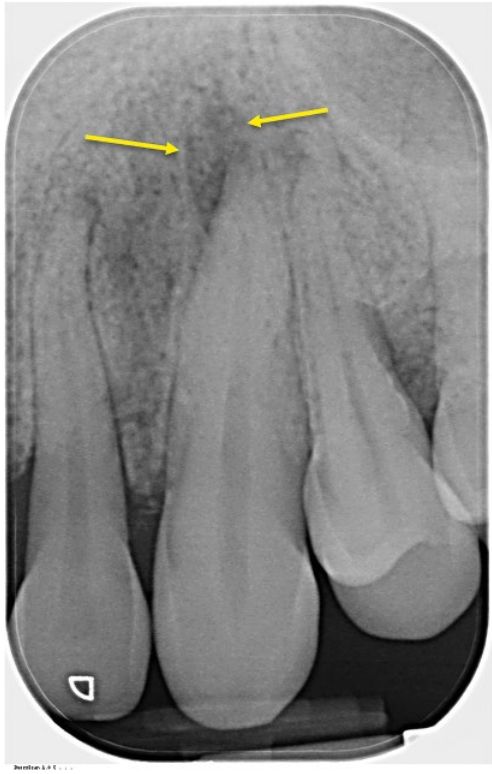

Figure 6.13 Incipient periapical bone loss. Periapical radiograph of the non-vital tooth 23. Demineralization of the bone around the apex of tooth 23 resulting in a "shotgun" appearance of the bone in the area (yellow arrows).

A widened periodontal ligament, which is directly related to an endodontic infection will, in the early stages of the disease process, be confined to the area surrounding the primary portal of exit of the infection. The periodontal ligament space adjacent to these areas will be unaffected and there will be a distinct transition between the affected and unaffected sites.

Another early indicator of AP is the disruption of the lamina dura. At the onset of the disease the lamina dura may appear less radio-dense and the continuity of the structure may be disrupted. Such changes in the lamina dura will be limited to the primary portal of exit of the microbes and their toxins. However, as an isolated finding, a breach in the integrity of the lamina dura should be cautiously interpreted. Channels in the lamina dura are necessarily present to permit the passing of vascular and neural supply between the cancellous bone and the tooth. These channels may be evident on some radiographs and not on others. In addition there will be some inter-individual variation in normal lamina dura thickness and density. The exposure angle of radiographs may also have a bearing on the appearance of these features of the lamina dura.

With the progression of AP the trabeculae of the cancellous bone become depleted of minerals and lose structural integrity. They appear thinner and less dense on conventional radiographs and the medullary spaces increase in size. The area of affected cancellous bone may develop a so-called "shotgun" appearance as a result of this apparent permeative bone destruction. This stage, although not always identifiable, represents a progression in the development of AP from the subtle changes in lamina dura and periodontal ligament space outlined above to the development of a clear-cut radiolucency.

Another possible antecedent to the appearance of a frank radiolucency in the evolution of AP is the development of condensing osteitis (Figure 6.14). Condensing osteitis is an inflammatory reaction in the alveolar bone around the root or roots of an infected tooth, which results in localized sclerosis of the affected bone. The sclerotic bone is apparent radiographically as a radiopacity, which, in its early stages at least, is confined to the periapical area (or area around the associated portal of exit) of the affected root. However, as it develops, the condensing osteitis can expand to involve the bone around unaffected roots in the same tooth or adjacent teeth. Furthermore, the area of inflamed bone may extend in an apico-coronal direction to involve bone some distance from the root end. The margins of any lesion of condensing osteitis may be diffuse or well-defined. The definition, shape, and extent of areas of condensing osteitis are, therefore, variable. The density of the sclerotic bone is also variable and in some cases it may be so

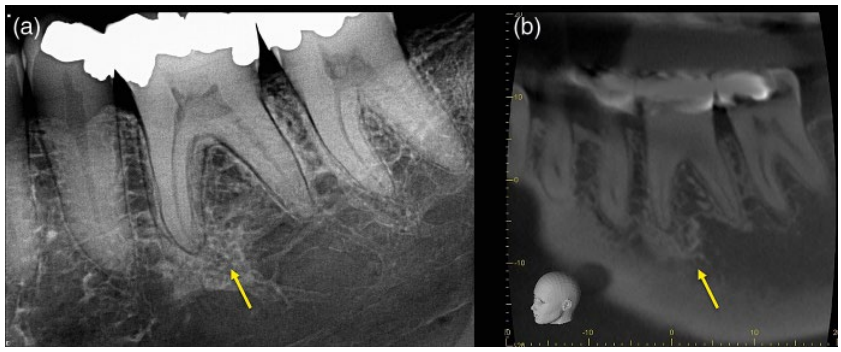

Figure 6.14 Condensing osteitis. (a) Periapical radiograph and (b) sagittal CBCT scan reveals increased radiopacity associated with the mesial root of the mandibular molar tooth, a sign of reactive osteosclerosis (yellow arrow) due to chronic irritation in the 46.

dense as to obscure the appearance of the anatomy of the tooth, which it surrounds.

6.3.3 Established Apical Periodontitis on Conventional Radiographs

When sufficient bone destruction has occurred a radiolucency will develop. The development of a periapical radiolucency may be a sequel to the preceding structural changes to the apical periodontium outlined already or it may accompany them. On occasion, when the disease process progresses quickly, the development of a frank periapical radiolucency may be the first radiographic sign of AP. Diagnosis of the disease process when it has developed to this point is less problematic. However, depending on the site of the affected tooth and the exposure angle of the diagnostic radiograph, the superimposition of adjacent anatomical structures may still obscure the bone destruction, such that it is not readily identifiable as a radiolucency.

In order for AP to be detected radiographically a threshold level of bone loss, relative to the surrounding healthy or unaffected bone, must occur. Early *ex vivo* investigations reported that simulated lesions of AP could not be detected if associated bone destruction was confined to cancellous bone i.e. the cortical bone was unaffected [11, 12, 96, 103]. Other studies of that time demonstrated that AP in the anterior maxilla affecting only the cancellous bone could be radiographically detected [20] even when the cortical bone was spared [105]. It is now generally accepted that the radiographic detection of AP is directly related to the ratio of mineralized to demineralized (caused by the disease process) bone, which itself is a factor of the extent of the disease process, the specific jaw involved, the position of the tooth within the jaw involved, and inter-individual variation.

Radiolucencies associated with AP vary in appearance. Their size is variable and related to the extent of the endodontic infection in the affected tooth and the tissue response to the insult presented. Their margins may be well or poorly defined (Figures 6.15 and 16.16). Occasionally, particularly in long-standing lesions, the margins may have a corticated appearance. Historically, it was considered that radiographic features of lesions of AP, such as size and the presence and nature of a radio-opaque corticated border were predictors of the histological nature of the lesion [14]. These associations have since been challenged, and a radiopaque rim is no longer regarded as evidence for the presence of a cyst [82, 83, 100].

Due to the variability in the radiographic presentation of AP and the subjectivity of subtle radiographic changes associated with

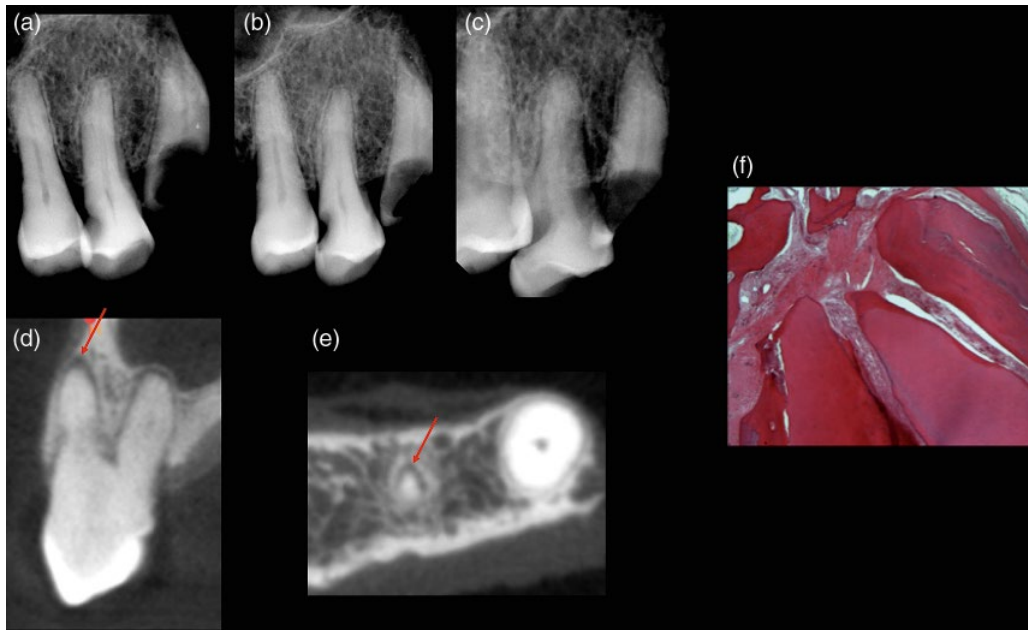

Figure 6.15 Apical periodontitis. The figure shows film PR, centered view (a), mesial shift (b) and distal shift (c) as well as digital PR, centered view (d), mesial shift (e) and distal shift (f). No PR technique detected any periapical lesion associated with the distal root of tooth 46. Histopathological examination of the distal root of tooth 46 (f) showed AP: decalcified root structure with necrotic apical pulp tissue and granulomatous tissue (magnification 4×; H & E staining). Yellow arrow showing area of inflammation with bone resorption, GT, granulation tissue, D: dentine, PDL, periodontal ligament. Reproduced with permission from [62].

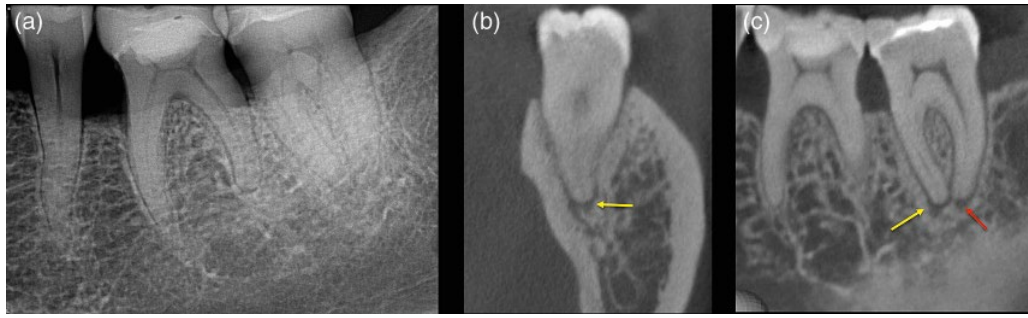

Figure 6.16 Apical periodontitis. (a) Radiograph of lower left quadrant in patient with poorly localized symptoms of irreversible pulpitis. (b, c) coronal and sagittal reconstructed CBCT views confirm a periapical radiolucency associated with the mesial (yellow arrow) and distal (red arrow) roots of the lower left second molar tooth.

incipient AP, a quantitative scoring system with a reference scale has been developed to improve the objective assessment of AP. The periapical index (PAI) is a scoring system, which uses a visual reference scale for assigning health status to a root [86].

6.3.4 Appearance of Apical Periodontitis on CBCT

The nature and size of AP is accurately represented by CBCT scans. Any expansion and/or perforations of cortical bone may be identified and related to clinical findings.

When compared to conventional radiography, a more objective and quantitative appreciation of the presence and extent of bone destruction associated with the disease can be obtained. Condensing osteitis is also much more accurately represented.

6.3.5 Apical Periodontitis Associated with Root-filled Teeth

Apical periodontitis is a dynamic process of bone destruction and remineralization, the ebb and flow of which cannot be appreciated from any single conventional or 3-dimensional radiographic exam. An isolated radiograph of a root-filled tooth with AP, taken at any given juncture in time, will only provide a snapshot of the size of the associated radiolucency at that time and will afford little, if any insight into whether the lesion is stable, healing or expanding. The healing of AP following root canal treatment may take years, even decades, to occur. Although expert consensus guidelines suggest that periapical lesions persisting four years after treatment are "usually considered to be associated with post-treatment disease" [40], there is no scientifically accepted upper time limit for the healing of AP following endodontic treatment. Indeed, several long-term outcome studies have demonstrated late healing of AP 5–10 years [110], 10–17 years [76, 77], and 20–27 years [75] after treatment. Molven et al. [74] reported that delayed healing in the majority of cases was associated with extrusion of root filling material into the periapical tissues during treatment.

6.4 Healing Characteristics

6.4.1 Healing of Apical Periodontitis After Non-surgical Root Canal Treatment

Very little is known about how the healing process of AP manifests radiographically; the healing timescale is highly variable. There is some evidence that a transient increase in bone density may occur over the first few weeks of healing [84, 85]. However, as outlined previously, radiolucencies associated with AP may partially resolve but ultimately persist for decades after treatment, before completely healing [76, 77, 110]. During healing the radiographic density of the periapical lesion increases as new bone is laid down within it. It is unknown if the bone is deposited concentrically from the lesion margins or if spiculae projecting towards the center of the lesion multiply to fill. If the cortical plate has been perforated, healing commences by the re-establishment of the integrity of the cortical plate and bone deposition then proceeds from there towards the center of the lesion [17]. The newly formed bone lacks maturity and may appear less organized than adjacent healthy bone. Eventually, in healed cases the periodontal ligament space and lamina dura will re-establish. Persistent widening of the periodontal ligament space and persistent bony rarefaction associated with excess filling material are radiographic characteristics of the healing process of root-filled teeth. Complete healing may develop with time [76, 110].

6.4.2 Healing after Endodontic Surgery

Healing after endodontic surgery differs from healing following non-surgical treatment in that the bony defect is excavated of granulomatous tissue as part of the surgical process, thus allowing immediate bleeding into the cavity and the subsequent formation of a blood clot, which provides a scaffold for new bone formation. The net effect is that healing generally occurs much more quickly following surgery when compared to non-surgical treatment. In order to obtain access to the body of the lesion during surgery, any overlying mineralized tissue is necessarily excavated. As such, the radiolucency appears more pronounced immediately postoperatively when compared to the preoperative situation. When healing occurs, the radiographic

appearance is more variable than the healing process following root canal treatment.

In situations where the facial and lingual/palatal cortical plates have been significantly eroded and cannot be re-established, bone formation commences from the intact lateral walls but the areas of cortical plate destruction are repaired with fibrous tissue, resulting in formation of apical "scar tissue". The formation of apical scar tissue is classified as "incomplete healing", but is considered clinically successful healing. The formation of apical scar tissue is characterized by a diminishing radiolucency extending at an angle into the periodontal ligament space. The radiolucency may be positioned asymmetrically around the root apex. Visible bone structure within the radiolucency may or may not be present. Continued healing may see the development of a lamina dura around the root apex, separating the radiolucency from the root end [78]. Scar tissue with similar characteristics may occur on occasion in teeth which have been treated non-surgically but the periapical lesion has caused extensive destruction to the facial and lingual/palatal cortical plates [78].

6.5 Conventional Radiography for Assessment of Apical Periodontitis

The 2-dimensional nature of radiographs, anatomical noise, and geometric distortion limit the accuracy of periapical radiographs to assess radiographic signs of AP).

The use of parallax radiograph views has been suggested to overcome some of the limitations of periapical radiographs [40]. Using block dissection and histopathological analysis of the periapical tissues as the reference standard, Kangasingam et al. [62] found that combination of two additional (parallax) images, with mesial and distal horizontal angulations increased the diagnostic accuracy of AP. However, it should be noted that multiple periapical radiographs do not guarantee the identification of all relevant anatomy or signs of disease [10, 70], and may not reveal much more than a single exposure.

Beam-aiming devices increase the likelihood of geometrically accurate images [46, 117]. A series of investigations by Forsberg [43–45] concluded that the paralleling technique was more accurate than the bisecting-angle technique for accurately and consistently reproducing apical anatomy.

Over-angulated or under-angulated radiographs (bisecting or paralleling technique) may increase or decrease the size or even result in the disappearance of periapical lesions [11, 13, 57].

Currently, periapical radiography is considered as the imaging technique of choice for the initial radiological assessment. The proceeding sections will describe some imaging techniques which have been used in an attempt to overcome these limitations for assessing AP.

6.6 Advanced Radiographic Techniques for Endodontic Diagnosis

Alternative imaging techniques have been suggested to overcome the limitations of periapical radiographs [80, 90].

Tuned Aperture Computed Tomography. Tuned Aperture Computed Tomography *(TACT)* works on the basis of tomosynthesis [121]. A series of 8–10 radiographic images are exposed at different projection geometries using a programmable imaging unit, with specialized software to reconstruct a 3-dimensional data set, which may be viewed slice by slice.

As well as less superimposition of anatomical noise over the area of interest [115, 120], the overall radiation dose of TACT is no greater than 1–2 times that of a periapical radiograph [81]. Additional advantages claimed for this technique include the absence of artefacts resulting from radiation interaction with metallic restorations (see

later section on computed tomography). The resolution is reported to be comparable to 2-dimensional radiographs [80].

Magnetic Resonance Imaging (MRI). MRI is a specialized imaging technique which does not use ionizing radiation. It is based on the behavior of hydrogen atoms within a magnetic field, which is used to create the MR image. Tutton and Goddard [114] claimed that the nature of periapical lesions could be determined as well as the presence, absence, and/or thickening of the cortical bone. Goto et al. [51] compared measurements taken from 3-dimensional reconstructed MRI and computed tomography images of a dry mandible and hemi-mandible. They concluded that the accuracy of MRI was similar to computed tomography. Cotti & Campisi [30] suggested that MRI may be useful to assess the nature of endodontic lesions and for planning periapical surgery.

Poor resolution, long scanning times, and high hardware costs mean that access to this type of imaging is only available in dedicated radiology units. Furthermore, specialized training is required to use the hardware and interpret the images.

Ultrasound. Ultrasound is based on the reflection (echoes) of ultrasound waves at the interface between tissues which have different acoustic properties [53].

Several research groups have suggested that ultrasound can differentiate between cysts and granulomas [6, 30, 53]. However, in none of these studies were the apical biopsies removed *in toto* with the root apex, therefore making it impossible to confirm whether a cystic appearing lesion was a true or pocket cyst. In addition, the lesions were not serially sectioned making accurate histological diagnosis impossible [99]. The ability of ultrasound to assess the true nature and type (for example, true versus pocket cyst) of periapical lesions is doubtful. Ultrasound is blocked by bone and is therefore useful only for assessing the extent of periapical lesions where there is little or no overlying cortical bone [6].

Computed tomography. Computed tomography (CT) produces 3-dimensional images of an object by taking a series of 2-dimensional sectional X-ray images.

Over the last five decades, there have been considerable advances in CT technology, resulting in high, soft, and hard tissue resolution with lower radiation dosages. Current CT scanners are called multi-slice CT (MSCT) scanners and have a linear array of multiple detectors, allowing "multiple slices" to be taken simultaneously, as the X-ray source and detectors within the gantry rotate around the patient, who is simultaneously advanced through the gantry. This results in faster scan times and therefore a reduced radiation exposure to the patient [111, 124].

CT has several other advantages over conventional radiography. These include the elimination of anatomical noise and high contrast resolution, allowing differentiation of tissues with less than 1% physical density difference to be distinguished compared to a 10% difference in physical density which is required with conventional radiography [124]. Velvart et al. [118] found that CT could more readily detect periapical radiolucencies and the location of the inferior alveolar nerve compared with periapical radiographs in mandibular posterior teeth scheduled for periapical surgery to the clinical findings at the time of surgery.

CT technology has now become superseded by Cone Beam Computed Tomography technology in the management of endodontic problems.

Cone Beam Computed Tomography. Cone beam computed tomography (CBCT) or digital volume tomography (DVT) was developed in the late 1990s to produce 3-dimensional scans of the maxillo-facial skeleton at a considerably lower radiation dose than conventional computed tomography (CT) [9, 79]. Its use has grown exponentially in endodontics [92].

With CBCT a 3-dimensional volume of data is acquired in the course of a 180° to 360° single sweep of the extraoral X-ray source and reciprocal sensor which rotate synchronously around the patient's head. The X-ray beam is cone-shaped, and captures a cylindrical or

spherical volume of data, described as the field-of-view (FOV). This has the advantage of reducing the patient radiation dose. Small (limited) high-resolution volume CBCT scans are indicated in endodontics.

Sophisticated software processes the collected data into a format that closely resembles that produced by medical CT scanners. Reconstructed CBCT images may be displayed simultaneously in the three orthogonal planes, allowing the clinician to gain a truly 3-dimensional view of the area of interest.

There is now evidence to suggest that adjusting the exposure parameters away from the manufacturer's default settings can result in CBCT images which are still of diagnostic use but at a significantly lower radiation dose [37, 60, 67].

CBCT have several limitations. These include poor spatial and contrast resolution [102, 128]. As well the generation of artefacts (for example, beam hardening and scatter) around highly radio-dense material (for example, enamel, gutta-percha, and metal posts), all these factors may reduce the diagnostic yield of CBCT [21, 41, 65].

6.7 Differential Diagnosis

Although AP is the commonest lesion of the jaws, other lesions be present in the jaws and may be mistaken for AP. The use of advanced imaging techniques such as CBCT and ultrasound [29] are helpful in the differential diagnosis. The next section briefly describes the most commonly associated radiolucencies of the jaws.

6.7.1 Concomitant Periodontal Disease

Advanced cases of chronic marginal periodontitis may result in the disease process advancing toward the apical third of the root(s). Radiographically, there would usually be a peri-radicular radiolucency and/or furcation involvement (Figure 6.17). Careful assessment is essential to confirm if the lesion has an endodontic component (perio-endo lesion), and this will determine if periodontal and/or endodontic treatment is required to manage the disease process. The reverse process of an apical inflammation spreading or draining coronally along the root may also occur, creating a probable, periodontal pocket-like sinus tract.

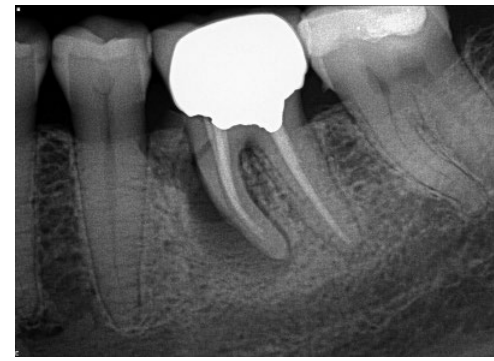

Figure 6.17 Vertical root fracture; a "j"-shaped periradicular radiolucency of the mesial aspect of the mesial root indicating a vertical root fracture of the mesial root.

CBCT scan may be required to determine the extent of alveolar bone involvement, and may also aid in the treatment planning.

6.7.2 Vertical Root Fracture

The prevalence of vertical root fractures (VRF) is higher in endodontically treated teeth than in vital teeth [25, 28]. It has been reported that between 20% and 32% of endodontically treated are extracted due to VRF [24, 27].

Early (incomplete) VRFs may not be readily detectable clinically or radiographically. However, as the VRF becomes more established and infected there will be widening of the periodontal ligament on one aspect of the root, in more advanced cases peri-radicular bone loss will be apparent [26]. A VRF will only be detected with conventional radiographs if the X ray beam is parallel to an incomplete fracture line (Figures 6.17 and 16.18). However, this is a rare occurrence [18].

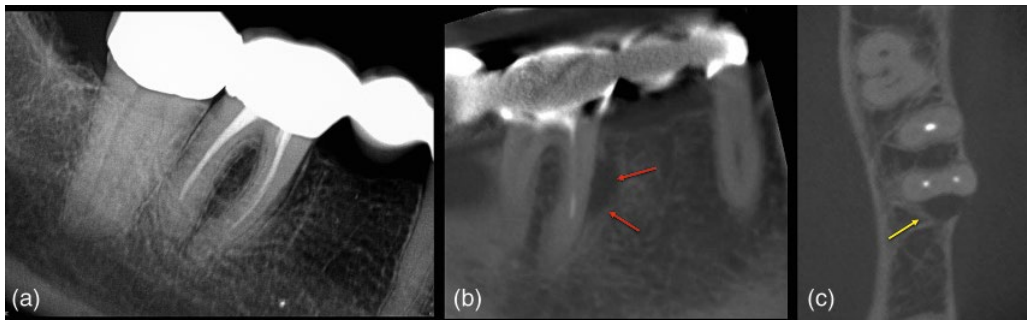

Figure 6.18 Vertical root fracture. (a) A periapical radiograph of the lower right molar teeth does not reveal anything untoward, however, the (b) sagittal (red arrows) and (c) axial (yellow arrow) CBCT slices reveal a clear radiolucency associated with the mesial root.

In cases where clinical and conventional radiographic examination are inconclusive CBCT may be useful in detecting subtle changes in peri-radicular bone adjacent to the site of a suspected VRF [91].

6.7.3 Osteomyelitis

Osteomyelitis may be a continuation of an AP, when the infection spreads and destroys the bone marrow [64]. It occurs more readily in the mandible. The radiographic appearance of the affected bone and periosteum is highly variable and age-related [123]. The adjacent periosteum may lay down new bone (periosteal reaction). Typical features include a moth-eaten (poorly defined) border, islands of radiopaque sequestra of necrotic bone, subperiosteal bone formation beyond the region of necrosis, and sclerosis of surrounding bone.

6.7.4 Occlusal Trauma

Injury to the periodontium due to excessive occlusal forces can result in widening of the periodontal ligament and thickening or discontinuity of the lamina dura [34]. The effects may be localized if it is associated with (non-) working side occlusal interference(s), or more generalized if there are parafunctional habits, orthodontic treatment, or an existing periodontal disease [59, 119].

6.7.5 Odontogenic Cysts

Radicular cyst. This is the most commonly found cyst in the jaw and usually originates from the epithelial cell rests of Malassez. These cysts most commonly occur in patients between 30 and 50 years old [122], and are associated with non-vital or root treated teeth, the most commonly affected tooth is the maxillary lateral incisor. It is uniformly radiolucent, round, unilocular, with smooth, well defined and corticated margins [123]. There may be cortical plate expansion, and displacement of adjacent teeth (Figure 6.19).

Odontogenic keratocystic tumor. This is a unilocular or multilocular, benign tumor, which emanates from the dental lamina epithelium and is most commonly detected in the posterior body and/or angle of the mandible or maxillary canine regions. It has well-demarcated borders, is uniformly radiolucent, and can expand considerably within the cancellous bone (Figure 6.20).

Dentigerous cyst. Dentigerous cysts are associated with the crowns of unerupted or impacted teeth. The cyst cavity is lined by epithelial cells derived from the reduced enamel epithelium It is usually detected in the second to fourth decade as an incidental radiographic finding and most commonly found in the mandible [36].

The cyst is usually well defined, unilocular and uniformly radiolucent, apart from the

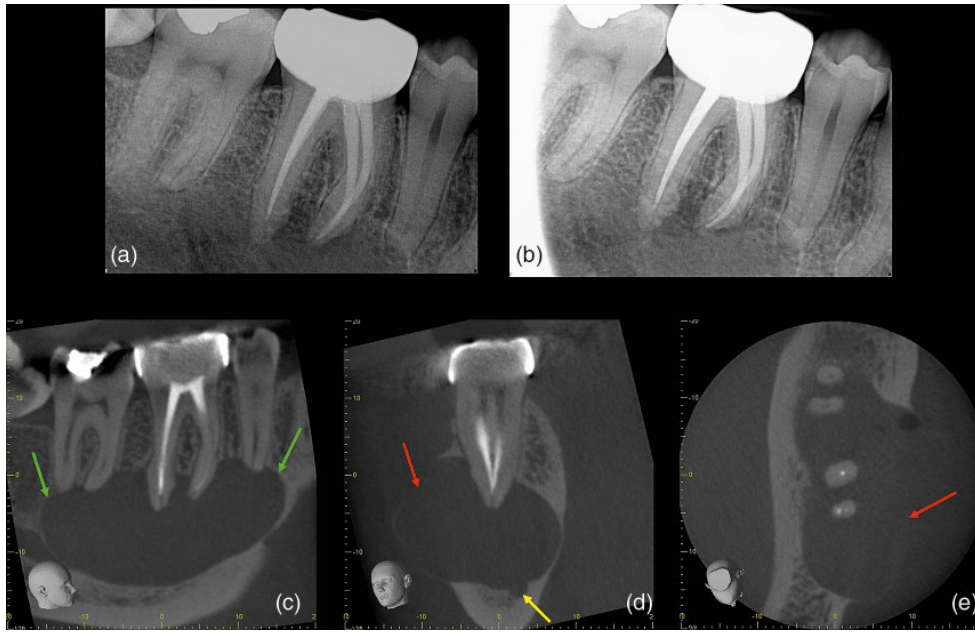

Figure 6.19 Radicular cyst. (a, b) Parallax periapical radiographs reveal a well-defined periapical radiolucency associated with the root treated lower right first molar tooth (green arrow). (c) Sagittal reconstructed CBCT scan reveals a well-defined periapical radiolucency extending from the lower right second premolar to the lower right second molar teeth which has resulted in apical resorption of the root apices. (d) coronal and (e) axial reconstructed CBCT slices reveal marked buccal expansion and perforation of the buccal cortical plate (red arrow). The inferior dental canal (yellow arrow) has been deflected inferiorly.

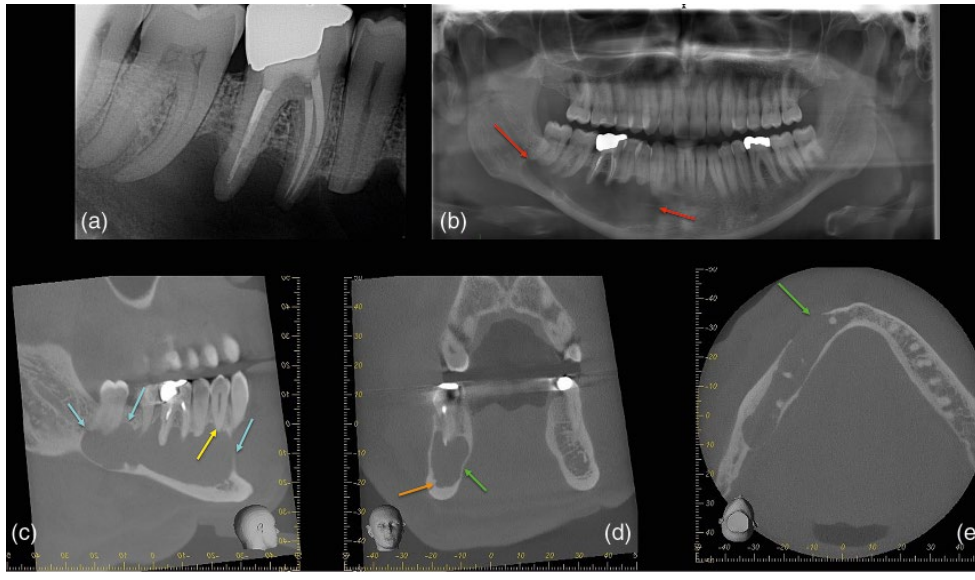

Figure 6.20 Odontogenic keratocyst. (a) Periapical radiograph reveals a large radiolucency associated with the lower right premolar and root treated molar teeth. (b) Dental panoramic tomograph reveals the extent of the lesion (red arrows). (c–e) Sagittal, coronal and axial reconstructed CBCT slices reveal a large well-defined pseudo-loculated radiolucency in the body of the right mandible extending from lower right incisor to the retromolar, which rises up between the roots. There are signs of external inflammatory resorption associated with the lower right premolar teeth, but not with the root-canal-treated lower right first molar tooth, indicating that the lesion is not inflammatory. The lesion has resulted in thinning out and buccal expansion of the cortices (green arrow), and is in close proximity to the inferior dental nerve (orange arrow).

associated tooth it envelopes. In some cases neighboring teeth may be displaced and there may be associated resorption of adjacent roots. Dentigerous cysts can expand extensively bucco-lingual and mesio-distally.

Lateral periodontal cyst. The origin of this cyst is unclear. It may arise from the epithelial cell rests of Malassez, the dental lamina, or from the reduced enamel epithelium. It is usually found in the mandible in the lateral incisor/premolar region. The affected tooth is typically vital, aiding differential diagnosis [104].

Radiographically, periodontal cysts present as well defined, unilocular radiolucency on the lateral aspect of the affected tooth; there may also be loss of the periodontal ligament and the associated lamina dura [71].

6.7.6 Non-odontogenic Cysts and Tumors

Nasopalatine (incisive) canal cyst. These cysts affects 1% of the population, typically males between 40 and 60 years old. It presents as a round or oval radiolucency with smooth and well-demarcated borders in the midline immediately posterior to the maxillary central incisor teeth [123].

It is uniformly radiolucent, and may cause the adjacent teeth to be displaced as it expands (Figure 6.21). The adjacent teeth normally respond positively to vitality testing, thus aiding differential diagnosis.

Traumatic bone cyst. This lesion is not a true cyst as it lacks an epithelial lining. It is usually detected as an incidental finding in the second decade and occurs more frequently in the mandible. These lesions are usually and irregular, unilocular shape with smooth borders, which arches up between roots of teeth. There is not direct effect on neighboring teeth or expansion of the jaw outline.

6.7.7 Bone-related Lesions

Giant cell granuloma. The etiology for giant cell granulomas include irritation and dental trauma. These lesions may be unilocular or multilocular in nature and have a high recurrence rate after surgical excision. The lesion has well-defined, smooth, and scalloped margins. Larger lesions may have a honeycomb appearance due to the presence of thin trabeculae. Adjacent teeth may be displaced and/or resorbed, and the adjacent jaw may be expanded. Giant cell granulomas may be either non-aggressive or aggressive in nature.

Periapical cemental dysplasia. A condition of unknown etiology, cemental dysplasia is lamentably often misdiagnosed as apical periodontitis. Typically diagnosed in early middle age and in association with lower incisor teeth, it is most common in people of African descent, and occurs in females more often than males. Unless otherwise affected, the associated teeth respond positively to vitality testing.

The lesions are round and approximately 5 mm in diameter, and associated with several teeth. The lesions are usually poorly defined (Figure 6.22). Depending on the stage of the lesion, it may be radiolucent (early), have radiopaque inclusions (intermediate), or be densely radiopaque (late). The lamina dura may not be visible.

6.7.8 Tumors

Ameloblastoma. An aggressive tumor, which can expand in all directions and loosen/displace adjacent teeth and typically detected between the ages of 30–60. It is more likely to be detected in the mandible than the maxilla [123].

Radiographically, it is commonly multilocular with bone trabeculae separating the lesions lobes. Less commonly it has a honeycomb appearance.

Malignant tumors. Malignant tumors are rare. The radiographic appearance is dependent on the type of malignancy and how long it has been present for.

Radiographic features range from widening a poorly defined, non-corticated radiolucency around one or more teeth to a poorly defined "moth-eaten" (ragged) non-uniform area of bone destruction. Resorption of

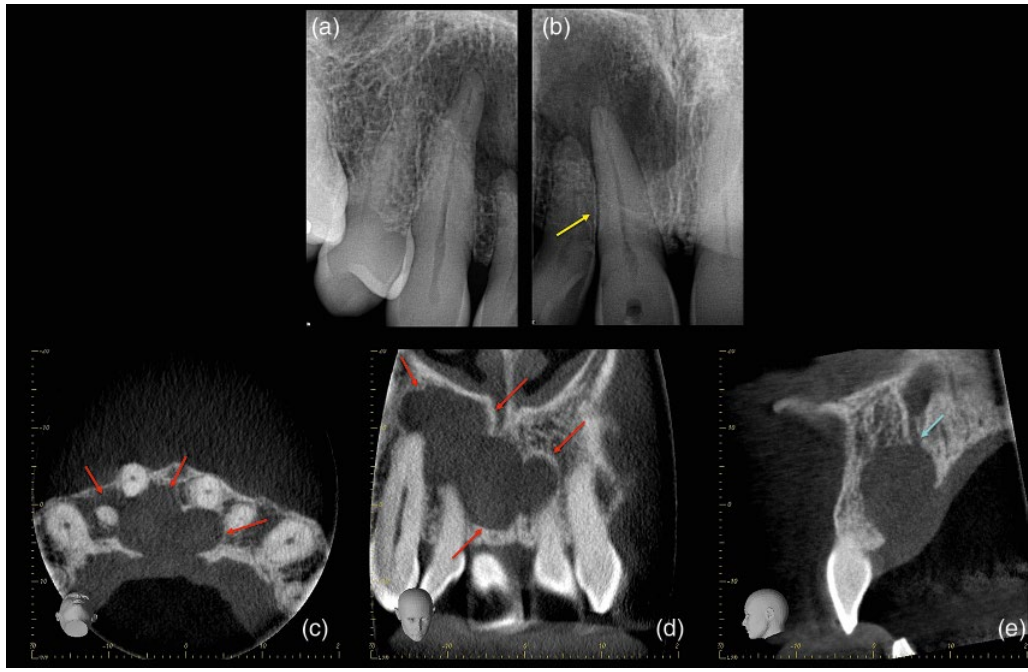

Figure 6.21 Naso-palatine canal cyst. (a, b) Periapical radiographs reveal attempted accessed cavity in the upper right central incisor. There is a large, well-defined and partially corticated radiolucency spanning UL1, UR1, UR2 and UR3. The lesion is displacing the UR1 (yellow arrow), and there is no evidence of root resorption. (c) Axial and (d) coronal CBCT reconstructed slices reveal lobulated, radiolucent radiolucency with minimal expansion of the cortical plates (red arrows), (e) sagittal view reveal that the radiolucency appears to be merging with the incisive foramen (blue arrow).

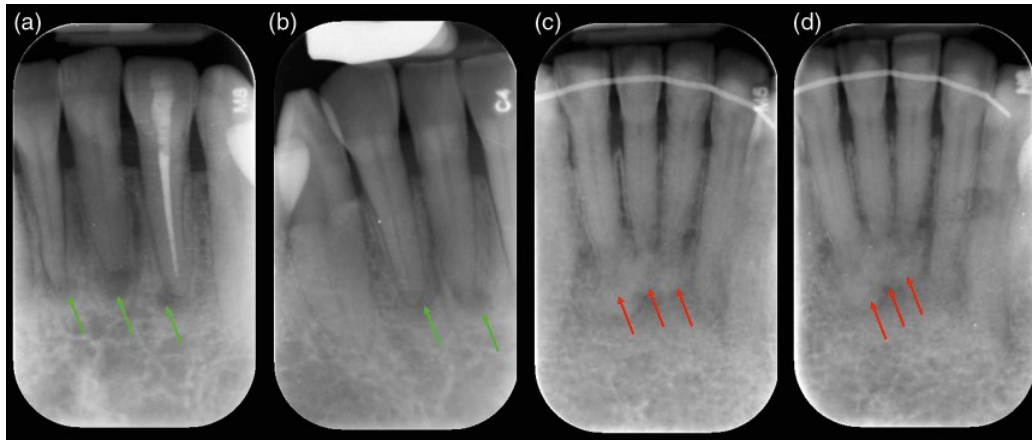

Figure 6.22 Periapical cemental dysplasia. (a, b) Early stage: well-defined radiolucencies (green arrows) associated with the all the lower incisor teeth. The teeth test vital, but tooth 32 was misdiagnosed to have apical periodontitis and was root treated. (c, d) Intermediate stage: periapical radiographs of the lower incisor region reveals a poorly defined radiolucency with patchy radiopaque inclusions (red arrows) associated with vital lower incisor teeth.

adjacent teeth may be a sign of invasiveness and suggest malignancy. Slower-growing, less aggressive tumors may displace the teeth rather than cause resorption.

6.8 CBCT for Assessment of AP

6.8.1 Diagnosis

Clinical studies have demonstrated 11–39% higher prevalence of AP with CBCT compared to periapical radiographs [5, 16, 32, 41, 69, 95]. In addition, 10% of teeth with irreversible pulpitis had AP when examined by CBCT [4].

The results of these clinical studies have been substantiated by *ex vivo* experiments in which periapical lesions have been intentionally created, i.e. the periapical bony status is known beforehand. Experimental studies of artificially created periapical lesions of varying sizes in pig mandibles have shown CBCT to be twice as sensitive as digital and conventional radiography [109]. Similar *ex vivo* studies using human jaws have also found CBCT to be more accurate than periapical radiographs for assessing the presence or absence of periapical lesions [87, 89, 107, 113]. Indeed, the diagnostic accuracy of CBCT has been confirmed in systematic reviews and meta-analysis of ex vivo experiments [66].

The correlation of radiographic and histological characteristics is essential for the distinction of diseased from healthy tissues [48, 62, 63] Apical periodontitis is an inflammatory disease characterized primarily by its cellular infiltrate, and the destruction of bone is a secondary consequence of the inflammation. As there are bony changes in otherwise healthy tissue, which may mimic pathosis, it is essential that the radiographic characteristics of CBCT, as well as PA, exposures be related to histology. An *in vivo* study in dogs where root canal treatment was done on teeth with induced apical periodontitis confirmed the the accuracy of CBCT with histology as the reference standard [35]. The specificity and positive predictive value (PPV) of radiographs and CBCT were 1, i.e. perfect accuracy for correctly determining the absence of periapical disease. However, the sensitivity of CBCT (0.91) was much higher than periapical radiography (0.77). This was also reflected in the negative predictive values (NPV) for CBCT and periapical radiographs, which were 0.46 and 0.25 respectively. The overall accuracy of CBCT and radiographs in the diagnosis of AP was 0.92 and 0.78 respectively [35].

Data are now emerging from human studies essentially establishing the strong correlation of CBCT features with histological tissue responses. Kanagasingam et al. [62, 63], using similar methodology to Brynolf [20], assessed the accuracy of single radiograph, parallax digital radiographs, and CBCT for diagnosing AP in fresh human cadavers using histology as the gold standard. In total, 86 roots in 67 teeth were analyzed. The specificity of all the imaging systems was excellent; i.e., when a diagnosis of AP was made by a radiographic technique, it was routinely confirmed by histology. However, the sensitivity (ability to detect the disease) of these imaging systems was 0.27, 0.38 and 0.89 for single view radiographs, parallax views, and CBCT respectively. The overall accuracy of these imaging systems was 0.63, 0.69, and 0.94 for single-view radiographs, parallax views, and CBCT respectively. Thus CBCT examinations may be used with confidence for detecting both the presence as well as the absence of AP.

A traditional view holds that radicular cysts, which develop from an apical granuloma, may need surgical excision for treatment. Radiographic signs that can differentiate cysts from granulomas thus become important for choice of therapy [31]. It has been suggested that CBCT may be able to differentiate "solid from cystic or cavity type lesions" [106], and even differentiate between granulomas and periapical cysts [54]. Such studies must be viewed with caution as the only way to determine this would be removing the periapical lesion *in toto* and

then serially section the specimen-to date this has not been done.

The increased accuracy of CBCT in the detection of PA lesions has been shown to be beneficial in the diagnosis and management of endodontic problems [33, 38, 101, 116], and also in cases where apical microsurgery is planned [16].

The interpretation of a PA lesion is also dependent of the training, knowledge, and experience of the clinician or radiologist assessing the CBCT scan [88].

The increased accuracy of CBCT for detecting PA lesions does not mean it should be routinely used for diagnosis and management of endodontic disease [66, 92]. Its use should be limited to specific cases where there is a potential overall gain from a diagnostic and/or management of apical periodontitis. The ESE CBCT position statement [91] suggests that in relation to AP, CBCT should be considered 1) in the diagnosis of radiographic signs of PA when there are contradictory symptoms/signs, and 2) for confirmation of non-odontogenic causes of symptoms/signs.

6.8.2 Treatment Outcome

An exciting area in which CBCT may be applied to in endodontics is in determining the outcome of treatment. Outcome is largely defined by the reduction or elimination of chronic AP, or by succeeding in preventing its development. Therefore, all the characteristics documented for CBCT accuracy in detecting AP come into play in follow-up studies of endodontic treatment. CBCT scans should result in a more objective and accurate determination of the prognosis of vital pulp therapy [56] and endodontic treatment [68, 94], and also have an impact on the further management of root treated tooth [33].

The much higher sensitivity of CBCT in detecting AP has as an inescapable consequence that the very high rates of healing following treatment of AP and monitored by PA radiographs are significantly reduced when the same teeth are followed by CBCT. Paula-Silva et al. [48] compared the outcome of endodontic treatment in dogs with periapical radiographs and CBCT using histological assessment of block dissections as the gold standard. Six months after endodontic treatment a favorable outcome was detected in 79% of teeth assessed with a periapical radiograph, but was only 35% when CBCT was used, i.e. half of conventional "successes" turned out to be failures. When teeth with preoperative lesions were studied separately, the success rate was even lower (25%) [48].

Liang et al. [68] compared the outcome of endodontic treatment after 2 years with periapical radiographs and CBCT. They found that a favorable outcome was reached in 87% of cases assessed with periapical radiographs and 74% of cases assessed with CBCT images; a much smaller difference than for teeth with lesions.

The higher sensitivity of CBCT in follow-up studies may be more pronounced when molars are assessed [33, 94] A higher failure rate was found for molar teeth assessed with CBCT, which may be attributed to a more complex root canal anatomy being more challenging to disinfect in primary and secondary retreatment, respectively.

Future research may show that periapical tissues which appear to have "healed" on conventional radiographs may still have signs of periapical disease (for example, widened periodontal ligament space, periapical radiolucency) when imaged using CBCT. This in turn may have implications for decision making and selection criteria when considering (re-)placing coronal restorations on teeth which have previously been endodontically treated and appear to have successfully healed radiographically [1]. Different outcome predictors may be revealed when assessing outcome with CBCT and this may help us understand the healing dynamics of endodontically treated teeth as well as revealing different outcome predictors [126].

The smallest FOV compatible with the clinical situation should be prescribed, as this will result in a lower radiation dose [93].

6.9 Concluding Remarks

Radiology is one of the cornerstones of successful diagnosis and management of AP. An in-depth appreciation of the limitations of conventional radiographs and knowledge of dentoalveolar anatomy is essential for an accurate interpretation of radiographs.

Users of CBCT must be familiar with the relevant position statement for the region, e.g., the ESE or the AAE/AAOMR CBCT position statements [2, 91], and regularly update their core knowledge on CBCT [19]. It is essential that the radiation dose is kept As Low as Reasonably Achievable (ALARA) when exposing patients to ionizing radiation [42]. Therefore, each radiation exposure must be justified in itself, after which the radiographic view, and the patient radiation dose, must be optimized.

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7

Clinical Manifestations and Diagnosis

Asgeir Sigurdsson

7.1 Introduction

Correct pulpal diagnosis is the key to all endodontic treatments. It is paramount to have established a clinical diagnosis of the pulp and the periapical tissue prior cutting access into the pulp chamber or to do any other treatment to a tooth. This clinical diagnosis has to be based on history of symptoms, presenting symptoms, diagnostic tests, and clinical findings. If it is not possible to establish the diagnosis or a differential diagnosis cannot be ruled out, therapy should not be initiated until further evaluation has been done or second opinion gained.

Unfortunately, it has been reported that there is a poor correlation between clinical symptomatology and pulpal histopathology [5, 31, 42, 107]. In the past, attempts were made to accurately diagnose the condition of the pulp based only on clinical signs and symptom, on electric pulp or thermal tests and radiography however most of those failed to show much of any correlation [25, 31, 83]. Many of those attempts used variety of different pulpal diagnostic terms where at least some were elaborate and almost all these classifications had in common of being very descriptive of the assumed histological appearance of the pulp. [5, 105]. Due to obvious reasons the assessment of the histology is impossible with current technology without removing and microscopically evaluating the pulp. In more recent times the trend has been to move away from these elaborate classifications and to use a somewhat modified version that Morse et al. suggested already in 1977 and later adopted with some minor simplification by the American Association of Endodontists [1, 82]. Even though this classification refers to some degree to histological status of the pulp, it helps the clinician to decide upon treatment because there is no crossover between categories in terms of treatment needs. More recently studies have been reported that using these more defined criteria for clinical and histologic classification of pulp conditions revealed a good agreement, especially for cases with no disease or reversible disease [101].

Since symptoms or test results can have somewhat limited predictor of pulpal and periapical status, as much information as possible should be collected about the presentation and history of symptoms and as many tests that are practical should be conducted prior to a formulation of final diagnosis [89]. Thus, clinical signs and symptoms, diagnostic tests in addition to a comprehensive knowledge of the reaction of the pulp to caries, operative manipulations, trauma, and periodontal disease, enable us to establish an empirical pulp diagnosis only.

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7.2 Pulpal Diagnostic Terms

7.2.1 Healthy Pulp

According to the definition, a healthy pulp is vital, asymptomatic, and without any inflammation. This diagnostic term is only used if the pulp needs to be removed and the tooth root-filled for restorative reasons, e.g., need for a post space or when shortening an extruded tooth. Additionally this term is used in case of dental trauma when a crown of a tooth breaks off, exposing the pulp, but then is treated within the first 30 to 48 hours [49].

7.2.2 Reversible Pulpitis

This implies that the pulp is vital, but inflamed to some degree. Symptoms can be very misleading, ranging from none at all to very sharp sensation associated with a thermal stimuli. Through information gathered during testing (see below), it is predicted that the inflammation should heal and return to normal once the irritant, like deep caries, is removed, or when an exposed dentin surface is sealed again. The mild trauma with subsequent inflammation can cause small regions of neurogenic inflammation and sufficient mechanical damage to stimulate nerve sprouting reaction [20, 21] and thereby possibly cause exaggerated response to vitality tests, indicating more severe inflammation than actually there is. As already stated, clinical signs or symptoms or diagnostic test do not always correlate with the histopathologic status of the pulp. There is, however, quite a high risk of diagnosing a pulp with mild symptoms as being reversibly inflamed, when actually it is irreversibly inflamed (see below). It is therefore very important to re-evaluate all patients with a diagnosis of reversible pulpitis few weeks after treatment and evaluate symptoms, including all possible changes in signs and symptoms as well as re-do appropriate vitality tests. A telephone consultation is not enough in these cases, because the pulp may have become necrotic and thereby give the false impression to the patient that the problem has been resolved. The history of symptoms for the diagnosis of reversible pulpitis will mainly reveal provoked pain or sensation, and the tooth will only bother the patient when the tooth is exposed to a hot and/or cold or other excitatory stimuli.

7.2.3 Symptomatic Irreversible Pulpitis

This term implies that the pulp is still vital but so inflamed that it will not be able to heal again even if the irritant or cause of the symptoms are removed. Therefore, the pulp needs to be removed and root canal therapy done. In almost all cases, if this condition is left alone the pulp will eventually become necrotic, and then bacteria will have an easy access to the apex and periapical structures. This is a clinical diagnosis based on subjective and objective findings and the patient will complain, in addition to pain associated with direct stimuli, lingering thermal pain, spontaneous pain, and/or referred pain [34].

7.2.4 Asymptomatic Irreversible Pulpitis

Symptoms can be rather misleading. It has been well documented that in many cases an apparent irreversible pulpitis is asymptomatic. Studies have reported that dental pulps can progress to necrosis without pain in 26 to 60% of all cases [4, 79]. It is of interest to note that neither gender nor tooth type seems to matter such cases of asymptomatic pulpitis [80].

This clinical diagnosis is therefore based on subjective and objective findings, indicating that the vital, but inflamed pulp, is incapable of healing because of long-standing exposure to bacteria and bacterial by-products, as in case of indirect/direct caries exposure or trauma [12].

7.2.5 Necrotic Pulp

When the pulp necroses, the necrotic part of the pulpal space is filled with sterile debris at best but no vital structures [11].

The distinction between partial and total necrosis can be very important in cases of immature teeth, where the presence of intact pulp tissue apically may allow continued root development. The only way to confirm vitality in those cases is to enter the pulp chamber and remove the necrotic debris down to a vital pulp stub because vitality test are not reliable in those cases even in healthy state [40].

7.2.6 Infected Pulp/Infected Pulpal Space

Infection of the pulp may affect only part of the pulp canal, such as in a recent pulpal exposure by trauma or caries, or when just one or two roots in multi-rooted teeth are involved. The space that is left once the pulp has become completely necrotic can be sterile in case of trauma where the blood supply to the tooth was cut off, but where there is no ingrowth of bacteria from the coronal part of the tooth [11]. In most cases, though, the pulp has become necrotic secondary to bacterial invasion through caries or crack/fracture in the crown and therefore the space is infected. It has been shown that all teeth with periapical lesions have infected pulp spaces [11]. Teeth that do not a have detectable periapical lesion may or may not be infected, as it is well known that there has to be a significant loss of bone structure before it becomes radiographically apparent [9, 10].

7.3 Symptomatology of Pulpal Disease

The dental training focuses primarily on diagnosing a problem by visual means, like assessing restorations, clinical signs of oral health or disease, and radiographs. However, when diagnosing origins of pain, most of the diagnosis is done by collecting anamnestic information, i.e., what we hear, and not what we can see [91]. In fact visual clues may throw us off track and lead to an incorrect diagnosis. Therefore, it is important to carefully listen to the patient and to systematically review all his/her present symptoms as well as the history of the symptoms prior to coming to a conclusion about the cause.

7.3.1 Presenting Symptoms

Presenting symptoms are, by definition, symptoms that are recognizable during the consultation. The presenting symptoms, while suggestive, cannot be used alone to make the final diagnosis.

Pain-free

It is a well-established fact that pulpitis can be painless [7, 31, 49, 79]. Lack of pain is therefore not a good indicator of the presence, severity, or reversibility of pulpitis. The reasons, however, for the variability of pain symptoms in pulpitis are not well known. At least in some cases the progression of the inflammation may either be so rapid that there is no pain, or so slow that the classical inflammatory mediators participating in the pain process never reach a critical level [79]. A more likely explanation would be that there is effective modification by local as well as centrally mediated systems. There are several local regulatory factors and systems in the pulp. Endogenous opioid, adrenergic sympathetic, and nitric oxide systems exist in the pulp [20, 55, 92], and there is a good indication that, for example, somatostatin may inhibit pulpal pain activation under certain conditions [23, 113, 114]. And more recently it has been shown in a study of pulps from patients diagnosed with irreversible pulpitis that several genes known to modulate pain and inflammation had different expression in asymptomatic and mild pain patients compared with those with moderate to severe pain; key inflammatory response like IL8, TNFA, and ILIB, which are all potent mediators of pain, were expressed at relatively lower levels in pulp samples from asymptomatic to mild pain patient compared to those who reported higher pain [41].

Sharp Pain

If the pain is short and only associated with a stimulus, like cold air or drink, it is likely to be mediated only by the A-delta neurofibers that are normally active throughout the dentin-pulp complex [7, 85]. Complaints of only provoked pain may therefore possibly indicate only mild (reversible) inflammation. Vital pulp therapy may be sufficient to treat the condition, e.g. by removing a shallow caries lesion, replacing a leaky restoration, or covering up exposed root surfaces.

Severe Pain that Lingers

There is building evidence that the classical inflammatory mediators that cause pain are released in the pulp in direct proportion to the insult. Serotonin (5-TH) can sensitize intradental A fibers, resulting in increased responsiveness [60, 92], and bradykinin has been shown to be in significant higher concentration in irreversibly inflamed human pulps [67]. It is not only the inflammatory mediators that are associated with pulpal pain. Recent studies have also demonstrated that neuropeptides from the nociceptive nerve fibers present in the pulp (calcitonin gene-related peptide (CGRP), neurokinin A (NKA), and substance P) are in significantly higher concentrations in symptomatic compared to asymptomatic pulps [17, 48, 86]. Initially these mediators and peptides will primarily affect the more peripheral A-delta fibers, but when the inflammation reaches deeper structures, the C fibers will be affected as well. This will cause their firing threshold to be lower and make the receptive field larger. Therefore, it is important when the patient is questioned about lingering pain after the stimulus has been removed to not only ask about the time it took the pain to go away, but also about the quality of the lingering sensation. A dull, throbbing, pain component of the lingering pain indicates that more C fibers are involved and that there is an increased likelihood of severe inflammation. This can be used, with caution, to predict if the pulp is likely to be irreversibly inflamed or not.

Pain to Hot, Relived by Cold

It is interesting and generally accepted that there are not nearly as much complaints about heat in normal pulp compared to cold sensitivity. However, when there is severe inflammation in the pulp there seems to be a strong tendency for heat sensitivity, especially in latter stages of the disease. Studies on this are scarce but clinical experience indicate that when a patient complains about severe heat sensitivity there is almost a certainty that the pulp is irreversibly inflamed. In the past it was theorized that this was due to increased intrapulpal pressure when heat was applied to the tooth and the increased pressure caused increased neural activity [14]. The same activity was not seen by similar degree of cooling [53] and therefore it was though that this was related to a pressure increase. It has become clear that this is an incomplete explanation at best. It is more likely that clinical heat sensitivity is due to a reaction of the heat-sensitive pulpal nerves. Heat-sensitive C fibers are not easily stimulated under normal circumstances, but they become more active with more extensive inflammation [20]. When the pulp becomes inflamed the nerves respond due to the influx of inflammatory mediators into the inflamed area as well as due to secretion of neuropeptides. This response will cause the nerves to undergo both local and centrally mediated changes, changes which are likely to explain the alterations in pulpal sensitivity seen in pulpitis. An example: symptoms of throbbing pain associated with pulpitis [20] could be allodynia in pressure-sensitive fibers. The same would hold for a tooth with heat sensitivity alleviated by cold. The firing threshold of heat-sensitive fibers may be lowered so much by the inflammatory mediators that in extreme cases the normal body temperature would activate the pulpal nerves, and cause pain: the only relief to the pain is then to cool the tooth down below ambient temperature [62].

Pain on Biting, When the Pulp has been Confirmed to be Vital

This may be indicative of a severe inflammation involving the pulp and periodontium,

and differential diagnosis to non-pulp-related conditions must be carefully considered. If the pulp is involved, the clinical finding suggests at least some necrosis within the pulp and an irreversible pulpitis of the vital tissue [10].

Referred Pain

As the inflammation progresses in the pulp there is an increased tendency for the pain to be referred to a site remote from the tooth. It has been shown that pain from one tooth can be referred to another adjacent tooth or even to the opposite arch as well as to remote areas like the ear, clavicle and temple. In a classical study on referral patterns from teeth with pulpitis, Glick [44] showed that there were certain tendencies in the referral patterns. Upper teeth tended to refer the pain to the zygomatic or temporal areas. Mandibular molars were more likely to refer the pain back to the ear or even to the occipital area. Pain from a tooth may be referred between the arches but never across the midline of the face. The mechanisms for referred pain is not fully understood but it is clear that both peripheral and central mechanisms are responsible [110]. It is interesting to note that soft tissue structures, like temporal and masseter muscles, can refer pain in similar fashion from the tissue to the teeth [115]. Therefore, it is important to palpate any area of pain that patient reports away from the teeth in the head and neck area. If the palpation of a muscles of the face and/or neck increases the pain response, then there is a strong possibility that the patient is suffering from muscular pain rather than dental pain (see later 7.10).

7.3.2 History of the Presenting Symptoms

A number of factors related to a history of the patient's chief complaint are important to predicting irreversibility of pulpal inflammation. These include the following:

Character of the Pain; Dental Versus Pulpal Pain

In dentinal pain, the sharp rapid pain in response to external stimuli is a reaction of the fast-conducting A-delta fibers. They extend 150 μm into the dentin and are normally active throughout the dentin-pulp complex [47, 74, 76]. The deeper-seated, slower, and unmyelinated C fibers are for the most part unresponsive to all but very intense stimulus in normal, uninflamed pulp [20, 86]. When a long and intense enough stimulus is placed on a healthy pulp, there is first sharp pain, mediated by the A-delta fibers, followed by second, poorly localized, dull pain sensation [58, 59]. Complaints of only provoked pain indicate therefore possibly only mild (reversible) inflammation that a vital pulp therapy would be sufficient to treat, like removing a shallow caries lesion, replacing a leaky restoration, or covering up exposed root surfaces. However, when bacteria or bacterial products start to affect the pulp, inflammatory mediators will affect nerve fibers, resulting in a lower threshold to firing especially the C fibers [48, 67]. The history of the pain can be very revealing if it follows the pattern of starting as primarily temperature sensitivity, with sharp defined pain episodes, and then changing to a more dull, throbbing ache that is increasing in severity. This is important for two reasons; first, it indicates a shift in pain consistent with more activation of the C fibers that would then indicate increased inflammation; second, it has been shown that self-reports of intensity and quality of dental pain are a valid predictor of whether or not the pulpal inflammation is reversible or not [109].

Severe Pain

Self-reports on the intensity and quality of toothache pain seem to be valid predictors of whether the pulpal inflammation is reversible or not [46]. It has also been shown that the more severe the pain is and the longer it has been symptomatic, the more likely it is that irreversible inflammation is present. [7]. However, the reported severity of pain can be misleading due to the subjectivity of the sensation. The fear of dentists, for example, has been shown to result in an exaggerated perception of pain and response to diagnostic stimuli [63].

Spontaneous (Unprovoked) Pulpal Pain

Probably though the clearest sign of an irreversibly inflamed pulp is the history of spontaneous pain, which will hit the patient without any thermal stimulation to the teeth, and may even wake the patient up from sound sleep [108]. Inflammation can cause spontaneous pain sensations in the affected area, and may at times prolong sensitivity to innocuous stimuli, which in the absence of inflammation would not cause any pain sensation (allodynia). This spontaneous activity is thought to be, at least in part, caused by the effects of inflammatory mediators on peripheral nociceptive nerve endings, primarily C fibers. [88]. These effects on the nerve endings will activate and/or sensitize them and cause local as well as central release of substance P and calcitonin gene-related peptide (CGRP) [30, 48, 117] These neuropeptides can then further increase the release of inflammatory mediators, creating a positive feedback loop or a vicious cycle. This vicious cycle is sometimes referred to as neurogenic inflammation.

7.4 Clinical Findings

The findings of the clinical examination, in addition to an extensive knowledge of the pulpal reaction to external irritants, are important for arriving at a correct diagnosis. A clinical examination is critical since pulpitis is frequently painless and also because of the lack of correlation between symptomatology, diagnostic tests and the histopathologic state of the pulp.

7.4.1 Carious Pulpal Exposure

Scientific evidence indicates that when the pulp is exposed directly to caries, bacteria have already penetrated the pulp ahead of the caries lesion and formed micro abscesses [65, 72, 102]. Therefore the pulp should be considered irreversibly inflamed if it has a carious exposure. It has been recommended that in cases where root development is incomplete an attempt should be made to estimate the level at which the pulp is inflamed or infected, that portion removed and apexogenesis attempted. With the advent of calcium silicate materials as capping agents a reasonable successes has been reported, especially in cases that did not have presenting pain indicating irreversible pulpitis [19]. Recently there has been a quite a bit of interest in pulp capping of carious exposures in teeth with fully formed apex using these new materials [68]. Overall the success seems to be good, especially if the patient reported no pain, the pulp did respond normally to sensibility testing and frank caries exposure was avoided [16] (see further Chapter 9).

7.4.2 Age-related Changes

With age, the pulp is reduced in size and volume due to continued dentin formation. Its content of cellular components decreases relative to the number and thickness of collagen fibers [29]. There is also a decrease in the number of blood vessels and nerves, and an increased incidence of calcification and pulp stones [51]. Although it has not been shown experimentally, these changes have been assumed to result in a pulp which is less likely to reverse an inflammatory response compared to a young pulp. On the other hand, the pulp may remain vital for indefinite time periods following extensive degenerative changes.

7.4.3 Periodontal Disease

It has been reported that a moderate to severe periodontal disease will result in a pulp which is "prematurely" aged [13, 66, 103, 107]. Thus, a pulp in a periodontally involved tooth is also thought to be less resistant to inflammation than that in a tooth with a healthy pulp. However, this view is not universally accepted since Mazur and Massler [75] found no difference in the pulp status of teeth with or without periodontal disease.

7.4.4 Previous Pulpal Insults

Previous pulpal insults such as caries, caries removal, and restorative procedures can all result in tubule sclerosis, reparative dentin formation, and fibrosis of the pulp. It has been hypothesized that this "premature" aging of the pulp may render it less likely to heal than an unstressed pulp [84, 108]. If a tooth that has large restoration or crown there is increased risk that the odontoblastic layer has been damaged if care was not taken to cool the bur and preparation done without adequate moisture [52].

7.5 Diagnostic Testing

Unfortunately, many clinicians solely rely on diagnostic tests for their diagnosis. It is important to remember that most commonly used test systems do not actually assess the vitality (blood circulation) of the pulp and they do not give much if any indication about presence or severity of inflammation in the pulp. The main reasons for doing a pulp test are to reproduce and, primarily, to localize the symptoms and to assess the severity of the symptoms. With every test it has to be remembered that the responses are subjective and some patients will have the tendency to exaggerate while others understate the pain felt [32, 64].

7.5.1 Sensibility Tests

Sensibility tests include the electric pulp test (EPT) and thermal tests. The primary function of these tests it to differentiate a vital from a non-vital pulp [56, 116]. By the use of these tests the nerve fibers in the pulp are activated, eliciting a reaction from the patient. The patient response is subjective, so that care must be taken to differentiate a "fearful" positive response from a true one [32, 64]. This is usually accomplished by comparing the patient's response with that of a contralateral or neighboring tooth and revisiting the tested teeth to ensure consistency. Care must also be taken to clean and dry the teeth so that conduction of the stimulus to the periodontal ligament or to adjacent teeth is minimized.

It is important to ask the patients prior to applying these sensibility test about recent intake of pain medications. This is because it has been shown that taking 800 mg of ibuprofen one hour before doing cold, percussion, palpation, and bite force tests significantly affected the diagnostic testing results in patients that had been diagnosed with endodontic pain [99].

As stated above, the main disadvantage of these tests, apart from their subjectivity, is the fact that an assumption has to be made that the presence of nerve fibers in the pulp correlates to a vital blood supply. While this assumption is mostly valid, in a number of cases the blood supply in the pulp will be lost before the degeneration of nerves in the pulp, resulting in an incorrect diagnosis of pulpal vitality [97]. Conversely it is known that, especially after luxation injury, vital pulps sometimes do not respond to sensitivity tests shortly after the trauma, but at a follow-up appointment a few weeks to few months later, normal responses are observed [78, 111].

7.5.1.1 Electric Pulp Test

The electric pulp testers activate the nerve bundle in the pulp, probably mainly the A-delta fibers. The unmyelinated C fibers of the pulp may [83] or may not respond [87]. The main problem with this type of test is the many variables that needs to be taken into account, some of which cannot be controlled. Key issues are location of the probe on the tooth (as far as possible from the gingival); conductivity between the instrument and the tooth; rate of stimulus intensity increase; isolation of the tooth tested from adjacent teeth and prevention of shunting to the gingiva by drying the crown. It is not possible to control for the thickness and electric resistance of the enamel and dentin, the presence of restorations and caries, and the functionality of the nerve complex in the pulp.

Procedure

The tooth and surrounding teeth are dried. The pulp test probe is placed on the incisal edge or cusp tip corresponding to the pulp horn of the tooth [8, 39] (Figure 7.1). Contact between the probe and tooth is facilitated by the use of conducting medium such as toothpaste or fluoride gel. The circuit must be completed with a lip contact or the patient holding the handle of the electric pulp tester. The amount of current is increased slowly with the patient instructed to indicate (e.g. by raising the hand) if a tingling or other sensation is felt.

Diagnostic Information

A response, within reasonable intensity, to a stimulus is an indication of a vital pulp tissue. The response level however does not indicate the health of the pulp or reversibility of inflammation that might be present in the pulp, because no correlation has been shown between the electric pain threshold in a pulp and histological condition [69, 83]. No response is, however, a strong indicator of a necrotic pulp in most teeth [97, 108]. Seltzer et al. found complete necrosis in 72% and localized necrosis of the pulp in 25.7% of those teeth that did not respond to EPT. Thus, if it is accepted that even localized necrosis is an indication for the need of pulpectomy in a mature tooth, 97.7% of cases with no response to EPT will require a pulpectomy or the debridement of a necrotic pulp. And a more recent study confirmed these findings from 1963 as it showed that EPT results had overall accuracy of 75%. However, the negative predictive value was 90%, indicating that if there is no response and there is a good contact with the tooth, the likelihood of the pulp being necrotic is high. The reverse was not true, because positive predictive value was only 58% [56].

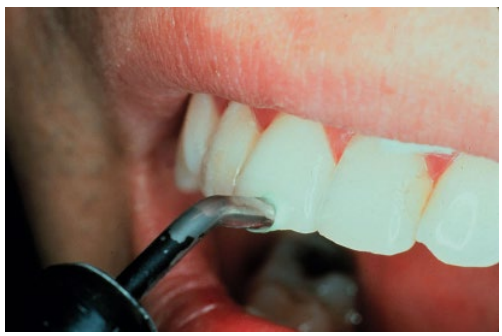

Figure 7.1 The electric pulp tester (EPT) probe is placed on the incisal edge of a maxillary anterior tooth. Theo tooth is thoroughly dried and contact between the probe and surface of the crown is facilitated by conduction medium like toothpaste or fluoride gel.

It is also important to remember that responses to EPT are unreliable shortly after dental trauma [111] as well as in teeth with incomplete root formation [39, 40]. This is likely due to the fact that the nerve plexus of Raschkow does not fully develop until very late stages of root formation. Thus the pulpal nerves do not reach the odontoblasts, predentin, or dentin, as in fully developed teeth that have reached normal occlusion. In young teeth the cold test, especially with CO_2 ice, is more reliable than EPT [39].

7.5.1.2 Cold Test

Probably the most commonly used test in dental offices is some form of a cold test [98]. It is important to realize that this testing method also, like the EPT and heat, only gives indication of functional nerve fibers, rather than information about the vitality status (blood flow) of the pulp.

The initial response to any cold stimulus is generated by cold-sensitive A-delta fibers [59] activated by the hydrodynamic forces. The temperature change causes a rapid movement of fluids in the dental tubules, a movement which then activates the intratubular nerves [17, 18]. Moreover, in animal models, the cold-sensitive A-delta fibers respond uniformly to rapid lowering of the tooth temperature [59], while the initial high-frequency discharge rate falls off as the rate of temperature change decreases and stops completely when the temperature reaches a steady level. This is a good indication that the sharp initial sensation when a cold stimulus is applied to the tooth is

caused by the A delta fibers and subsides when those fibers stop firing once the movement of the intratubular fluid stops. The C fibers also show a quite uniform but very different reaction. The C fibers start to discharge after a short latency and then the discharge rate is low [59]. In an experimental study in human [77], the subjects reported sensations that could be interpreted as being comparable to these phasic type of activation of the A and C fibers in the animal study. There was a distinct, sharp, and short-latency (1.6 sec) pain felt when the tooth was rapidly cooled. The latter pain was described as dull, burning pain, which was difficult to localize. These findings support the assumption that the response behavior of the human pulp nerves are comparable to those of the animal model: the first sharp pain is evoked by intradental A delta fibers and then later, once the interdental temperature is raised sufficiently, the C fibers are activated and are responsible for the dull, throbbing, and aching sensation. Subsequently other research groups have come to similar or same conclusions, though with somewhat different research approaches. It is important to note, however, that the studies providing the data for these mechanisms were done on relatively normal, healthy pulps [2].

Very few studies have been done to investigate pain sensation in inflamed human pulps. There appears to be only a poor correlation between estimates of pain magnitude and the total A delta nerve activity in patients that were clinically diagnosed with pulpitis [3]. However, abnormal but positive responses are equally distributed among the pulps of teeth with varying degree of inflammation. Therefore a positive cold test response only indicates vitality but not if the pulp is reversible or irreversible inflamed; however, no response is very indicative of a necrotic pulp.

Historically, ice and ethyl chloride spray have been used for cold testing but both of those have several problems that have led to new approaches. Both are not very cold; ethyl chloride is only about –4° C and ice is at or just above 0° C. An additional problem with water is that once it melts very cold water can drip on adjacent teeth and thereby give false positive responses. Carbon dioxide (CO_2) ice pencils have now been available for over twenty years. They are safe to use on vital pulps and will not cause damage to enamel or pulpal tissue in vivo. [54, 95, 96]. The CO_2 ice may also be more dependable than ethyl chloride and water ice in producing a positive response, and in young patients with incomplete root formation CO_2 ice seems to be more reliable than EPT [40]. Additionally the CO_2 ice appears more reliable than EPT in patients undergoing orthodontic movement of teeth [24].

More recently refrigerant spray, 1,1,1,2 tetrafluoroethane, has been introduced. It has the advantage that it is supplied in spray cans; it can be stored at chairside; and it has less start-up cost compared to the CO_2 ice. It is, however, not as cold as CO_2 ice (–26.2° C, –15.4°F). It appears that there is no difference between tetrafluoroethane and CO_2 ice in producing a pulp response regardless of tooth type or presence of restoration [57].

If several vitality tests are planned on a patient, it seems that the sequence and interval between electric pulp testing and cold vitality testing with refrigerant spray does not affect the reliability of pulpal diagnostic testing [93].

Procedure

As with the EPT, care needs to be taken to dry the tooth and surrounding teeth. The cold object should be placed on the incisal edge or close to a pulp horn for optimum results. The cold test should be administered gently, as a placement of the very cold ice or object could cause rapid and severe pain, especially in the anterior teeth (Figure 7.2).

Diagnostic Information

Abnormal but positive responses are equally distributed among the pulps of teeth in all diagnostic categories [31, 108]. Therefore a positive response is an indication that the pulp is at least partially vital, but it does not indicate if inflammation is reversible if

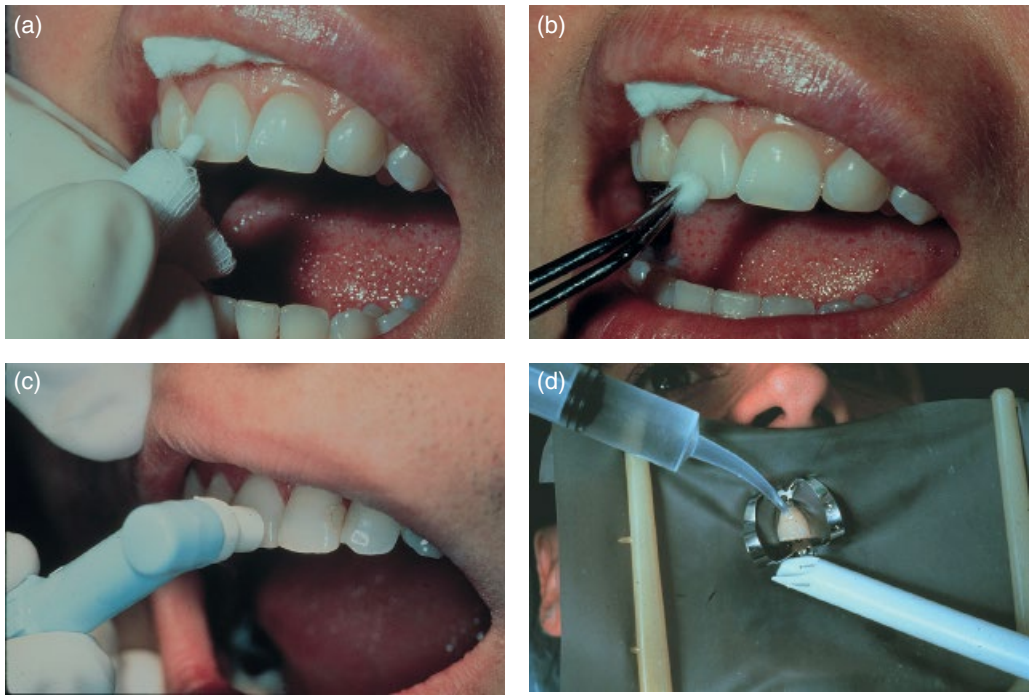

Figure 7.2 Thermal sensitivity test with (a) CO_2 frozen stick (–70°C), (b) cotton pellet sprayed with 1,1,1,2 tetrafluoroethane, (–26°C), (c) prophy rubber cup without any lubrication used to heat the tooth surface, (d) single-tooth rubber dam isolation and irrigation with hot water. Courtesy of Dr. Sigurdsson.

present. Despite the lack of an apparent clear correlation between cold response and histological appearance of the pulp, clinical experience indicates that an exaggerated response, which quickly turns into a dull aching pain, coupled with other signs, like history of pain and spontaneous pain strongly indicates a pulpal inflammation that is most likely irreversible.

7.5.1.3 Heat Test

A less common, but probably a more clinically informative, temperature test is some form of heat test. The main problem with this approach is that many commonly used tests can cause excessive heat that may damage the pulp or adjacent tissue, and others are cumbersome to apply. Therefore, the clinical use of heat tests is somewhat limited.

The normal pulp reaction and sensation to heat is similar to cold. It is in most cases biphasic where initially the A delta fibers are activated and then later, if the stimulus is maintained, a dull, radiating pain follows [18, 86]. It has been shown in vivo human studies that hot gutta-percha induced a more complex neural response pattern than ethyl chloride. The hot gutta-percha evoked a response in three phases, where the third phase was a slow spontaneous emerging activity in the absence of physical stimulus, and this activity was not felt by the test subjects, indicating more C-fiber activity [3].

Procedure

Several heat tests have been advocated. One is to heat gutta-percha over flame and then place it on a lubricated surface of the crown towards the incisal edge [83]. The problem with this approach is that there is little control over the heat and the gutta-percha tends to stick on the tooth and thereby cause continued heating of the tooth after the bulk materials is removed. Using a prophy cup or

a rubber wheel without lubrication has also been advocated, but again there is no control over the heat generated. A better, but cumbersome, approach is to put the patient in supine position, isolate a single tooth with rubber dam and then bathe the exposed tooth in hot water. Once a response has been noted, the rubber dam is moved mesially by one tooth and the procedure repeated. One starts distal to the suspected tooth so that if there is a leakage thorough the dam, the hot water will not drip on the suspected tooth and thereby give a false positive response.

Diagnostic Information

No correlation has been found between an abnormal response to heat and histologic diagnosis [31, 108]. It is, however, generally accepted that when there is severe inflammation in the pulp there seems to be a tendency for heat sensitivity. Studies on this are scarce but clinical experience indicates that when a patient complains about severe heat sensitivity, the pulp is usually irreversibly inflamed. This is confirmed to some degree in animal studies, where it has been shown that the nerves stimulated by heat are located in similar areas as markers for pain responses in symptomatic pulpitis. [26, 27].

7.5.2 Mechanical Tests

Percussion and palpation test are not technically vitality tests but rather give indication about periodontal and/or periapical inflammation. It has been stated that pain is more likely to be elicited on percussion when there is a partial or total necrosis present in the pulp [106] and as such an indirect assessment of the status of the pulp. Other causes for percussion sensitivity, like recent traumatic occlusion, high filling etc, obviously need to be ruled out. The same seems to be the case when the periapical area is sensitive to palpation but the pulp is still vital. Presence of percussion and/or palpation sensitivity in conjunction with a vital pulp with hypersensitivity to thermal stimulation is indicative of a pulp that is severely and thus most likely irreversibly inflamed [106]. However, if the tooth is not sensitive to percussion and/or palpation, inflammation is not necessarily absent [10].

Percussion

This test is properly performed with the handle of a mouth mirror. The aim is to determine the presence/absence of inflammation in the apical periodontium.

Procedure

The mouth mirror handle is used to percuss not only the occlusal, but also facial and lingual surfaces of the teeth. The teeth should be percussed in a random order so that the patient does not respond to "anticipates" rather than real pain.

Diagnostic Information

As stated before, a positive percussion test indicates inflammation of the periradicular tissues. Care must be taken, when interpreting the results of the percussion tests, to rule out a positive response due to periodontal diseases or cuspal fracture. This is particularly difficult in cases where the pulp vitality tests indicate a vital pulp. The results of other diagnostic tests and presenting symptoms need to be used to differentiate periodontitis of marginal or endodontic origin.

Palpation

This test is used to detect inflammation in the mucoperiosteum around the root of the tooth. It may be possible to detect tenderness, fluctuation, hardness, or crepitus before extensive swelling is present.

Procedure

The index finger is pressed against the bone through the mucosa. When pressure is felt, the finger is rolled causing sensitivity if inflammation is present. As with percussion, the test should be performed in a random fashion and the results obtained should be compared to a contralateral tooth or neighboring teeth.

Diagnostic Information

Similar to the percussion test, a positive response when palpating over the root tip is a reliable indicator of periapical inflammation. However, if a positive response is not elicited, inflammation is not necessarily absent [10].

7.5.3 Radiographic Examination

The radiographic examination is one of many tests, and the findings should always be evaluated together with those of presenting symptoms, clinical examination as well as with those of the other tests. All radiographs should be taken using holders which allow parallelism and standardization. If comparative radiographs will be required on follow up, it is useful to fabricate a rubber bite-block so that the angulation of the follow-up radiographs will be as similar as possible (Figure 7.3).

Diagnostic Information

The radiograph cannot detect pulpal inflammation directly. However, caries or defective restorations seen on the radiograph will indicated pulp inflammation [73]. Condensing apical periodontitis is a near pathognomonic sign of pulpitis (Figure 7.4). Signs of obliteration and calcification (diffuse or as pulp stones) may be considered but are not directly correlated with inflammatory reactions in the pulp. Also, the presence of an apical radiolucency of endodontic origin may be a good indication that necrosis or a necrotic zone is present in the pulp space.

7.5.4 Experimental Testing Methods

Sensitivity test of pulp vitality require functional nerves to respond to a stimulus. Pulp with effective circulation and vital cells, but with severed or compromised nerves, may be misdiagnosed as being necrotic or non-vital by these tests. Therefore, attempts have been made to demonstrate pulpal vitality based on blood circulation. Several experimental methods have been proposed, like crown

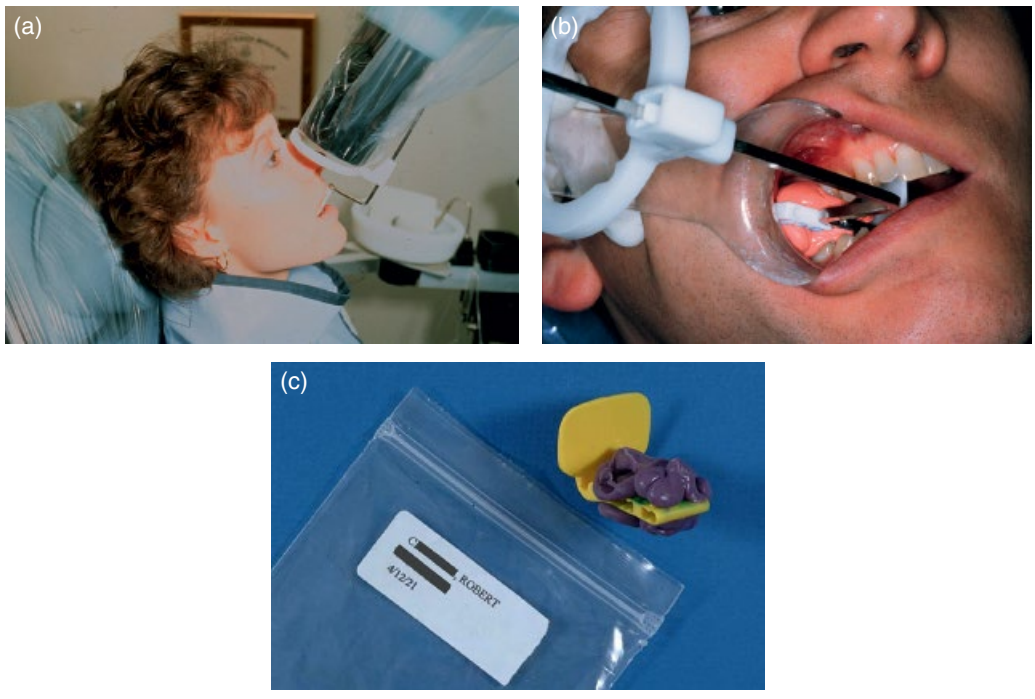

Figure 7.3 (a) All radiographs should be taken using holders which allow parallelism and standardization. (b) If comparative radiographs will be required on follow up, it is useful to fabricate a customized rubber bite-block using putty impression material. (c) The bite registration is then stored along with the film holder. Courtesy of Dr. Sigurdsson.

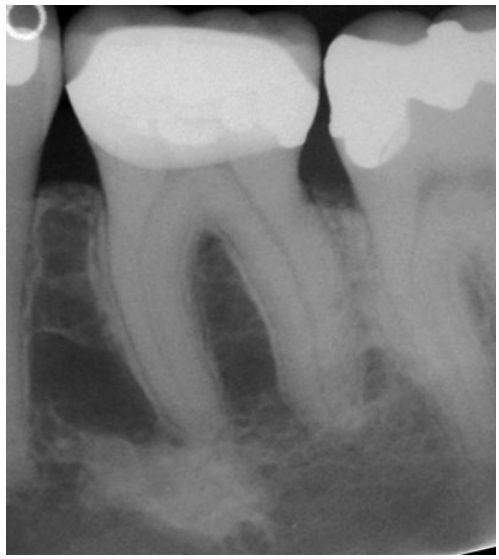

Figure 7.4 A radiograph showing condensing apical periodontitis associated with the mesial root of a lower molar. The tooth had been restored some years earlier, with what appears to be direct pulp capping. The patient reported hypersensitivity to cold for some weeks prior to seeking help.

surface temperature measurements [36, 37], Xenon-133 radioisotope injection [61], pulse oximetry [104], dual wavelength spectrophotometry [90], and laser Doppler flowmetry [43]. Of those alternative methods the laser Doppler flowmetry and pulse oximetry seems to be most likely to be accurate in determining the vitality of the pulp [71]. However, and very unfortunately, they have a limited ability to assess the level of inflammation that might be present in the pulp, and most of these approaches, despite experimental demonstration of very accurate and reliable findings, have never been made available commercially for pulp vitality assessment.

7.6 Formulation of a Pulpal Diagnosis

The diagnosis is made using the information obtained above (see Table 7.1).

Table 7.1 The formulation of a pulpal diagnosis.

Symptom, test, supporting information	Necrotic pulp	Vital pulp	
		Irreversibly inflamed	Reversibly inflamed
Pulp test	Negative	Positive	Positive
Key factors			
Pulpal exposure		Present	Absent
Pain to percussion		Present	Absent
Related factors			
Severe pain		Present	Absent
Spontaneous pain		Present	Absent
Past history of pain		Present	Absent
Pain that lingers		Present	Absent
Pain to hot, relieved by cold		Present	Absent
Factors related to treatment plan			
Age, periodontal disease, previous pulpal insults		Questionable (complex treatment plan)	Questionable (simple treatment plan)

7.6.1 Key Factors

Necrotic Versus Vital

Using the patient's presenting symptomatology and the results of as many diagnostic tests as possible, it should be possible to accurately determine if the pulp is necrotic or vital. If the pulp is necrotic, the choice of treatment is root canal therapy if the tooth is to be maintained for an extended period.

It is a much greater challenge to differentiate a reversible from an irreversible pulpitis. For this determination, the presenting symptomatology and its history (subjective) and clinical findings are used.

Pulp Exposure

As already discussed, if on excavation the pulp is found to be exposed to bacteria, an irreversible pulpitis can confidently be diagnosed. Treatment is then extirpation of the whole pulp and root canal filling as a preventive measure. An exception would be a case of a very young tooth with incomplete root formation. In those cases, a temporary (few months to a year) partial pulpotomy would be indicated in an attempt to allow the apex to fully form prior to initiation of complete root canal therapy.

7.6.2 Related Factors

A history of severe pain, spontaneous pain, a past history of pain in the same tooth, or referred pain, are all indications of an irreversible pulpitis.

All other related factors, such as age, periodontal status, and previous pulpal and treatment history, must be considered but are less suggestive.

7.6.3 Treatment Planning

It has been continually stressed that with methods of diagnosis available today, the diagnosis of an irreversible pulpitis is at best an "educated guess" and mistakes are inevitable. Because of this fact, the overall treatment plan for the patient should play a role in the choice of pulpectomy or conservative therapy.

For example, if a tooth is the only tooth in the arch needing treatment and the long-term restoration is to be a simple amalgam or resin (Figure 7.5), a conservative approach can be attempted without pulpectomy, even though some of the related factors suggest a moderate inflammation is present. With the same presenting signs and

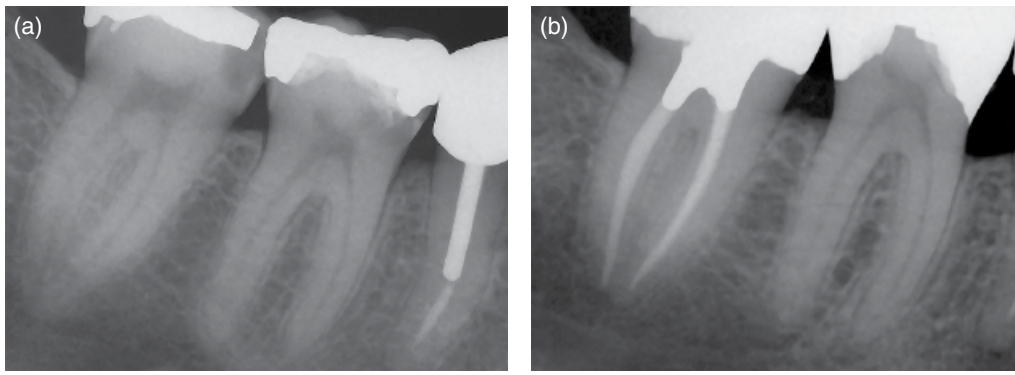

Figure 7.5 Radiograph (a) showing two lower molars both with extensive and deep carious lesions. Patient complained about sever pain of few days duration but was unable to determine which tooth was causing the pain. Pulpal test were inconclusive on which tooth had more severe pulpal inflammation. After caries excavation a caries perforation was found in the second molar but not the first. The second molar was treated with root canal therapy. The first with vital pulp therapy and restoration. (b) At one year re-evaluation the first molar responded normally to pulpal tests and both teeth were normal to percussion and palpation.

symptoms, a tooth planned as an abutment for a bridge which would be difficult to treat endodontically subsequent to possible failure of conservative treatment, might preferentially be subjected to immediate pulpectomy without an attempt at conservative treatment.

7.7 Periapical Diagnosis

The term "apical periodontitis" implies that there is an inflammation in the periodontal ligament caused by infection of the pulp or of the necrotic pulp space. The noxious material and bacterial byproducts have passed to the periodontium through the apical foramina. If communication between the pulp space and surrounding periodontium is present through furcation or accessory canals, periodontal inflammation can result in these locations as well. Histologically the lesion is predominated by chronic inflammatory cells with the overall appearance of a granuloma or cyst [15].

Like pulpal inflammation, the periapical inflammation can be symptom-free and then only diagnosed in a chronic phase on a periapical radiograph. However, if there is a periapical lesion detectable on a radiograph it is almost certainly caused by an infection in the root canal system, irrespective of the tooth's history or the occurrence of symptoms [11]. As always, if the patient is symptomatic it is important to be able to diagnose the source prior to treatment. Treatment is always to remove the irritant that causes the symptoms or lesion. This could be accomplished by simple occlusal adjustment in cases of occlusal trauma, but as the cause is usually bacteria in the root canal system, the only predictable treatment is to completely disinfect the canal space followed by obturation and a good coronal seal. Antibiotic therapy alone is not effective [38].

7.7.1 Normal Apical Tissues

7.7.1.1 Symptomatic Apical Periodontitis (SAP)

This term usually implies that the apical inflammation started in the acute phase causing clinical symptoms like biting and percussion sensitivity and/or palpation sensitivity. Often there are minimal or no radiographic changes associated with SAP. However, this diagnostic term might be associated with an apical radiolucent area. There can be several causes for this inflammation. Most benign would be occlusal trauma. If that is the case, the pulp should be vital and unaffected. However, in case of bacterial infection, the pulp is either severely or irreversibly inflamed and usually partly or totally necrotic. An acute apical periodontitis can also be superimposed on a previously chronic lesion (see 7.7.1.3).

7.7.1.2 Asymptomatic Apical Periodontitis (AAP)

This term implies that the apical periodontitis is of some duration without symptoms. The condition may be suspected when the pulp is necrotic with radiographic signs of apical periodontitis (radiolucency or rarely a radiopacity).

7.7.1.3 Acute Apical Abscess (AAA, Phoenix Abscess)

Here the periapical inflammation has caused a purulent breakdown of periapical tissues with accumulation of pus in the periodontium, subperiosteally, submucosally, and/or subcutaneously, often characterized by rapid onset of pain, and tenderness to pressure and swelling. Commonly, this acute inflammation is superimposed on a previous chronic apical periodontitis and has been termed a Phoenix abscess.

7.7.1.4 Chronic Apical Abscess

This term is used when there is a slow and gradual inflammatory reaction due to pulpal necrosis and ensuing infection. Most often there is little or no discomfort and there is a

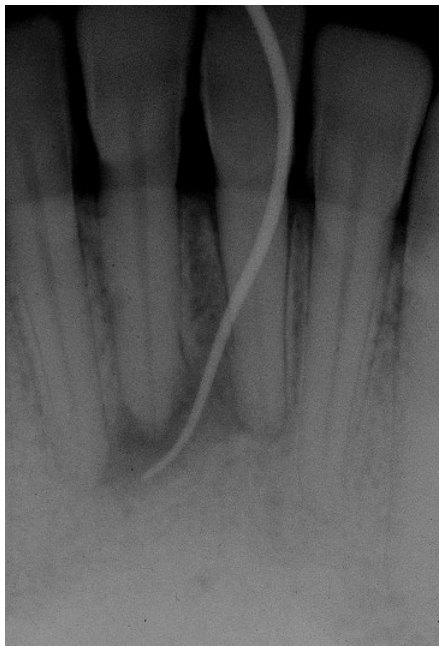

Figure 7.6 A radiograph of a sinus tract that has been traced with size 40 gutta-percha. Note that the exit of the tract was distal to the left central incisor but traced to the right central incisor.

traceable sinus tract associated, where the periapical exudate discharges onto a body surface (intra- or extra-orally), establishing a traceable sinus tract with periapical drainage (Figures 7.6 and 7.7).

7.7.1.5 Condensing Osteitis

These are usually asymptomatic findings that are associated with a diffuse radiopaque area usually close the apex of the tooth (Figure 7.4). It is thought that this is associated with a low-grade inflammatory stimulus on the pulp and from evaluating human cadavers exhibited areas of inflammation or no inflammation, occupied by connective tissue bordered with a rim of varying widths of dense lamellar-type bone replacing the cancellous bone and marrow [45].

7.8 Symptomatology of Periapical Disease

The same diagnostic steps should take place for the diagnosis of periodontal pathosis as described above for the pulp. The patient's

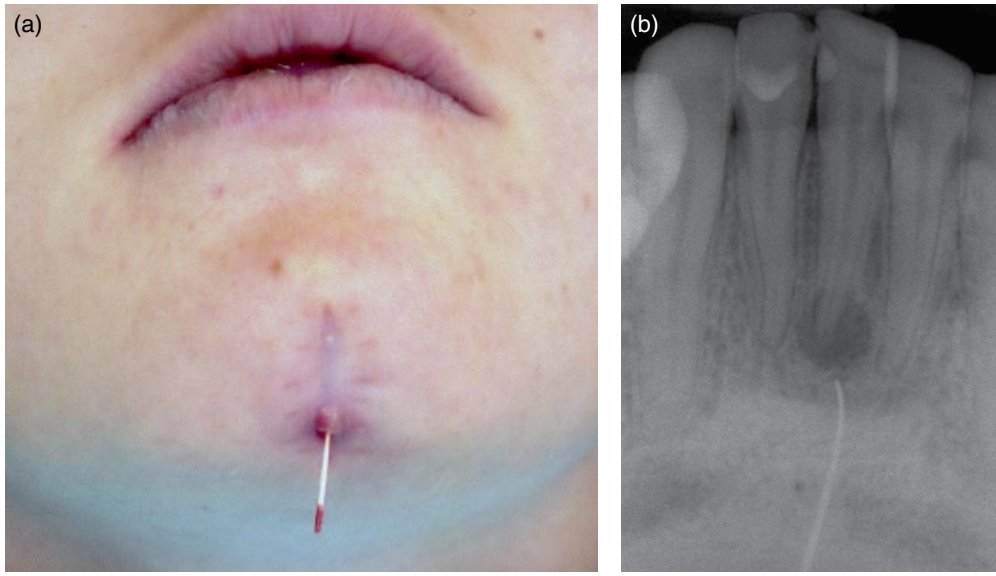

Figure 7.7 (a) The patient with that has epilepsy (grand mal) had been treated by multiple physicians for a "chin cyst", which led to the scar tissue on the chin as well as a localized paresthesia. Eventually she was seen by an endodontist that traced the sinus tract with gutta-percha point. (b) The point traced to lower left central incisor. The pulp did not respond to sensibly tests and there was a periapical lesion evident on the radiograph. After first appointment where the canal was debrided and $Ca(OH)_2$ the sinus tract closed and was still closed at two-year re-evaluation. (Courtesy of Dr Buttke.)

Table 7.2 Differential diagnosis of an endodontic sinus tract versus a periodontal pocket.

Test applied	Periodontal pocket	Endodontic sinus tract
Vitality testing	Within normal limits	No response
Perio probing	Wide pockets	Narrow tract
Clinical status of the tooth	Minimal caries/restorations	Evidence of caries/restorations
General periodontal condition	Poor	Within normal limits
Dark field spirochete count	>30%	<10%

presenting symptoms are carefully evaluated, diagnostic tests are performed, clinical findings recorded, and the information is compiled to make the tentative diagnosis (see Table 7.2).

7.8.1 Symptomatic Apical Periodontitis (SAP)

Traumatic Occlusion

Presenting symptoms: Patients complain of pain on biting, eating or "when the teeth come into contact".

History: Commonly the patient has recently had a dental procedure performed which has resulted in a restoration where the occlusion is not balanced, leaving high contact.

Clinical findings: There is often evidence of a new restoration(s) in the area.

Diagnostic tests: Response to thermal and electrical sensitivity testing are normal. Pain is elicited to percussion, and on rare occasions to palpation as well. Radiographic findings are usually non-specific.

Treatment: The occlusion should be adjusted so that premature contacts are removed, including all interference in acentric contacts. The patient should return for a follow-up visit to ensure that the acute apical inflammation has subsided and the pulp has remained vital.

Symptomatic Apical Periodontitis with Acute Pulpitis

This condition has already been discussed in pulpal diagnosis. Acute apical periodontitis in conjunction with an acute pulpitis indicates an irreversible pulpitis.

Treatment: Pulpectomy/endodontic therapy (see Chapter 10). A permanent restoration should be placed as soon as possible after obturation to prevent coronal leakage with subsequent chronic apical periodontitis [70].

Acute Exacerbation of Asymptomatic Apical Periodontitis

Presenting symptoms: Patient complains of pain on biting, eating, or "when the teeth come in to contact". There may also be episodes of spontaneous and intense pain, as well as swelling and malaise.

History: The history in these cases is varied. In some cases, the patient reports episodes of previous pain or there is a recent restoration placed in the tooth. In other cases, the patient may tell a previous history of pulpal pain which later subsided. Sometimes root canal therapy has previously been performed on the tooth.

Clinical findings: These are consistent with a tooth with a necrotic pulp or a previous root filling. Examples would be a deep carious lesion, a previous pulp capping, a large restoration, or full-coverage crown.

Diagnostic tests: There is no response to thermal and electrical sensitivity tests. There will be pain to percussion and/or palpation. An apical radiolucency is present on radiographic examination, indicating the presence of chronic apical periodontitis in addition to the acute exacerbation.

Treatment: The treatment involves complete root canal instrumentation and disinfection with antibacterial irrigation and intracanal medication of the root canal system [22] (see Chapter 10). Since abscess formation has not yet occurred, drainage is not possible. For the same reason antibiotic therapy is not required. Pain medication is prescribed as needed. Re-evaluation should

be after 1–4 weeks for confirmation that the apical inflammation has subsided, after which the canal is obturated. A permanent restoration is placed as soon as possible for prevention of coronal leakage.

7.8.2 Asymptomatic Apical Periodontitis

Presenting symptoms: By definition the patient is asymptomatic or at least reports very minimal discomfort associated with the tooth. The condition may be diagnosed on a routine recall radiograph, or a restoration may have been required on a tooth which when tested for vitality shows no response.

History and clinical findings: These are as in acute exacerbation of AAP, and pain may have been completely absent.

Diagnostic tests: There is no response to thermal and EPT tests. Percussion and/or palpation tests are usually negative, although slight sensitivity may be present.

Radiographic findings: Since these cases are largely asymptomatic, the diagnosis of chronic apical periodontitis is made primarily on radiographic evidence of the presence of radiolucency (or rarely on opacity) (see Chapter 6) (Figure 7.8). It is possible for an apical periodontitis to go undetected radiographically [9, 10]), so that pulp necrosis and infection is evident only after initiation of endodontic therapy. A revised diagnosis of probable AAP should then be made and the tooth treated accordingly (Chapter 11).

Treatment: Effective disinfection of the root canal system will result in reversal of the chronic apical periodontitis.

7.8.3 Acute Periodontitis with Abscess

During acute phases of apical periodontitis (primary acute or an exacerbation of chronic lesion), the infection may develop to produce an accumulation of pus and formation of an abscess. From this point, the inflammation may become chronic, but in some cases continues with formation of a clinical abscess. There is an increase in tissue pressure, bone resorption is initiated by inflammatory mediators, and the pus escapes first through the bone and underneath the periosteum and then, if the periosteum is penetrated, into the tissue spaces.

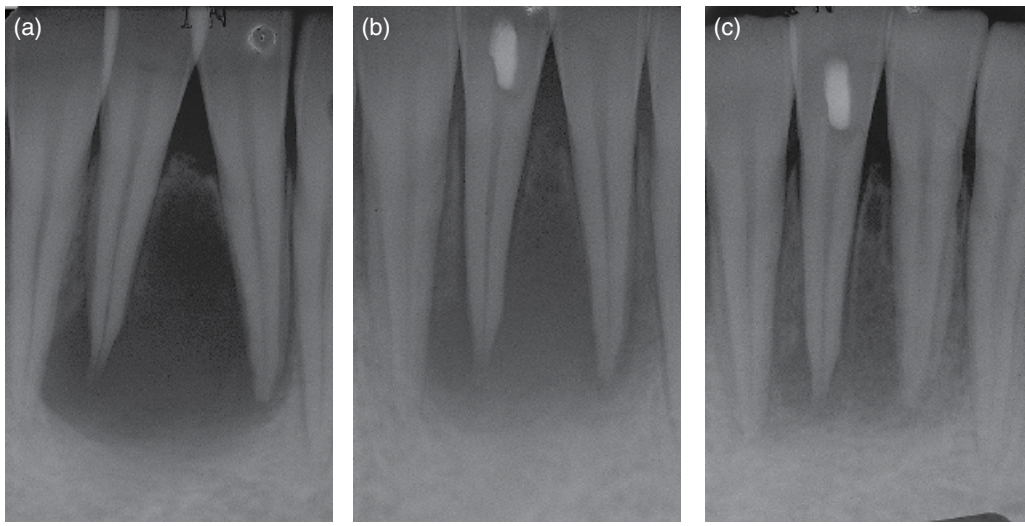

Figure 7.8 13-year-old patient asymptomatic apart from noticing some firm enlargement facial to his lower anterior teeth. Radiographic evaluation revealed large periapical radiolucency with the roots of the central incisors pushed distally (a). The right central incisor did not respond to cold, but the left one did. Diagnosis of necrotic pulp with chronic apical periodontitis (CAP) was made. Six weeks after placing calcium hydroxide in the root canal system there was significant improvement of the periapical lesion (b). After three months of calcium hydroxide treatment there was almost complete resolution of the lesion (c).

The exact location of the accumulation of fluids is dependent on the anatomical location of the root apex relative to the facial muscles [81]. Most commonly the drainage is buccal into the oral vestibule. A rare complication is spread from maxillary and mandibular third molars via the pterygoid plexus, causing a cavernous sinus thrombophlebitis and impairment of cerebral vascular drainage [50] (Figure 7.9). In the mandibular teeth the most serious spread would be from the premolar or molar teeth lingually under the mylohyoid muscle and into the retropharyngeal space. Ludwig's angina is a bilateral retropharyngeal spread which may become serious through a blockage of the airway [112]. In most cases the pus will break through the intraoral mucosa to a surface. Occasionally the abscess will drain extraorally on or under the chin or below the mandible.

Presenting symptoms: Symptoms will vary according to the stage of progression of the inflammatory process. Initially, the symptoms may be minor with pain on biting, eating or occlusal contact. As pus builds under the periosteum, the pain increases in intensity and can in time be excruciating. When the periosteum is penetrated by the pus, diffuse swelling may develop (Figure 7.9). The penetration of the periosteum by the pus is usually accompanied by a release of pressure and a considerable reduction in the pain.

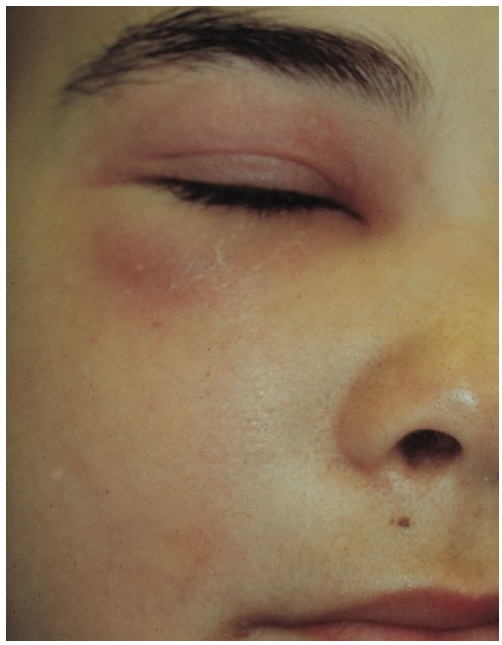

Figure 7.9 Severe swelling due to an abscessed maxillary first premolar tooth. Pus has broken through the periosteum and caused severe and very serious swelling around the orbit.

History and clinical findings: These are the same as in SAP.

Diagnostic tests: There is no response to thermal or electrical sensitivity tests. Pain on percussion and/or palpation is elicited which can sometimes be severe, depending on the stage of the process.

Radiographic findings: The stage of development of the abscess determines the radiographic picture. At the early state of abscess formation radiographic signs of inflammation may be minimal if any. As the abscess spreads and more bone is destroyed, the radiographic signs of apical periodontitis will be more obvious. In the case of an acute exacerbation of a chronic lesion, the signs of apical periodontitis are present throughout.

Treatment: Treatment principles are the same as for all teeth with apical periodontitis, i.e. the disinfection of the root canal (see Chapter 9). Additionally, in the case of an acute abscess, concerns of spread of the infection in the tissue spaces (cellulitis) and pain control must be addressed in the treatment protocol. Emergency treatment is primarily aimed at eliminating the source of the abscess, the infection of the pulpal space, and antibiotics are only used as supplemental treatment if the patient is showing systemic effects of the infection. In severe cases when a spread is suspected in to the retropharyngeal space or orbit an emergnecy consultation with oral surgeon or emergency room is recommended.

7.8.4 Chronic Apical Abscess

This occurs when the apical inflammatory exudate drains to a body surface, intra- or extra-orally. The formation of a sinus tract may be the mechanism with which the

body controls the infection, or it may indicate a specific infection of some – as yet unknown – bacterial combination. It may develop as a result of an abscess as described above or be without preceding symptoms, and the diagnosis may be made without the patient even being aware of its existence. When long-standing, the tract can become completely epithelialized [6]. With adequate disinfection of the root canal and resolution of the periapical inflammation, the epithelium, if present, will in most cases disintegrate. Commonly, the sinus tract will drain on the mucosa adjacent to the offending tooth. However, the opening may be at some distance from the involved tooth (Figure 7.6), drain through the periodontal ligament mimicking a periodontal pocket or even extraorally, where it might be misdiagnosed (Figure 7.7). For these reasons, it is important to perform a thorough diagnostic examination and not rely on the presence and location of the sinus tract opening.

Presenting symptoms: Generally, when a sinus tract is present in conjunction with the apical periodontitis, pain is absent or very mild and swelling is minimal. This is due to the lack of pressure build-up under the periosteum or adjacent tissues.

History and clinical findings: A history consistent with that described for AAP or SAP

Diagnostic tests: The pulp is nonresponsive to thermal or EPT tests. There is no or only light pain to percussion and/or palpation.

Radiographic findings: Radiographic evidence of chronic apical periodontitis is usually present, but the location of the sinus tract on the mucosa does not always correspond to the offending tooth. Therefore, it is important to trace the sinus tract with an opaque object, for instance a size 35 or 40 gutta-percha cone, to determine the origin of the tract. Care must be taken to not force the cone and thereby create a false tract, which could lead to an incorrect diagnosis.

Treatment: Disinfection of the root canal system should result in reversal of the chronic apical periodontitis and the sinus tract will disappear within days to few weeks. In rare cases the sinus tract will not heal because the infection is primarily located extraradicular and antibiotic therapy and/or surgical treatment my become necessary.

7.8.5 Condensing Osteitis

As stated before, this is a diffuse radiopaque area usually close to the apical area of a tooth [118].

Presenting symptoms: Generally, this is an asymptomatic identity and the pulp is viatl in most cases. However, it can be hypersensitive to sensibility tests.

History and clinical findings: It has been theorized that there is a long standing chronic irritation to the pulp, like deep and/or leaky fillings that causes a low-grade inflammation in it, along with the periapical bony changes where there is replacement of marrow spaces and cancellous bone by dense, compact bone along with areas of fibrosis replacing fatty marrow [45].

Diagnostic tests: The pulp is usually responsive to thermal or EPT tests. There is no pain to percussion and/or palpation.

Radiographic findings: Diffuse radiopaque areas that are concentrically arranged around a root apex that may or may not have a radiolucent inflammatory lesion that does not alter the PDL space [35].

Treatment: No treatment is recommended, except ruling out source of the irritation to the pulp by evaluating the restoration(s) in the tooth.

7.9 Formulation of a Periapical Diagnosis

The diagnosis is only possible to make after obtaining all the information listed above, see summary in Table 7.3.

7.9.1 Key Factors

Periapical diseases associated with the root canal system are nearly always caused by bacteria and/or bacterial by-products. Therefore, elimination of those should ensure resolution of the disease and healing.

Table 7.3 The formulation of periapical diagnosis.

	Presenting symptoms			
	Pain	Swelling	Radiographic signs	Other findings
Acute apical periodontitis	Yes	No	Normal PDL	
Chronic apical periodontitis	No	No	Periapical radiolucency	
Apical periodontitis with abscess	Yes/No	Yes	Initially normal PDL Late stage some widening	
Apical periodontitis with sinus tract	No	No	Usually periapical radiolucency	Draining sinus tract

7.9.2 Related Factors

Diagnosis of periodontal versus endodontic causes for periapical diseases is crucial for correct and proper treatment of the disease (see Table 7.2).

7.10 Future of Pulpal and Periapical Diagnosis

Recently it has been suggested that various biomarkers, like for example matrix metalloproteinase, could be a useful tool to assess the level of pulpal inflammation [119]. This is because it has been shown, that at least in general, irreversible pulpitis is associated with different expression of various biomarkers compared to healthy pulps and many of those could be even collected non-invasively from crevial fluid or from dentinal/interdentin fluids collected prior to accessing the pulp in case of caries or other restorative procedures [100]. The interplay of these biomarkers could not only be used to assess the level of pulpal inflammation but also have the potential to provide enough information to differentiate between periapical cysts from granuloma [28, 33, 94, 100]. However, we are not quite there yet, because according to a recent systematic review on biological markers for pulpal inflammation the authors concluded that the main current challenges in the clinical application of biomarkers lie in the identification of biomarkers or biomarker subsets that reliably correlate with pulpal inflammation, the improvement of sample collection, and the interference of the biomarkers with inflammation of other than pulpal origin [100].

Determining the endodontic diagnosis prior to any invasive endodontic, or even any dental, procedure is paramount. It is clear that it takes clinical skills, good understanding of clinical signs and symptoms, as well as recognition of the limitations of commonly used sensitivity tests to make a clinical diagnosis – an acquired skill that does take some time to develop and always is at risk of subjectivity or bias.

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8

Biological Basis for Endodontic Repair and Regeneration

Kerstin M. Galler

8.1 Principles of Regeneration and Repair

Root canal treatment can prevent tooth loss by eliminating pulpal and periradicular disease; however, it is not a biology-based approach. Advances in pulp biology and tissue engineering challenge the traditional concept of replacing lost pulp with inert synthetic materials. Regenerative strategies aim at the production of new vital tissue that resembles the original in architecture, structure and function. As for the dental pulp, it has been known for decades that this tissue possesses regenerative capacity, and dentistry has been pioneering regenerative approaches with the use of agents such as calcium hydroxide to promote healing after pulp-capping procedures [41]. Such therapeutic measures are mainly empirical, but as we unravel the biological basis and start to comprehend the mechanisms underlying regeneration and repair, regenerative medicine offers great scope also for the field of endodontics.

Definition of Terms

Repair is the general term for the replacement of dead or damaged cells in a body tissue or organ by healthy new cells.

Repair by regeneration – activation of stem cells for the natural renewal of a structure; tertiary dentine formation in the pulp

Repair by substitution – scar tissue; fibrous/osteoid tissue growing in pulp space, as in current revitalization procedures

Repair by tissue engineering – creating human tissue from stem cells; experimental creation of pulpal tissue

The term 'regeneration' refers to the recreation of the original architecture, form, and function of a specific tissue type; 'repair' denotes healing by formation of a tissue that has partially lost the biological function of the original tissue. The distinction between repair and regeneration is often difficult in the clinical setting. Moreover, as biological processes, the two overlap in most situations. A complex fracture of the long bones will typically heal by virtually complete regeneration of the bone itself, but the surface wound will heal by fibrous tissue formation and a skin scar.

8.1.1 Pulp and Periapical Regeneration Processes

Periradicular bone possesses the capability to regenerate fully after removal of the inflammatory trigger. Healing of bony lesions is a major goal of endodontic therapy and it occurs predictably if the clinician succeeds in eliminating or sufficiently reducing bacterial activity within the root canal system. Thus, the connective tissues of the periapical bony structures have an innate capacity, with an adequate supply of multipotent cells, for

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complete regeneration of the periodontal ligament and the alveolar bone in the right circumstance.

Pulp tissue can also regenerate; however, it remains a challenge to elucidate exactly under which circumstances this may take place. It is important to identify the cell sources that may be activated for regeneration. Remaining islands of vital pulp can form the point of origin for regenerated pulpal tissue [77]. Stem cells of the apical papilla in immature teeth is another source of cells that can proliferate, differentiate, and form odontoblasts and pulp tissue [44]. These cells may cause regeneration of the pulp after revitalization procedures. In teeth with incomplete root formation and pulp necrosis, revitalization can be performed as an alternative treatment to apexification, where provocation of bleeding into the canal flushes in the respective cells from the apical papilla [54]. Even in teeth with complete root formation, the periradicular tissues comprise mesenchymal stem cells that can be delivered into the canal [14]; however, it is uncertain whether formation of a pulp-like tissue is biologically possible after pulp necrosis in mature teeth. The tissue formed inside the root canal after revitalization procedures contains elements of pulp tissue (fibroblasts, connective tissue, blood vessels, collagen), but other cell types are missing, among them notably odontoblasts, whereas non-targeted cell types or tissue such as osteoblasts and cementum may be present [56, 97]. Thus revitalization procedures may not so much give healing by regeneration but rather healing by repair.

8.2 Vital Pulp Therapy

Measures such as indirect and direct pulp capping or pulpotomy aim at maintaining tooth vitality and supporting the pulp's inherent capability of regeneration and repair. In either case, the pulp will respond with the formation of tertiary dentine. The generation of tertiary dentine is an active defense mechanism to create a mineralized barrier that separates the tissue from the site of injury; as such, it is a measurable parameter of healing. Tertiary dentine can be either reactionary or reparative. During tooth development, odontoblasts secrete primary dentine at a rate of 4–8 μm per day [49, 57], but they adopt a resting state after the completion of root formation where secretion is reduced to about 0.5 μm per day [18, 79]. Mild stimulation induces an upregulation of odontoblast activity, where primary odontoblasts increase their secretory activity to the original level, leading to rapid deposition of reactionary dentine, which displays a tubular structure [16, 81]. After therapeutic intervention, reversible inflammatory processes within the pulp tissue are expected to heal by regeneration. With increasing intensity of the stimulus and delayed intervention, healing will more likely take place as repair. If the original odontoblast layer is lost, e.g. after intense stimulation or pulp exposure, it can be replaced after differentiation of stem cells into secondary odontoblasts [16, 22]. These cells deposit reparative dentine, a mineralized matrix that may not exhibit the characteristic tubular structure, but resemble bony tissue and often display cellular inclusions [33]. It remains unclear whether osteodentine formation is particular to the cell source, which is not comprised of original odontoblasts, or to the intensity of stimulation, which leads to hasty deposition of a mineralized tissue that is less organized. Furthermore, tertiary dentine is also characterized by increased peritubular dentine deposition [81], a vital process which has to be differentiated from intra-tubular-calcifications due to physicochemical precipitation of mineral crystals [5]. Thus, the structure of tertiary dentine is highly variable.

8.2.1 Bioactive Materials

Bioactive materials have long been used to induce tertiary dentine formation and to support healing of the dentine pulp complex. A material is termed bioactive if it exerts a

positive influence on vital tissues and elicits a desirable biological response at the interface [39]. The response can be indirect, through antibacterial activity, or direct, by interaction with adjacent cells, e.g. stimulation of proliferation, differentiation, and/or biomineralization. Calcium hydroxide was introduced in root canal therapy nearly a hundred years ago [40], and studies with this material in contact with the pulp have demonstrated that it enables the tissue to remain vital and to form a mineralized barrier [41]. Calcium hydroxide has been extensively used in endodontics and dental traumatology, and it has been the material of choice for vital pulp therapy for several decades. Due to its high alkalinity, it not only exerts antibacterial and antifungal activity [80], but induces necrosis of adjacent cell layers and an inflammatory reaction in the underlying tissue. Pro-inflammatory cytokines and chemokines recruit immune cells, which promote healing by clearance of the injury site, and stem cells, which differentiate into secondary odontoblasts. Furthermore, calcium hydroxide releases growth and differentiation factors that are bound in the dentin matrix [34, 94], which affect and modulate cellular behavior [94, 99]. The use of calcium hydroxide for direct pulp capping has been shown to result in the formation of a mineralized barrier [2, 29], its thickness increasing with longer postoperative periods [29]. Histological analysis after pulp capping shows a superficial layer of tissue debris beneath the calcium hydroxide [29] and an adjacent, mineralized barrier. This barrier may display a tubular structure continuous with the original dentin, only with fewer and more curved tubules [29, 67], but more often it presents as an amorphous and atubular calcified tissue with cellular inclusions [33, 67, 71].

Application of calcium hydroxide thus has several effects that enable healing by regeneration. Negative properties, however, include high solubility and low mechanical stability. Moreover, the mineralized barrier formed may be porous and exhibit tunnel defects [19, 60], and calcium hydroxide appeared to be inferior regarding the thickness of newly formed mineralized tissue compared to Portland-cement-based materials such as mineral trioxide aggregate (MTA) [2, 95]. Due to these drawbacks, calcium hydroxide is progressively displaced by such materials, collectively referred to as hydraulic calcium silicate cements. These cements form a stable calcium-silicate-hydrate matrix; calcium hydroxide and calcium ions are side products of this reaction and create the bioactive effects [9, 10]. Similar to calcium hydroxide, MTA solubilizes dentin matrix proteins [91], which is likely to contribute to the material's bioactivity. Besides increased stability of these materials compared to calcium hydroxide, hydraulic calcium silicate cements offer a wide range of applications in endodontics due to their capability to seal and disinfect and to induce dentinogenic or osteogenic mineralization [21]. Several studies provide evidence that the use of hydraulic calcium silicate cements results in a less distinct necrotic zone, hyperemia, and inflammatory reaction compared to calcium hydroxide, to a more homogenous and solid layer of tertiary dentin, and to a superior clinical performance regarding failure rates and pulp vitality [1, 42, 64, 89].

8.3 Cell Types Involved in Pulp Healing

Among the cells that constitute the pulp tissue, the odontoblasts are the first target for external stimuli due to their peripheral localization in the dental pulp and their extension into dentine. Odontoblasts are post-mitotic cells, i.e. they are not replaced during the life of the organism under physiological conditions. As such, they share certain features with neurons and myocardiocytes as static cell populations [70, 92]. Stimuli include thermal variations and biomechanical forces, but also molecular products derived from microorganisms.

8.3.1 Tertiary Dentine

An essential feature of pulpal defense is the formation of tertiary dentine. As discussed above, reactionary dentine has to be distinguished from reparative dentine, as they arise from two different populations of cells and thus their genesis and nature are distinct. Reactionary dentinogenesis refers to the secretion of a tertiary dentine matrix by surviving post-mitotic odontoblasts, which increase their secretory activity in response to an appropriate stimulus. Implantation of dentin extracellular matrix components into unexposed cavities in ferret teeth leads to a localized stimulation of reactionary dentine [85]. This can mainly be attributed to the presence of transforming growth factor β-1 (TGF β-1), the most abundant growth factor in the dentine matrix, which is known to markedly upregulate odontoblast secretory activity [81]. Additionally, secretion of matrix at the mineralization front and along the odontoblast process leads to a progressive increase in thickness of the peritubular dentin, and the gradual occlusion of the dentinal tubules by centripetal deposition of calcium phosphate crystals leads to sclerosis and thus a decreased permeability of dentine [91]. In carious lesions, the demineralization of dentine induced by bacterial acids and the subsequent solubilization of bioactive molecules, in particular TGFβ-1, is considered responsible for initiating the stimulatory effect on the odontoblasts and thus the main cause of reactionary dentine formation. In contrast, reparative dentinogenesis is a more complex biological process. Stronger stimuli will lead to the death of the odontoblasts, but if conditions are favorable, a new generation of odontoblast-like cells may differentiate from stem or precursor cells within the pulp.

8.3.2 Stem Cells: Sources and Activation

A major cell source for regeneration or repair is the pool of stem cells that is present in the dental pulp, papilla and periapical tissues. Stem cells have been isolated from the pulp of permanent [35] as well as deciduous teeth [59] and furthermore from the apical papilla of teeth with incomplete root formation [44, 87]. Most commonly, stem cells are separated from the population of ex vivo cultured primary cells based on presence or absence of specific glycoproteins on the cells' surface. Thus, patterns characteristic for mesenchymal stem cells can be recognized, and cells can be sorted by use of specific antibodies.

By definition, stem cells are characterized by their capacity for self-renewal and their ability to differentiate into different cell types. In general, stem cells are involved in regular tissue turnover to replace aged cells, and during repair and regeneration. Located in the perivascular niche [78], where they are kept in an undifferentiated stage, stem cells in the dental pulp remain quiescent until an insult occurs. Stem cells undergo asymmetric division, meaning that one cell gives rise to an identical cell to keep the pool of stem cells constant, whereas the second daughter cell enters the path of differentiation. Chemotactic signaling recruits stem cells to the site of injury, they leave their niche, migrate, and differentiate into secondary odontoblasts [22], cells that can produce a mineralized barrier at the interface of soft and mineralized tissue. Thus, stem cells fulfil an essential role during regeneration and repair of the dentine pulp complex. See Figures 8.1 and 8.2.

8.3.3 Neurovascular Components in Regeneration

Pulpal inflammation and subsequent healing are also influenced by a complex neurovascular relationship and the interplay between the inflammatory process and sensory nerve fibers. Besides nociception, nerve fiber functions in normal and injured tissues include vasodilatation and neurogenic inflammation. Noxious stimuli trigger the release of neuropeptides, in particular substance P (SP), which induce vasodilation, increase pulpal blood flow and vascular permeability, and activate immune cells. The expression of SP in dental pulp is significantly upregulated in carious

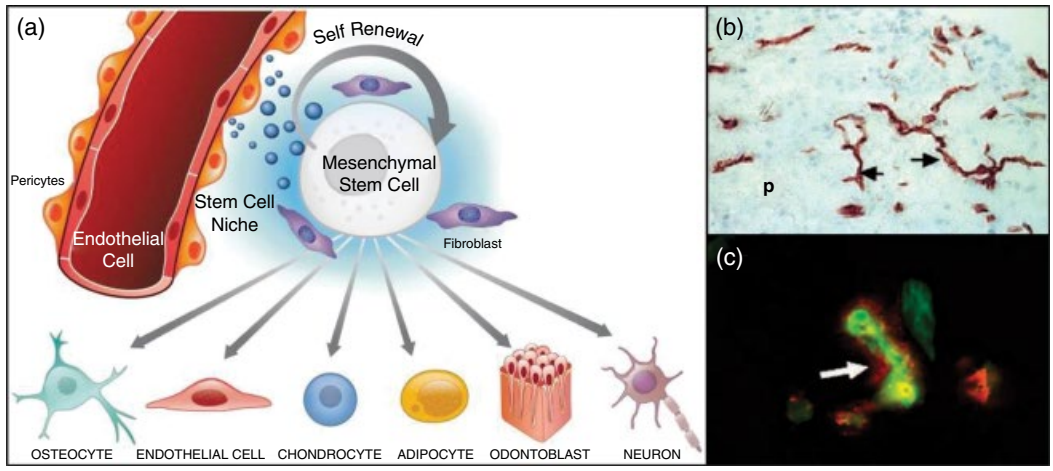

Figure 8.1 Perivascular niche and multipotency of mesenchymal stem cells. (a) Mesenchymal stem cells (MSC) reside in perivascular niches where they undergo self-renewal and maintain the surrounding cells or tissue. Under specific signaling conditions, MSC can undergo differentiation into different lineages. From [65]. (b) Immunolocalization of the CD146 antigen, an endothelial surface marker, to blood vessel walls in human dental pulp tissue. (c) Co-localization of blood vessels and mesenchymal stem cells in dental pulp. Dual immunofluorescence staining showing reactivity of an antibody to the mesenchymal stem cell marker STRO-1 labeled with Texas red to a blood vessel to the endothelial marker CD 146 labeled with fluorescein isothiocyanate. From [78].

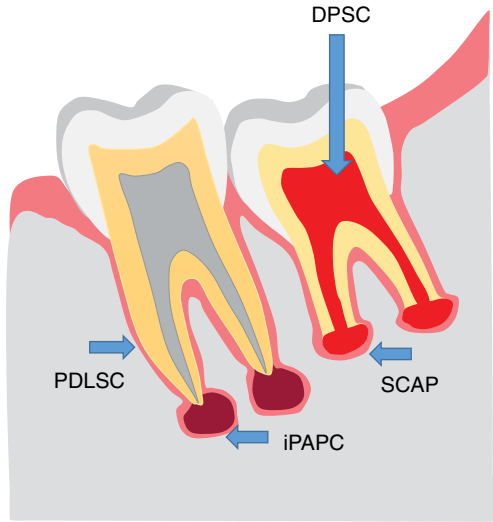

Figure 8.2 Stem cell types relevant for regenerative processes in the pulp and periapex. BMSC: bone marrow stem cells; iPAPC: inflamed periapical progenitor cells; DPSC: dental pulp stem cells; SHED: stem cells from human exfoliated deciduous teeth; SCAP: stem cells of the apical papilla. Modified from [38].

teeth [74]. Neuropeptides may exert stimulatory effects on odontoblast-like cells by increasing their expression of bone morphogenetic protein 2 (BMP-2), thus inducing tertiary dentin formation [12]. Furthermore, dental sensory fibers react to damage and inflammation by sprouting of their terminal branches into the surviving pulp beneath the injured site [51]. Increased neuropeptide levels from sprouting fibers can enhance inflammation through the recruitment of immune cells and the increase of vascular permeability [7, 37]. Denervated teeth show less survival of vascular pulp and accelerated loss of pulp tissue in comparison with innervated pulps [7]. Thus, if the sensory innervation is removed before injury, the pulp is less able to defend itself, to trigger neurogenic inflammation and to heal after pulpal exposure.

The role of pulp fibroblasts in the process of defense and regeneration has also been explored. Pulp fibroblasts produce all components of the complement system and can form the membrane attack complex (MAC), which is effective against cariogenic bacteria [48]. Furthermore, stimulation with bacterial toxins enables pulp fibroblasts to guide nerve sprouting during pulp regeneration through complement system activation and the production of neurotrophic factors [8, 13].

Immune cells are naturally involved in regeneration and repair of the pulp tissue, which will be discussed in more detail below.

8.4 The Role of Inflammation

As carious lesions progress, bacterial toxins and eventually bacteria themselves enter the pulp space via the dentinal tubules [53]. Odontoblasts sense bacterial toxins (such as cell wall components of bacteria) through receptors of the Toll-like (TLR) and nod-like (NLR) families [88], and contribute substantially to the pulpal immune response.

The dental pulp reacts to an infection with an inflammatory response as part of the – initially protective – host response. The most common cause for an invasion of microbes towards the pulp is tooth decay. Owing to its tubular structure, dentine is a penetrable barrier, where permeability increases due to an increase in both tubule density and diameter with decreasing distance from the pulp tissue. Thus, bacterial toxins or components and eventually intact bacteria reach the pulp via the dentinal tubules, and their penetration accelerates with increasing depth. The initial invasion of microbial components and toxins activates innate immunity, which in general is not antigen-specific, but recognizes molecular patterns that are specific to bacteria and initiates their attack and elimination by phagocytotic killing. Due to the unique anatomical location of bacteria in carious lesions, phagocytosis will not take place until the carious front has reached the pulp. However, the innate response of the dentine-pulp complex mobilizes a variety of protective measures, including the reactions of odontoblasts, the production of neuropeptides, the activation of immune cells, and the production of chemokines and cytokines [36].

Generally, the outward flow of dentine liquor due to the positive intrapulpal pressure prevents sporadic migration of oral bacteria from exposed dentine surfaces into the pulp. Thus, the rate of bacterial invasion is significantly lower in vital compared to non-vital teeth [61]. As an initial protective mechanism against caries progression, the rate of outward flow can be increased by vasodilatation after the neuropeptide release of intradental sensory afferent nerves [55, 58].

8.4.1 Pulp Responses to Bacterial Challenges

The odontoblasts are anatomically located in the periphery of the pulp, and their cellular processes extend well into the dentinal tubules. Therefore, they are the first cells to encounter bacterial antigens. In order to sense potential threats, odontoblasts constitutively express so-called pattern recognition receptors (PRRs), which recognize and can bind to various bacterial components. Receptor binding leads to intracellular activation of signal transducers and the secretion of interleukins, pro-inflammatory cytokines, and chemokines. These messengers stimulate the diapedesis of leucocytes and recruit immature dendritic cells [25, 43], which process and present antigens and thus act as messengers between the innate and adaptive immune systems.

The production of the acute-phase, lipopolysaccharide-binding protein (LBP) by odontoblasts contributes to the protective mechanisms. LBPs neutralize bacterial cell wall components and can attenuate the immune response by inhibiting the production of pro-inflammatory cytokines [26]. Challenged with bacterial toxins, odontoblasts also produce vascular endothelial growth factor (VEGF), a potent inducer of vascular permeability and vasculogenesis [90]. Furthermore, antibacterial peptides called defensins are secreted [23]; these exert a broad spectrum of antimicrobial activity as they generate channels or micropores in the cell membrane of microorganisms. Moreover, odontoblasts secrete TGF-β1, which stimulates dentine matrix secretion as well as acting as an anti-inflammatory mediator; its expression increases in irreversible pulpitis [68]. Dental pulp cells may also produce anti-inflammatory

signaling molecules, which attenuate the immune response to restrict tissue damage, while simultaneously stimulating odontoblast differentiation and formation of new dentin [26].

The remaining dentine thickness appears to be critical. Studies assessing the immune response of pulp tissue due to caries show the full spectrum of the cellular host response if the layer of dentine falls below 0.5 mm [15]. Apparently, the progression rate is also of importance, as slowly progressing caries is characterized by a different bacterial flora, color, and consistency compared to rapidly progressing caries [6]. Slow caries progression allows for defensive measures as described above as well as for peritubular mineral deposition leading to dentine sclerosis in order to shut the gates for invading microbes.

8.4.2 The Link between Inflammation and Regeneration

Thus the innate immune response offers a repertoire of protective mechanisms that aim at the restoration of pulp homeostasis. Therapeutic intervention and removal of caries and other sources of infection can thereby result in resolution of inflammation and healing. The initial inflammatory response, however, promotes regeneration, and the general importance of this close link was established with the observation that healing after myocardial infarction was compromised by corticosteroids in animals [52]. A prerequisite for healing is the elimination of bacterial noxa as well as the production of pro-inflammatory mediators by the host. The immune system produces both pro-inflammatory as well as anti-inflammatory signaling molecules. Whereas pro-inflammatory mediators initiate the immune response, anti-inflammatory molecules prohibit an excessive response and lead back to tissue homeostasis. Pathogen removal results in attenuation of the response and remaining toxins will eventually be neutralized [26]. Undoubtedly, if the damage to the dentine pulp complex is severe enough, it cannot be resolved by healing. Increasing numbers of immigrating immune cells induce extensive collateral tissue damage. They produce proteases, which on the one hand enable passage of immune cells through the tissue, but on the other lead to tissue dissolution. Immune cells furthermore secrete reactive oxygen species and enzymes, which not only damage bacteria but also local cells. Signals derived from necrotic cells promote the inflammatory process further and lead to exacerbation. Prolonged stimuli evoke chronic inflammation characterized by moderate immune cell infiltrates, collagen fibrosis, and premature aging of the tissue with reduced host response, and/or it may lead to necrosis and spread of microorganisms into the root canal system and eventually the periapical region.

In contrast to a slow invasion of microorganisms towards the pulp in tooth decay, the pulp can be exposed to microbial contamination and infection rapidly after dental trauma and crown fractures. In that case, a healthy tissue with a fully functional immune response is confronted directly with microorganisms. A classic study in monkeys found that the zone of inflammation one week after pulp exposure by grinding or fracture did not extend beyond a depth of some 2 mm [20] without significant differences compared to the penetration depth after 48 hours. The healthy pulp thus has a remarkable resistance to bacterial invasion.

8.5 Signaling Molecules in Dentine

During tooth development, neural crest-derived cells of the dental papilla undergo terminal differentiation into dentine-forming odontoblasts. Upon completion of cytodifferentiation, odontoblasts start their secretory phase and produce an organic template of collagenous and non-collagenous proteins, which later mineralizes with hydroxyapatite crystals to form calcified dentine. During this synthesis process, the odontoblasts not only lay down the predentine, but also express a

variety of bioactive molecules, which are secreted into the extracellular space [28, 73, 100]. During mineralization, these bioactive factors become embedded and immobilized in the dentine matrix. Whereas proteins and growth factors in an active form have a short half-life, binding to extracellular matrix components may serve to maintain their bioactivity by protecting them from proteolytic degradation and thus prolonging their life span. Growth factor-binding compounds include mainly proteoglycans [72, 96], but also specific binding proteins [3], glycoproteins such as fibronectin [69], and different types of collagen [66, 86].

As there is no turnover in dentine extracellular matrix, bioactive regulatory molecules can be reactivated much later in life upon release from their bond. During caries, bacterial acids, such as lactic acid, expose the organic component of dentine and release bioactive factors, which may modify the immune response, cell recruitment and differentiation (Figure 8.3) (Table 8.1) [24]. Application of dental materials onto dentine, e.g. calcium hydroxide or hydraulic calcium silicate cements, but also self-etching dental adhesives, release bioactive factors [27, 34, 93]. Organic acids or chelating agents such as ethylenediaminetetraacetic acid (EDTA), which is commonly used in endodontic treatment, are also suitable for dentine demineralization. A multitude of bioactive molecules have been identified in dentine extracellular matrix (for review see [84]). These include non-collagenous proteins that regulate mineralization, as well as growth and differentiation factors, cytokines, chemokines, and neurotrophic factors. Growth factors present in human dentine extracellular matrix include transforming growth factor β1 (TGF-β1), basic fibroblast growth factor (bFGF), bone morphogenetic protein 2 (BMP-2), platelet-derived growth factor (PDGF), placenta growth factor (PIGF), and epidermal growth factor (EGF), in addition to angiogenic factors such as vascular endothelial growth factor (VEGF) [11, 28, 73]. These molecules are effective at very low concentrations and

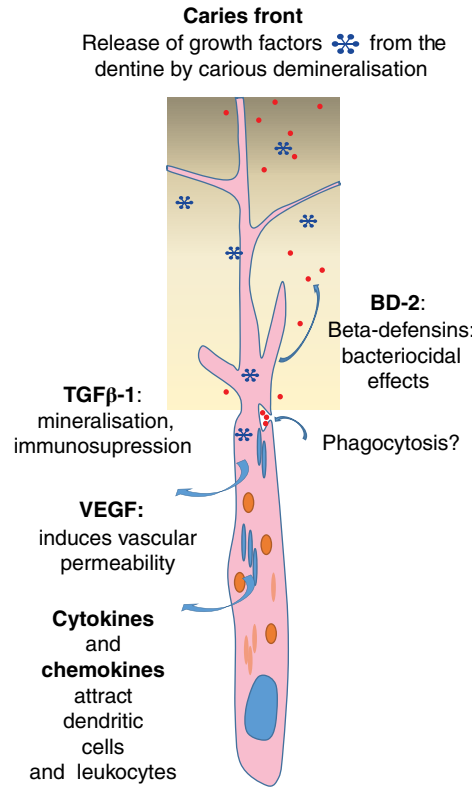

Figure 8.3 Simplified schematic drawing of odontoblast responses to carious lesions. Odontoblasts express TLRs (Toll-like receptors) to recognize various bacterial products (red). Receptor binding activates intracellular signaling pathways. Odontoblasts produce antibacterial peptides such as beta-defensin 2, cytokines and chemokines to attract immune cells, growth factors such as vascular endothelial growth factor to increase vascular permeability and transforming growth factor β-1 to induce mineralization. A phagocytotic activity of odontoblasts has been suggested. The response is likely to be modulated by growth factors released from the dentine matrix by demineralization caused by bacterial acids. Based on data in [36].

elicit cellular responses even at the picogram level. They modify immune reactions, stimulate angiogenesis, exert chemotactic effects to recruit cells to the site of injury, and promote proliferation, differentiation and mineralization [4, 83, 98]. Dentine matrix proteins and their roles in healing and regeneration are under continuous study, and their bioactive properties are explored for tissue engineering approaches.

Table 8.1 Bioactive dentine matrix components and their putative functions, a non-exhaustive overview.

Growth Factors and Cytokines	Function
Transforming growth factor beta 1 (TGF-β1) (Isoforms 1, 2, 3)	Anti-inflammatory; induction of odontoblast differentiation and matrix secretion; chemotaxis
Basic fibroblast growth factor (bFGF)	Induction of cell proliferation; chemotaxis; angiogenesis
Vascular endothelial growth factor (VEGF)	Angiogenesis
Bone morphogenetic proteins (BMPs) (BMP 2, 4 and 7)	Differentiation; mineralization
Adrenomedullin (ADM)	Anti-inflammatory; odontoblastic differentiation
Insulin-like growth factor (IGF-1 and -2)	Proliferation; differentiation
Platelet-derived growth factor (PDGF)	Regulation of cell growth and division; angiogenesis
Placenta growth factor (PGF)	Angiogenesis; chemotaxis; modulation of odontoblastic differentiation
Hepatocyte growth factor (HGF)	Proliferation; migration; cell survival
Epidermal growth factor (EGF)	Cell growth and neurogenic differentiation
Interleukins (IL-8, IL-10)	Anti-inflammatory
Neuropeptides and neurotrophic factors	**Function**
Calcitonin (CT)	Ca-metabolism
Calcitonin gene-related peptide	Vasodilation,; nociception
Neuropeptide Y (NPY)	Neurotransmitter
Substance P (SP)	Neurotransmitter; neuromodulator

Note that bioactive molecules are often multifunctional. From [82, 84].

8.6 Tissue Engineering Approaches to Dental Pulp Regeneration

Tissue engineering is an interdisciplinary field of research, which involves the use of materials and cells with the goal of understanding tissue function and eventually enabling specific tissues to be made *de novo*. According to the classical tissue engineering paradigm, stem cells are seeded into a suitable scaffold laden with growth factors, which, after transplantation into a host, induce stem cell differentiation and tissue formation (Figure 8.4). The isolation of pulp–derived stem cells has opened new avenues for tissue engineering approaches in dentistry. Stem cells have been isolated from permanent [35] and deciduous teeth [59], and from the apical papilla of teeth with incomplete root formation [44].

Dental pulp tissue can also be engineered by the transplantation, in experimental animals, of dental pulp stem cells in a suitable carrier system. Tooth slices, dentine cylinders, and whole tooth roots laden with scaffolds and dental stem cells can be transplanted subcutaneously in the back of mice, and vascularized tissue similar to dental pulp forms after few weeks *in situ* with cells adjacent to the dentine differentiating to deposit tubular dentine [17, 31, 45, 76]. Other animal models can more closely mimic clinical regenerative endodontic treatment procedures: following pulpotomies and pulpectomies in dogs' canines, filling the void with a scaffold material containing stem cells and recombinant growth factor resulted in the formation of

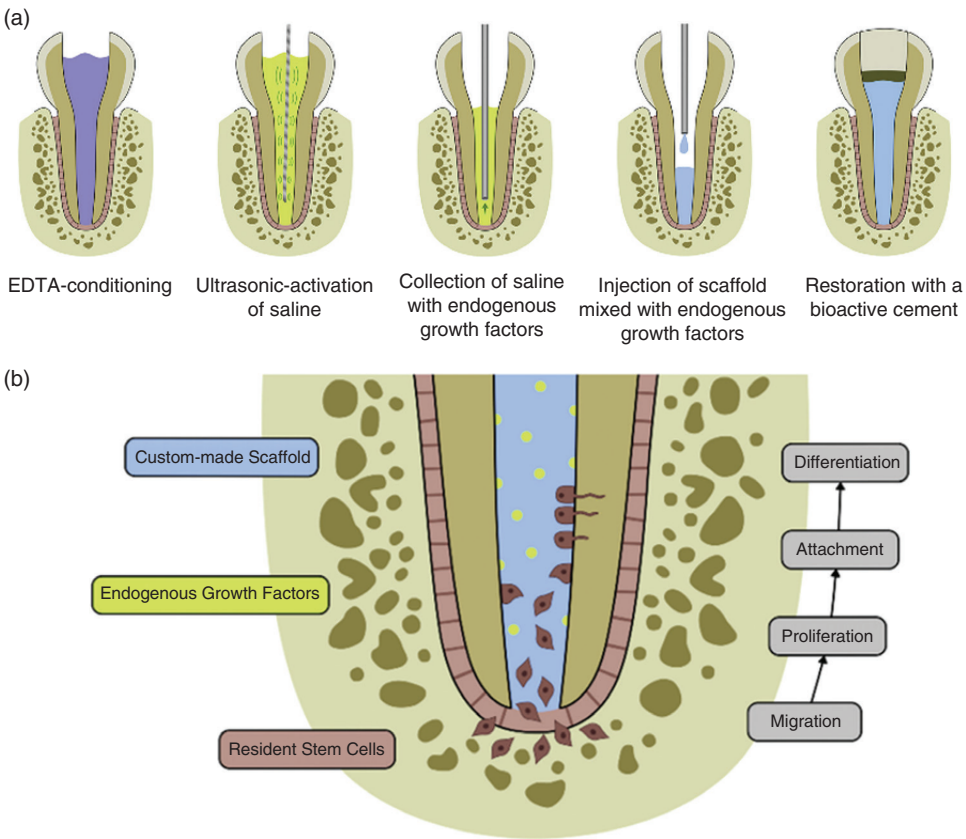

Figure 8.4 Illustration of selected steps of a cell-homing approach for pulp tissue engineering. (a) After conditioning with EDTA, endogenous growth factors are released into saline by ultrasonic activation. The scaffold is laden with isolated growth factors and injected in the canal space; the tooth is restored with bioactive cement and a tight coronal seal. (b) Schematic illustrating potential actions initiated by exposed or supplemented endogenous growth factors in a cell-homing approach in mature teeth. Bioactive molecules may induce chemotaxis of resident stem cells (DPSC if remnant pulp tissue is present, PDLSCs or iPAPCs), their proliferation and attachment as well as odontoblast-like cell differentiation. From [32].

vital, pulp-like tissue [46, 47, 62]. There is also evidence that functional recovery of pulp tissue after transplantation of autologous dental pulp stem cells into the root canals of humans is possible [63]. These cell-based experiments follow the tissue engineering concept described above, which involves the delivery of stem cells and recombinant growth factors in a carrier material. However, transplantation of stem cells has several problems, including limited availability of cell sources, technical issues of cell harvest and expansion, logistics, high cost, nd regulatory hurdles for approval and clinical translation.

Alternatively, cell-free approaches to dental pulp tissue engineering have been proposed [30, 50, 75]. Following the principle of cell-homing, resident stem cells from remnant pulp or periapical tissues can be recruited into specifically designed biomaterials via recombinant or endogenous, dentine-derived growth factors. Evidence is accumulating that a regenerative endodontic procedure following the principle of cell homing might be a feasible and affordable alternative to cell transplantation [32, 50, 75].

General principles of regenerative mechanisms and tissue engineering approaches in treatment modalities for pulpitis or pulp necrosis are listed in Table 8.2.

Table 8.2 Current and envisioned treatment modalities for pulpitis or pulp necrosis.

Current treatment modalities					
Type of assault	**Type of tooth**	**Treatment**	**Cell type activated/recruited**	**Healing**	**Proposed Mechanism**
Infection – mild stimulus (Reversible pulpitis)	Mature tooth	Indirect pulp capping Restoration	Primary odontoblast	Regeneration or repair	Upregulation of odontoblast secretory activity
Infection – intense stimulus (Irreversible pulpitis)	Mature tooth	Direct pulp capping or pulpotomy Restoration	Dental pulp stem cells	Repair	Differentiation of stem cells into odontoblast-like or mineralizing cells
Pulp necrosis/apical periodontitis	Immature tooth	Revitalization	Stem cells of the apical papilla or Ectopic cells (bone, PDL)	Regeneration, more likely repair	Differentiation of stem cells into odontoblasts, completion of root formation or Apposition of cementum, ingrowth of bone
	Immature tooth	Apexification	Bone cells/periodontal ligament cells	Regeneration of bone Repair in contact with a synthetic material	Induction of a mineralized barrier by a bioactive material
	Mature tooth	Root canal treatment	—	Regeneration of bone Repair in contact with synthetic material	Healing in contact with a biocompatible material
Proposed tissue engineering-based treatment modalities					
Type of assault	**Type of tooth**	**Treatment**	**Cell type activated/recruited**	**Healing**	**Proposed Mechanism**
Irreversible pulpitis or pulp necrosis/apical periodontitis	Mature and immature	Tissue engineering by cell transplantation	Transplanted cells (e.g. dental pulp stem cells)	Regeneration	Differentiation of transplanted stem cells Ingrowth of vasculature
	Immature tooth	Tissue engineering by cell-homing	Dental pulp stem cells and/or Stem cells of the apical papilla	Regeneration	Differentiation of resident, recruited stem cells
	Mature tooth	Tissue engineering by cell-homing	Dental pulp stem cells or mesenchymal stem cells from the periapical region	Regeneration	Differentiation of resident, recruited stem cells

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9

Prevention

Treatment of the Exposed Dentine Pulp Complex

Lars Bjørndal

9.1 Diagnostic Challenges of Deep Caries and Traumatic Pulp Exposure

Prevention of apical periodontitis begins with the evaluation of whether the pulp can be preserved or not. The task of assessing the actual stage of pulpal infection and inflammation has been and still is today a major challenge [61]. Stringent means of pulpal diagnosis are largely lacking. It is particularly important to: 1) establish an exact threshold for unwanted inflammation that inevitably leads to necrosis; 2) estimate whether an exposed dentine pulp complex pulp should be avoided; and 3) in case of exposure, decide if a pulp capping or pulpotomy procedure is possible or if a pulpectomy is necessary.

This essential diagnostic problem of estimating degrees of pulp inflammation and infection has led to different treatment concepts for deep carious lesions. These concepts range from a strictly conservative approach, leaving affected dentine and the pulp underneath the restoration, to radical pulp removal and root filling. Questionnaire surveys based on radiographic illustration of deep carious lesion have shown that there is a huge variation among dentists in their choice among the treatment modalities for a given situation [64, 76, 85, 86]. In clinical practice, the decision whether to save the pulp or not varies even when supplementary subjective and objective diagnostic data are added [85]. The majority of dentists lean toward an invasive approach suggesting that a pulpectomy should be carried out [64, 86]. The variation in choice of treatment may also be a consequence of an unclear definition of what constitutes a deep carious lesion, as well as of a lack of unambiguous clinical signs and symptoms for defining the status of the pulp. Clinical research should strive to improve the evidence base both for the diagnostic procedures and for the choice of therapy associated with the different clinical situations. In practice, this means that we need clearer definitions of deep lesions based on solid clinical evidence, and we need sophisticated technologies for measurements of inflammation. While there is active clinical research in this field, many questions are still unanswered.

9.1.1 Deep Caries as an Endodontic Problem

Endodontics as a discipline holds the key for providing optimal "pulpal care". Endodontology has a natural focus on aseptic strategies that is fundamental and mandatory for a successful preservation of pulp vitality. This includes the preparation of an aseptic working field using rubber dam isolation and application of a disinfection agent. Lack of asepsis and use of contaminated instruments

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

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in deep caries treatment, as well as the potential introduction of carious dentine fragments into an exposed pulp, is likely to be the main reason why conservative approaches to treatment so often fail. In general dental practice, focus on an aseptic working field using rubber dam in these situations is variable at best. A great majority of practitioners treat deep caries and associated pulpal exposures without regard to asepsis [15, 44, 70, 82], which makes pulp-capping procedures a vulnerable modality from the outset. Clear guidelines are needed, and efforts should be made to ensure that protocols for treatment of deep caries and pulp capping are recognized as strictly aseptic procedures with predictable outcomes, rather than simple and easy methods just hoping to avoid a pulpectomy [49].

This chapter deals with the dilemma of correctly diagnosing the pulpal status in deep caries. Also addressed is the biological platform for understanding why the dentine pulp complex is able to produce a hard tissue barrier. Moreover, the various modalities for managing the disease of the inflamed pulp are reviewed, including approaches to avoid pulp exposure.

9.2 Discerning Pulpal Diagnosis

Most often the cause of a pulp exposure and subsequent endodontic intervention is caries [14]. Therefore, the diagnostic dilemma is best illustrated by the scenario of a deep carious lesion. From a classical endodontic viewpoint, the consideration of the actual status of pulp inflammation immediately emerges. It is generally recognized that signs and symptoms as presented by the patient or during an objective examination do not permit an accurate diagnosis of the histologic status of the pulp; consequently, it is difficult to assess whether a pulpal inflammation is reversible or not [26, 78, 79] (see also Chapter 10). From a histopathological/bacteriological viewpoint, the point of no return for unwanted and irreversible inflammation in the pulp can be defined as the stage where the invading microorganisms have entered the pulp space either through tertiary dentine or directly into the pulp [66, 69]. However, this critical threshold of infection is difficult to detect clinically. Particularly, deep lesions with risk of pulp exposure during caries removal should be better defined by added information on progression stage and penetration depth, lesion activity and estimated length of progression time (patient age). These elements of defining the carious lesion are further detailed below.

9.2.1 The Penetration Depths of Carious Lesions

Clinically, defining the depth of a deep carious lesion is usually based on dental practitioners' expectations of reaching pulp exposure following excavation [18]. Radiographically, a deep carious lesion is in this context defined as a lesion penetrating into the pulpal quarter of the dentine, but still with a well-defined zone of radiopaque dentine separating the lesion from the pulp [7]. With further development of the carious process, demineralized dentine may be seen to affect the entire thickness of the dentine; caries may then be defined as extremely deep [17]. Examples of deep and extremely deep lesions are shown in Figure 9.1.

Based on knowledge from caries histopathology, the infected carious dentine is initially restricted to the so-called outer zone of the demineralized dentine. This outer zone is non-remineralizable and decomposed with degraded collagen [31], whereas the inner zone being the most advanced part of the carious process includes a radiopaque/hypermineralized zone, which is actually keeping the microorganisms from directly entering the pulp. When this hypermineralized zone is broken down, the carious lesion has microorganisms penetrating into the critical area of tertiary dentine and the pulp [66]. At this stage clinical symptoms may occur that are useful in defining a diagnosis of irreversible pulpitis, corresponding well with the presence of bacteria within the pulp [69].

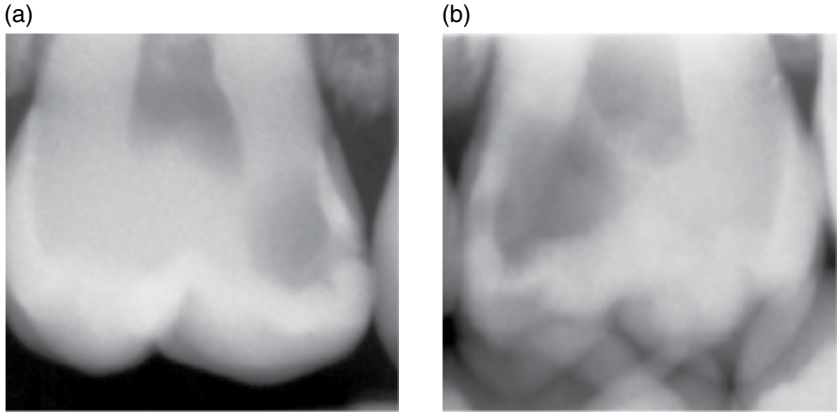


Figure 9.1 (a) A deep carious lesion defined with a lesion penetration into the pulpal quarter of the dentine. A well-defined zone of radiopaque dentine is separating the carious lesion from the pulp (arrow). (b) An extremely deep carious lesion extending through the entire thickness of the dentine, no evidence of a radiopaque zone of dentine, but there may be thin radiopaque lines within the pulp chamber (arrows).

Therefore, the careful examination of lesion depths on bitewing radiographs becomes a substitute for evaluation of the risk of bacterial invasion into the pulp. The exact degree of carious lesion progression has seldom been described in literature dealing with pulp capping [10]. This means that there is probably a wide variation among cases with regard to the extent of dentine and pulp infection, which could be one explanation of the difficulties in comparing studies and in predicting the outcome following direct pulp capping. It is definitively important whether the capping procedure is performed within a deep or extremely deep carious scenario versus an iatrogenic exposure. In the latter case, the capping procedure is done after preparation for a filling or a crown that has exposed the pulp, e.g., a pulp horn, and the local pulp has not been affected by caries. Such information is seldom specified in clinical reports [10, 28], but it probably represents a major difference in outcome. A pulp exposed during carious tissue removal would have a much higher risk of becoming infected during treatment, whereas an iatrogenic exposure stands a fair chance of remaining free from infecting bacteria (see also later).

9.2.2 Pulp Inflammation – a Two-edged Sword

Caries is the common reason for performing root canal treatments, particularly in vital cases [14], and is a main cause of pulp inflammation. An update on pulp inflammation and caries pathology is briefly presented. The view of seeing pulp inflammation under caries as an irreversible process that cannot be stopped is no longer tenable. It was based on the belief that if carious dentine was left behind intentionally, it would represent a strong, permanent challenge to the subjacent pulp, and sustained inflammation was to be expected. Even if carious tissue removal and pulp capping was performed on such an inflamed pulp, the inflammation was expected to run its own course and eventually lead to necrosis and clinical failure [63]. Pulp inflammation does not necessarily lead to the pulp becoming necrotic and infected. Rather, a low-grade inflammation provides a platform for repair and regeneration [23]. When the source of injury is removed, the pulp may still have the capacity and potential to provide an upregulation of hard-tissue-forming cells including odontoblasts [81]. In addition, pulp reactions to caries are dynamic in nature and

differ in response to slowly versus rapidly progressing carious lesions. The dentine-pulp complex lays down reactionary, tertiary dentine formed by primary odontoblast cells in response to a low-grade/slowly progressing lesion environment (typically adult/older patient, lesion penetration half into the dentine) [8]. In contrast, subjacent to a rapidly progressing lesion (young patient, lesion in inner quarter of dentine), the tertiary dentine, if present at all, is characterized by a decreasing presence of dentinal tubules, eventually being completely atubular, by a process called fibrodentinogenesis [6]. Here the primary odontoblasts have decayed and died, and the reaction should be seen as repair and not regeneration [68].

9.2.3 Untreated Caries a Model to Understand Reparative Dentine

Figure 9.2, using different teeth for the different stages, illustrates the progression of untreated occlusal lesion in molar teeth. The carious dentine shrinks due to the extensive loss of mineral, and a gap or slit is created laterally along the enamel-dentine junction, which provides the pathway for a massive infection. This gap, that is undermining sound enamel, comprises a biofilm [12]. Clinically the lateral spread of infection [9] appears as a milky change of dentine translucency surrounding the cavity (Figure 9.2b arrows). During mastication, the undermined enamel breaks off and a large dentine surface is exposed to the environment (Figure 9.2c). This in turn creates a change in the growth conditions for the cariogenic biofilm. Even though the lesion is deep, the large, exposed surface causes it to change into a more slowly progressing lesion, and a massive formation of reparative dentine formation is often noted subjacent to these open deep lesions. Histologically, a new layer of tubular dentine is laid down on top of the atubular dentine [8]. Unfortunately, while the pulp may be only reversibly inflamed, when deep lesions progress to the extent where the enamel has been broken severely down, these teeth may no longer be salvageable.

The key message of this "natural history" of caries progression is that when the outer lesion environment changes, reducing the cariogenic load, the pulp may react with reparative dentine formation. In principle, this knowledge may enable us to convert lesion activity by conservative treatment approaches to deep carious lesions: if we can change the environment over an active lesion, we may be able to support the production of effective reparative dentine. Another message is that the formation of reparative dentine *per se* is not a permanent sign of biological, or treatment, success. New bacterial infection will always challenge any type of new tertiary dentine barrier that has been laid down. Thus, while the formation of a dentine barrier is a step in the right direction

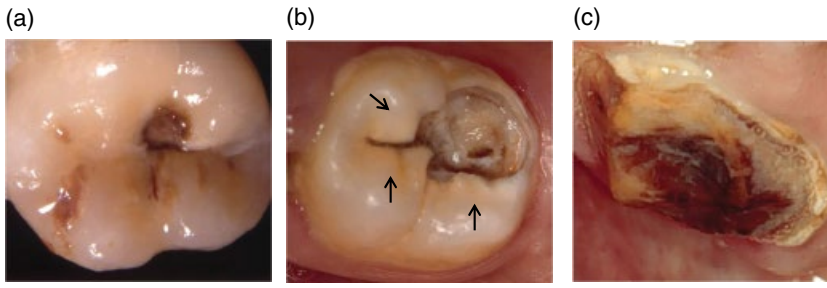


Figure 9.2 (a, b, c) The "natural history" of untreated caries. Three progressing stages of a deep lesion, where (a) undermined enamel eventually (b) breaks off due to mastication (arrows), converting the lesion environment from a "closed" to (c) an "open" environment converting the central part of the lesion into a slowly progressing environment. Changing the outer environment during the course of the carious process turns the active, rapid lesion into a slower or even arrested phase. Courtesy of Dr Lars Bjørndal.

Table 9.1 Clinical and radiographic characteristics of reversible and irreversible pulpitis.

	Pulp condition	
Diagnostic factors	**Reversible pulpitis**	**Irreversible pulpitis**
Spontaneous toothache	No	Yes
Pulp response to cold and electricity	Yes	Yes
Lingering pain to cold	No	Possible
Lingering pain to heat	No	Yes
Pain relief from cold	No	Possible
Swelling	No	No
Increased mobility	No	Possible
Tenderness to biting/percussion	Possible	Yes
Radiographic signs of apical bone destruction	Possible	Possible

of maintaining pulpal vitality, permanent elimination and control of dentine infection is essential for a lasting preservation of the pulp.

Table 9.1 lists some variables describing features of clinical caries. Combined with subjective, objective and para-clinical information, a pragmatic approach is presented for dividing the status of pulp into normal or reversible pulpitis versus irreversible pulpitis.

9.3 The Pulp Biology Associated with Pulp Capping

9.3.1 Hard Tissue Formation

Numerous experimental, animal studies have provided evidence that the dentine pulp complex has the biological capacity of laying down tertiary dentine formation following sealing of a pulp exposure, with the prerequisite that the pulp *per se* is without infection. The classical Kakehashi study [45] is well known for documenting the fundamental role of root canal infection for the development of apical periodontitis; but it also showed that the exposed pulps of germ-free rats, i.e., in the absence of bacteria, healed with the formation of tertiary dentine. It is beyond the scope of this chapter to further detail the basic biological interactions causing this to happen, but the following items will be briefly discussed: the origin of dentinal repair cells, hemostasis, biological and sealing properties of the capping material.

9.3.2 Stimuli Causing Tertiary Dentine and Origin of Dentinal Repair Cells

Tertiary dentine is defined as new dentine produced in relation to an external injury [6]. The subdivision of tertiary dentine into reactionary dentine (dentine laid down by primary odontoblast cells) and reparative dentine (hard tissue laid down by other cells than the primary odontoblasts) simply reflects the type of the external stimuli that has caused it to happen and was illustrated above in relation to caries [8, 68]. A completely atubular pattern is typical for a very rapidly progressing carious lesion, but it can also be seen in the pulp underneath an acute, iatrogenic trauma, caused by, for example, an insufficiently water-cooled rotating bur during cavity preparation. The atubular dentine or fibrodentine may also occur as a physiological phenomenon in teeth without signs of external injuries [6], e.g., at the tip of a pulp horn or on the floor of the pulp chamber [19], and reflect a mineralization potential of pulpal stem cells, not only the odontoblast.

Indeed, tertiary dentine can be produced by non-primary odontoblast cells, as seen following pulp exposure, where the re-establishment of a dentinal bridge formation can be expected following a successful pulp-capping procedure (Figure 9.3). Dentine bridge formation following a pulp-capping procedure reveals many non-mineralized parts or so-called "tunnel defects" that could easily be invaded by microorganisms [24]. Therefore, the placement of a bacteria-tight, coronal seal securing long-term control of contamination is mandatory after a capping procedure.

9.3.3 Hemostasis

For any pulp capping (or pulpotomy) procedure to be successful, it is commonly held that hemostasis is obtained and blood clot formation between the capping material and the pulp tissue is avoided. This is important for the proper placement of the capping material on a dry cavity base; moreover, the presence of a blot clot has been linked to higher risk of infection [71, 72]. The means of obtaining hemostasis seems of little importance, particularly for capping of a sound pulp. A clinical trial investigated different hemostatic protocols using either saline, sodium hypochlorite, or chlorhexidine digluconate prior to pulp capping with calcium hydroxide, and found no difference among the liquids in the expression of specific gluco-proteins related to pulpal repair [4]. Moreover, a randomized clinical trial has shown improved outcome if a disinfection agent is applied in the hemostatic protocol prior to the application of a capping material [88]. On the other hand, it may also be seen as a contradiction that the blood clot should in fact be removed. It contains numerous potentially bioactive molecules, which could contribute to and benefit a repair process. In fact, revascularization protocols actively

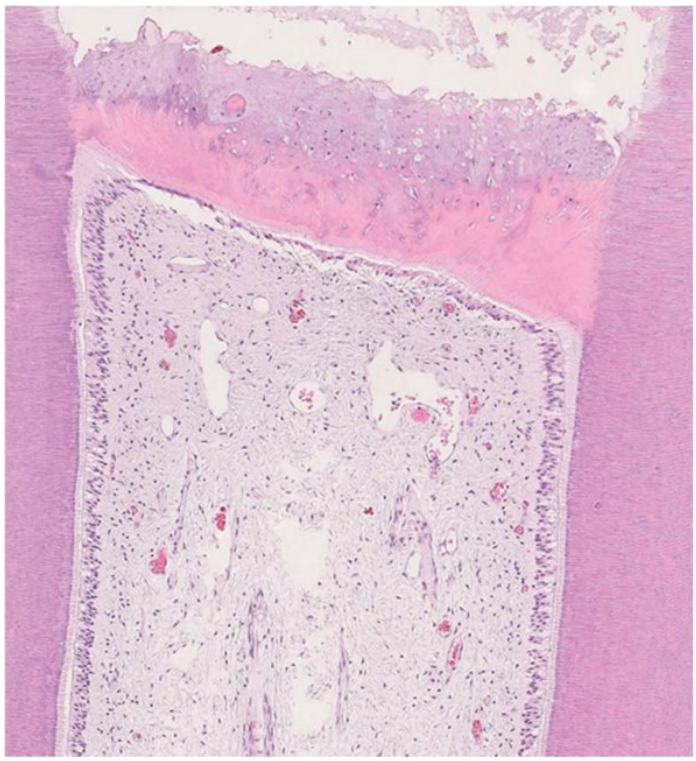


Figure 9.3 Histological evidence of reparative dentine in human following direct pulp capping.

induce bleeding [32, 33]. Current pulp-capping protocols are most probably far from making a full biological benefit of the healing potential of the pulp.

9.3.4 The Pulp-capping Materials

The majority of evidence is from calcium hydroxide in various preparations, both in animal as well as in human studies. However, many of the studies in human suffer from a low level of evidence. For one thing, a control group is missing in many of these studies [28]. Furthermore, numerous pulp-capping studies have tested the materials in an uncontaminated environment, *i.e.,* using teeth with non-inflamed pulps and no caries. This is relevant for various trauma and/or iatrogenic scenarios where a relatively sound pulp is exposed. However, capping the cariously exposed pulp may represent the most common clinical situation, and capping procedures should be tested under these more realistic conditions. In particular, whereas bioengineered anti-inflammatory pulp-capping materials have been around for quite some time [49], the lack of realistic clinical testing is an impediment to clinical acceptance. Marketing for new pulp-capping materials often runs ahead of robust clinical evidence. However, recent reviews provides some evidence for a superior outcome with the hydraulic calcium silicate cements, in particular various forms of the mineral trioxide aggregate material (MTA) [52, 91]. However, as high-quality randomized clinical trials comparing and testing capping materials are still lacking, the relative performance of different materials for capping is largely unknown (see also below).

9.4 Criteria for Assessing Success of Vital Pulp Therapies

A number of signs and symptoms converge to form a definition of successful treatment. As patients are well aware, absence of clinical symptoms, such as pain or discomfort, spontaneous or induced, is essential. Recording of symptoms is therefore one important part of success/failure assessment. A necrotic pulp may be a sequel to treatment/infection, but does not necessarily lead to symptoms; therefore, assessment of pulpal sensibility by thermal or electrical testing is necessary. Finally, the pulp may become infected with development of apical periodontitis, which is reflected in periapical control radiographs. It should be noted that in some trauma cases, inflamed pulps may induce periapical radiographic changes known as transient apical breakdown [3], and the pulp is salvageable. Radiographs may at times also be suitable for monitoring dentine bridge and tertiary dentine formation. The use of cone beam CT may represent a refinement for detecting changes also in the tissue responses to pulp-capping procedures. It appears that cariously affected, vital pulps may have radiographically visible changes at the apex more easily detectable by CBCT, and that monitoring treatment by CBCT may be a more sensitive technique for observing differences among pulp-capping methods [37].

9.5 Indirect Pulp Capping and Stepwise Excavation

Traditionally, complete caries removal was seen as appropriate for any and all carious lesions. However, clinical and experimental evidence has gradually been building up a far more nuanced concept for dealing with deep and extremely deep lesions [39, 47, 67, 74]. Complete carious tissue removal to sound dentine (non-selective carious tissue removal) is clearly contraindicated in several instances [41, 75]. Randomized clinical follow-up studies have shown that a stepwise excavation approach to deep carious lesions as defined in this chapter (Figure 9.1) is preferable to complete carious tissue removal procedure in one visit: the incidence of pulpal exposure was reduced, and the patients had more vital teeth with less pain and without apical periodontitis after 5 years. A partial carious tissue

removal procedure with a permanent filling placed at the same appointment may perform successfully [21, 29]; for deciduous teeth, this concept has been taken to its extreme by the so-called Hall technique, where a stainless steel crown is cemented directly over carious teeth, which shows similar, high success rates [40, 42]. However, from a clinical, endodontic viewpoint, a clear definition of lesion depth is difficult, and the evidence gained on well-defined deep carious lesions as well as on an adult population is limited. Therefore, the classical, indirect pulp-capping procedure [47], permanently leaving carious dentine behind based on one-stage carious removal, cannot currently be recommended for deep carious lesions *in adults*. It is unknown to what extent retained carious dentine will shrink, which may impair the coronal restoration and increase the risk for pulpal complications. With the less invasive carious removal strategies, an insufficient temporary or permanent coronal seal may also lead to failure including pulpal and apical pathosis [18, 56].

As discussed above, conversion of the outer lesion environment to one less conducive to caries progression can explain why a less aggressive, stepwise carious tissue removal concept is possible. The aim of the first stage of stepwise excavation is therefore to change the cariogenic environment (Figure 9.4a–b).

Active carious dentine is clinically recognized as a soft, discolored, and wet tissue (Figure 9.4c), becoming darker, harder, and drier when arrested (Figure 9.4d). After the first carious tissue removal procedure the active carious dentine is left under a calcium hydroxide base material followed by a glass-ionomer

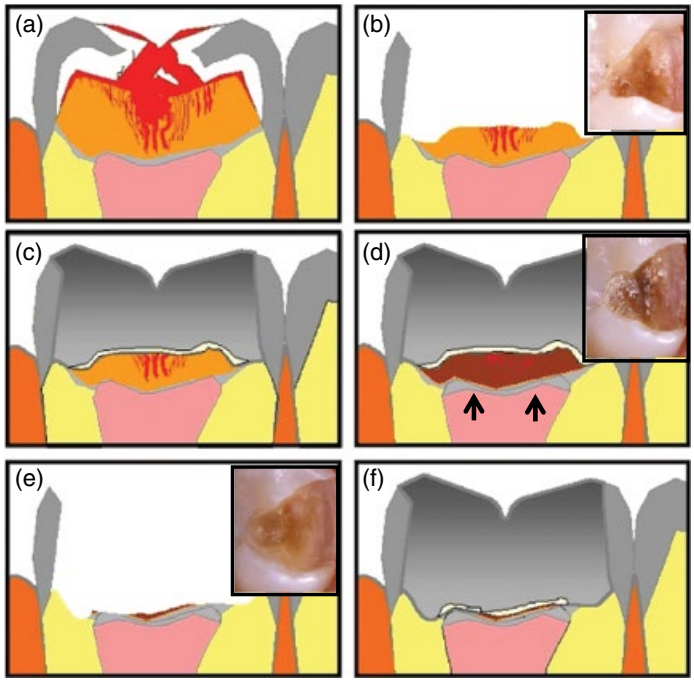


Figure 9.4 The principal changes during stepwise excavation. (a) The deep lesion has undermined enamel and an undisturbed cariogenic biomass. The active carious dentine is light brown/light yellow in color, and it is relatively wet and soft. (b) After first stage carious tissue removal leaving light and soft dentine close to the pulp (insert is a clinical example) (c) A calcium hydroxide base material and a temporary seal is made. (d) After the treatment interval (2–9 months later), the carious dentine appears darker, drier and harder (insert is a clinical example). (e) Non-selective carious tissue removal during the second stage leaves hard, tertiary dentine in the cavity floor (insert is a clinical example). (f) The permanent restoration can now be placed and on tissue that do not shrink further.

temporary restoration (Figure 9.4c). On re-entry after 8 to 12 weeks, remaining caries is removed non-selectively until dentine with hardness comparable to unaffected dentine is encountered. While it is difficult to judge, in radiographs, the quality and extent of the tertiary dentine being laid down between visits (arrows, Figure 9.4d) [54], it appears that this is not clinically relevant for the procedure.

The stepwise excavation procedure reduces the risk of pulpal exposure significantly compared with immediate, complete caries excavation [16]. The clinical result of leaving behind carious dentine in between two stages is that carious dentine changes into signs of more chronic or arrested caries [13, 55]. Arrest of carious dentine after a treatment interval is associated with a reduction in the degree of infection [13, 65]. The arrest also induces underlying changes stimulating hard tissue formation, which may add to the fact that the incidence of pulp exposures is lower for teeth treated by stepwise excavation compared with complete excavation at the first sitting. Moreover, if exposure should occur during the second excavation stage, there is evidence that the outcome following a pulp capping is improved compared to capping of exposures occurring during the first visit [51]; this may be related to the different level of infection in the first and second stage of a stepwise excavation approach. During the process of carious dentine arrestment the retained dentine shrinks; this creates a gap subjacent to the temporary restoration. The second stage is therefore also aimed at optimizing the final cavity for the permanent restoration.

9.6 Pulp Capping of the Uninflamed Pulp (Class I)

This is the conventional pulp-capping procedure done after a complicated trauma leading to superficial exposure of the pulp or after an accidental perforation. The pulp is judged to be clinically sound and without inflammation prior the treatment. The perforation is small (preferably <1 mm in diameter) and is located in the coronal third of the crown pulp chamber, e.g., a pulp horn. It is essential that the cavity be effectively sealed with a coronal permanent restoration. Given the presumption of a clinically sound pulp, the tooth has been asymptomatic before the perforation. Perforations occurring after excavation of deep caries cannot be treated by this modality. Randomized studies have documented dismal results for capping of pulp exposure after excavation of deep caries, with only around 5% pulp survival after some five years [11], confirming earlier retrospective data [5].

9.7 Pulp Capping of the Cariously Involved Pulp (Class II)

This modality applies to carious lesions where the preservation of a vital pulp is especially important, such as in teeth with incomplete root formation. The carious lesion has caused demineralization into the pulpal one fourth of the dentine or more. For pulp capping to be indicated, the pulp must be vital with no reported pain indicative of irreversible pulpitis. This is a more refined treatment protocol, because a severe microbial challenge is expected. There are promising results with this method, but it is currently based on observational data only [20, 53, 57], and randomized studies are yet lacking. Furthermore, the data on outcome with this procedure has been gathered primarily in children, and even though the carious lesion has been extremely deep, the clinical diagnosis has always been "reversible pulpitis" with no spontaneous, lingering pain.

9.8 Partial Pulpotomy

Partial pulpotomy, widely known as the "Cvek" pulpotomy, was first applied to traumatic pulp exposures in young teeth. The superficial part of a contaminated and possibly infected exposed coronal pulp is removed

aseptically and a calcium hydroxide dressing or capping material is placed over the exposure [25, 30]. Partial pulpotomy has also been applied to deep carious lesions [58–60, 92], but with a limited number of reported cases with long-term follow-up data [1]. The retention of the capping material may be improved following this modality, e.g. with complicated crown fractures in single-rooted teeth [25], but the important rationale for the procedure is that the superficial and expected necrotic and infected pulp tissue at exposure site is removed. In the young patient with incomplete root formation, the advantages are two-fold: (a) root formation may run to completion; and (b) secondary and some tertiary dentine is formed, whereby the cervical part of the tooth is strengthened and the risk of cervical fractures may be decreased. The added opening of the exposure site for the removal of potential inflamed tissue should be as small as possible, because a higher risk of failure may be expected in children with larger exposure sites [22]. However, clinical trials comparing direct pulp capping with partial pulpotomy in adults with carious exposures could not show a difference between the two treatment modalities in this clinical situation [11].

9.9 Pulpotomy

The rationale for this procedure is to remove completely the coronal pulp tissue, thereby increasing the chance of eliminating any infected pulpal tissue. It is a standard method for vital pulp treatment in deciduous teeth, where pulp chamber dimension makes pulp-capping procedures more difficult to perform. The case selection criteria for full pulpotomy in non-carious teeth are complicated crown fractures and teeth with incomplete root formation. See Figure 9.5.

As for other vital pulp therapies, signs and symptoms of irreversible pulpitis, e.g., lingering or persistent pain following thermal provocation (this may be difficult to estimate in children) are absolute contraindications. Radiographically, care must be taken to differentiate the radiolucency of a follicle associated with a root-open tooth from a chronic apical periodontitis [89].

A serial case report found that pulpotomy could be a permanent treatment option in adults in cases of reversible pulpitis [80]. There is also evidence to suggest that irreversible pulpitis can be treated successfully with pulpotomy [27, 90]. Pulpotomy can certainly be considered as an intermediate

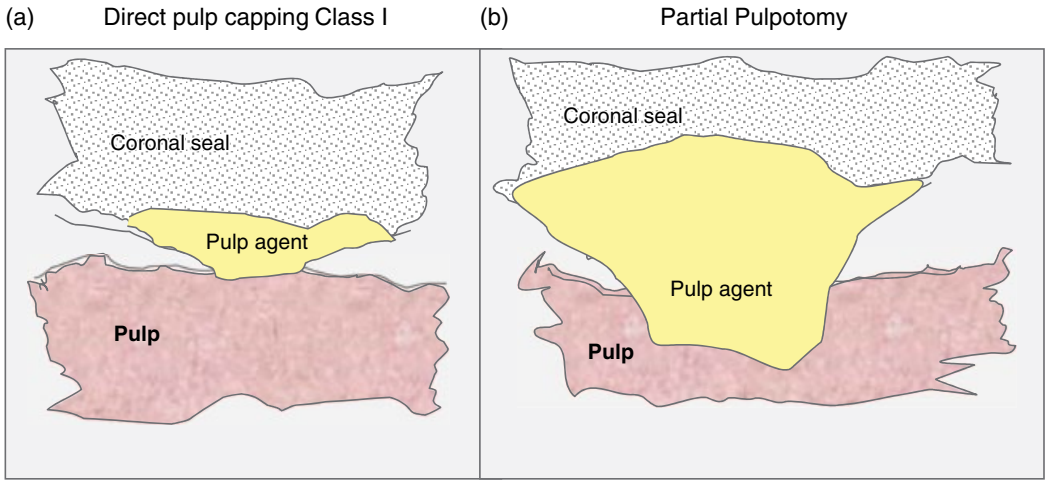


Figure 9.5 The difference between (a) direct pulp capping and (b) partial pulpotomy. Partial pulpotomy entails active removal of inflamed/infected superficial pulp tissue, and provides improved retention of the capping agent.

treatment option in managing carious vital pulp exposures of permanent teeth with closed root apices serving as a substitute for extraction when root canal treatment for some reason cannot be performed [2]. Clinical testing comparing pulpotomy with conventional pulpectomy is needed for a complete assessment of the indications for this procedure.

9.10 Treatment Details for Pulp-preserving Techniques

General: Isolation by rubber dam, peripheral caries removal, and chemical surface disinfection is mandatory in all clinical situations with deep caries and/or when an exposure has already occurred. After surface disinfection, sterile instruments are used for removal of carious tissue and cavity preparation. Hemostasis must be obtained without a thick coagulum. This is usually achieved within 5 min. by rinsing with sterile saline or sodium hypochlorite. If hemostasis is not obtained, pulpectomy should be performed. Calcium-hydroxide- or calcium-silicate-based cements are used for capping as well as pulpotomy procedures, with a minimum thickness of 1.5 mm. A temporary or permanent filling placed under aseptic conditions completes the procedure. Clinical and radiographic follow-up is mandatory.

Stepwise excavation: After rubber dam isolation and surface disinfection, the superficial discolored and wet tissue (Figure 9.4c) is selectively removed to the extent that a temporary restoration can be made. Light and softened dentine is left under a calcium hydroxide base material followed by a glass-ionomer restoration (Figure 9.4c). After 8 to 12 weeks, the cavity is re-entered and the darker, harder, and drier dentine closer to the pulp is removed non-selectively with sterile excavators, leaving only dentine that is firm with a hardness comparable to sound dentine (Figure 9.4d). This process also eliminates the potential problem associated with shrinkage following arrestment of carious dentine, as the base of the cavity following second stage should not be able to shrink further, optimizing the interface between the permanent restoration and cavity.

Accidental or traumatic perforations (Class I): After rubber dam isolation and surface disinfection, the cavity and the perforation are gently rinsed with sterile saline to remove debris and to establish a clean and non-bleeding wound. Spraying of the exposure site as well as application of pressure by a cotton pellet may provoke hemorrhage. If hemostasis is not achieved after 5 minutes, the pulp-capping option is discarded and pulpectomy initiated. After placement of the capping material, the permanent restoration is placed, preferably immediately after capping but at any rate within days, as this improves the outcome significantly [5].

Capping of the cariously involved pulp (Class II): After rubber dam isolation and surface disinfection, carious tissue is removed with the guidance of a detector dye, and the use of loupes or an operating microscope as well as enhanced illumination is mandatory. Excavation continues until no dye is visible at the dentine margins of the exposure. No enlargement of the exposure site is attempted. After application of the capping material, it is covered by a glass-ionomer cement, followed by a bonded coronal restoration. A two-step procedure is recommended if necessary for clinical control of setting of the capping material. A case following the principles for a Class II pulp cap is shown in Figure 9.6.

Partial pulpotomy: After rubber dam isolation and surface disinfection, an end-cutting diamond operating at high speed with copious irrigation is used to remove approximately 2 mm of the underlying coronal pulp tissue. When the superficial pulp cutting is accomplished and hemostasis is obtained, the procedure is identical to that used for pulp capping of a carious pulp exposure. See Figure 9.5.

Pulpotomy: After rubber dam isolation and surface disinfection, a sterile diamond bur in a high-speed hand piece under water spray is used to remove the entire coronal pulp tissue,

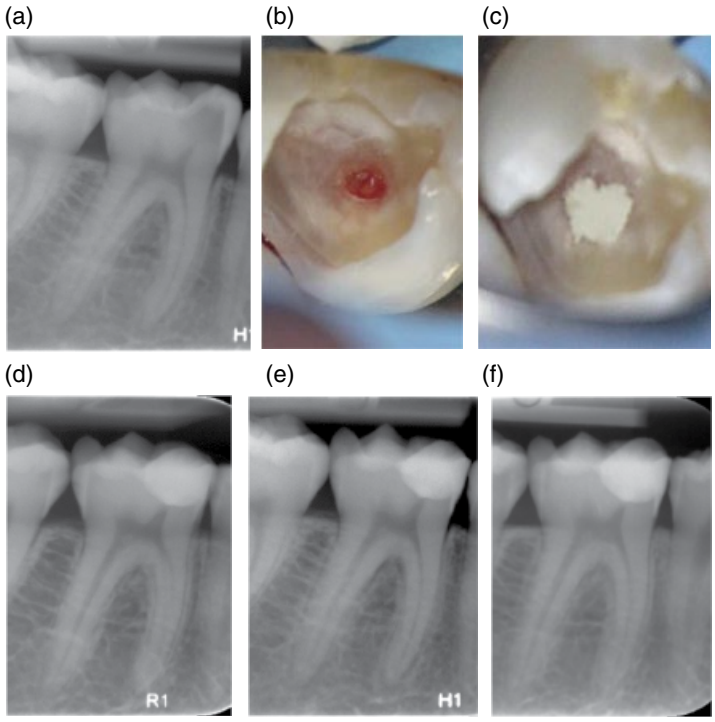


Figure 9.6 Clinical example of a Class II direct pulp capping. (a) Preoperative radiograph reveals a deep lesion and no apical pathosis. (b) After non-selective carious removal using the operating microscope, no carious dentine is retained, and hemorrhage is stopped. (c) The capping agent (MTA) is placed in increments. (d) Postoperative radiograph with permanent restoration in place (e) 1-year follow-up and (f) 2-year follow-up. Case courtesy of Dr Phu Le.

with the amputation wound located at the orifice 1–2 mm into the root canal. For single-rooted teeth, the amputation wound should be placed in the cervical region 2–3 mm below the collar of the tooth. A paper point (blunt end toward the capping material) is a simple tool for compacting the capping material for proper placement and sealing of the material. A thickness up to 4–5 mm of the cement is desirable, if possible. A two-step procedure is recommended if clinical control of setting of the amputation material is needed, but may not be necessary [48]. A clinical case is demonstrated in Figure 9.7.

9.11 The Available Evidence for Relative Merit of Treatment Procedures for Vital Pulps

9.11.1 Scenarios for Multiple Outcomes and Follow-up Treatment

Based on published data, it is possible to make simulated scenarios for establishing a cost-effectiveness analysis of various vital pulp therapies [77]. Such an analysis does not improve on existing data but can expand our interpretation of them. In an example on the outcome of vital pulp treatment [77] a scenario was created by the following assumptions: (i) a deeply carious molar with a sensible, non-symptomatic (painless) pulp exposed during caries excavation, subjected to (ii) either direct pulp capping using calcium hydroxide or MTA followed by direct restoration, or root canal treatment (vital pulpectomy) with a cast coronal restoration. The carious lesion could be approximal as well as occlusal. Various potential scenarios were followed using a state transition diagram simulating the lifetime of such a tooth (Figure 9.8). Based on data collected before 2014, the model suggests that an optimal scenario for successful direct pulp capping is younger patients (< 40 yrs) with exposures occlusal in posterior teeth, whereas older patients (>40 yrs) with approximal exposures in anterior teeth represent the other extreme,

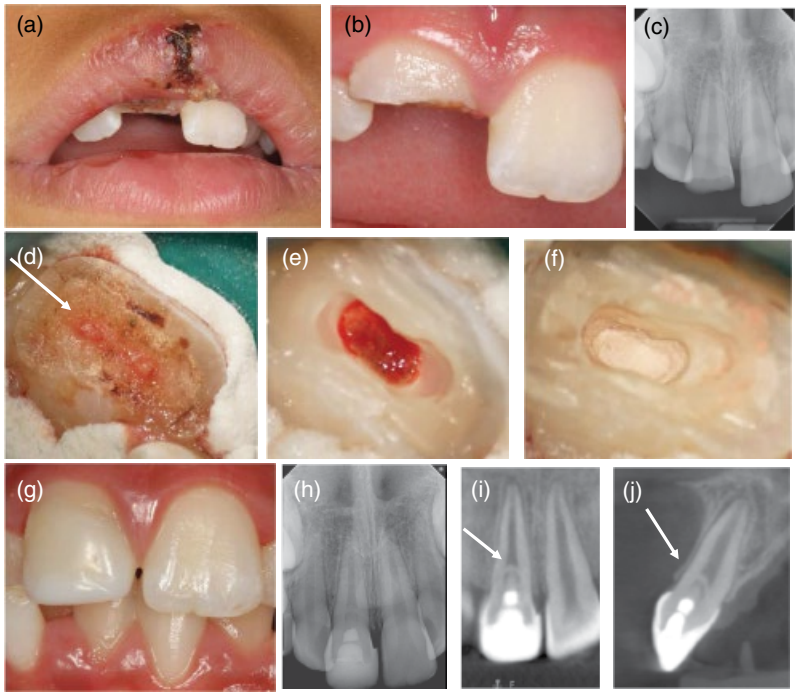
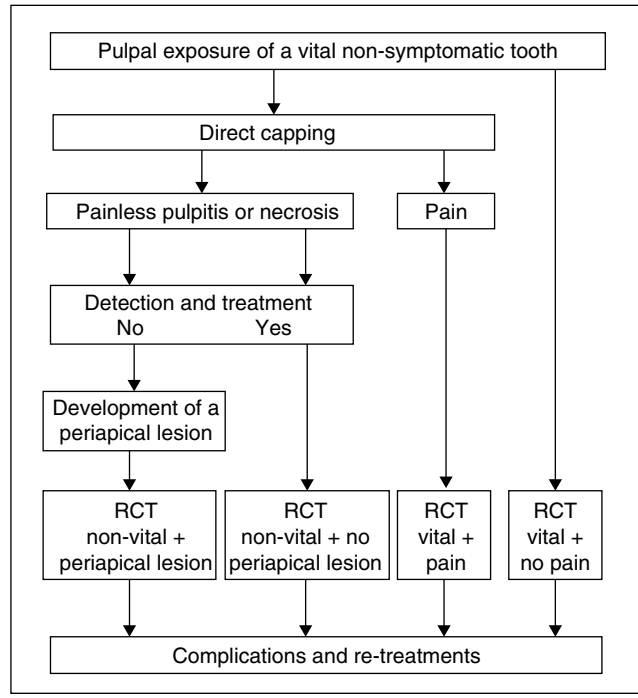


Figure 9.7 (a) A 10-year-old girl presenting for endodontic treatment 4 days after emergency treatment of the lacerated lip with sutures, but no dental treatment. (b) Clinical and (c) radiographic examination showed a complicated crown fracture with (d) exposure of pulp horns (white arrow). The patient had an exaggerated non-lingering response to cold test and a positive response to electric pulp testing reflecting a diagnosis of reversible pulpitis. (e) After adequate dental dam isolation, approximately 3 mm of coronal pulp was removed with copious water spray with a diamond-coated bur. (f) A calcium silicate-based cement was placed immediately over the pulp tissue, followed by a layer of glass ionomer and a composite restoration. (g) After 1-year follow-up, the patient remained asymptomatic and the tooth responded to electric pulp test without any signs or symptoms of discoloration or (h) radiographic signs of apical pathosis. (i, j) Small-volume cone beam computer tomography (CBCT) revealed a dense mineralized zone. Case courtesy of Dr Anibal Diogenes.

Figure 9.8 State transition diagram illustrating the lifetime of a tooth with an exposed, sensible, non-symptomatic pulp given various event options.



where many cases more quickly needed endodontic treatment after complications.

Figure 9.9 illustrates a failed direct pulp capping (Class II) where capping of an approximal exposure, while technically well performed, resulted in pulpal necrosis, infection and apical periodontitis. Given the poorer prognosis of treatment of established apical periodontitis compared to pulpectomy, the choice of capping the inflamed pulp in this case increased the cost and probably shortened the life span of the tooth.

9.11.2 Strategies for Obtaining Optimal Clinical Evidence

Treatment options for pulps affected by well-defined deep caries are stepwise caries removal or direct pulp capping, partial or full pulpotomy, or pulpectomy. For extremely deep carious only pulp invasive tretaments are recommended. Current evidence point to a more conservative approach in the sense that the pulp may survive the less invasive techniques better than previously thought. Further evidence for selection of the appropriate technique must rely on data from carefully performed clinical studies,

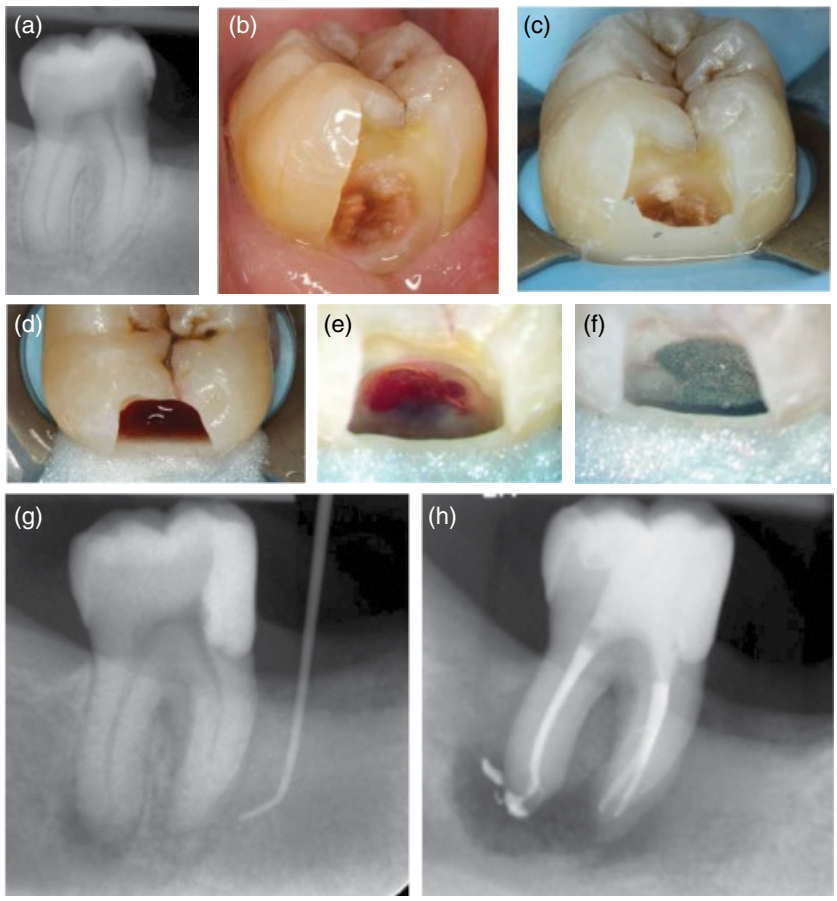


Figure 9.9 Clinical example of a Class II direct pulp-capping procedure (male, 48 years). (a) Preoperative radiograph reveals a deep lesion and no apical pathosis. (b) Carious tissue removal shows that the lesion is located deep down at the approximal site. (c) An small build-up filling is placed at the margin to facilitate asepsis. (d) A bleeding exposure is noted. (e) Hemostasis is about to be reached. (f) Application of calcium silicate cement. (g) After 3 months, the patient presents with a sinus tract and apical periodontitis. (h) An immediate postoperative radiograph of completed root canal treatment. Case courtesy of Dr Pim Buurman.

preferably with a randomized design. Important aspects in designing such trials include well-defined inclusion criteria; e.g. a better definition of lesion size and lesion activity may lead to more accurate data analysis (Table 9.2, Figure 9.10); power calculations for sample size estimation; central randomization; and unbiased, i.e. blinded recording of outcome.

9.11.3 Lack of Standardization in Clinical Trials

Table 9.3 is a list of recent, randomized clinical trials covering the topics presented in this chapter. Although these studies were not meant to be compared, they all have the same inclusion criteria: a deep carious lesion and signs of reversible pulpitis. However, the treatments vary from pulpotomy to indirect pulp capping and stepwise excavation, reflecting that no global consensus or tradition as yet dominate the choice of treatments of vital pulp therapy. Moreover, details vary widely even when the treatment is supposedly the same, indicating the need for more standardization for the performance of clinical trials.

Table 9.2 Carious dentine status and prognosis following a pulp-preserving treatment.

Clinical signs of dentine surrounding a pulp exposure	Prognosis following a direct pulp capping Class II in relation to carious activity status of suggested event
Soft, wet, and yellowish discolored dentine	+ (Performed in relation to a deep or extremely deep carious lesion)
Firm, dry, and dark discolored dentine	++ (Performed after second stage during stepwise excavation)
Hard, dry, and gray dentine	+++ (Performed in medium-sized lesion or as an iatrogenic exposure (pulp horn))

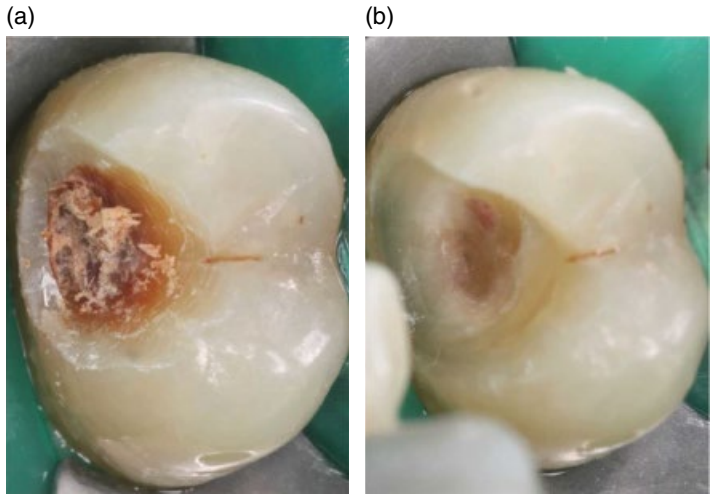


Figure 9.10 Clinical criteria for inclusion in trials. More precise data of the lesion environment that is going to be capped will lead to a better platform for inclusion criteria into trials, hence a better interpretation of data (a) Carious tissue removal in a lesion with signs of arrested lesion activity, reflecting a darker and more firm appearance of dentine. (b) When pulp exposure takes place the prognosis has been reported to be better than for exposures under an active lesion environment with a much lower level of infection than an active lesion environment [51]. Case courtesy of Dr Miguel Marqeus.

9.11.4 Choice of Capping Materials

Even though some studies suggest a histologically better response beneath MTA compared with calcium hydroxide, (i.e. a more homogenous dentine bridge and less or no inflammation) [87] and clinical cohort studies also favor MTA [62], calcium hydroxide is still considered suitable for successful pulp-preserving treatments. Several studies have failed to show a significant difference between the two types of capping agents. It appears that the size of the exposure is more important than the choice of capping material [22]. A randomized, multicenter practice-based study designed to reflect better the real clinical situation also did not find a significant difference in treatment outcome with the two materials, although, again, the tendency was for MTA to give better results [38].

Adherence to protocol and the establishment of a bacteria-free cavity and pulp wound is probably a key issue [20, 24], and the translation of this basic knowledge to clinical practice is probably the greatest challenge.

9.11.5 Status of Current Clinical Evidence

Data may be collected from all levels of the evidence pyramid, from simple case series to cohort and comparative studies of varying quality. However, of the many randomized, controlled clinical trials in humans (Table 9.3) comparing different capping materials, several may be underpowered, which makes inferences to clinical practice questionable [43, 46, 84]. In addition, an adequate randomization procedure is not always done, but is critical for adequate interpretation of the intervention effect [34]. Even well-designed randomization procedures run the risk of ending with case characteristics (in casu, lesion depths) having an uneven distribution between the groups tested. [50]. Thus high-level, scientific recommendations can hardly be made for proper choice of capping materials based on current studies [73].

Table 9.4 is a suggested cross-tabulation relating each intervention procedure to its current level of evidence.

Table 9.3 Examples of randomized clinical trials of capping agents and pulp-preserving interventions.

	Jang et al. 2015 [43]	Kang et al. 2015 [46]	Hashem et al. 2015 [37]	Bjørndal et al. 2017 [11]
Study material				
Teeth	Permanent	Deciduous	Permanent	Permanent
Caries	y & n	y	y	y
Pulp status	Reversible pulpitis	?	Reversible pulpitis	Reversible pulpitis
Age	>19 yrs	3–10 yrs	Median 28 yrs	Median 29 yrs
Trial				
Intervention	15%	?	20%	20%
Power	80%	?	80%	90%
Group size	23	47 to 48	36	156–158
Material	ProRoot MTA vs Endocem	ProRoot MTA vs OrthoMTA vs RetroMTA	Glass ionomer vs Biodentine	Dycal/Ketac cem
Variable	Age; occlusal vs axial surface	—	Cavity size; CBCT	Procedure

Table 9.3 (Continued)

	Jang et al. 2015 [43]	Kang et al. 2015 [46]	Hashem et al. 2015 [37]	Bjørndal et al. 2017 [11]
Protocol				
Indirect pulp capping			x	
Stepwise excavation				x
Immediate capping	x			x
Pulpotomy		x		
Outcome assessment				
Observation time	1 yr	1 yr	1 yr	5 yrs
Pulp test	x	x	x	x
Radiography	a	a	x	x
Clinical pulpal diagn	a	a	x	a
Outcome variable				
Material	nsd	nsd	nsd	
Method			CBCT more sensitive	
Treatment procedure				Stepwise better than immediate
Other	axial cavity worse			

a: moderate precision of registration
nsd: no significant difference

Table 9.4 Cross-tabulation of treatments and diagnostic factors with estimation of evidence when applicable (++ treatment comparison RCT-based), (treatments observation-based +).

	Diagnostics factors						
Treatments	**Sound dentine (trauma)**	**Age young**	**Age old**	**Radiograph deep carious lesion (pulpa 1/4)**	**Radiograph Extremely deep carious lesion (pulpa 4/4)**	**Reversible pulpitis**	**Irreversible pulpitis**
Direct pulp capping Class I	yes (+)	yes	yes	no (++)	no	yes	no
Direct pulp capping Class II	no	yes	yes	yes (+)	yes (+)	yes	no
Partial pulpotomy	yes (+)	yes	no	no (++)	no	yes	no
Pulpotomy	yes	yes	yes	? (+)	?	yes	yes
Stepwise excavation	—	yes	yes	yes (++)	no	yes	no
Indirect pulp capping (ad modem Kerkhove et al.)	—	yes	yes	yes (+)	no	yes	no
Partial excavation/ selective carious tissue removal.	—	yes	?	?	?	yes	no

9.12 Future Perspectives of More Advanced Biological Approaches

The cells involved in dentinal repair of reparative type are often described as secondary odontoblast-like cells, and they originate from differentiated stem cell/progenitor cells. These cells can be expressed in various places (niches) around the pulp and are typically associated with blood vessels [36]. Thus, repair processes are not restricted to the area close to the original dentine, and the ubiquitous presence of these cells explains why both a superficial pulp capping as well as a deeper pulpotomy can be carried out with a successful dentine bridge formation. However, it is still unclear exactly which cells of the stem cell/progenitor cell populations actually participate in dentinal repair [35]. These cells express numerous mesenchymal and embryonic cell markers, reflecting a variation in the stem cells/progenitors in the pulp [83]. In short, more research is needed before clinical practice can truly benefit on a larger scale.

Improved information on the origin of cells involved in dentinal and pulpal repair will obviously guide the content of future treatment protocols on revitalization and regenerative endodontic procedures, including techniques and materials for pulp capping and pulpotomies (see Chapter 10).

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10

Vital Pulp Extirpation

John Whitworth

10.1 Introduction

Teeth with vital pulps contain perfused pulp tissue, which in most circumstances responds clinically to thermal and electronic sensibility tests. This includes everything from the pristine tooth with a completely healthy pulp, to the grossly carious tooth with extensive, irreversible pulpitis. The important inference is that teeth with vital pulps contain little or no microbial infection, at least in their apical regions [95]. As such, their clinical management differs from those with completely necrotic, infected pulp systems and established apical periodontitis.

Assuming the desired outcome of endodontic treatment is to prevent or heal apical periodontitis, teeth with no preoperative infection or radiographically-detectable periapical lesion enjoy higher levels of treatment success than those with necrotic/infected pulps and periapical inflammation [76]. Against this background, dentists may approach the treatment of teeth with vital pulps as a simple technical exercise – a rapid and predictable mechanical task, rewarded by instant pain relief, a pleasing postoperative radiograph and guaranteed long-term periapical health. Often overlooked is the attention to detail necessary to manage the vital pulp space under aseptic conditions, to prevent clinically-acquired (nosocomial) infection during treatment procedures and to safeguard against microbial colonization during a lifetime of function. In order to promote the long-term survival of root canal treated teeth, dentists should also minimize the risks of unduly harsh mechanical and chemical treatments on structurally important hard tissues, and restore them appropriately after treatment [74].

Although an account of this sort must contain some discussion of instruments, materials and techniques, emphasis is placed wherever possible on biological principles for the infection-controlling and tissue-preserving management of teeth with compromised, vital pulps.

10.2 Pulpectomy – Definition and Rationale

Pulpectomy is quite simply the removal of vital pulp tissue from a tooth, severing the soft tissues close to the apical foramen, in a site that is likely to be sterile. The empty canal space is then sealed, and a coronal restoration applied to protect the pulp system from the oral environment and to safeguard the tooth against fracture.

The most obvious indication for pulpectomy is symptomatic irreversible pulpitis, where vital pulp tissue is removed for pain relief and to prevent the cascade of events that will ultimately lead to complete pulp necrosis, established pulp-space infection and the development of apical periodontitis.

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

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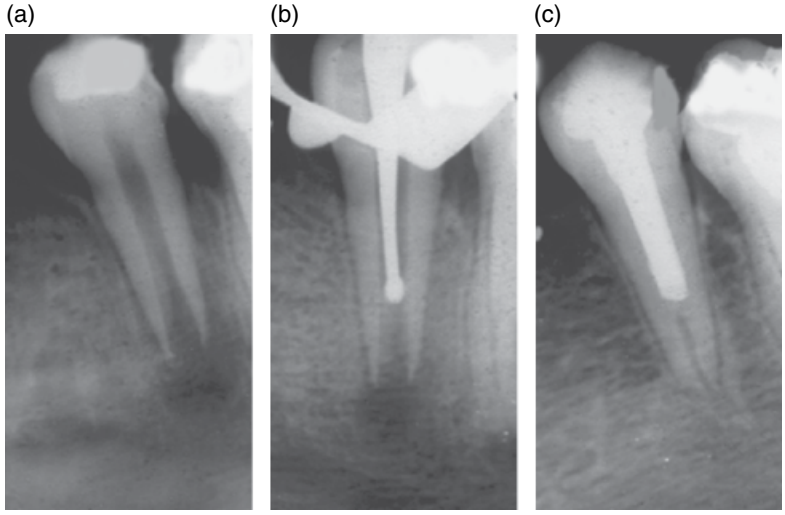

Figure 10.1 Deep pulpotomy in an immature tooth with partial pulp necrosis. (a) Tooth at presentation with recurrent caries and apical periodontitis. Root formation is incomplete. (b) After isolation of the tooth, removal of caries and restorative materials, bleeding pulp tissue was identified 5 mm from root end with a long-shanked round bur. The wound was dressed over an extended period with multiple changes of non-setting $Ca(OH)_2$. (c) One year after treatment was initiated, the tooth apex is complete, the walls of the root have thickened and a mineralized bridge divides the pulp from overlying restorative materials.

Carious pulp exposure in an asymptomatic tooth is considered by many as an example of asymptomatic irreversible pulpitis and unfavorable for conservative pulp-capping or pulpotomy procedures. In this situation, pulpectomy currently has a stronger base of evidence for the preservation of periapical health than vital pulp therapies, particularly when followed up in the long-term [10]. A notable exception is the immature permanent tooth, where there may be stringent efforts to preserve the radicular pulp, at least until root apices have fully formed (apexogenesis) and root walls have gained thickness and strength. Here, a deep pulpotomy is usually performed, resecting pulp tissue at a level judged to be sterile, before applying a wound dressing and sealing coronal restoration in an effort to exclude new infection and preserve vital pulp functions (Figure 10.1).

Extrapolating from this and from the evidence of successful pulpotomy outcomes after pulp-exposing trauma [21], interest has re-surfaced on the potential of carefully-conducted pulp capping [14] and pulp chamber pulpotomy procedures [59, 94, 106, 119] as predictable treatments for cariously involved permanent teeth. Many attribute the apparent success of these procedures to the properties of calcium silicate cements [65], yet reports also point to the meticulous attention to detail required at all stages of clinical management if favorable outcomes are to be predicted. Attractive though they may appear, these are not quick and easy fixes and long-term outcome data are currently lacking. Patients may be offered such treatment options, but only after open discussion of the potential benefits and risks. They are experimental interventions at this stage with less certain outcomes than pulpectomy.

Another form of asymptomatic irreversible pulpitis is internal inflammatory root resorption, where pulpectomy is the only sure way of arresting the process.

Pulpectomy may also been indicated for teeth with normal, healthy pulps that are at risk of serious damage from restorative procedures. Examples include the decoronation of teeth as overdenture abutments, and heavy crown preparations to compensate for tooth misalignment or rotation. Elective

pulpectomy may also be considered when teeth cannot be restored to long-term function without using the pulp space for posts or other retentive devices.

Elective pulpectomy has also been suggested in teeth with signs of progressive root canal obliteration following trauma. Clinical data suggest that between 10 and 20% of these may become infected and necrotic within 20 years [41, 99] and some have seen this as justification for intervention, removing the pulp before it becomes impossible to access. Relevant to this discussion is that the hard tissue deposition in such cases does not always represent an organized diminution of the pulp space by concentrically-retreating odontoblasts; a situation that inevitably leaves a central, though reduced pulp space for instrumentation (Figure 10.2). In many circumstances, the hard tissue deposited is rather chaotic in structure, sometimes even osteodentine, with no central lumen to negotiate with endodontic instruments (Figure 10.3). Others use the same data to suggest that the risk of pulp breakdown is relatively low and does not justify prophylactic treatment. Reports from the pre-microscope era suggest that 80% of such teeth could be successfully managed if apical periodontitis did subsequently develop [22], and it is likely that the situation will have improved since then. The advent of 3D scanning and guide-sleeves to control bur alignment open further opportunities for safe, conservative, and efficient entry to diminished pulp spaces [137], and undermines further the case for routine prophylactic intervention.

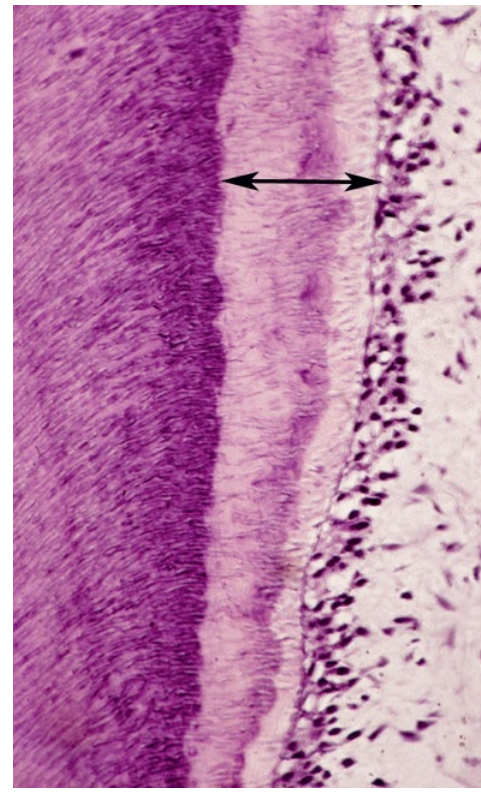

Figure 10.2 Photomicrograph showing the locally accelerated deposition of tertiary reactionary dentin matrix in response to cavity preparation. This physiological response to mild trauma will continue for as long as the stimulus is present. It will not lead to pulp necrosis or the complete mineralized obliteration of the pulp space.

10.3 The Challenge of Effective Local Anesthesia

The acutely inflamed vital pulp presents a recognized challenge for effective local anesthesia. Most clinicians will have encountered patients with a profoundly numb lip after inferior alveolar nerve block injection, but with a tooth that remains exquisitely sensitive to operative intervention – the so-called "hot pulp" [81]. Explanations are complex and incompletely understood, though peripheral sensitization caused by increased excitability of sensory nerves in the pulp and central sensitization caused by changes in pain processing within the central nervous system play important parts. Approaches include the administration of additional injections to increase the dose, and expose greater lengths of sensory nerve trunks to anesthetic solution, such as higher, Gow-Gates [35] and Akinosi [3] nerve blocks in the mandible. Supplementary infiltrations with articaine have also received considerable interest in the mandible [56]. Some of the most effective

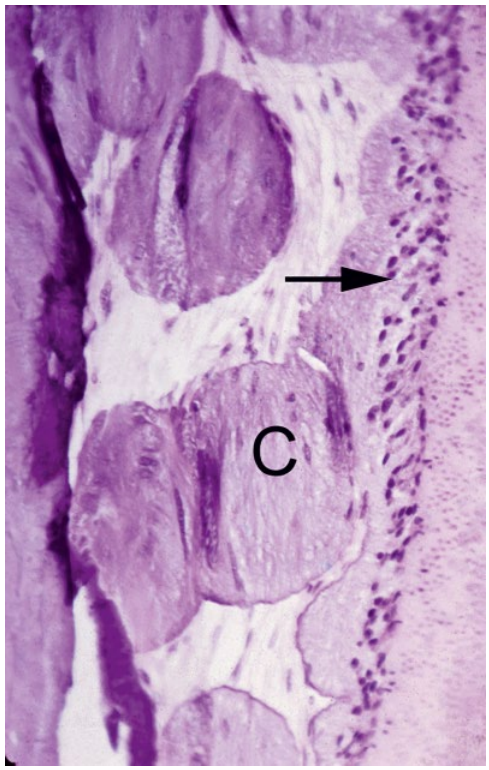

Figure 10.3 Photomicrograph showing mineralized obliteration of the pulp space. The mineralized deposits (C) are not associated with odontoblast function, but a sign of poor pulpal health. Note that the odontoblast layer (arrowed) has been included in the mineralized tissue. The progress of mineralization will be unpredictable, may be associated with pulp necrosis, and may leave a pulp space that is difficult or impossible to negotiate with endodontic instruments.

supplementary techniques involve local, intraosseous delivery of anesthetic solutions, including intraligamentary, intraseptal and frank intraosseous delivery through a perforation in the buccal cortical plate [81]. The practice of medicating acutely inflamed pulps with "mummifying pastes" to induce necrosis is discouraged, as case reports have shown extensive tissue damage from the local actions of these highly toxic materials [112]. On occasion, it is still necessary to inject directly into inflamed pulp tissue in order to secure effective anesthesia, a procedure associated with momentary pain, but usually rewarded with comfort as sensory nerves are concussed by the entry of solution into the non-compliant pulp space.

Investigators have also explored the potential of premedication with a variety of non-steroidal and steroidal anti-inflammatory agents as a means of enhancing anesthetic success [103]. No premedication regime has yet achieved widespread acceptance. Occasionally, patients may require sedation to supplement local anesthesia for the comfortable extirpation of a pulp.

10.4 Principles of Effective Pulpectomy

A central tenet of vital pulp management is the exclusion of microbial infection, principally from the oral microbiota, but also from commensal and environmental pathogens derived from the patient, dental personnel, clinical materials, and equipment [78]. Standard measures are summarized in the following paragraphs.

10.4.1 The Aseptic Working Environment

10.4.1.1 Preparing the Tooth for Treatment

Pulpectomy should generally be limited to teeth that can be isolated from the oral environment during treatment and are capable of restoration when it is complete. Ideally, all restorations should be removed from teeth before endodontic treatment and the remaining tooth tissue assessed for caries, cracks, volume and distribution [1]. In this way, unrestorable teeth are identified early, teeth that need support from an orthodontic band and/or preoperative restoration can be appropriately managed, and final restorations can be planned from the outset. Defective restorations must be removed and caries excavated to the brink of pulp exposure before penetrating the

pulp chamber with fresh, sterile burs, reducing the risk of pulp-space contamination. It is also good practice to eliminate supragingival calculus and plaque from teeth before isolation with rubber dam and entry to the pulp space.

10.4.1.2 Isolation with Rubber Dam

Isolating teeth from the oral environment with a well-fitting rubber dam is a recognized prerequisite for safe and effective endodontic practice. This standard measure has the support of professional bodies [27, 28], yet its adoption in general practice may vary [5, 73, 132]. The rubber dam is an integral part of the efforts to exclude infection from the operating field [20]. It also protects patients from pungent and caustic irrigating solutions such as NaOCl. Published reports have shown improved healing of periapical lesions after treatment including rubber dam isolation, though the effect is not strong [58, 128]. Routine rubber dam isolation will also protect the oropharynx from mislaid instruments. While very few endodontic instruments are likely to be swallowed or inhaled in general practice [118], the avoidance of even one such incident is probably sufficient justification for the routine application of this simple and inexpensive measure. The benefits of using rubber dam, including improved vision, improved patient comfort and the improved working environment for the dentist, should also be emphasized. Given the unequivocal, causal association of infection with periapical inflammation [70], randomized controlled studies that compare endodontic outcomes with and without the use of rubber dam are unlikely to be conducted and would be unethical to perform. The timing of rubber dam isolation should be tailored to clinical circumstances. On the grounds of infection control, it is probably wise to isolate teeth before the pulp space is entered, though this must be balanced against the risks of excessive tissue removal and catastrophic tooth perforation if the dam impairs bur alignment and depth orientation.

10.4.1.3 Disinfection of the Operating Field

Removal of caries and defective restorations and the application of rubber dam are mechanical means to prevent contamination of the pulp space during treatment. As an additional safeguard, surface or field disinfection of the isolated tooth and rubber dam is mandatory. Documented procedures include the use of hydrogen peroxide, iodine or chlorhexidine preparations that are applied before entering the pulp space. Field disinfection is common in many surgical disciplines and was promoted in endodontics by researchers taking microbiological samples from root canals [71]. The relative effectiveness of different regimes remains uncertain [77].

These preoperative measures represent small infection-controlling steps that build an environment compatible with optimal infection control, thus swinging the balance of probabilities in favor of clinical success. Practitioners who adopt these measures are likely to be focused on infection control as a key outcome determinant and carry that approach through all of their decision making and actions.

10.4.1.4 Sterile Instruments

Surgical instruments must be sterile before use on patients. In many instances, endodontic instruments are pre-sterilized by the manufacturer, and often marked for single use only. For instruments supplied in a non-sterile state, contemporary washer/disinfector and autoclaving protocols allow them to be satisfactorily cleansed of environmental contaminants before use. The decontamination of used endodontic instruments is more problematic. Practices of "sterilizing" endodontic instruments during treatment by chairside immersion in hot salt, glass beads or even molten tin [29] are now largely historical. Even with contemporary and rigorously controlled sterilization practices, the intricate blades of endodontic shaping tools are notoriously difficult to clean and effectively sterilize. The specter of prion-based

disease has presented a special challenge, and the impossibility of eliminating such materials from endodontic instruments led in 2007 to the recommendation that all endodontic reamers and files should be considered as single-use instruments [131]. Despite these potential concerns, there have been no reported cases of prion-based disease linked to dental interventions.

Handling the blades of endodontic instruments and points of filling materials must be avoided to reduce the risks of nosocomial infection with microorganisms derived from skin and from the clinical environment [78]. The negative potential of nosocomial infection was highlighted in two CBCT-based endodontic outcome studies in which approximately 20% of teeth without apical periodontitis developed lesions within 1–5 years of pulpectomy [31, 87]. The true impact of nosocomial infection remains a hot topic for research, as does the use of 3D imaging in assessing endodontic outcomes.

10.4.2 Tissue-preserving Access

To eliminate tissue from the entire pulp space, it is necessary to unroof all canal entrances. Shaping, cleaning and filling will then be optimized. At the same time, endodontic treatment aims to provide reliable foundations for effective long-term restoration, and it makes sense to adopt principles of minimally invasive dentistry and preserve as much dentin as possible. This may be particularly important in the cervical regions of teeth, where large access cavities and extensive pre-flaring of canal entrances may inadvertently weaken teeth and increase the risk of fracture [34].

10.4.3 Pulp Tissue Resection and Elimination

The term pulpectomy may conjure images of clean surgical resection, yet the procedure is rarely so precise. Although the notion was discussed more than 90 years ago [23] and modified instruments for the purpose have been suggested [67], microscalpels are not currently available to neatly divide the apical pulp tissue with its rich network of neurovascular bundles. After unroofing the chamber, vital pulp tissue in young teeth may sometimes be removed by engaging a barbed broach with a half-turn and pulling to remove an apparently intact pulp from the canal (Figure 10.4). Alternatively, endodontic files can be extended to the desired working length in rotational or rasping motions to sever the pulp and shape the surrounding hard tissues. This sort of resection inevitably involves the compression, twisting, stretching, and tearing of apical soft tissues (Figure 10.5) [82], including the afferent trigeminal sensory nerve fibers that will be preserved within the apical wound. Few patients experience significant persistent symptoms after vital pulp extirpation [80], though lasting symptoms associated with endodontic deafferentation injury are occasionally encountered [79].

Figure 10.4 Extirpation of a young vital pulp with a barbed broach.

In the necrotic, infected case, it is logical to extend instrumentation as close to the root canal terminus as possible in order to remove microbial biofilm, infected dentin and decomposing pulp tissues from an avascular and defenseless environment [111]. In such cases, the practice of securing and maintaining apical patency has also been advocated as a means to preserve access to the deepest parts of the canal system throughout instrumentation and disinfection [75]. In teeth with vital pulps, the situation is somewhat different. Since the radicular pulp and dentin

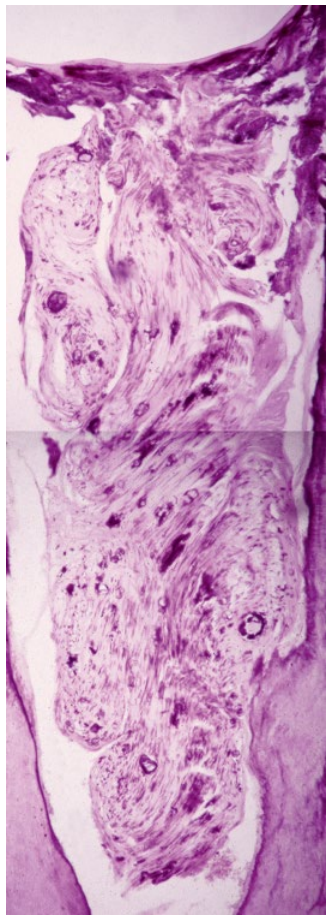

Figure 10.5 Remaining pulp tissue after pulpectomy with a root canal reamer. The pulp tissue was twisted all the way to the apical foramen. (Courtesy of Dr H. Nyborg.)

walls are uninfected, it is suggested that the wound may legitimately be placed 1–2 mm from radiographic root end [48, 51] with the expectation that strict asepsis will allow the apical pulp stump to survive. A shorter section of disturbed apical pulp stump may re-vascularize more readily after the traumas of resection than a longer one, whereas an apical pulp stump greater than 2 mm may not recover [110]. Experimental studies in the 1970s provided evidence that if the pulp was removed aseptically and the canal was subsequently filled 2–4 mm short of working length, healing would occur [40, 83] presumably by reorganization of a sterile blood clot. Contemporary electronic apex locators offer good length control and facilitate our ability to contain instrumentation within 0.5–1.0 mm short of the point of maximal constriction [72]. At this point, the severed pulp wound is likely to be as small in cross-section as possible, and this will not be influenced by the degree to which the canal walls are enlarged (Figure 10.6a). Extending instrumentation beyond the point of maximal constriction may open the apical wound to a greater cross-section and promote bleeding into the canal that may compromise apical seal. In the case of apical stop-preparations (see later) it may also promote the over-extension of filling materials (Figure 10.6b). Although it is necessary to touch periapical ligament with a small file in order to secure

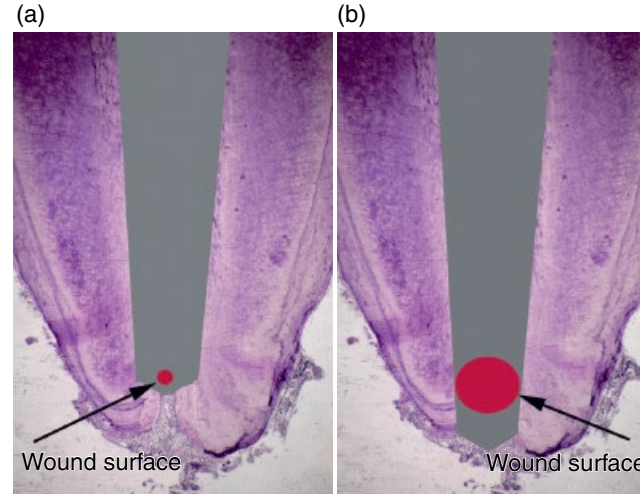

Figure 10.6 (a) Apical pulp resection at a level that minimizes the cross-sectional area of the wound. The surface area of the soft tissue wound will not increase, regardless of how widely the canal walls are instrumented. (b) Over-extension of the same instrument greatly enlarges the wound surface, as well as promoting bleeding into the canal. With a modestly tapered instrument, apical resistance form may be lost, risking the over-extension of filling materials.

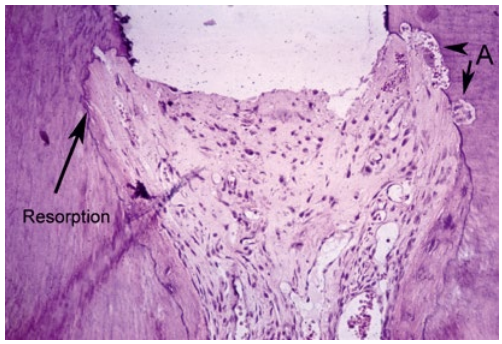

Figure 10.7 Tissue in the apical 2–3 mm of a root canal subsequent to pulpectomy. The lateral walls of the root canal have undergone resorption on a broad front (arrow) and in localized areas (A); no inflammatory cell infiltrate can be seen.

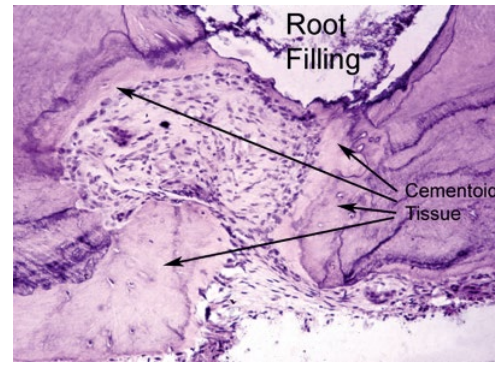

Figure 10.8 Tissue in the apical 2–3 mm after pulpectomy and root canal filling. After the initial dentin and cementum resorption following pulpectomy (Figure 10.7), the tissue has undergone repair. Apposition of cementum-like tissue can be seen around the entire pulp space (arrows).

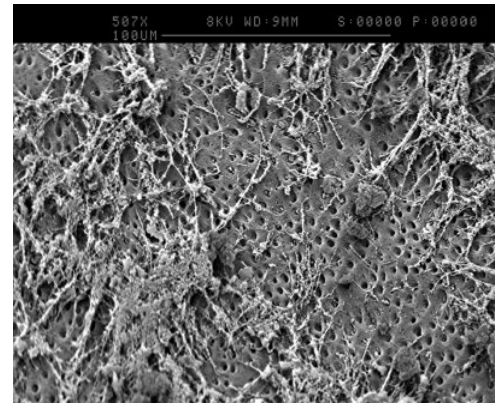

Figure 10.9 SEM image of debris-covered root canal wall immediately following pulp extirpation with a broach.

an electronic apex locator "zero" reading, the maintenance of apical patency may be unwise in vital cases, where an already disrupted apical pulp stump would be repeatedly impaled with instruments during canal instrumentation. Yet the apical part of the pulp and surrounding periodontal tissues have great ability to recover following non-infectious tissue damage. This is normally characterized by an initial resorption of apical root dentin and cementum (Figure 10.7), providing access for periapical connective tissues to the narrowly enclosed apical pulp that is severely damaged during pulpectomy [82]. The pulp that may survive will form an osteodentin wall that will close off the vital pulp tissue from the subsequent root canal filling. In most instances, the damaged pulp tissue is re-vascularized and a fibrous connective tissue replaces the pulp. When the apical resorption is arrested, cementum will form to replace lost dentin and further close off the pulp space (Figure 10.8) [30, 83]. Damage caused by over-instrumentation will heal with apical repair provided the root canal is adequately and aseptically filled after pulpectomy [8, 40]. Maintaining asepsis remains the key to successful treatment.

Even when the pulp appears to have been removed intact, it should not be anticipated that it has been completely eliminated from complex webs, fins, cul-de-sacs, isthmuses, and lateral canals. Neither should it be expected that all odontoblast processes will have slid neatly from their tubules along with the main body of pulp tissue. The walls and ramifications of freshly-extirpated pulp systems contain variable amounts of cellular material, blood and micoorganisms (Figure 10.9). Key to the successful management of this situation is that the apical pulp stump is maintained in a healthy and uninfected condition, and that as much cellular debris/avascular pulp material is removed from canal walls and ramifications as possible.

Such material may compromise the seal of the root canal filling and provide substrate for any microorganisms that may find their way into the canal system during a lifetime of function.

10.5 Canal Shaping

Most root canals require some mechanical enlargement if they are to be adequately cleaned and filled. The prepared canal should facilitate easy placement of the root filling while limiting periapical extrusion. The optimal root canal shape for this purpose is a smoothly tapering conical form, with its narrowest point apically and widest point coronally. Issues that are currently discussed include:

- Degree of taper: what is sufficient for effective cleaning and filling but without unnecessary sacrifice of tissue?
- Apical shape: is there an optimal apical shape for cleaning during pulpectomy and for containment of the root filling within the tooth?
- Wall contact: is it necessary or even desirable for instruments to shape all walls of the root canal during pulpectomy?

Degree of taper (Figure 10.10a): The classical, standardized root canal instruments had a taper of 0.02 mm mm^{-1} or 2%. Some of the most popular shaping techniques have involved step-back or crown-down instrumentation with such instruments. Applied sequentially at 1 mm increments from the root apex, they created nominal tapers of 0.05 mm mm^{-1} or 5%. The apparent success of such techniques suggests that this may have been sufficient for effective cleaning and filling, though other techniques have been successful with considerably smaller (e.g. Lightspeed [93]) and larger (e.g. GT rotary [17]) degrees of taper.

Apical shape: Resistance to the apical displacement of root filling materials during compaction can be accomplished with a stop preparation or with tapering resistance form

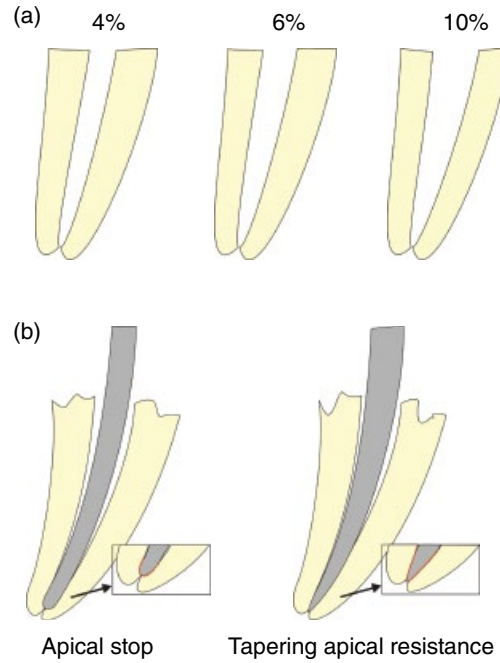

Figure 10.10 (a) Differing degrees of canal taper, but how much is sufficient? (b) Apical stop and tapering resistance form.

(Figure 10.10b). Traditionally, advocates of preparing vital cases short of the root-terminus have practiced stop-type preparations [48], while others may have advocated preparation up to or slightly beyond the point of maximal constriction with tapering apical resistance form (Figure 10.10b). While proponents of each approach may be able to support their views with successfully-treated cases, there is little high-level clinical evidence to advocate one approach over the other. During pulpectomy, apical canal enlargement must be sufficient to resect pulp tissue, but in contrast with infected, necrotic cases, there is probably no need to enlarge the apical canal dimensions greatly, since the canal walls are unlikely to be colonized by microorganisms.

Wall contact: Mechanical instrumentation undoubtedly plays a part in cleaning canal walls during pulpectomy, and it is also necessary for the development of taper and apical resistance form.

It is, however, fallacious to imagine that reaming and filing instruments will evenly

contact all root canal walls, particularly those of wide, oval, ribbon-shaped, or irregular cross-section [91, 133]. Even with efforts to guide them into canal irregularities, instruments cannot be controlled as they work around canal curvatures and many areas of canal wall will remain untouched. Instruments will equally not enter complex secondary anatomy, including lateral canals, webs, fins and isthmuses or areas of internal resorption. This may present limitations on the capacity of instruments to remove cellular debris in the case of pulpectomy and necrotic material/microbial biofilms and contaminated dentin in the case of on-vital cases. Irrigating solutions with antimicrobial and soft tissue dissolving properties are therefore necessary to compensate for the limitations of instrumentation.

10.5.1 Instrument Motion

Shaping instruments should be advanced into root canals in a manner that will optimize their cutting efficiency, while minimizing risks of tooth or instrument damage. Specific instrument motions are often recommended by manufacturers on the basis of instrument design (see section 10.5.2). During initial canal negotiation, small hand instruments are usually advanced with gentle reciprocating or watch-winding motions between index finger and thumb (Figure 10.11). This may be punctuated by short periods of low-amplitude up-down filing motions to open the coronal portion of the canal and allow instruments to advance more freely. Further canal enlargement with hand instruments may then continue with watch-winding and outward, rasping motions against canal walls. Balanced Force motion [98] is a development of watch-winding in which an instrument is first lightly engaged into the canal by gentle clockwise rotation. The instrument is then rotated anticlockwise with sufficient axial pressure to balance the force with which the instrument is seeking to reverse out of the canal. With the tip of the instrument engaged in dentin, stress builds within its shank until the canal walls are unable to restrain it, and dentin is cut, often with an audible and palpable click. The instrument is then advanced lightly clockwise once again to pick up dentin chippings before removal from the canal for cleaning and inspection. Balanced Force motion has proved to be safe and effective [19], though it is not the easiest method to conceptualize or master. Great tactile sense is needed to gauge the degree of rotation and the axial force that can be safely applied. This varies greatly for instruments of different sizes and for instruments manufactured from stainless steel and nickel titanium.

Hand pieces such as the Giromatic, Endocursor, Endolift, and M4 [38, 60, 61] delivered reciprocating file movements analogous to watch-winding, with equal clockwise/anticlockwise rotation (60° clockwise/anticlockwise in the case of EndoCursor).

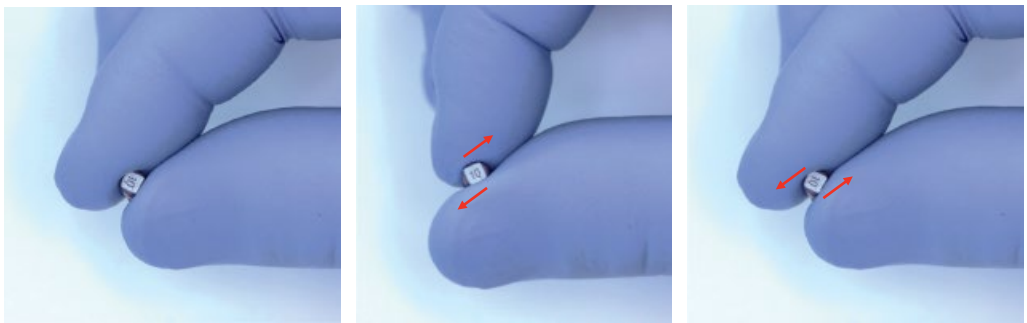

Figure 10.11 Manual, reciprocating, or watch-winding motions for initial canal negotiation with small, precurved stainless steel files.

Since the introduction of nickel titanium (see section 10.5.2), most instruments have been driven in continuous clockwise rotation. Instruments rotating clockwise have a tendency to engage walls and screw into the canal, risking damage to the instrument and the tooth. The application of engine-driven instruments to root canals is defined by the engineering terms speed and feed. Speed refers to the speed of instrument rotation and is specific to the instrument system (e.g. 300 rpm for ProTaper Universal; 600 rpm for BioRaCe, 800 rpm for XPEndo Shaper). Feed refers to manner in which the instrument advances into the canal. For many systems, this is a so-called pecking motion, applying momentary episodes of light axial pressure to advance the instrument by cutting the canal walls. Pecks are typically limited to 3 or 4 before removing the instrument for cleaning and inspection and to irrigate the canal. An alternative method is brushing, where the rotating instrument is inserted into the canal and dragged or brushed outwards against the walls. By flaring the canal in this way, space is created for the instrument to enter more deeply on its next insertion. This method of feed is less likely to engage canal walls and allow instruments to screw in, but may sacrifice greater amounts of tooth tissue. Interest has re-emerged in reciprocation, but with motors that deliver unequal clockwise/anticlockwise motion. The net result is full instrument rotation, but punctuated by short periods of reversal which reduce the risk of screwing in. Reciprocating instruments are also less likely to fracture than those in continuous rotation [88], and may be fed into the canal with pecking or brushing motions.

10.5.2 Shaping Instruments

Root canals are generally enlarged with instruments manufactured from stainless steel or nickel-titanium (NiTi) alloys, while other areas are cleaned by the mechanical and chemical actions of irrigants and medicaments.

Stainless Steel

Stainless steel engine reamers and ISO specification hand files are not ideal tools for the optimal shaping and cleaning of root canals, especially those with any degree of curvature (Figure 10.12).

Mechanically-driven Gates-Glidden drills and Peezo reamers have been routinely used to enlarge the straight coronal portions of root canals. But they are aggressive and inflexible, and may rapidly work harden and fracture if flexed or driven into canal curvatures.

Stainless steel instruments become less flexible as their diameter increases, and their stiffness may cause transportation errors, even in the most expert hands. Up-down filing motions with stainless steel instruments have a special propensity to transport, while rotational watch-winding and Balanced

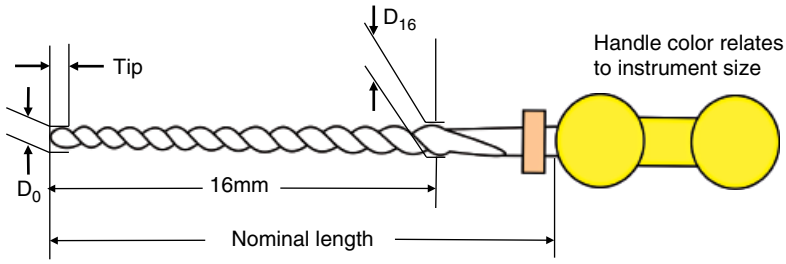

Figure 10.12 Schematic representation of an ISO endodontic hand instrument. D_0 denotes the instrument diameter close to the tip. This determines the nominal size of the instrument: size 10, D_0 = 0.01mm; size 30, D_0 = 0.03mm. All have a 16 mm bladed region, with a taper from D_0 to D_{16} of 0.02mm/mm or 2%. Thus a size 20 instrument is 0.52 mm in diameter at D_{16}. Nominal length denotes the overall length of the instrument, typically 21, 25 or 31 mm. Handle color relates to instrument size: yellow = 20 or 50; red = 25 or 55.

Force motions are considered safer [98] (see section 10.5.1). Reports indicate that stainless steel files of size 25 and greater will transport curved canals and for this reason, it is common to limit apical preparations with stainless steel instruments to around size 25 or 30 in the curved canals of molars [75]. Paradoxically, classic morphometric studies on the apical dimensions of root canals [47] may suggest that this would leave many vital pulps incompletely resected and many root canals under-enlarged for optimal cleaning, especially if prepared short of the canal terminus. Some of this may be compensated by the lengthy preparation times required for stainless steel instrumentation and the extended periods of time during which canals are bathed with tissue-dissolving NaOCl solution.

Nickel-titanium

Most dentists are now well acquainted with the benefits of NiTi instrumentation. The rapid evolution of NiTi shaping instruments and engine-driven systems has been driven both by clinical need and by the commercial interests of manufacturers. All stakeholders, whether patients, dentists or commercial companies, have an interest in efficient systems that will quickly and safely shape canals without undue risk of instrument fracture or damaging teeth. Files are often packaged in systems with size-matched paper points for drying and gutta-percha points for filling. The pace of change in this field makes it necessary to seek up-to-date information from sources including current scientific literature and the web sites of leading manufacturers.

The following is a brief summary of NiTi alloys, instrument design, and key milestones in their evolution. This will allow future developments to be placed in context.

10.5.3 Fundamentals of NiTi Alloys

The unusual properties of equiatomic nickel-titanium alloys were observed by Beuhler at the US Naval Ordinance Laboratory in the 1960s [18]. Challenges in the processing and manufacture of objects from these alloys limited their use in endodontics until 1988 when Walia described the first NiTi root canal file [130]. NiTi alloys are stronger (higher compressive and tensile strength) and less stiff (greater flexibility, lower modulus of elasticity) than stainless steel alloys [139]. This means that NiTi instruments can be used to negotiate and enlarge curved canals with less risk of transportation than stainless steel instruments of equivalent size. Above room temperature, the metal alloy generally adopts an austenitic or "parent" crystal structure (Figure 10.13a) [125]. Endodontic instruments are usually manufactured to be straight in this austenitic form. The application of stress by bending or twisting NiTi instruments changes their metal structure by a process of stress-induced martensitic transformation (Figure 10.13b). In this state, they are able to absorb considerable energy without permanent deformation and spring back to their original, straight, austenitic form when stress is released (Figure 10.13b–a). This super-elastic behavior is observed when NiTi instruments emerge from curved root canals straight and with regular cutting flutes after episodes of shaping activity. For this reason, it is difficult to pre-curve NiTi instruments before use, and pre-curved stainless steel instruments are often necessary for initial scouting and bypassing ledges. Unlike NiTi, bending of stainless steel alloys results in permanent slippage of the metal grain structure and any attempt to straighten a bent stainless steel instrument runs the risk of work hardening and fracture.

NiTi instruments are not, however, indestructible. Excessive stress may cause permanent deformation of the metal structure, which does not spring back fully when forces are removed. The clinical manifestation is an instrument that emerges from the canal bent or with irregular cutting flutes. Some, if not all, of this may be restored by heating NiTi instruments above a threshold temperature in a process of reverse transformation (Figure 10.13c). This is an example of shape memory, as the distorted instrument "remembers" and returns to parent austenitic state (Figure 10.13a). Such restoration may be

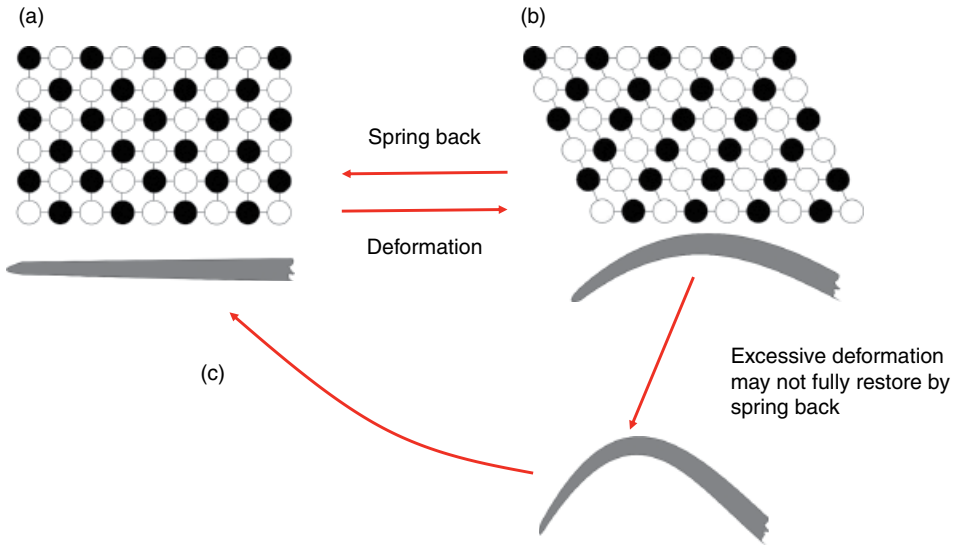

Figure 10.13 Schematic representation of stress and temperature-induced phase changes in NiTi alloys. (a) Non-deformed NiTi file in its parent, austenitic form. (b) Flexion of the instrument results in stress-induced martensitic transformation to detwinned, deformed martensite. Provided instrument deformation is not excessive, the release of stress allows springback to the parent austenitic form; an illustration of super-elastic behavior. Excessive deformation may not fully restore by springback. (c) The application of heat above a threshold temperature may allow partial or complete restoration of the alloy to its parent austenitic form by reverse transformation; an illustration of shape memory. (Adapted from Thomson 2000.)

claimed by manufacturers to allow the safe-re-use of NiTi instruments. What the user does not know is whether cracks or other unrestorable changes have occurred within the crystalline framework of the metal, and if the instrument will fracture on its next use. It is probably wise, if economically possible, to regard endodontic shaping instruments as single use [46] and certainly to discard them at the first signs of distortion.

The phenomenon of shape memory has been utilized more recently to produce instruments of radically different designs and behaviors. Shaping tools have been produced that are straight when chilled in cool water or with a pulp-testing refrigerant, but which adopt innovative helical or other forms when warmed to body temperature and adopt their parent form (see section 10.5.5: Innovations since 2013).

As the understanding of these immensely complex alloys continues to grow, proprietary thermomechanical treatments have been developed to manipulate them into still more helpful forms. R-phase NiTi represents an intermediate stage between austenite and martensite, and in this form, instruments may be expected to exhibit greater flexibility and still greater cyclic fatigue resistance than conventional austenitic forms. M-Wire is another thermomechanically derived variant, containing both austenite and martensite at room and body temperature and with a concomitant increase in flexibility and fatigue resistance. Martensite/austenite hybrid alloys are also available with controlled memory, and are thus capable of pre-curving before clinical use, while once again restoring to parent state after heating [139].This is a rapidly developing area, and detailed accounts of the latest technology will quickly date.

From a clinical perspective, the unique properties of NiTi alloys allow the manufacture of instruments with:

1) Increased flexibility: virtually eliminating the classic shaping errors of transportation, even with larger instrument sizes. This permits greater enlargement of canals apically, potentially improving vital

tissue resection, canal debridement and deep irrigant exchange without the risk of apical transportation.
2) Reduced fracture risk: NiTi instruments are able to absorb considerably more energy than stainless steel files before they fracture. They are also far more resistant to fracture in cyclic fatigue than stainless steel alloys.

Despite these improvements, the risk of instrument fracture remains a concern. Risks may be minimized by following the manufacturer's instructions, receiving adequate training, using instruments patiently and gently, and learning to feel when instruments are failing to advance. Any instinct to push should be resisted. Many NiTi instruments have the propensity to screw into root canals and can reach a point where they are locked tightly into dentine. They may reach a locked position where they are incapable of further rotation and advance, while the motor continues to turn, risking torsional overload and fracture. This is the phenomenon of taper lock, and can be avoided by gentle, progressive work, and using a torque-controlled motor that stops turning or may even reverse when the instrument is not free to rotate. Work-hardening or cyclic fatigue is caused by repetitive cycles of stretching and compression at metal grain boundaries as instruments are rotated around curves, with the development of microcracks that propagate through the instrument and result in failure. Measures to control this include rotating the instrument at the correct speed, avoiding the use of mechanically-driven NiTi instruments in acutely curved canals where the degree of repetitive compression and stretching is excessive, keeping the instrument moving up and down in the canal to avoid the concentration of flexure cycles at one level on the instrument, and keeping the instrument rotating in the canal for the minimum time possible. The disposal of instruments after a single use is again helpful.

The concluding note is that current NiTi alloys have allowed unprecedented opportunities for the safe, rapid and predictable manual and mechanized shaping of curved root canals during endodontic treatment, including pulpectomy.

10.5.4 Fundamentals of Instrument Design

Contemporary NiTi instruments are manufactured by a variety of sophisticated milling and twisting techniques and can be produced in an almost limitless range of sizes, tapers and cutting-flute designs. The quest for quicker, sharper, safer and more efficient instrument systems has resulted in a large succession of tools, each claiming to achieve their goals in a distinctively superior way than rivals. Figure 10.14 illustrates a selection of variously configured NiTi endodontic instruments, all designed to perform similar shaping and cleaning tasks.

Some features that have been the focus of innovation are described in the following paragraphs.

1) *Tip shape:* Almost all NiTi endodontic files are now manufactured with non-cutting tips. Not only is the leading point rounded, but the transitional angle between the smooth head and bladed region are gently blended (Figure 10.15). These features reduce the risk of engaging and perforating canal walls and of canal transportation during shaping.
2) *Taper:* Because of their increased flexibility, NiTi instruments can be supplied with increased tapers of 6, 8, 10 or even 12% without significant risk of transportation. Sometimes, instruments of greatly increased taper have relatively shorter bladed regions in order to avoid excessive dentin removal in the cervical third of the canal. Equally, some manufacturers have designed instruments with short cutting heads and no taper, while others have justified the benefits of making taper variable throughout the length of the instrument in an effort to limit the risks of instruments screwing into canals,

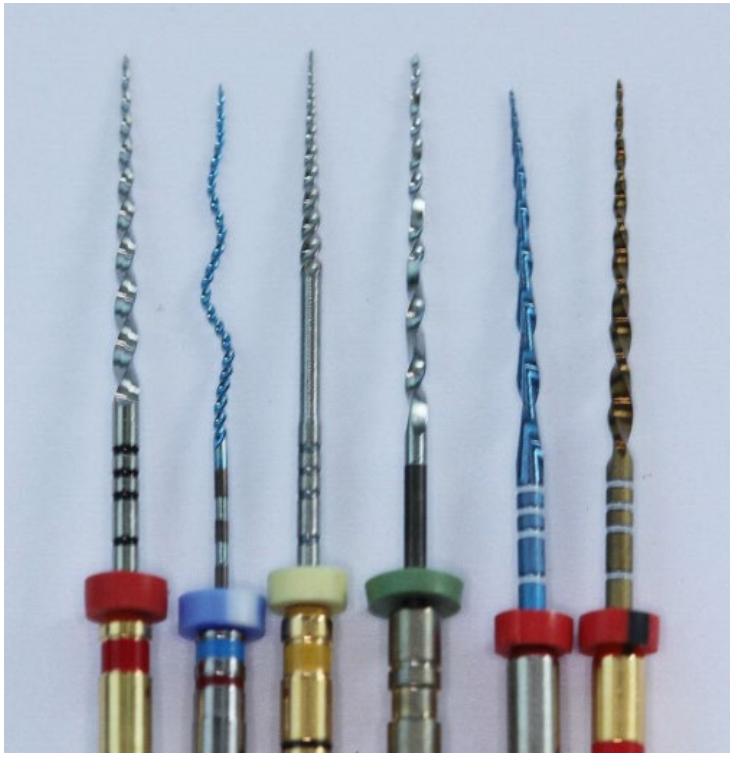

Figure 10.14 NiTi canal shaping instruments of varying design. All bring many benefits compared with stainless steel instruments. Which of their specific features have the greatest bearing on safety and performance remains uncertain.

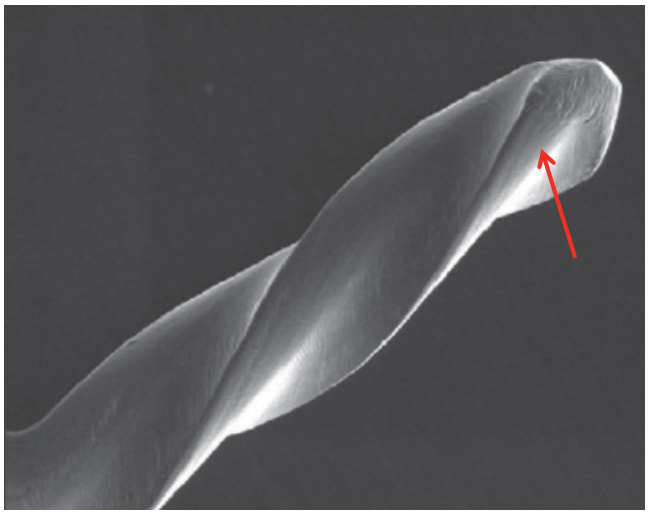

Figure 10.15 Instrument with non-cutting tip and smoothened transition angle between tip and bladed region (arrow) to minimize risks of transportation and canal wall damage (image courtesy of Paul Dummer).

engaging too much of the canal wall at any given time or removing excessive volumes of dentin.

3) *Rake angle:* This is the angle formed by the leading edge and the cross-sectional radius of the instrument as it rotates in the canal (Figure 10.16a). Many early instruments had negative or neutral rake angles to gently plane root canal walls, while others were more active and apparently aggressive, or – depending on your view – efficient, at dentin removal.
4) *Helical angle:* This is the angle formed between the blade and the long axis of the instrument (Figure 10.16b). When this is large, the blades of the instrument are close together, creating a greater density of cutting blades, but less space between them into which the cut chippings of dentin and other debris can gather. The space available between instrument blades into which debris may gather is termed the chip space (see 6 below). The relative benefits of the number of blades and space for material to be removed can be hotly debated. A constant helical angle throughout the length of an instrument can promote its tendency to screw into a canal, and this has prompted some manufacturers to vary the helical angle throughout the length of the instrument or at points along its bladed region.
5) *Core diameter and flute depth:* instruments milled with a narrow core of metal at their center are likely to be more flexible than comparable instruments with a thicker core (Figure 10.16c). A thin central core also allows the blades to be deeper, which may make them more efficient (aggressive) at cutting and less liable to clogging with cutting debris, provided they are regularly cleaned.
6) *Cross-sectional chip space:* Many file designs have emphasized the cross-sectional shape of the instrument, not only in terms of metal mass and flexibility, but again to emphasize the open space available for cutting debris to gather (Figure 10.16d). Instruments that provide little chip space may begin to cut efficiently, but as soon as their blades are clogged with debris, their action is compromised. This may impede the progress of instrumentation and even promote stress and instrument fracture. All instruments should be used for just a few seconds at a time before removing from the canal for cleaning of the blades and inspection for distortion. During this interval, the canal should be thoroughly irrigated to remove cutting debris and refresh tissue-dissolving and antimicrobial activity.
7) *Surface treatment:* The manufacture of most NiTi instruments involves milling shapes into sections of wire and leaves surface imperfections. These may become the focus of stress-concentration during use and predispose crack propagation and premature fracture. Strategies to improve the surface quality and consistency of endodontic instruments have included electro-polishing, and surface implantation with nitrogen ions. In a quite different approach, the manufacturer of at least one instrument system has roughened their surface by bead blasting to facilitate scrubbing of the root canal walls as they vibrate gently up and down.

The design features of instruments have complex interactions and cannot be considered in isolation. A tool with a sharp, positive rake angle may, for example, be expected to cut efficiently, but if the cross-section allows little chip space, and the helical angle is unfavorable, performance may not be so efficient. This remains a highly active area for research and development.

10.5.5 The Evolution of NiTi Engine-driven Instrument Systems

The evolution of NiTi shaping instruments has seen shifts in focus to address emerging challenges. Several generations of instruments to 2013 have been succinctly summarized [37].

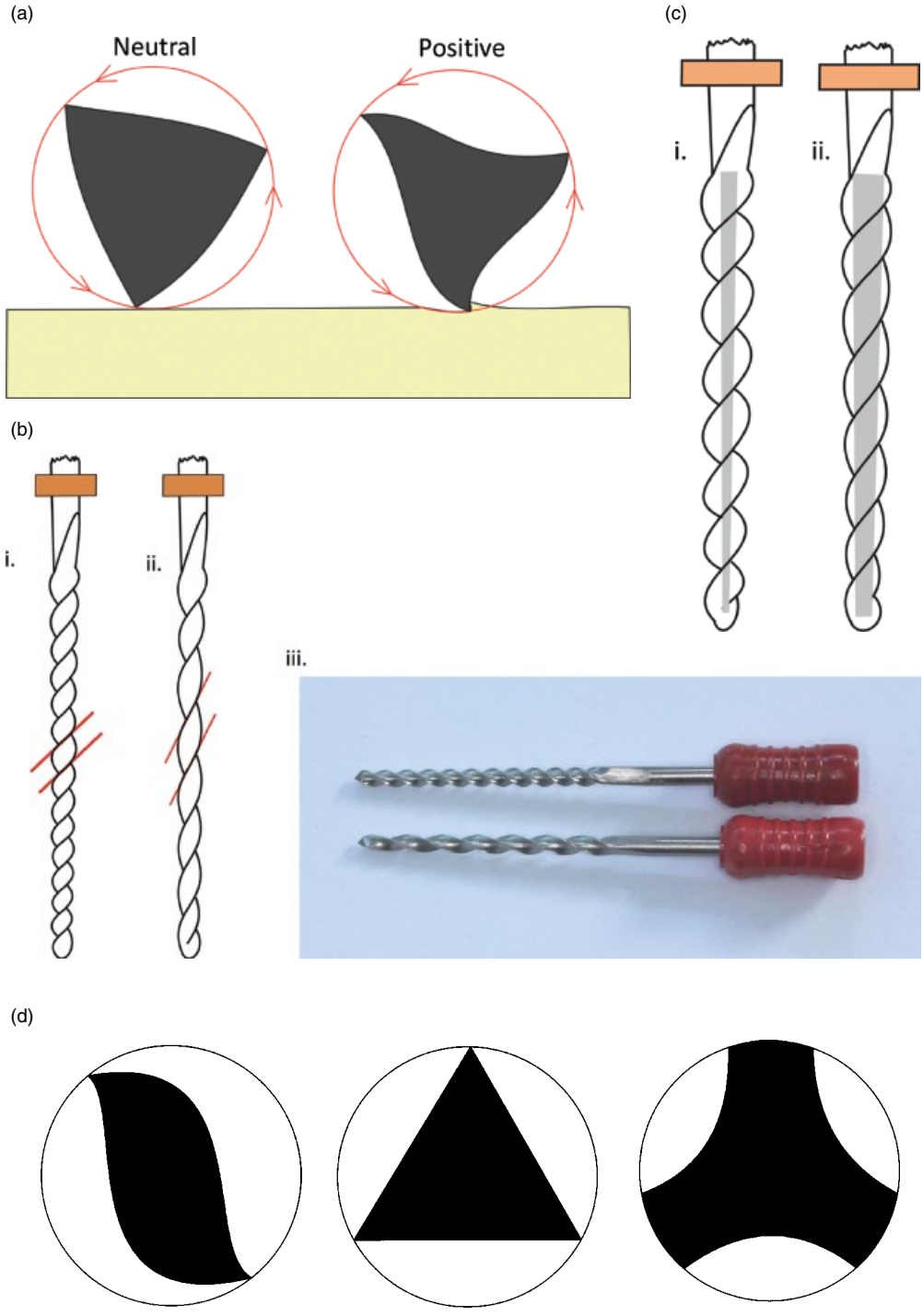

Figure 10.16 Design features of NiTi shaping instruments. (a) Rake angle influences the angle of engagement between instrument and dentin. Neutral angles plane the root canal surface. Positive angles cut more aggressively. (b) Helical angle: i) Shallow angle results in closely spaced blades, while steeper angle ii) results in greater spacing iii) The differing helical angles of traditional hand files (above) and reamers (below). (c) Core diameter: Instruments with a narrow central core of metal i) are more flexible than those with a thicker core ii) They also have deeper flutes and greater chip space and may therefore cut dentin more efficiently. (d) Chip space: the cross-sectional shape of an instrument may influence the amount of space available for cutting debris to accumulate.

First Generation: Increasing Taper and Radial Lands

Credit should be given to Drs John McSpadden in 1992 and Ben Johnson in 1994 for introducing mechanically-driven NiTi instruments to dentistry. The first were 2% taper and somewhat prone to fracture. These were rapidly followed by 4% and 6% tapered ProFiles and accompanying orifice shapers of even greater taper. In parallel, Dr Steve Buchanan was developing Greater Taper files of 6, 8, 10 and 12% taper, while Wildey and Senia were taking a different tack with their taperless Lightspeed instruments – a series of 22 instruments with small cutting heads reminiscent of Gates-Glidden drills, in sizes and half sizes from 20 to 140 [33].

A common feature of this first generation was a triple U-shaped cross-sectional design, creating three chip spaces, neutral or slightly negative rake angles for gentle plaining of root canal walls, and radial lands which were believed to keep instruments centered in the canal. All tapered instruments had a consistent taper throughout their length and constant helical angles.

Second Generation: Positive Rake Angles and Variable Taper and Helix

Key developments included positive rake angles for more active and efficient (aggressive) dentine removal while still avoiding transportation, smaller helical angles for more spaced cutting blades and a reduced tendency to screw into the canal. The ProTaper system incorporated variable tapers along the length of the shaping instruments to promote the enlargement of different parts of the canal by different instruments, and diminish the risk of instruments engaging along their entire length (taper lock). ProTaper Finisher instruments maintained fixed tapers for final apical enlargement. Other instruments including K3 and Quantec incorporated changes in their radial lands and chip-spaces in an effort to optimize the efficiency and safety of canal enlargement. BioRaCe instruments exhibited discontinuous helical angles to diminish screw-in effects. They were also electro-polished in an effort to smoothen out manufacturing imperfections. BioRaCe instruments were labeled as "bio" because the relatively large apical sizes and modest tapers of standard apical finishing instruments (size 35 and 40, 4% taper) were considered to fit the most common anatomy of teeth, while avoiding unnecessary tapering and tissue removal coronally.

Third Generation: Improvements in Metallurgy

The period from 2007 saw NiTi alloys undergoing thermomechanical manipulation and endodontic instruments with even greater flexibility and fracture resistance. M-Wire, comprising 508 Nitinol which has been subject to specific tensile stresses and temperatures [43], was the first, with early examples including Dentsply ProFile GT Series X, ProFile Vortex and Vortex Blue (the blueness created by oxides generated by the closely-guarded manufacturing process). In 2008, SybronEndo developed R-phase instruments by twisting rather than milling NiTi wires, with examples including K3XF and Twisted File. Controlled Memory Wire appeared in 2010, with HyFlex and Typhoon instruments as leading examples. These could be pre-curved before insertion into the canal, offered great flexibility, and could be restored to original shape by autoclaving – a benefit to those re-processing and re-using their instruments.

Fourth Generation: Reciprocation, Single-file and Single-use Instruments

Until 2011, most mechanically-driven NiTi instruments were driven in constant clockwise rotation. In 2008, experimentation revealed that a single ProTaper Finisher 2 file (size 25, 8% taper) could safely and efficiently shape many canals without the need for further instrumentation [134]. Out of this grew reciprocating file systems WaveOne and Reciproc, both manufactured from M-Wire, both involving the selection of a single instrument to shape the canal in question, and both

being driven in clockwise/anticlockwise rotation – WaveOne 50° clockwise/170° anticlockwise; Reciproc 30° clockwise/150° anticlockwise. The net outcome is anticlockwise rotation, but punctuated by short reversals to diminish the tendency to screw in. The blades of both instruments are milled in the reverse direction to most endodontic instruments, so that the larger, anticlockwise element is the advancing, cutting motion. Additional innovations included the use of discontinuous tapers – greater in the apical 3 mm of the instruments and moderating towards the shank in order to minimize coronal third tissue loss. Thermally modified versions followed in the form of WaveOne Gold and Reciproc Blue, both offering greater flexibility, improved fracture resistance and the capacity to pre-curve.

Fifth Generation: Off-center Axis

This generation of instruments was characterized by an off-center axis of rotation, which results in a snaking or swaggering action of the instrument as it advances into the canal, reducing engagement and probably reducing stress on canal walls, creating more space for debris elimination, and reducing the tendency for instruments to screw in and lock. Key examples include ProTaper Next and Revo-S.

Innovations Since 2013: Exploiting Shape Memory to Create Less Aggressive Instruments

2016 saw the introduction of instruments that take advantage of *thermally-induced shape-memory* effects. Examples include the XPEndo file, which, despite its modest 1% taper when cold, adopts a helix with a taper of at least 4% when warmed. Running at a relatively high spin speed of 800 rpm, the instrument lightly contacts canal walls and is likely to induce less stress in root dentin than traditional tools that bore a path along the canal. Its relatively minimal mass may also theoretically result in better movement of irrigating solution and clearance of dentine chippings and other canal contents.

Other Designs and Areas of Use

Development continues along other innovative lines, including the Self-Adjusting File. Constructed by laser-cutting a fine meshwork into a NiTi tube, instruments are bead-blasted for surface roughness and are driven in low-amplitude up-down vibration with a constant sodium hypochlorite feed. Their unusual design, analogous to stents for expanding narrowed coronary arteries, allows them to expand into irregular spaces, and to lightly remove debris and dentine from greater areas of the canal wall than traditional rotating tools.

Alongside advances in instrument systems for canal shaping, modestly tapered, engine-driven NiTi instruments have become established for safe and effective glide path development [13], and may reduce the stresses on subsequent shaping tools [12].

Many of the well-established frustrations, risks, and inefficiencies associated with stainless steel shaping instruments appear to have been addressed by NiTi systems. Yet innovation invariably raises further questions, and the advent of single-instrument shaping systems has led to new questions and concerns. It is conceivable that many single-instrument approaches result in excessive stress on root canal walls and increase the risk of developing microcracks [89] that may propagate with time into longitudinal root fractures. It remains to be determined whether this risk is real and if so to what extent in influences the outcome of treatment.

It has also been argued that single-instrument systems in rotary, especially reciprocating, systems may increase the risk of apical extrusion of excessive amounts of cutting debris [126].

The answers to these questions remain unresolved, but highlight the need for continued investigation, and for clinical vigilance.

In concluding this rather technologically-focused discussion, it is important to regain the biological perspective. The benefits of NiTi instrumentation have empowered clinicians who can now safely and quickly shape

curved root canals that were until recently very difficult or impossible to treat. Many of the challenges caused by instrument straightening have been overcome, but issues of instrument fracture, weakening and stressing of teeth and the extrusion of debris require further research. Their impact on long-term pulpectomy outcomes is incompletely understood. It is still not known what degree of canal enlargement, both apically, and in terms of taper, is optimal for clinical success, and the limitations of mechanical shaping must still be managed by effective irrigation. The focus of this chapter is on pulpectomy, and it is not known which, if any, instrument system results in a better, more complete, or less traumatic apical pulp resection or in cleaner and better-shaped canals. The variations in technique associated with the many different instrumentation systems make it imperative that clinicians gain training and experience of any given system they choose to use, not least in order to understand the limitations of their mechanical efforts in addressing clinical endodontic problems.

10.5.6 Principles of Canal Shaping

In young vital cases, pulp tissue may preferably be extirpated with a barbed broach before shaping the canal. In the case of older, fibrotic pulps, negotiation with the help of an EDTA gel lubricant may allow instruments to glide through pulp tissue, rather than compact it apically and create a troublesome apical blockage. The tell-tale sign is an obstruction that feels "springy", "rubbery", or "spongy", resisting the passage of small instruments, and requiring considerable work, sodium hypochlorite irrigation, and ultrasound to disrupt and bypass.

Most manufacturers provide detailed guidelines for the optimal use of their instruments in uncomplicated cases. Enlargement generally follows a pattern of:

1) Scouting into the coronal thirds of canals with small stainless steel hand instruments or engine-driven NiTi pathfinders.
2) Flaring the coronal 1/2–2/3 of the canal.
3) Determining working length by electronic and/or radiographic means.
4) Flaring the apical 1/3–1/2 of the canal.
5) Gauging apical root canal diameter and finishing the apical region as required.

Instrumentation should be accompanied by frequent, deep irrigation to flush away cutting debris, lubricate file movement, kill microorganisms, and dissolve organic matter.

In canals that are already wide, it may be unnecessary to undertake much, if any, mechanical enlargement, and instrumentation may be limited to developing appropriate apical resistance form to prevent over-extension of the root filling. In this case, the canal is cleaned predominantly by the mechanical and chemical effects of irrigants rather than instruments.

10.6 Canal Irrigation and Medication

10.6.1 Irrigant Delivery and Exchange

Instrumentation creates significant cutting debris, in the form of dentine chippings, remnants of pulp tissue and often microorganisms. At the very least, irrigation is necessary to flush away the cutting debris that would otherwise result in canal obstruction and loss of working length. As an aid to instrumentation, it will lubricate and prevent clogging of cutting flutes that would compromise shaping efficiency and increase the risk of instrument fracture. Flushing efficiency is influenced by the depth of irrigant delivery and by fluid dynamics within the canal. Delivery is usually through a narrow needle with a Luer Lock connection to a small (3–10 mL) syringe. The needle is extended as close to the canal terminus as possible without binding against the canal walls or over-extending. Needles of 30 gauge (0.25 mm diameter; ISO size 25) are often considered optimal, though many still opt for wider needles of 27 gauge (0.36 mm diameter; approximately ISO size 35) or

greater, probably to minimize the risk of over-extension during the use of sodium hypochlorite solutions [36]. Irrigating solutions adopt characteristic flow patterns, depending on the design of the needle tip and the position, shape, and orientation of the opening [104]. Generally, needles with closed ends and side vents are regarded as optimal to balance effective flushing with safety. One consistent observation is that even with open-ended needles, irrigating solutions do not exchange more than 2–3 mm beyond the needle tip [2], and simple flushing with a needle and syringe cannot be relied upon to effectively flush debris from the full length of canals, especially when deep penetration is compromised by curvature or narrowing [15]. It is also recognized that the chemical actions of irrigating solutions are optimized by frequent refreshing of solution against the canal walls.

For these reasons, interest has developed in "activated" irrigation, where mechanical energy is applied to encourage deep, high-volume exchange of irrigating solutions, but without increasing the risk of extrusion into the periapical tissues [4]. Approaches have included manual agitation, mechanical brushing actions, sonic and ultrasonic vibration, laser irradiation and negative-pressure suction.

Manual Activation

'Manual dynamic activation' describes the low-amplitude pumping of a gutta-percha cone up and down in an irrigant-filled canal, typically at a frequency of 2 Hz (approximately 100 strokes per minute). Extending close to working length, the moving gutta-percha cone is believed to exchange irrigant in the apical third of the canal where the needle may not penetrate. Its rhythmic action generates rapid fluid movement along the canal walls as the film thickness is repeatedly reduced and expanded by the advance and withdrawal of a tapered cone [42]. The same phenomenon may be created, to a lesser degree, by the insertion and removal of shaping instruments.

Mechanical Brushing

Canal brush is a tapered plastic instrument which is advanced to canal length in a slow-speed hand piece, scrubbing canal walls and creating turbulence and exchange in the irrigant solution [45]. In the same way, small "bottle brushes" of the sort employed for interdental plaque control may be applied in particularly wide canals.

Thermally-induced shape memory effects (see Section 10.5.4) allow taperless NiTi instruments (eg: XPEndo Finisher) to adopt a helical form at mouth temperature and create both turbulence in irrigating solutions, and repeated light contact with irregular canal walls as they are advanced and withdrawn while rotating at speeds of 600–1000 rpm. *In vitro* investigations suggest some promise, particularly in apical canal debridement and the management of internal resorption [7, 50].

Sonic and Ultrasonic Vibration

Sonic vibratory methods include vibrating syringe systems [100] that create turbulence during canal flushing, and devices including the "Endo Activator" [63] that agitate the irrigant at sonic frequency (<10,000 Hz) with a non-damaging plastic tip (Figure 10.17).

Ultrasonic activation (20–45 kHz) is well-established [66], delivering energy to create complex acoustic microstreaming patterns within irrigating solutions, with rapid fluid flow and whirlpool effects that are believed to promote deep cleaning. Additional benefits include warming the solution, which may promote tissue dissolution and antimicrobial effects, and occasional reports of cavitation [62] that may tear deposits from canal walls as bubbles in the solution implode. This was for some time described as passive ultrasonic irrigation (PUI), with a typical recommendation to flood canals after shaping was complete and activate the irrigant for 20 seconds with an ultrasonically-activated size 15 file before refreshing the solution and repeating twice more [127]. The process was described as passive because it was assumed that ultrasonically-activated files could be held within

Figure 10.17 Endo activator for the sonic activation of irrigants with a vibrating plastic tip.

the irrigating solution and prevented from making canal wall contact. It has since become clear that activated instruments make frequent and sometimes damaging contact with canal walls [16]. It is also recognized that wall contact dampens vibration and diminishes the beneficial effects of activation. This process is now described simply as ultrasonically-activated irrigation, and fears about the potential damage caused to canal walls by ultrasonically-activated metal instruments has promoted interest in plastic alternatives.

Laser Activation

Photon-induced photoacoustic streaming (PIPS) represents a further approach to irrigant activation [53]. Here the canal system is flooded with irrigant, before applying an E:YAG laser through a specially designed tip that reaches only into the pulp chamber. Activation of the laser results in photoacoustic shock waves that are believed to transmit through the entire pulp system, disrupting debris, smear layer, and biofilm. The principle appears exciting, yet the hardware costs are considerable.

Negative Pressure Irrigation

Negative pressure approaches involve the placement of narrow suction catheters, first in the coronal 2/3 of the canal, and then in the apical third, while constantly feeding irrigant into the pulp chamber (Figure 10.18). Here, the solution moves rapidly as it is drawn into the canal, producing shearing forces that are expected to enhance wall cleanliness [108] and penetrate anatomical complexities, yet without the risk of apical extrusion that always accompanies positive pressure methods [68]. A variation on this theme is GentleWave, which combines negative pressure technology with multiple-frequency sonic activation [69]. Early reports show considerable promise, with high levels of clinical success at 6 months [105].

The current focus on activated irrigation, and the burgeoning technology for its delivery seems rational from a biological and therapeutic standpoint. Many laboratory studies can evidence improved dislodgement of debris, tissue dissolution and antimicrobial actions from irrigant activation, yet this has not currently translated into demonstrably improved clinical outcomes, either for necrotic/infected cases or after pulpectomy [55]. Clinical trials that are able to control for single variables such as irrigant activation may not be forthcoming, and practitioners should diligently apply methods that they believe will optimize cleanliness, without doing harm, whether canals are heavily infected or not.

Although canals should be constantly flushed with irrigant throughout instrumentation, their deepest elements and lateral

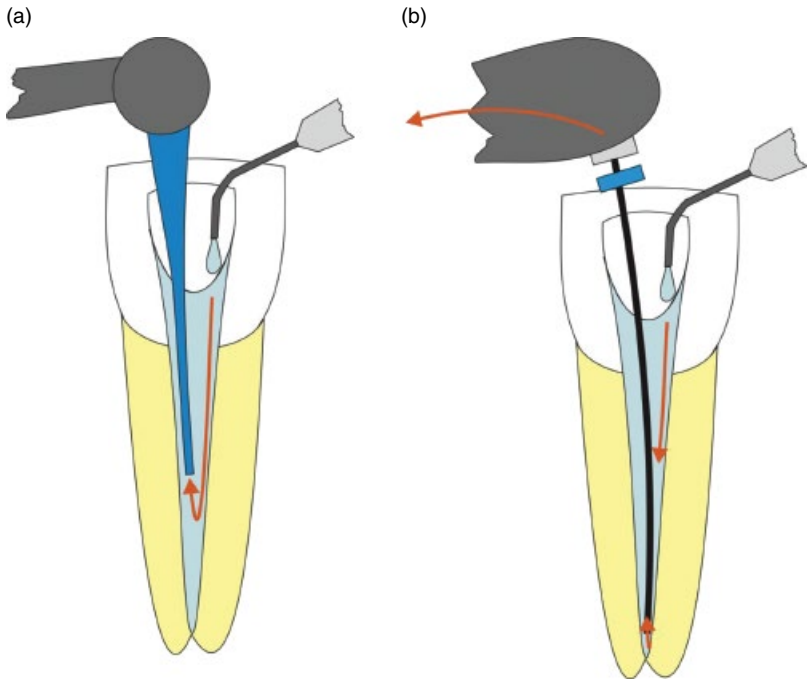

Figure 10.18 Schematic representation of EndoVac, negative pressure irrigation. (a) Irrigation to mid-root, with solution flowing into the pulp chamber and suctioned away through a plastic cannula extended to mid-root. (b) Apical fluid exchange following the extension of a metal micro-cannula to the canal terminus.

complexities are most effectively flushed only after shaping is complete. In pulpectomy cases, it may be tempting to dismiss the need for post-instrumentation irrigation, since the canals are unlikely to be heavily infected. Yet the removal of cutting debris and pulp remnants from uninstrumented regions of the canal system suggest that a period of activated irrigation may be wise before preparing to fill. To emphasize this sequence of events, and the benefits of post-instrumentation cleaning, the term "shaping and cleaning" may be more helpful than "cleaning and shaping" for both infected and uninfected cases.

10.6.2 Solutions for Debris and Soft Tissue Removal

In pulpectomy, the focus is on asepsis, keeping the apical pulp stump alive and dissolving organic matter within canal ramifications. This contrasts with necrotic/infected cases, where the focus is on disrupting and eliminating established biofilms and large masses of necrotic tissue. Fortunately, many irrigating solutions have properties that make them suitable in both situations.

Sodium Hypochlorite

Evidence dating back 100 years to Dakin suggests that dilute (0.5%) NaOCl solutions are effective for cleaning wounds and preserving the health of vital tissues [57]. This observation is echoed in pulpotomy and pulp-capping wounds, which have been managed effectively, at least in the short term, with strong (5%) NaOCl solutions [14], and it may be reasonable to regard pulpectomy as the creation of a (very) deep pulpotomy wound.

NaOCl is unusual among irrigating solutions with its combination of hemostatic/tissue-dissolving effects and antimicrobial actions stemming both from cell disruption and lysis of polymers within the biofilm matrix [121]. In addition to dissolving pulp

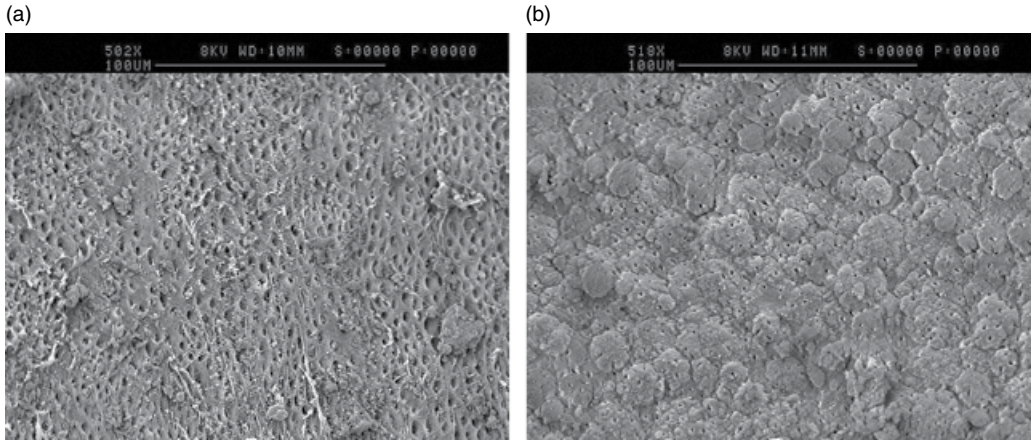

Figure 10.19 (a) Canal wall covered by predentin and small amounts of tissue debris. (b) Exposure to 5% NaOCl for 10 minutes removes cellular debris and predentin and exposes calcospherites of fully mineralized dentin on canal walls.

debris, NaOCl will remove variable quantities of poorly mineralized predentin from the surfaces that it contacts (Figure 10.19). The optimal concentration for use in pulpectomy is not established and its effectiveness in key roles will be influenced not only by concentration but temperature, volume applied, depth of exchange, nature of activation and time [107]. It may be rationalized by some that less concentrated solutions of 0.5–2% may achieve their goals with good activation and time, yet others prefer more concentrated solutions (3–5%) for all of their cases whether infected or not. Strong solutions, particularly if heated, may increase the risks of damaging dentin by deproteination [120, 138].

NaOCl, usually at 0.5–3% concentration, remains the gold standard agent for irrigation in pulpectomy cases.

Chlorhexidine

Chlorhexidine (0.5 to 2%), displays broad-spectrum antimicrobial activity, and may be expected to be well tolerated by the apical pulp stump, but has none of the tissue-dissolving and hemostatic effects that may be important in pulpectomy. Chlorhexidine may be a legitimate alternative to NaOCl, but is generally regarded as a second choice to NaOCl. The combination of NaOCl and chlorhexidine results in an immediate, sticky precipitate of chloroaniline, which may not only cause blockages, but be toxic to vital tissues [11].

Alternative agents, including iodine potassium iodide and benzalkonium chloride are of particular interest for canal disinfection and there can be few indications for their use in pulpectomy.

10.6.3 Agents for Smear Layer Removal

Shaping instruments leave a smear layer on surfaces they touch. Some have argued that this should be removed, especially in infected cases, where the access of NaOCl to microorganisms residing in dentinal tubules may be compromised, and where there may be fears that the seal provided by the root filling against biofilm and debris-smeared walls may be compromised. Others have concluded that the impact of smear layer on clinical outcomes remains uncertain [129]. Common solutions for the removal of smear layer include EDTA (typically 17%) and citric acid (typically 5%) and their effectiveness in this role is recognized. Concerns have, however, been expressed about the potential of such agents, especially if applied warm or in higher concentrations, to demineralize and

etch dentine. Their alternating use with strong NaOCl solutions has been a particular concern, creating repeated cycles of demineralization and de-proteination and the potential for chemical damage to dentine. Combining NaOCl with EDTA also diminishes the antimicrobial activities of NaOCl. A recent alternative incorporates the reportedly gentle chelating actions of etidridic acid [86] or (1-hydroxyethylidene)bisphosphonic acid (HEDP) which is mixed with NaOCl without disrupting its properties to form a "dual rinse" solution.

Combination products such as the antibiotic-containing MTAD (Mixture of Tetracycline isomer, Acid and Detergent) and related Tetraclean are claimed to combine antimicrobial activity with gentle smear removal later. Yet the use of broad-spectrum antibiotics as topical agents against biofilm infections seems ill-advised as a global crisis of antibiotic resistance unfolds. It is particularly difficult to identify a role for such agents in minimally infected or uninfected pulpectomy cases. QMix is an alternative product, combining EDTA with chlorhexidine and a detergent to enhance canal wall wettability and tubular penetration. Much of its evaluation has focused antimicrobial activity [114] and the specific benefits of QMix in pulpectomy are unclear.

Hard scientific evidence is once again not available on the optimal irrigating solution for use during pulpectomy. To date, NaOCl solutions are probably the best evidenced.

10.6.4 Intracanal Medicaments

Discussion around the merits of single or multiple-visit endodontic treatment apply usually to necrotic/infected cases [64], and even here the case in support of multiple-visit treatment is not strong. For vital cases, there is general consensus that wherever possible, treatment should be completed in a single visit, primarily to safeguard asepsis [32].

Treatment of vital cases over more than one visit may be indicated if the patient is unable to tolerate further time in the chair, if there is insufficient time available for the dentist to complete treatment to the requisite standard, or if the canal continues to fill with blood and cannot be dried.

The standard inter-appointment medication is a soft slurry of calcium hydroxide in an aqueous or sodium hypochlorite vehicle [136] which will help to eliminate further organic matter from the canal and promote hemostasis, while acting kindly to the pulp stump. Calcium hydroxide may also disinfect and help to preserve canal asepsis until the canals can be filled.

The use of non-setting calcium hydroxide may be of particular value during pulpectomy for internal resorption, where the medicament may play a valuable role in cleaning tissue remnants from large regions of the canal that will be untouched by instruments.

In some countries, the application of steroid/antibiotic pastes is popular, with the supposed benefits of anti-inflammatory and antimicrobial activity. The value of such materials in pulpectomy, either in terms of postoperative pain control or wound healing, cannot be evidenced from the literature, and the topical application of broad-spectrum antibiotics is probably an unwise stewardship of a precious and diminishing resource when antimicrobial resistance is growing.

What is critical in such cases in the provision of a tight coronal seal, protecting canal entrances with a soft and easily-retrieved material such as Cavit Grey, or with a small increment of sterile cotton wool, foam sponge, or ball of polytetrafluoroethylene (PTFE) tape [102] before sealing with at least 3 mm thickness of well-adapted cement.

10.7 Preserving the Aseptic Environment: Root Canal Filling and Coronal Restoration

Kakehashi showed that mechanically injured and exposed pulp tissues were able to reorganize themselves and remain healthy, provided their environment was microbe-free [44]. Similar observations have been made

after traumatic pulp exposure and pulpotomy, and in numerous studies on the effects of dental materials on the pulp [9]. The positive impact of a microbe-free environment on periapical health after endodontic treatment has also been observed in classic literature [109]. Extrapolating from this, the root canal filling and coronal restoration should at the very least provide a microbe-free environment to promote the health of the apical pulp wound and hence the periapical tissues. Whether the root canal filling material that makes direct contact with the apical soft tissue wound must have any special "bioactive" properties is less certain. It may seem logical to speculate that materials based on calcium hydroxide and particularly hydraulic calcium silicate cements may have special properties to promote health in the apical pulp stump as they do in aseptically managed pulp wounds elsewhere. Yet the favorable treatment outcomes observed for pulpectomy procedures where the canals were filled with gutta-percha and a chemically diverse array of sealer cements suggests that clinical success may rely more heavily on the exclusion of infection rather than the active induction of biological healing processes. It follows from the principles of asepsis that all materials and instruments for root canal filling must be applied in a sterile or effectively disinfected condition.

A no-touch policy should be adopted, and it may be wise to soak gutta-percha points in 2% chlorhexidine for at least a minute before insertion in the canal [115]. Other items, including mixing pads, mixing spatulas, and foam sponges for instrument storage may be further sources of contamination. Quite how much the recognized and profound antimicrobial properties of most freshly-mixed root canal sealer cements [101] have on such low-level contamination is not known, though once again, small acts to preserve asepsis may cumulatively favor treatment success.

10.7.1 Preparing to Fill

After shaping and cleaning, it should be confirmed that the canal is free from significant exudate from the apical pulp wound and can be dried. The incorporation of blood or tissue fluid within a root filling is not consistent with optimal sealing, and filling should be postponed to a later date, after further canal irrigation and medication. Canals are usually dried with absorbent paper points and care should be taken not to penetrate the apical pulp stump and provoke unwanted bleeding.

10.7.2 Master Cone Selection and Fit

Master cone fit is an essential first step in most root canal filling techniques. Gutta-percha remains the commonest filling material, with contemporary variants including points containing "bioceramic" particles for use with calcium silicate sealer cements. Alternative materials based on polycaprolactone (eg: Real Seal, Sybron) have gained some popularity amid claims of better-sealing and root-strengthening, bonded root canal fillings. The theory may, however, be simpler than the reality of optimally etching, priming and bonding to dentine walls deep in the canal system [122]. This type of product may also be susceptible to enzymatic breakdown and alkaline hydrolysis by contaminating microbes [123, 124]. The unfavorable ratio of bound and unbound surfaces (configuration, or "c" factor) in such a deep and narrow cavity as a root canal also promotes pulling away of materials from canal walls as they undergo polymerization shrinkage [135].

Another innovation is C point, a product constructed from similar materials to soft contact lenses, and which expands laterally in the presence of an aqueous-based root canal sealer to provide a seal [25].

Many of the tapered NiTi instrument systems come with matched master cones, and the expectation that in many circumstances, the canal space can be filled with a single cone and sealer. In complex and ribbon-shaped canals, this may be fanciful (Figure 10.20). ISO master cones are also available for those who finish their apical preparations with ISO instruments, and these modestly tapered master cones provide the apical seal before filling the remainder of canal space, often by

Figure 10.20 Irregularly shaped canal system incompletely instrumented, incompletely debrided and incompletely filled with a single gutta-percha cone and sealer.

cold lateral condensation with an appropriate sealer cement.

Master cones may be trimmed with a sharp scalpel to provide apical tug-back, which is believed to demonstrate snugness of fit at full working length or 0.5 mm short if the cone is to be compacted.

10.7.3 Choice of Sealer Cement

Gutta-percha cannot be relied upon to seal canals without the help of a sealer cement, and the same is true for all other core filling materials currently available. Despite their critical role, sealer cements have traditionally been regarded as the vulnerable element of the root canal filling, with the potential for air entrapment and voids, shrinkage during setting and instability/solubility, particularly when used in large volume. This, added to concerns about length control and retrievability in the case of retreatment or post-space preparation, has discouraged the use of cement-only and cement-heavy root canal fillings.

Decisions on the choice of sealer cement may be influenced by a range of factors, some of which can be reasonably evidenced from scientific literature and others which may be related to convenience and local practice.

Much research on sealer cements during the last 30 years has focused on dye leakage and fluid filtration methods to determine the sealing potential of root filling materials and methods. The lack of correlation between such studies and clinical outcomes [84, 117] and their subsequent restriction from mainstream endodontic research-intensive journals [24, 26] has largely eliminated this method of evaluation and comparison in recent times. The thrust of current root canal filling research remains largely laboratory-based, with evaluations of the biocompatibility of materials in contact with cultured tissues, antimicrobial properties, bond strength to dentin, ability to wet dentin and capacity for tubular penetration. These are all potential proxy markers for clinical performance, with few if any *in vivo* studies of sufficient scale to control for the impact of individual sealer cements on clinical outcomes in pulpectomy. It should also be recognized that parameters such as bond strength do not necessarily correlate with sealing effectiveness.

The desirable properties of a root canal sealer cement relate to:

1) Antimicrobial properties, to kill or imprison residual microorganisms [116] and prevent canal infection in the long-term.
2) Sealing ability, to prevent the entry of microorganisms and nutrient fluids into the canal system, and the exit of biologically significant materials such as microbial endotoxin into the periradicular tissues. Cements should be inert and insoluble within the canal system. Their seal should not be compromised in the long term by adverse interactions with core root canal filling materials and coronal restorative materials.

3) Interactions with host tissues, including adequate flow into anatomical complexities, wetting and adaptation against canal walls, biocompatibility on contact with apical soft tissues, and the absence of unsightly staining to hard and soft tissues.
4) Handling properties, to ensure ease of mixing and application, adequate working and setting time, and sufficient radiopacity to judge the extent and quality of fill.

Economics will also be a significant consideration for material selection, unless the properties of an individual material make it stand out significantly from alternatives.

A detailed appraisal of all of these parameters for all classes of root canal sealer cements, and an attempt to balance their relative importance in pulpectomy is not realistic. When used in combination with established gutta-percha compaction techniques, there is currently no convincing clinical evidence that superior pulpectomy outcomes will be achieved with zinc oxide-eugenol, epoxy resin, calcium hydroxide, polyvinylsiloxane, glass ionomer, methacrylate resin, or calcium silicate sealer cements. When the sealer cement may exert a systematic effect on the clinical prognosis, it appears to be small and quickly overridden by other biological and technical factors associated with a complex intervention such as endodontic treatment [85]. What is critical is preserving a sterile apical pulp wound and avoiding large-scale extrusion into the periapical tissues. For this reason, techniques that are more prone to drive larger volumes of gutta-percha or sealer into the apical pulp stump or beyond, such as thermoplastic and carrier-based methods [90], may seem less desirable in pulpectomy [54, 96]. Yet there is some evidence that vital apical stumps of pulp tissue may provide greater resistance to material extrusion than necrotic/infected tissues, and certainly the demonstration of fine anatomical complexity is less likely after pulpectomy [97]. Care should always be taken to avoid the damaging over-extension of endodontic materials into adjacent structures including the inferior alveolar nerve [92] and maxillary sinus [39].

10.7.4 Condensation

Despite growing interest in systems-based endodontics and the potential of single matched-cone cementation with an appropriate sealer, concerns about filling the canal space in its totality encourages the use of compaction techniques to optimize the density of gutta-percha and drive sealer cement into as many ramifications as possible.

Cold lateral condensation remains popular and widely taught, requiring no expensive equipment and enjoying a reputation for clinical effectiveness [52]. Cold lateral condensation is a generic term that embraces a variety of approaches, including the use of ISO or tapered master cones, ISO or tapered spreaders and accessory cones, stainless steel or NiTi spreaders, variable degrees of spreader penetration and loading, and variable methods for coronal cut-off and vertical compaction. If the master cone is tapered, it may be difficult to enter the canal deeply with a spreader and accessory cones and condensation may be compromised. A particular concern is that the wedging forces applied to the canal interior during lateral condensation could promote the cracking or crazing of dentin, or aggravate defects that have arisen during instrumentation. This relatively slow technique demands a slow-setting sealer cement, and the relatively low risk of large-scale apical extrusion means that canals can be loaded heavily with sealer, with excess displaced progressively in a coronal direction as the filling is compacted and built-up.

Cold lateral condensation often forms the foundation for further consolidation with heat, and several options are described. In thermomechanical compaction, a rotating instrument is applied to the cold-condensed material, and swept to mid-root, thermoplasticizing the material by the creation of frictional heat and driving the molten material apically on the instrument flutes [49]. A small, ultrasonically-energized file may also

be advanced within a mass of cold-compacted gutta-percha, softening the material as the energized file warms, and providing after withdrawal a pathway for the re-insertion of a cold spreader and further accessory cones [6]. Alternatively, a heat carrier, warmed electronically or in a Bunsen flame may be used to remove increments of cold-compacted material to a deep level in the canal, softening the material ahead of its tip and providing the opportunity for vertical condensation with a cold plugger.

In the case of a tapered master cone that nominally matches the final shaping instrument, warm vertical condensation is the customary method of condensation. This may be accomplished in a single wave of heating and compaction, or in multiple waves, taking small increments of thermally softened material from the canal before compacting with cold pluggers and repeating the cycle until reaching 5–7 mm from working length. This approach demands a sealer with adequate working time in the presence of heat. The risk of apical extrusion is considered greater than with cold lateral condensation and it is usual to advise the sparing use of sealer.

Special considerations are necessary during pulpectomy for the management of internal root resorption. Here, the ballooned area of the canal does not lend itself well to filling by cold lateral condensation or single cone techniques and thermoplastic methods are positively indicated (Figure 10.21).

Once again, there is little compelling evidence that any particular filling method outperforms any other in pulpectomy cases. The evidence we do have points to well condensed root canal fillings, extended to within 2 mm of radiographic root end and with no significant extrusion of materials as those which will perform best [54, 76].

All techniques may be performed well or not so well, and practitioners should work diligently to execute filling techniques with predictable quality, within the constraints of the environment in which they work. It seems more important to perform a technique predictably well rather than to agonize over which technique is "best".

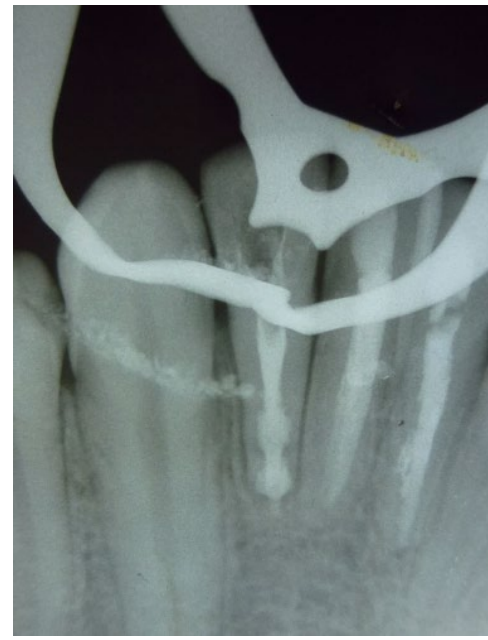

Figure 10.21 Thermoplastic filling of an internal resorption following pulpectomy (image courtesy of Dr Geoff Seccombe, Newcastle).

10.7.5 Protecting the Root Filling and the Tooth

Endodontically treated teeth are expected to perform for many years, if not decades, and the long-term prevention of infection after pulpectomy is critical. It may be advantageous to cut back root canal filing materials 2–3 mm below canal entrances, and seal the openings with a well-adapted cement canal plug [113] (Figure 10.22). Similar canal plugs may be placed after deep cut-back for an intraradicular post. It is probably wise to avoid the use of hard and well color-matched materials such as flowable composite for this purpose, as later re-entry may be greatly complicated.

All such procedures, including post-space preparation, whether conducted immediately after root canal filling or many years after initial treatment should be protected from oral contamination with a well-sealing

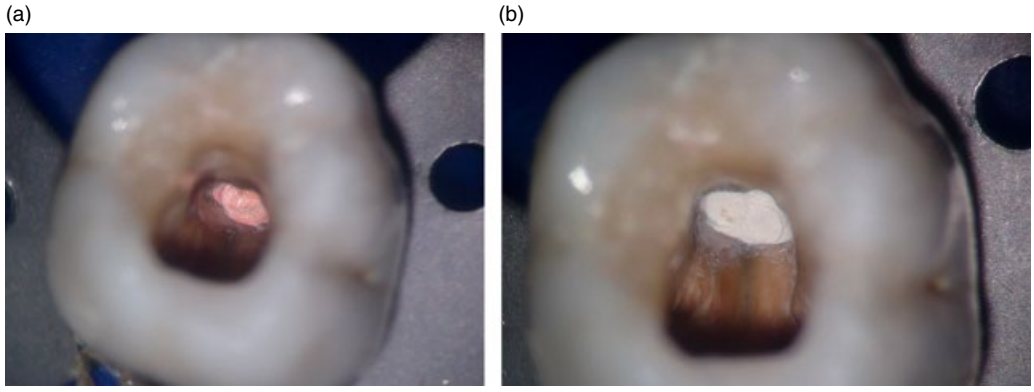

Figure 10.22 Canal plugs, a second layer of defense to protect root canal filling materials from the oral environment. (a) Cut-back of root canal filling material 2–3 mm below level of pulp chamber floor. (b) Canal plug of IRM cement.

rubber dam. The placement of this internal barrier may be logical to protect the root canal filling from oral fluids and microorganisms in the event of leakage around provisional or permanent restorations. All efforts should then be made to optimize the seal of all provisional and permanent coronal restorations by the meticulous cleaning of cavity walls, adapting materials carefully against dentin and optimizing material bonding. In the case of posterior teeth with a marginal ridge missing, cuspal coverage is strongly indicated as a means of promoting tooth survival [74] in the long term by safeguarding against catastrophic fracture.

10.8 Concluding Remarks

Pulpectomy may seem to be a simple and predictable treatment to preserve teeth with injured or endangered pulps. Outcome data, both in terms of periapical health and tooth survival, suggest that dentists should have confidence in the management of such teeth, but this should not make them complacent. The importance of strict asepsis at all stages of the procedure cannot be over-emphasized, and hard and soft tissues should be handled with care. In a complex intervention of this sort, it is impossible to identify from well conducted clinical trials which elements of treatment have the greatest bearing on outcome. Equally, it is virtually impossible to discern which subtleties of materials and techniques are responsible for optimizing success. In the final analysis, it is probably attention to detail and asepsis in a host of small acts that wins success. It is important to recognize that the behavior of dentists is greatly influenced by the community of practice in which they work and that this can have positive and negative effects on treatment quality. Educators have great responsibility to promote optimal practice, and the principles outlined in this chapter encapsulate current best practice for the management of teeth with vital but irreversibly inflamed pulps.

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11

Endodontic Treatment of Apical Periodontitis

Dag Ørstavik

11.1 Introduction

11.1.1 Rationale for Treatment

The diagnosis of any form of apical periodontitis is generally considered a need to treat the condition. Acute/symptomatic or chronic/asymptomatic apical periodontitis, periapical abscesses with or without a sinus tract, as well as radicular cysts, start from an infected root canal system, which is the common target for therapeutic measures in these situations.

A chief complaint of pain or discomfort brings its own indication for intervention, and if apical periodontitis is the source of the pain, then curing the disease is the obvious treatment plan. Asymptomatic, chronic apical periodontitis, on the other hand, needs a rationale for treatment by itself. Patients do not necessarily feel any need for a costly and complicated treatment without having any pain or discomfort. There are three good reasons for attempting to cure also chronic, asymptomatic apical periodontitis.

11.1.1.1 Pain Control

A chronic inflammation caused by bacteria has the potential to exacerbate. Chronic apical periodontitis may develop insidiously to form large cysts without any symptoms, or it may remain stable and unchanged in size. The risk of such a lesion changing into an acute inflammation with toothache, swelling and abscess formation is a primary reason why one advocates treatment of the disease. It must be recognized, however, that predicting the incidence and severity of such exacerbations is difficult [7, 97, 198, 207], and is influenced by many variables that may be outside the control of patients and dentists.

11.1.1.2 Local Spread of Infection

A second rationale for treatment is the possibility of the infection spreading to tissues and organs in the immediate and regional vicinity. The medulla of the jawbones may be infected to cause osteomyelitis, which in many cases is not easily treated. The maxillary sinus is frequently involved, and many cases of sinusitis have apical periodontitis as a contributing or causative factor [249, 250]. Brain abscesses have been reported with tooth infection as the source in over 30% of cases, but the type of tooth infection is often not known [109]. Acute infections follow tissue spaces or fasciae and may reach the mediastinum with life-threatening complications, particularly in immunocompromised individuals [100, 156].

11.1.1.3 Association with Systemic Diseases

Finally, the association of local dental infections or inflammation with heart and circulatory disease is an issue of concern (see also Chapter 4). While the risk posed by dental infection is low, there is no longer doubts about its existence [20, 112]. Patients with

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heart valve problems may be susceptible to infections from bacteremia following endodontic procedures. Here elimination of apical periodontitis traditionally is considered mandatory [42].

11.1.2 Purpose and Challenges of Treatment

The microbial etiology of the disease itself and of its sequelae defines the purpose of treatment: the elimination of microbes infecting the root canal system and occasionally the periapical tissues. All other efforts made during treatment are subsidiary to this primary goal, and one cannot compensate for failure to control the infection by other technical or clinical aspects of the procedures, such as pain control, use of antibiotics, or a visually pleasing root filling.

There are multiple protocols for treatment of apical periodontitis. This chapter does not aim to set out in detail the practicalities of treatment, but to highlight the principles and biological underpinnings of the elements of treatment.

11.1.2.1 Elimination of Infecting Microbes

The complexity of the root canal system makes treatment difficult: biofilms formed on surfaces in isthmuses and canal ramifications are poorly accessible by physical and chemical means; and antibiotics hardly penetrate dental tissues in sufficient concentrations for clinical effect. In addition to the treatment principles for root filling after vital pulp extirpation (Chapter 10), treatment of the infected tooth needs to ensure maximal antibacterial effect by mechanical instrumentation and application of disinfectants.

The classical works of Sundqvist and associates from the 1970s through the 1990s remain the backbone for our concepts and principles for reducing or eliminating endodontic infections. They and others established that apical periodontitis in humans does not develop in the absence of root canal infection [18, 225]. They went on to document how a systematic approach with instrumentation [26], irrigation [27, 28], and dressing [24, 212] had the potential to render the canal bacteria-free, and that subsequent treatment with root canal obturation was followed by success rates similar to those found after vital pulp extirpation [25, 213].

11.1.2.2 Outcome of Endodontic Treatment

These clinical bacteriological studies suggested that a treatment protocol with predictable curing of apical periodontitis could be established. Moreover, it lent support to a concept of a qualitative approach to disinfection: it should be possible to eliminate the infection and thereby re-establish a healthy apical periodontium completely free of microbes. However, subsequent clinical experiments did not reproduce the same level of disinfection, and follow-up studies of treatments performed according to the same principles did not always show the same good clinical-radiological results. It is also recognized that culturing of samples from root canals may give both false positive and false negative results, especially when sampling is performed with suboptimal techniques [195]. Thus, it remains a challenge to get the same high success rate for treatment of established apical periodontitis as for root fillings after pulpectomies.

11.2 Anatomic Location of the Microbes

11.2.1 Infection of the Root Canal System, Dentin and Cementum

The anatomical location of the infecting microbes (Figure 11.1) is a major reason why disinfection of root canals and the periapical area is so difficult. The main bulk of microorganisms are located inside the root canal itself, and even in long-standing and post-treatment apical periodontitis it is the bacteria inside the root canal that are mainly responsible for the inflammatory response. These pulpally located bacteria should be readily susceptible to mechanical and

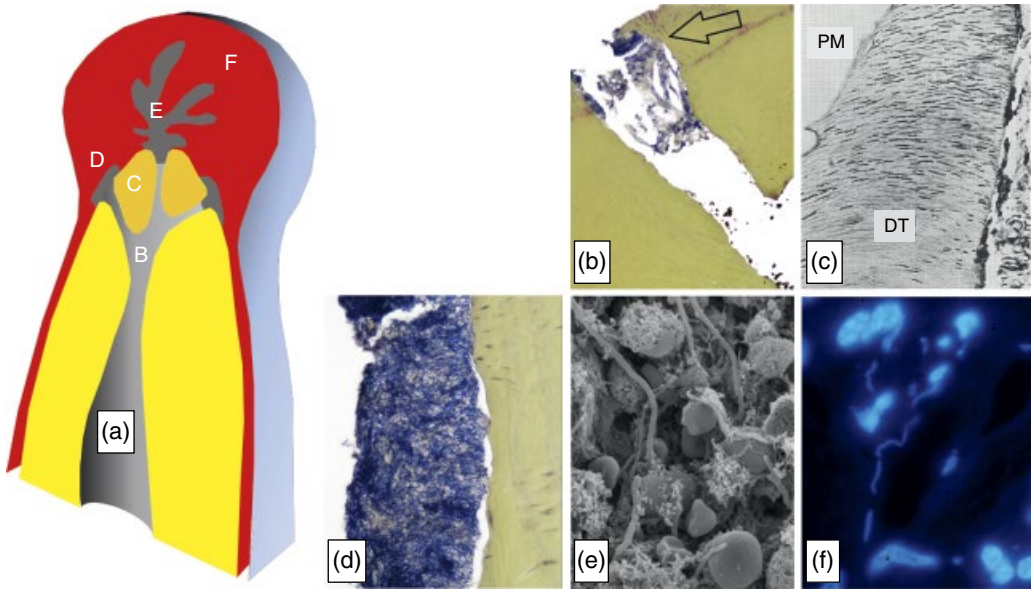

Figure 11.1 Anatomical location of infecting microbes in apical periodontitis. (a) Overview. The bulk of bacteria are located inside the pulp lumen. (b) Microbes in pulp canal ramification (reproduced with permission from [176]). (c) Bacteria in dentin tubules (DT) infected through to the periodontal membrane (PM) (reproduced with permission from [244]). (d) Biofilm on external root surface (reproduced with permission from [174]). (e) Filamentous and coccoid bacteria and red blood cells from the center of a granuloma (courtesy of Dr Pia T. Sunde). (f) Specific fluorescent staining of bacteria extraradicularly in the connective tissues of an endodontic lesion (reproduced with permission from [223]).

chemical debridement, but the complexity of the pulp ramifications limits the accessibility of instruments and medicaments even here. Moreover, the microbes are organized in biofilms colonizing the least accessible areas on the internal root surface [259].

With long-standing infection of the pulp space, microorganisms also occupy lateral and furcal side canals. This will further complicate efforts to disinfect the space.

Infection of dentinal tubules also occur. When the overlying cementum is intact and vital, bacteria penetrate the tubules to a limited depth. With longer-standing infections where the destructive processes have reached the periodontal membrane causing necrosis of the cementum, a "through and through" infection of the dentinal tubules may occur [244]. In such cases, there is often an irregular resorption of the dentin surface on the periodontal side of the apical foramen, caused by clast cells attempting to get at the microbes infecting the tubules and the pulp space. Fortunately, successful elimination of the infection leads to remodeling of the periodontal tissue even after extensive apical resorption [179].

11.2.2 Extraradicular Infection

Modern concepts of bacterial infections emphasize the importance of a foothold or surface for bacteria to attach to and multiply on [63, 105]. Skin, mucosal surfaces, and the interior of blood and lymphatic vessels offer such surfaces for adhesion. At the root apex there is limited opportunity in the surrounding tissues for attachment and growth of bacteria with limited pathogenicity and virulence. In acute phases, the balance of bacterial virulence and tissue resistance is shifted in favor of the bacteria, and their interaction with tissue and immune cells results in liquefaction and abscess formation. When a sinus

tract develops, the infecting organisms are kept at bay by constant drainage through the tract; but the tract's interior surface may be colonized by the bacteria.

A bacterial biofilm may cover the cementum itself in chronic apical periodontitis [118, 134, 175]. This biofilm is similar to bacterial plaque with a tendency for calcification [165]. It is reasonable to speculate that conservative measures to eliminate the infection are much less likely to succeed when such a plaque is established.

The extraradicular bacteria in abscesses, sinus tracts, and expanding cysts may not be easily susceptible to the body's immune defenses. Moreover, in other situations, particular types of microorganisms manage to survive within the soft tissues of the apical granuloma without the presence of an external, solid surface [180]. *Actinomyces* and *Propionibacterium* colonies are the classical example. The cells cluster and grow from the inside, and when the aggregate is large enough, even massive attacks from phagocytic cells on its outside fail to limit growth in the central area. Moreover, also other cells may join forces to create clusters within the soft tissues, which help them survive the host defenses [50, 68, 180, 222, 223, 238–240].

While systemic antibiotics may limit growth and expansion of the infection, long-term success is questionable at best, and surgical removal by apicectomy is the preferred treatment modality [177]. It should be noted, however, that a clinical diagnosis of an extraradicular infection is always a speculative one until verified by a biopsy.

11.2.3 Infection in Endodontically Treated Teeth

The bacterial flora of infected, previously root-filled teeth may be different from that of primary infections (see Chapter 4). The microenvironment at the apex was disturbed during the first treatment, and the presence of root filling materials at or beyond the foramen provides niches and physical surfaces different from the original. Thus bacteria may also grow on surfaces of the root filling material, core material, and sealer [87].

This may induce a selection of a more facultative flora, with streptococci and particularly *Enterococcus faecalis* increasing in relative numbers [130, 278]. Yeasts also have been associated with persistent apical periodontitis [256], but initial findings of relative dominance in post-treatment disease have not been confirmed [160]. On the other hand, if the post-treatment inflammation is associated with a root filling ending at a distance from the apical constriction, the residual space provides an environment similar to the pretreatment state, and the flora is most likely similar to that of a primary infection.

The differences in bacterial composition may be one reason why the success rate of retreatments is generally poorer than primary treatment of apical periodontitis [130, 226], and attempts at elucidating mechanisms for the persistence of these infections are still being made [89, 231]. However, molecular studies of the microbial flora in different endodontic infections do not show any clear-cut differences among them [182], and it appears that generally, the microbial profile of post-treatment endodontic infections [274, 275] is similar to that of primary infections [191]. While there is still the possibility that the relative proportions of species or taxa may differ systematically, such differences have not yet been translated into differential treatment strategies with documented efficacy.

11.3 Bacteriological Status During Treatment

Given the microbial etiology of apical periodontitis, it makes sense to monitor the presence or extent of infection during treatment, from entry into the pulp chamber to the time of root canal obturation. The infection follows a course as outlined in Figure 11.2 (modified from [148]). Given the proven concept of "no bacteria, no lesion", a bacteria-free canal ensures treatment success, and a

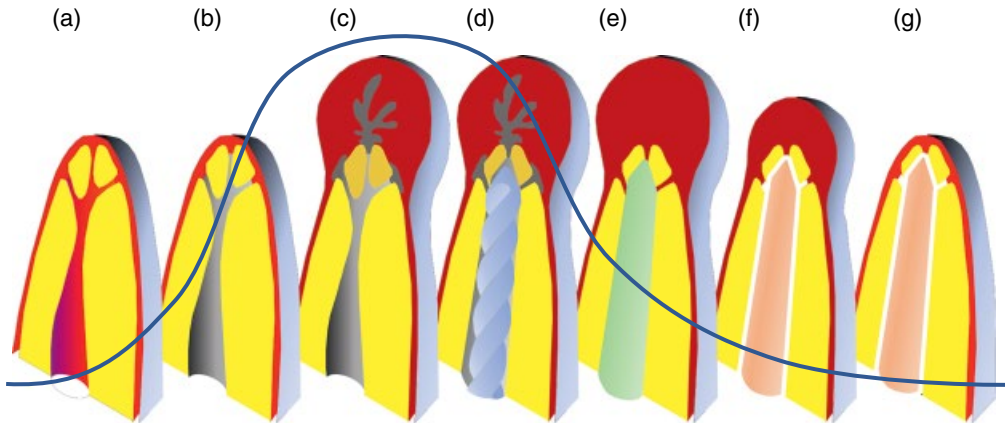

Figure 11.2 Development and healing of apical periodontitis related to the level of infection. Microbes in the root canal system (a, b) initiates and sustains the periapical inflammation (c); mechanical instrumentation (d), irrigation and medication (e), and root filling (f) reduce the level of infection sufficiently for repair and regeneration of the apical periodontal structures (g) [148].

bacterial sample taken at the time of filling should not show any growth.

11.3.1 History and Status of Microbiological Sampling from the Root Canal

The bacteriological sample may be seen as a surrogate measure of treatment success, and was advocated as a routine procedure to be applied before a root canal is considered ready to be filled [69]. For many reasons, however, this procedure is no longer applied in everyday practice. It is cumbersome; available techniques are often error-prone; and evaluating results does not give an accurate bacteriological picture at the filling session. However, bacteriological sampling remains an essential tool for experimental and comparative studies on the efficacy of treatment procedures, and bacterial growth from the canal is the only parameter that has an etiology-based, direct correlation to treatment outcome. Chairside, real-time methods for assessing the bacteriological status have been developed, and some techniques hold a potential for use in clinical practice [74].

There may be good reasons to assess techniques and materials for their ability to adhere to dentin walls, to eliminate necrotic or vital tissue, to penetrate tubules; to limit extrusion of debris etc., but these parameters are all subordinate to the effect of microbial infection on the healing or development of apical periodontitis. Therefore, over the decades, a standardized way of checking for infection during treatment has been developed and applied for assessments of the performance of endodontic treatment methods [26, 51, 153, 181, 225]. An initial sample from the root canal is taken on first entry into the canal space. This is to ascertain that the root canal is indeed infected, and provides a reference for subsequent samples. A second sample is taken at the end of the appointment, and the reduction or elimination of the bacterial content is a measure of the efficacy of the mechanical and/or chemical/biochemical methods used. If a second session is scheduled, another sample is taken on re-entry, reflecting the effect of the temporary medication; and a final sample is again taken just before filling. These samples are usually labeled S1, S2, S3, and S4.

Methods of sampling vary with the clinical state of the tooth, but the general principle is to collect as much of the infecting flora as possible. Multiple paper points usually collect initial samples and samples of the instrumented canal. Various modifications may

apply to, e.g., root-filled teeth and for sampling of dentin filings. A sterile file retrieving root-filling material or with dentin chips in its flutes may be cut off and collected in transport medium for growth [153, 275]. False positive samples can be avoided by strict adherence to asepsis during sampling, but false negatives remain a significant problem for research as well as clinical applications: many organisms cannot be cultivated with current techniques, and biofilms may be located distant from the site of sampling.

The technique initially recorded growth/no growth of the microbes. Sensitivity increased with improved conditions for growth of, especially, anaerobic bacteria. For research purposes, sampling also included characterization of the genera and species that could be determined by cultural and biochemical means. Molecular methods for detection of genetic material from microbes have expanded the sensitivity even further. But the association of treatment outcome with bacteriological status rests so far with bacteria detected by culture, i.e., live microbes. Moreover, the association is qualitative. While there may be a relationship between the quantity of organisms in the root canal and treatment prognosis, the established, significant association is with cultivable bacteria at the time of filling [55, 131, 214, 246, 254]. Whether and to what extent genetic material from dead bacteria and/or non-cultivable organisms play a role in the pathogenesis of primary and post-treatment apical periodontitis remains to be established, but it is generally recognized that for maintenance of disease, live bacteria in sufficient numbers and organization are necessary [209].

The concept of root canal infection as a surrogate measure of treatment efficacy has been applied to ex vivo models: extracted teeth or root segments are infested with microbes, typically *E. faecalis*, and surviving organisms are monitored after various mechanical and chemical disinfection procedures. There is a long way from such ex vivo studies to proven clinical efficacy, but the methodology may have value in finding differences in efficacy among products and techniques and thereby provide a rationale for clinical studies with these variables.

11.4 Infection Control During Treatment

The basic principles and practices of asepsis outlined in Chapter 10 for vital, uninfected teeth fully apply also to infected teeth with apical periodontitis, as do the principles of instrumentation and root canal filling. Endodontic treatment of the infected root canal has the added purpose to maximize the possibility of completely disinfecting the root canal system and to maintain asepsis for the lifetime of the tooth.

11.4.1 Field Isolation and Disinfection

Practitioners with limited skills and insight repeatedly question the need for tooth isolation by rubber dam. Given the infectious nature of pulpitis and apical periodontitis, any means of reducing the microbial burden, including application of the rubber dam, are of course helpful. A research protocol omitting such procedures would be ethically inappropriate, and in comparisons of treatments done with or without rubber dam the rubber dam cases have shown better prognosis [116]. Furthermore, patients run a greater risk of aspirating or swallowing sharp objects or harmful chemicals applied during the procedure [2]. Judicious, often extensive, removal of existing restorations that may harbor bacteria and of residual carious dentin is obligatory. This may create a need for complex reassembly of structure to facilitate placement of the rubber dam (see Figure 11.3)

Traditionally, chlorhexidine or iodine solutions have been used for field disinfection of the isolated tooth and its surroundings. Five percent iodine tincture alternating with full-strength hydrogen peroxide was shown by Möller to be effective [133]; and satisfactory disinfection by swabbing with 3% hydrogen peroxide followed by 2.5% NaOCl has also been documented [275].

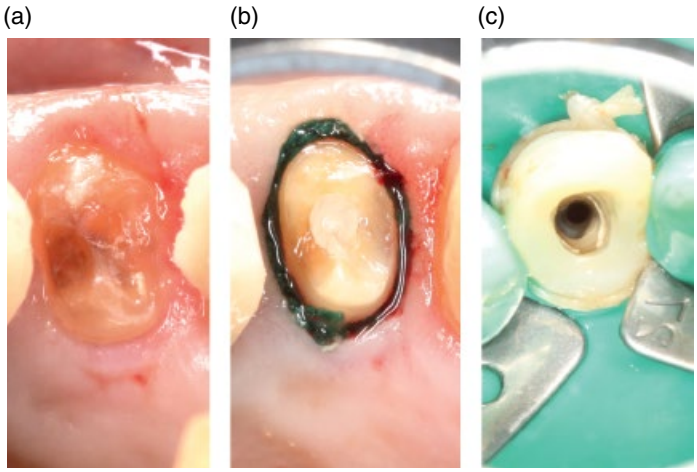

Figure 11.3 Pre-endodontic build-up: (a) on admission; (b) after complete removal of caries with protection of the exposed pulp and hemorrhage control; and (c) bonded build-up in situ with rubber dam fixed with clamps on neighboring teeth to limit stress on the interim restoration. (Courtesy of Dr Nikola Petronijevic.)

11.4.2 Mechanical Instrumentation

Quantitatively, mechanical instrumentation by any means, hand- or machine-operated, stainless steel or nickel-titanium instruments, removes the vast majority of cultivable microbes from the infected root canal in vivo [26, 51, 153]. The greater the initial load of root canal infection, the greater the likelihood that more efforts are needed for optimal reduction or total elimination of the microbes [26, 275]. Moreover, even if the quantitative reduction is impressive, residual bacteria are usually detectable in all or almost all teeth subjected to mechanical instrumentation only [26]. Therefore, supplemental, antimicrobial efforts need to be applied for effective disinfection.

11.4.3 Antimicrobial Irrigation

Irrigation solutions are used for various purposes during instrumentation or preparation of root canals. Solubilization of soft/necrotic tissue, solubilization of dentin, lubrication/wetting for ease of instrumentation, and opening of dentinal tubules are functions considered important for treatment. Keeping the primary treatment goal in mind, all of these functions serves the purpose of minimizing the microbial presence in the root canal.

Irrigating solutions range from sterile saline to strong disinfectants. Sodium hypochlorite, in concentrations ranging from 0.5 to 5.25%, remains the standard for root canal disinfection. It kills a broad spectrum of bacteria; it dissolves necrotic tissues; it disrupts and remove biofilms; and it contributes to the cleansing of the canal system [72]. Chlorhexidine in concentrations from 0.5 to 2% also has strong antibacterial activity and its substantivity to hard tissues gives it prolonged activity in dental tissues, but it is less effective on biofilms [67, 72]. Ethylene diamine tetra-acetate (EDTA) is used not for its antibacterial activity, which is is low, but for dissolving and eliminating the smear layer and dentin debris in the instrumented canal [251]. Old and new formulations are regularly proposed and tested for suitability in endodontics. Formaldehyde and glutaraldehyde may match the antibacterial effect of NaOCl, but their use has been abandoned due to their toxicity and carcinogenicity. Other compounds tested are less antibacterial than NaOCl and are at best adjuncts in the disinfection of the root canal.

11.4.4 Principles of Chemical Disinfection and Mechanisms of Action

Disinfection is the process of killing pathogenic organisms or rendering them inert. Most commonly, this is achieved by thermal or chemical means. There are general principles for the process of disinfection, principles that apply to root canal disinfection as well. Primarily, the disinfectant must be effective against the pathogenic organism or organisms. Apical periodontitis is a non-specific disease, so the disinfectant must reach a broad range of potential pathogens.

Second, the agent must penetrate relevant tissues and physically touch the microbes. The solution or suspension must have surface and chemical properties that allow penetration through vital and necrotic tissues, biofilms, and dentin structures.

Third, disinfection takes time. Time is required for the agent to reach the target organisms, to break down extracellular matrix that protect them, and for the direct destructive action of individual cells. Classical studies on the reference disinfectant, phenol, showed that full strength phenol required 30 minutes to kill *Escherichia coli* even in planktonic suspensions [82]. The time-to-kill issue may have bearing on the single-visit controversy (see below).

Fourth, disinfectants generally show increased activity against microorganisms at higher concentrations. However, tissue toxicity of the disinfectant may limit the concentration that may be safely applied. The high relative toxicity of the most potent agents, the aldehydes, has made them poorly suitable for endodontic use [216]. Because there may be consumption of the active ingredient by adsorption to tissues and debris and by chemical reactions, adequate concentration is maintained as much by replenishment during use as by having an initially high, but potentially toxic, concentration of the agent.

Fifth, the disinfectant may be energized through physical/thermal means. Ultrasound activation is generally acknowledged to increase the antimicrobial effect of irrigants in the root canal [139], particularly as so-called passive ultrasonic irrigation (PUI). Instruments for and methods of applying ultrasound to endodontic irrigation fluids have been developed and extensively studied [71, 136, 253, 271, 273]; however, the effect may be marginal and have limited clinical significance [155], particularly as the most apical part may not be much affected [252].

11.4.5 Principles of Biomechanical Instrumentation

Biomechanical instrumentation in endodontics means the mechanical preparation of the root canal with due respect for the biological and anatomical features of the canal system. Mechanical instrumentation serves three purposes: it should shape the canal to accommodate and fit a root filling; it should remove residual pulpal tissue, debris, and microbes from the canal lumen; and it should facilitate and support the antimicrobial action of the irrigating solution. The latter two purposes link directly to the biological purpose of eliminating pathogenic microbes as the overall goal of treatment.

Standardization of instruments: Harmonizing instruments with root filling core materials has a long history in endodontics. Championed by Ingle in the 1950s [81] and formalized with the basic 2% taper in international standards, the concept was revitalized in the 1970s with supporting clinical data by Kerekes and Tronstad [91–94]. Many, but not all, root canals may be instrumented to standard apical sizes with elimination of pulpal tissue and infected debris in a circular preparation in the apical 3 to 5 mm region. The standardized technique also showed good clinical results, as good as or better than the step-back technique used traditionally [90, 94]. Two per cent taper is optimal for hand instrumentation: it limits extensive contact with canal walls coronally, thereby preventing fastening of the instrument and risk of fracture, while allowing effective dentin removal in the apical part.

Greater taper has become the norm with machine-operated instruments. One perceived drawback with the standardized, hand

instrumentation was the danger of enlarging the apical part too much, creating ledges and causing perforations. Step-back hand instrumentation [257] reduced this risk, but produced a non-standardized apical preparation that did not correspond to prefabricated cores. Rotary and reciprocating instruments with greater or varying taper produce, at least in theory, a canal that can be filled completely with cores of corresponding sizes, thus combining the theoretical advantages of the two classical hand instrumentation techniques.

Mechanical efficacy: The tissue-removing ability of instruments is dependent on their size and ability to touch canal walls. This is traditionally tested by scanning electron microscopy [29, 77, 135, 159, 168, 190, 200, 248], more recently also by micro-computed tomography (micro-CT) [17, 22, 35, 49, 157, 158, 162–164, 247, 279]. While results vary and some instrumentation systems may perform better than others, it is a universal finding that instruments do not touch a significant part of the canal surface area (some 30 to 50%). Therefore, mechanical instrumentation alone cannot ensure disinfection during treatment of apical periodontitis. In bacteriologically controlled clinical studies, most teeth remain infected after instrumentation in the absence of a disinfectant [26, 153].

Facilitation of disinfection: Instrumentation supports the activity of disinfectants by facilitating contact of the agents with biofilms, and in the case of sonic and ultrasonic instrumentation by energizing the antibacterial agent. Intentional dentin erosion by ultrasound is currently not a preferred method, and energizing the disinfectant by ultrasound is mostly done by passive ultrasonic activation, where the instrument is not supposed to act on the dentin walls. Any hand- or machine-driven instrumentation technique will drive the accompanying irrigation liquid towards the dentin walls and the apical area. As more debris and infected material are removed by mechanical action, the distance between the disinfectant and residual microbes becomes shorter and the volume of cells to be killed becomes smaller. These considerations formed the basis for maintaining that the apical preparation should be made as large as possible (without compromising the integrity of the root end). This would more likely eliminate bacteria in a greater part of the apical delta and facilitate diffusion of the irrigation liquid into spaces inaccessible to the instrument [153], possibly improving the incidence and rate of healing [127]. The counter-argument is that extensive instrumentation weakens the root and renders it more prone to cracks that may lead to fracture, and carries a high risk of perforation.

11.4.6 Intracanal Medication

An extraordinary range of substances has been placed in the root canal with the purpose of curing apical periodontitis: creosote, formaldehyde, glutaraldehyde, iodine formulations, chloramine, cresols, even radioactive substances [137] are among them. Concerns of toxicity and carcinogenicity and simple lack of demonstrable effectiveness have eliminated most of these.

The infectious nature of apical periodontitis was unequivocally demonstrated in the 1960s and early 1970s [85, 225, 235, 236], but a concept of medication as a means of supporting the host's healing ability remained. The pioneering work of Cvek [43–48] indicated that a thick slurry of calcium hydroxide was effective on both fronts: he demonstrated a reduction or elimination of bacterial growth in teeth thus treated, and after long periods of dressing (months to more than a year), healing was seen with formation of a hard tissue barrier (apexification) in immature teeth.

Buffered formaldehyde and camphorated chloro-phenol compounds were routinely used as dressing at that time, based in part on their favorable antibacterial-to-tissue toxicity ratio [216]. Iodine potassium iodide is more effective than chloro-phenols, but its action is short-lived and it was used for short intervals of 2 to 4 days only. When comparative tests favored calcium hydroxide as medication over both camphorated chloro-phenol [24]

Figure 11.4 Microbial control during endodontic treatment. Percentage of cases positive for bacteria. S1, sample on entry; S2, after instrumentation and irrigation in first session; S3, after an inter-appointment dressing with calcium hydroxide. Data from [123, 153, 161, 201, 212, 275]. Zandi and Rodriguez data are with molecular techniques and from retreatment cases.

and iodine potassium iodide [189], $Ca(OH)_2$ became established as the medicament of choice for inter-appointment dressing.

From the onset, calcium hydroxide was supposed to support the healing processes as much as being an effective disinfectant. It was applied for months while watching for healing by sinus tract closure, elimination of pain or discomfort, and radiographic signs of lesion reduction. As healing is difficult to assess before three months [95], this became the standard duration for this treatment modality. However, focus shifted to an emphasis on the antimicrobial effects. Using microbiological monitoring, the time for calcium hydroxide to complete its antibacterial effect came down from many months [47] to 4 weeks [24] and, finally, 7 days [212]. When applied for 10 minutes at the end of the first session no benefit over irrigation was observed [212].

11.4.7 Infection Control in One and Multiple Visits

The rationale for a dressing period is based on first, the surrogate measure of root canal infection as a predictor of success, and second, the improved incidence of healing when canals are bacteria-free at the time of filling. Several subsequent studies have used a similar methodology to optimize the disinfection process [1, 31, 37, 51, 84, 123, 124, 126, 140, 155, 161, 173, 181, 184, 185, 201, 206, 254, 258, 264, 270] (Figure 11.4), but it has proven difficult to establish a protocol that will predictably eliminate bacteria from the root canal. Rather, the importance of an added antibacterial effect of calcium hydroxide dressing has been questioned [107, 161]. If a dressing does not improve infection control to a clinically significant level, then an interim period with a temporary filling carries a risk of new contamination that may outweigh any small benefit [275].

Treatment of apical periodontitis in one appointment saves time and cost, and many well-designed, randomized clinical experiments have been done comparing the short and long-term clinical outcomes after treatments in one or two visits. These include symptomatic as well as asymptomatic cases. As would be expected, there are differences in results among individual clinical experiments, some favoring two-visit [186, 241], others one-visit treatments [61], and many finding no difference of importance [21, 64, 262, 266, 268]. It seems safe to draw the conclusion that in terms of subjective (pain experience, analgesic usage) and conventional objective (radiographic) signs of healing after treatment, there is virtually no difference between the

two treatment modalities. This has been repeatedly documented for symptomatic as well as asymptomatic teeth in almost all of several systematic reviews [59, 193, 194, 197, 221, 267].

While clinical and conventional radiographic criteria are the practical yardstick for success, the histopathological appearance and CBCT may be more sensitive in detecting differences. Data from animals [76, 88] and humans [245] certainly suggests that fewer bacteria may be seen and healing appears better after a dressing with calcium hydroxide, and a randomized study using CBCT for assessment showed a somewhat greater lesion volume reduction for teeth treated in two sessions [52].

It is in the interest of all parties to limit the time and effort necessary for treatment success. There is no arguing against prolonged disinfection, by any means, for the purpose of maximal reduction of canal bacteria. But achieving complete asepsis may require several visits or longer intervals [24], and the protocol to confirm it is complicated [138]. Given the results of systematic reviews and meta-analyses, it is becoming increasingly difficult to maintain two or more visits as the standard protocol for treatment leading to success by conventional clinical-radiographic criteria. On the other hand, a dressing with calcium hydroxide remains the effective procedure when dictated by time constraints or particular clinical considerations.

11.4.8 Special Techniques

Alternative approaches to mechanical instrumentation with rigid files are sought and developed (see Chapter 10). A "non-instrument" cleaning of root canals was developed where an airtight seal was established over the tooth after access to the pulp, whereby a vacuum or reduced and variable pressure could be established and activate irrigating solutions [119, 120]. Recent developments of this concept has been tested ex vivo and clinically in cases of chronic apical periodontitis with favorable results in terms of both bacterial reduction and clinical-radiographic outcome [32, 73, 132, 202, 203, 261, 265], but randomized comparisons with other techniques in clinical studies are as yet lacking.

11.5 Root Filling Phase

11.5.1 Purpose and Functions of the Root Filling

The primary function of the root filling is the prevention of new infection of the root canal system. There are two ways of fulfilling this task: either to prevent microbes from entering or to kill them when they try to penetrate the barrier created by the material. The first depends on the physical integrity and adaptation to tooth substance by the materials; the second requires some form of biological activity, which in turn is dependent on a degree of solubility of the material. Any antimicrobial properties of the materials may also support disinfection of the root surface and canal system by the biomechanical procedures [149].

In mature teeth with closed apices, the area of contact of the filling material with soft tissue is so small that biocompatibility is of limited importance. However, in other clinical situations, e.g., pulp exposures, perforations, and surgical approaches, the area of contact between the material and soft tissue is much larger. Here the material must possess properties that permit, at best promote, healing and regeneration of the relevant tissues [60], in addition to preventing bacterial activity.

There are thus three properties necessary for the clinical performance of endodontic materials: sealing ability, antimicrobial activity, and biocompatibility. In addition, the materials must have properties that allow their proper placement and which ensures durability [70].

These properties of the root filling reside mainly with the sealer cement, and there is a large body of literature characterizing the chemistry and physical properties of the various types of sealer. Some comparative

Table 11.1 Sealer types, examples and clinical tests.

Material	Subgroup	Examples	Clinical testing on cases with apical periodontitis
ZnO-eugenol		ProcoSol, Roth 811	Eriksen et al., 1988 [56] Trope et al., 1999 [241]
Resins	epoxy	AH26, AH plus	Conner et al., 2007 [14]
	methacrylate	EndoRez, RealSeal	Barborka et al., 2017 [14]
$Ca(OH)_2$		Apexit, Sealapex	Waltimo et al., 2001 [255]
Silicone		RoekoSeal, GuttaFlow	Huumonen et al., 2003 [78]
Ceramic	Ca-Si	BioRoot	
	Ca-Si-P	Endosequence	
Gutta-percha	beta	generic	
	alpha	GuttaFusion, GuttaCore, Herofill	
Resin cores	complete	Resilon	Barborka et al., 2017 [14]
	resin coated	EndoRez, Endosequence	

clinical studies have compared the long-term performance of sealers, and almost all have shown very small if any differences [56, 78, 150, 152, 255]. However, the finding of long-term failure of some new products [14, 263] is a reminder of the need for vigilance in the process of accepting and promoting new methods and materials.

Core materials are developed and designed to be inert and stable, yet must possess some plasticity to adapt to the instrumented canal. They act as pistons for the sealer, which determines the functional properties of the root filling. Table 11.1 lists some sealer material types used for root filling.

11.5.2 Antibacterial Properties

Antibacterial properties are usually associated with some degree of tissue toxicity [217, 218], and there is limited incentive to develop sealer materials with strong antibacterial activity. However, most products are antibacterial before setting; many have remaining activity towards bacteria also after setting and into dentinal tubules [39, 86, 167, 169]; and additions of antibacterial components to sealers are commonplace [11, 16, 38, 187]. Bioceramic sealers create a strong alkaline environment locally, which acts as a long-lasting inhibitor of microbial growth [4, 260].

11.5.3 Biological Properties

Root-filling sealers need to be tolerated by the tissues, and even if they may be strongly cytotoxic before setting, most are quite bland when set [117, 146, 147, 280]. Bioceramic sealers have properties that may stimulate repair [34, 269]; this may be valuable in situations where there is a large contact area with the periodontal tissues; e.g., root-end fillings and perforation repair [149].

11.5.4 Basic Clinical Guidelines for Root Filling

Apical level. While the apical extent of the root filling has little bearing on the the prognosis in pulpectomy cases [213], it is clear from follow-up studies that it is of crucial importance for treatment of apical periodontitis [142, 145, 213] (Figure 11.5). Radiologically, this means that the root filling should end 0 to 2 mm short of the radiographic apex in infected cases, probably

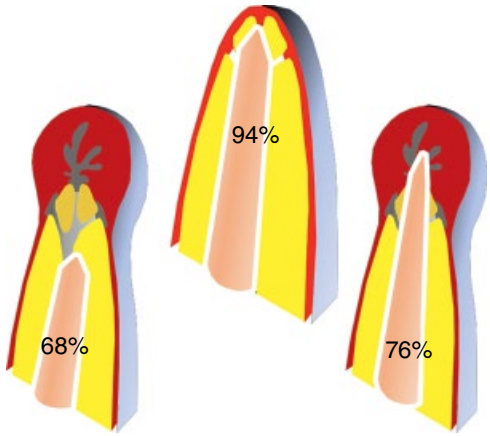

Figure 11.5 Critical influence of root-filling-to-apex distance on outcome of treatment of apical periodontitis. Root fillings ending within 2 mm from the radiographic apex may give results comparable to root fillings of vital pulps. Too short fillings or overfills strongly reduces the success rate. Modified from [213].

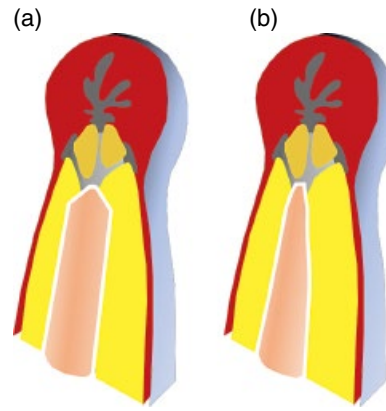

Figure 11.6 Apical box and tapered preparations. (a) the apical box philosophy emphasizes wide instrumentation for maximal removal of infected dentin and access of disinfectant; (b) a tapered preparation is designed for better conformity with the original canal shape.

reflecting that the bulk of the infection is coronal to the apical constriction.

Width of apical preparation. One treatment philosophy argues for extensive apical instrumentation to eliminate as much as possible of infected dentin [31, 94]; other investigators stress the importance of selecting the final file size after gauging the actual diameter of the apical root canal [143, 257] and avoid the risk of ledging and transportation (Figure 11.6). There is little data on the clinical importance of apical preparation size. Large case series show successful results with the extensive approach [94, 152], others have reported good, maybe better, outcome with the step-back, apical conservative technique [143]. Irrespective of method chosen, clinicians should be aware that infected teeth with lesions generally have larger apical foramen diameters than teeth with vital pulps [62].

Patency. The concept of patency is controversial [79]. Maintaining apical patency by repeatedly securing access beyond the apical constriction with very small files would seem to favor access of the irrigating solution to the most apical areas. It may also prevent accumulation of infected debris and be beneficial in producing a good apical seal of the filling. There are data to suggest that patency may have a positive effect on prognosis [108, 128, 142, 230]. On the other hand, the procedure carries the risk of transporting infected material into the periapical area with potentially negative consequences for development of exacerbation and for the long-term prognosis [80, 121, 128]. It seems safe to conclude that if maintenance of patency is desirable in a given case, then very small files (ISO 008 or 010) should be used, and mainly in cases where the apical constriction is sufficiently large to allow unimpeded passage of the patency file [79].

Compaction of filling. The quality of the root filling as judged by radiographic homogeneity appears to affect the prognosis after root filling of teeth with apical periodontitis [94], but results vary among studies and may be of particular significance for retreatment procedures [142, 213].

Coronal seal. The long-term success of treatment also depends on the quality of the coronal restoration [101, 103, 104, 171, 215, 237]. More specifically, a tight coronal seal extending below the marginal bone level is associated with less apical periodontitis [219]. This may also prevent bacteria from

entering the filled root canal from lateral canals and dentinal tubules exposed to a periodontal pocket.

11.6 Clinical Issues During Diagnosis and Treatment of Primary Apical Periodontitis

Successful disinfection and effective filling of an infected pulp canal ensures success to the level achieved for vital pulp extirpations [94, 152, 213]. However, there are situations where the disinfection becomes especially difficult or impossible, and the infection may pose special problems not encountered in pulpectomies.

11.6.1 Fracture

If a fracture or crack involving the pulp and extending to or from the gingival margin is present, one cannot predictably treat the root canal. Disinfection and filling procedures may mask symptoms for a time, but eventually the crack will allow ingress of microbes to renew the infection and inflammation.

11.6.2 Marginal Periodontitis

The so-called endo-perio-problem (Figure 11.7) has been extensively discussed and reviewed [3, 196, 277]. The pulp status governs the endodontic treatment options: pulpal sensitivity strongly suggests that there is no apical periodontitis as part of the diagnostic issue. However, if a periodontal pocket is established with communication to the apical area and the root filling, the continuous seeding of bacteria from the plaque in the pocket will maintain the apical inflammation. While there are case reports of pulpal infection from a periodontal pocket [276], these situations are rare and usually do not interfere with successful endodontic treatment of the ensuing apical periodontitis. However, secondary infection from deep periodontal pockets may contribute to post-treatment apical periodontitis in some cases [188].

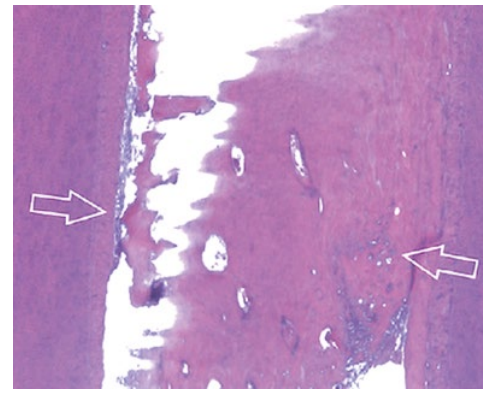

Figure 11.7 Histological analysis of tissues generated in the root canal of a human tooth after revitalization treatment. Detail from the middle third portion of the canal. The canal is partly occupied by a newly formed calcified tissue (between arrows; original magnification 950). Reproduced with permission from [115].

11.6.3 Exacerbations

Subjective symptoms, if present, normally subside quickly after instrumentation with disinfection, but the procedures carry some risk of initiating an acute exacerbation of the infection. The environmental change in the root canal may favor growth of more virulent organisms, and instrumentation beyond the apex may transport microbes to that area, giving rise to a transient, but sometimes painful, even dramatic, clinical condition. Treatment procedures follow general recommendations for acute oral infections. Significant inter-appointment pain hardly occurs in extirpation procedures [9], testifying to infection, not physical effects of over-instrumentation or chemical irritation by irrigation solutions, as the sole cause of so-called flare-ups [208, 210].

11.6.4 Anatomical Variations and Anomalies

Isthmuses between root canals in the same root is a source of persistence of infected material [98], and particular efforts are made to clean and disinfect these areas [5, 54, 205]. Simple forms of dens invaginatus can be

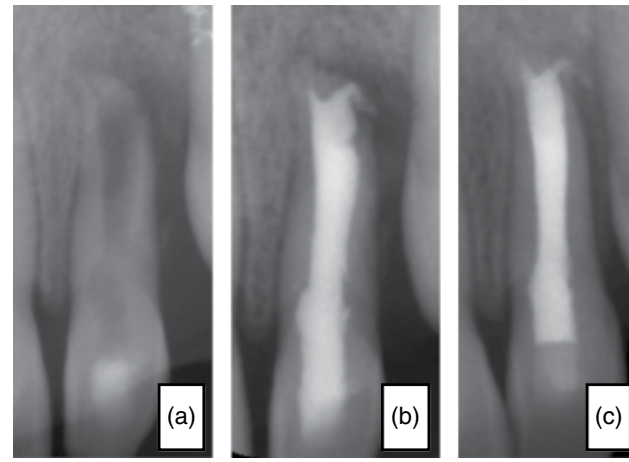

Figure 11.8 Apical periodontitis in a tooth with dens invaginatus successfully treated despite highly irregular apical anatomy: (a) on admission; (b) immediate postoperative; (c) 5 months follow-up. (Courtesy of Dr Line Hardersen.)

fairly easily treated by conventional methods (Figure 11.8), while more complex types require combinations of surgical and operative adjunctive treatments.

11.6.5 Influence of Systemic Disease

Patients with certain chronic diseases may have a healing pattern that varies from the healthy individual. They may also be more prone to exacerbations. Diabetes is the typical case. Apical periodontitis seems to occur with higher frequency in diabetic patients and healing after treatment may be slower [75, 113, 192, 199, 228].

11.7 Treatment of Persistent or Recurrent Apical Periodontitis

All treatment procedures in retreatment cases have the same aim as for treatment of primary apical periodontitis, but the presence of a root filling complicates the issue. The root filling itself, sealer or core material, may be the substrate for biofilm formation, which typically occurs in the interface between filling material and the dentin wall [178]. Residual bacteria in association with remnants of root filling material are difficult to reach and eliminate, which makes it crucial that one removes all root filling material.

11.7.1 Instrumentation and Removal of Filling Material

Any of several types of rotary or hand instruments are used for removal of the root filling. The Hedstrom file is useful for manual approaches, and many commercial file systems include rotary or reciprocating instruments designed for root filling removal [30, 83, 96, 125, 204, 272].

Chemical solvents may assist in the removal of gutta-percha. Chloroform is highly effective, but toxic and potentially carcinogenic [229]. Xylene is quite effective, but also toxic. Limonene or orange oil is a substance derived from citrus fruits and is available commercially [170, 242, 243], which while less effective than chloroform or xylene is also less volatile and toxic.

Given the range of compounds and of the chemistry that go into the sealers setting around the gutta-percha cone, there is no general solvent for these materials. Essentially, one must rely on the mechanical procedures for maximal removal of the sealer, and by current methodologies, complete removal may be virtually impossible [10].

11.7.2 Biofilms in Recurrent Apical Periodontitis

The bacteria associated with filling material and the difficulty in physically eliminating the residual material offer one

explanation for the reduced prognosis of retreatments [19, 141, 144, 145]. It also helps explain the experience that retreatment of root fillings of poor quality has a better prognosis [53], as material removal is easier and the infecting organisms reside in the previously unfilled regions of the pulp canal space.

It has been proposed that the microbial composition in persistent lesions differs from that of primary apical periodontitis (see Chapter 4). While there may be more facultative organisms, particularly streptococci and enterococci, attempts to augment disinfection procedures in retreatments by targeting this group of organisms [8, 129, 211, 227] have so far not resulted in proven clinical protocols.

Bacteria may establish a foothold in the tissues beyond the apical foramen and outside the reach of medicaments in extraradicular infections [68, 165, 174, 175, 180, 183, 224, 238, 240]. Attempts to treat such cases with antibiotics [15] have not been shown to reliably lead to healing, and root-end surgery is routinely applied [180].

11.8 Treatment of Immature Permanent Teeth with Apical Periodontitis

Apical periodontitis in immature teeth with open apices could be successfully treated with conventional methods, and formed part of the basis for calcium hydroxide dressing as a modality [48]. However, teeth thus treated have thin walls and may be prone to fracture. The risk of a new trauma is increased in children with a previous history of oral trauma [166]. Therefore, the finding of hard tissue formation in immature teeth with apical periodontitis treated with an antibiotic paste (Figure 11.9) opened for an improvement of the fracture resistance of such teeth [13] and stimulated extensive research in regenerative endodontics [99] (See Chapter 8).

11.8.1 Definition of Terms

As with other regenerative or tissue engineering methods in dentistry, procedures are not yet standardized to a point where the outcome is predictable. Moreover, there are many biological processes involved that are poorly understood [99], and the clinical aim of treatment is often only vaguely formulated. The cells involved and mechanisms for their activation are described in Chapter 8. In cases of apical periodontitis in immature teeth, the primary goal is to stimulate completion of *apexogenesis* [66] for root length and strength. Other methods also predictably achieve this: a dressing with calcium hydroxide was the standard procedure, today, bioceramic materials applied in a single visit give similar results [41].

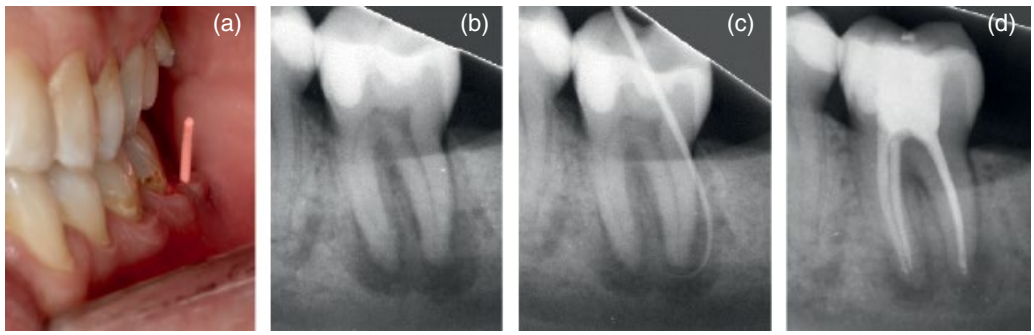

Figure 11.9 A gutta-percha point is inserted in a periodontal pocket buccal to a lower left second molar (a) with diffuse radiolucency (shown in b). The point traces to the apex of the distal root (c). The lesion has healed 12 months after endodontic treatment (d). (Courtesy of Dr Dyveke Knudsen.)

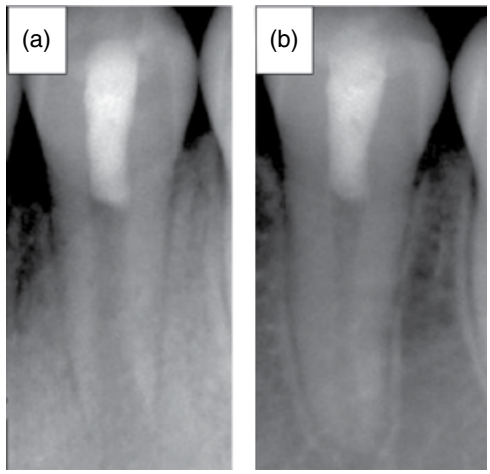

Figure 11.10 Preoperative radiograph of tooth #35 with open apex (a), and 12-month postoperative radiograph (b). Continuous root extension and canal wall thickening, relative to the preoperative image. Reproduced with permission from [110].

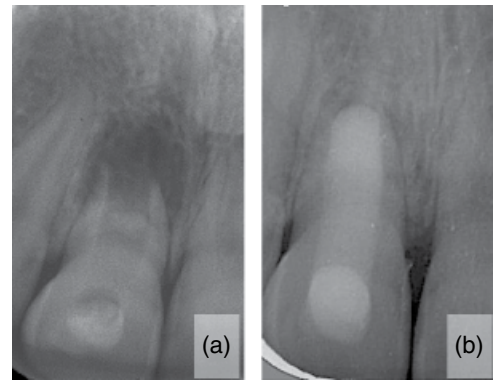

Figure 11.11 Apexification. The infected canal (a) was cleansed with light instrumentation and copious irrigation, followed by a dressing of calcium hydroxide. Four mm of MTA was packed in the apical part and the tooth root-filled with a resin restoration coronally. The 15-year follow-up radiograph shows dental hard tissue formation with generation of a complete periodontal ligament (b). (Reproduced with permission from [172].)

Revascularization. This is the simplest and least biologically demanding process applied to pulpless teeth. First attempted in 1961 [154], it aims basically to achieve ingrowth of a vascularized tissue into the vacated pulp space, supporting and promoting apexification or apexogenesis [6]. Revascularization is the first step towards repair or regeneration.

True *regeneration* may be said to occur when vital pulp tissue with functioning odontoblasts is established in the canal. This is still experimental at best, and the practical aim in clinical practice is biological repair by induction of some form of hard tissue in the root canal [115]. If integrated with the pre-existing dentin, the root may be strengthened by the osteoid tissue, but new dentin integrated with pre-existing dentin is currently not achieved (see Figure 11.10).

11.8.2 Case Selection

The first cases with impressive hard tissue formation were in premolars with dens evaginatus and no history of trauma [13]. Teeth with apical periodontitis caused by caries affecting the convolutions in the occlusal enamel may respond better to regenerative treatments than traumatized teeth [114], which often have concomitant damage to other dental tissues. If apexification (Figure 11.11) or apexogenesis is the main clinical purpose of treatment, then classical protocols with applications of calcium hydroxide or bioceramic materials followed by conventional filling of the coronal pulp space have predictable results [41].

11.9 Monitoring Healing, Prognostication

Chapter 6 gives an overview of prognostic factors and the fate of endodontically treated teeth. Many follow-up studies do not separate the outcomes for initially healthy and diseased periapices. While one may assume that most factors are common for all diagnoses, it is known that some are exclusively linked with the presence of a preoperative lesion, e.g., the apical end-point of the filling [213].

There are essentially three aspects of healing apical periodontitis: relief from pain, resolution of clinical signs (swelling, sinus tract), and radiographic assessment of replacement of the lesion's soft tissue with bone. In

addition, tooth survival is often used for recording the clinical success of treatment.

11.9.1 Signs and Symptoms

Signs and symptoms, if present at start, are generally eliminated or reduced by most treatment protocols, but may persist or develop in up to some 10% of cases [143]. While some changes in the nervous system due to the inflammation and/or manual severance of pulpal nerves may account for some altered sensitivity, some of these minimally or symptomatic teeth probably harbor a residual infection that causes inflammation.

11.9.2 Radiographic Monitoring

Most studies of treatment outcome in teeth with apical periodontitis focusses specifically on radiographic healing. Classical success/failure-analysis utilizes a comparison of preoperative, periapical radiographs with radiographs taken at control, but stresses the importance of a re-established lamina dura and normal periodontal structures [143]. These so-called Strindberg criteria [220], with modifications, have been and are widely used. Interpretation of radiographs is very prone to personal bias, and the success/failure approach is no exception [65]. The periapical index (PAI) [151] is an attempt to reduce bias by relating experimental, test images to histologically verified references [23] and has been used extensively in recent years.

Irrespective of the scoring system used, it is essential that observers are trained and calibrated for results to be comparable over time and geographical area. Such calibrations should be standardized and related to a common set of images with recognized status of healthy or diseased. Few studies document global calibration and caution must be exercised in comparing results from one study with others.

11.9.3 Cone Beam Computer Tomography

CBCT offers a more sensitive and probably more specific detection of apical disease (see Chapter 5). Comparison of PAI scores with the lesion volume show a clear and significant correlation, but the same study highlights the limitations of a 2-dimensional radiograph compared to the 3-dimensional CBCT images [122]. Indices have been proposed also for CBCT recording of apical lesions [57, 233], which may become important for comparisons of results across studies.

Follow-up data on treatment suggest that many teeth considered healed by conventional radiography may show residual lesions and other factors may prove relevant for prognostication when monitored with CBCT [111]. The associations of prognostic factors observed by conventional radiographs remain, but they cannot automatically be transferred to similar studies with CBCT. Moreover, some lesions detected by CBCT may reflect fibrous, not infected and inflamed tissue, at least in surgical cases [106].

11.9.4 Tooth Survival

Tooth survival is a crude measure of follow-up of endodontically treated teeth. It is frequently used in studies comparing endodontic treatment with other treatment options, particularly implants [33, 234]. Tooth survival after endodontic treatment is, however, not only a reflection of the endodontic treatment itself. It is as much, or more, dependent on the periodontal conditions and on the degree of loss of coronal tooth substance [12]. Assessing the survivability of teeth is of course essential for treatment planning, especially for complex clinical cases, but treatment of apical periodontitis is generally so successful that it rarely is a decisive factor in such considerations.

11.10 Concluding Remarks

11.10.1 Principles of Case Selection and Treatment Decisions

Prevention is better than cure. This is true for endodontology as for other diseases. Successful treatment of many teeth with large lesions [143] does not alter the fact that

in all prospective outcome studies the prognosis is significantly better for root filling after vital pulp extirpation [36]. Moreover, the larger the lesion, the poorer the prognosis [58, 102]. A traditional, wait-and-see approach to endodontic treatment runs the strong risk of reducing the prognosis when treatment eventually is initiated. Moreover, investigations with CBCT detect approximately twice as many lesions as periapical radiographs [111], magnifying the difference in prognosis between teeth with and without preoperative apical periodontitis.

Furthermore, the generally poorer prognosis of retreatments [213, 232] points to the need for high-quality endodontic treatment of pulpitis and primary apical periodontitis. As dental students [94, 152] as well as specialists [143] routinely obtain more than 90% success by conventional radiography for teeth without a lesion, early intervention with optimal aseptic, disinfection, and obturation techniques is the key to a general improvement in periapical health.

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12

Surgical Endodontics

Frank C. Setzer and Bekir Karabucak

12.1 Introduction, Including History

Surgical treatment of teeth with apical periodontitis has a history that spans centuries. The 1880s saw the introduction of approaches to resect a root with the intention to remove necrotic portions of the apex [17, 34, 70], as well as of apical curettage to remove diseased periapical tissues, but without addressing sources of infection from inside the root canal system [199]. In the 1890s Partsch published various reports on root-end resection [181–183], leading to wider acceptance in Europe [71].

A great variety of surgical techniques have been suggested and put to use to make the procedure safer for the patient, easier to perform, and more predictable [79]. The standard approach for many years included access and root-end resection with surgical burs and the use of amalgam as root-end filling material [43, 64, 103, 176].

With improvements in chemical and mechanical cleaning for both initial root canal treatment as well as non-surgical retreatment, the usefulness of endodontic surgery was called into question as some clinical studies demonstrated extremely low outcome [7, 76].

Unsuccessful outcome of surgical endodontics is caused by the failure to address the biological issues responsible for causing apical periodontitis. For the majority of the situations the primary reason for failure of either non-surgical and surgical endodontic treatment is the continued presence of intra- and/or extraradicular infection. Success of either treatment will rely on eliminating this infection, or at least lowering the number of microbial cells below a threshold that will allow the body to resolve the apical periodontitis, and entomb them within the harsh environment of a sealed root canal system.

Persisting intracanal infection will be a source of failure after surgery if the method and materials used did not provide an effective seal. This may be aggravated if an apicoectomy leaves open a large number of tubules and canal ramifications, through which microorganisms may penetrate and cause periapical inflammation [51]. An operating microscope to identify these anatomical details together with new materials for root-end filling provides for better treatment and more predictable outcomes [79].

Modern endodontic surgery has evolved into endodontic microsurgery [125]. The surgical operating microscope gives high magnification and direct illumination. This reduces the need for beveling of the resected root and reduces the number of exposed dentinal tubules [51]. Ultrasonic instruments for in-axis root-end cavity preparation limit damage and achieve better cleaning [239], and the use of biocompatible and stable root-end filling materials support healing

Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition. Edited by Dag Ørstavik.

Companion website: www.wiley.com/go/orstavik/essentialendodontology

processes better than conventional cements [203]. This chapter seeks to provide an evidence-based overview of the state-of-the-art procedures with a focus on surgical retreatment, while contrasting the differences with traditional, historic techniques and other surgical endodontic procedures.

12.2 Surgical Endodontic Procedures

Procedures recognized as endodontic surgery include apical curettage, periradicular surgery, crown and root resections, as well as tooth replantation. Periradicular surgery includes, historically, apicoectomy (simple root-end resection without retrograde filling), in its modern context procedures such as exploratory surgeries and surgical perforation repair, as well as classical root-end surgery combining root-end resection and root-end filling. Crown and root resections include root amputations, complete root resections, hemi- or trisection, and bicuspidization. Replantation includes transplantation and intentional replantation, of which the latter properly belongs to endodontic procedures.

12.3 Indications

12.3.1 Tooth-related Factors

Surgical intervention is a valid alternative in cases where non-surgical retreatment is unfeasible or has failed. The general indication for non-surgical or surgical retreatment intervention is the presence of apical periodontitis. Prior to choosing endodontic surgery as a treatment option, the etiology for any persisting pathology must be carefully assessed [120], including appropriate follow-up periods for pre-existing asymptomatic lesions. Non-surgical retreatment should be the preferred option if the quality of the initial root canal treatment is deemed insufficient, if there are indications of missed canals or need for a new permanent restoration. Most odontogenic periapical lesions, including granulomas [22, 228, 236], cysts [195], and abscesses will respond positively to appropriate non-surgical retreatment. The following situations may, however, prevent periapical healing after non-surgical retreatment or present an indication for surgical retreatment after initial endodontic therapy has failed:

Complicated Root Canal Anatomy

Teeth with extreme root curvatures (>30°) or s-shaped canals, canal division in the middle or apical third, very long roots (>25 mm), or open apices (>1.5 mm in diameter) may present with challenges for non-surgical retreatment that may be difficult or cannot be overcome. Moreover, calcified canals as well as internal and external resorptions may tip the balance in favor of surgical rather than non-surgical retreatment. Iatrogenic changes to the original canal anatomy, such as blockages, transportation, ledges, or perforations may prevent proper biomechanical disinfection [87] and leave microorganism in recesses of the root canal system in proximity to the constriction [274] or the apical foramen [227].

Pathophysiology of the Periapical Pathology

The overwhelming majority of periapical lesions are inflammatory in nature [18]. Nair et al. [195] classified 35% of lesions on extracted teeth as periapical abscess, 50% as granuloma, and 15% as cysts. Periapical cysts may be pocket cysts, which have direct connection with the infected root canal system, or apical true cysts, which are separated from the root [164, 195]. Of all periapical lesions, 9% were described as apical true and 6% as apical pocket cyst [195]. True cysts may be less likely to resolve by primary endodontic treatment or non-surgical retreatment [195], and may thus require surgical intervention. Periapical foreign body reactions, initiated by amalgam remnants, gutta-percha, and other endodontic filling and sealer materials, pieces of paper points, or cholesterol clefts associated with cystic lesions also occur [163–165].

Extraradicular infection may occur in the form of biofilms attached to the external root surface [257], as well as colonies of Actinomyces and Proprionibacterium species within the soft tissue lesion itself [194, 200, 229, 240]. If the initial size of the periapical defect exceeds 5 mm in diameter healing by non-surgical treatment may be impaired [168, 169]. All these scenarios are unlikely to be resolved by non-surgical retreatment, and may result in the need for endodontic surgery.

Altered Canal anatomy Impeding Non-surgical Instrumentation

Many retreatment cases display a microbiological spectrum that may be more difficult to eradicate (see Chapter 5). Moreover, as a consequence of the initial treatment, there may be alterations to the original root canal anatomy that may prevent instrument and irrigant access to all areas of the root canal system. These alterations include transportations, ledges or perforations and separated instruments. While some of these obstacles may only be obvious upon initiating a conventional retreatment attempt, some may be apparent in the treatment planning stage. The success rate of non-surgical retreatment cases with apical periodontitis together with altered root canal anatomy that could not be renegotiated is only about 40% [87]. Then, depending on the situation, including residual tooth structure and the existing coronal restoration, a choice between non-surgical retreatment followed by surgical intervention or surgical retreatment only must be made. Non-infected instrument fragments may not need to be removed, but in the presence of apical pathology removal attempts should be considered. In general, non-surgical retreatment should be attempted first for stainless steel or nickel-titanium fragments, however, it must be considered if the loss of tooth structure is justifiable [219]. Particularly at or beyond a curvature, the superelasticity of conventional nickel-titanium instruments may wedge them towards the outer curvature of the canal and make some removal attempts very difficult or impossible [179], so that sometimes a direct surgical option may be less invasive [120].

Hard Root Canal Filling Materials

A variety of root canal filling materials, including hard pastes and silver points may prevent progress during non-surgical retreatment. Resorcinol-formaldehyde pastes ("Russian Red") [6], formerly often used in East European countries may be impossible to remove from the root canal system, requiring endodontic surgery. Newer calcium silicate based sealer materials are also known to set very hard, however, no data exists to date in regard to clinical retrievability.

Posts and Build-ups

Long, prefabricated metal posts, cast post and core build-ups and crowns [120] may require a disassembly of the existing restoration that may carry risks of root fracture, excessive tooth tissue loss or perforation, favoring a surgical approach.

Resorptions, Perforations and Root Fractures

The repair of internal or external resorptions, as well as perforations, depends on the location within the root canal or the root [219, 226]. The more coronal any resorptive defect is located, the more likely a non-surgical retreatment is to succeed [155, 170, 193, 219], the more apical, a surgical approach will more likely be favorable. Apical resorptions usually arrest after non-surgical retreatment, however, if a resorption progresses they may need surgical treatment as well. Vertical root fractures may require exploratory surgery for clinical confirmation. In multi-rooted teeth surgical resection of the involved root may salvage the remaining roots and tooth crown, depending on the size and location of the defect. Horizontal root fractures will not require surgical intervention for vital teeth as the apical segment stays vital even if coronal fragment is necrotic [111]. However, a surgical approach may be necessary if the tooth was infected prior to the trauma [72].

12.3.2 Patient Participation in Treatment Selection

Evidence-based practice implies that the patient is informed about treatment alternatives with associated prognoses and risks. For endodontic surgery, it should be explained that it is a true surgical procedure with potential risks of damage to adjacent anatomical structures, postoperative swelling, discomfort and impaired wound healing. The patient will make the final decision based on her/his perception of advantages and disadvantages of the suggested procedures, the value they place on the tooth, as well as their willingness to undertake lengthy dental procedures, and the cost [138].

12.3.3 The Dentists' Role in Decision Making

The clinician's decision making must include a detailed medical and dental history, and clinical examination. The type of coronal restoration, presence or absence of posts, and the practitioner's skill level may influence treatment planning decisions [39, 238]. These are known to be different between endodontic specialists and general practitioners [230]. The dentist may strive for the academic success to heal a periapical lesion, whereas the patient may be more concerned about tooth survival and functionality. The decision to recommend treatment will be easier with the presence of symptoms, but more intricate for asymptomatic apical periodontitis. In particular, if surgical treatment is recommended, the clinician should have the adequate knowledge, skills, experience, and armamentarium to confidently render the procedure. Surgical procedures should only be undertaken with adequate training, though they may provide a highly predictable and expedient outcome in appropriately selected cases.

12.4 Contraindications

There may be situations where endodontic surgery may be compromised or contraindicated. This may include proximity of the surgical site to anatomical structures that could suffer severe or permanent damage, for example the mental and infra-alveolar nerves, the nasal or sinus cavities, or the palatal neurovascular bundle. Teeth with unfavorable crown-to-root ratio, increased mobility or advanced periodontal disease will have a less favorable prognosis. Systemic diseases, such as cardiovascular disease prohibiting the use of vasoconstrictors with the local anesthesia, congenital bleeding disorders, a history of intravenous bisphosphonate therapy that puts the patient at high risk of bisphosphonate-related osteonecrosis of the jaws, may not allow a surgical procedures [219]. Other situations, such as diabetes, immune deficiencies, or anticoagulant therapy may put the patient at elevated risks of postoperative complications or impaired wound healing. Cooperation with the patient's physician is then mandatory [219].

12.5 General Preparations for Surgery

12.5.1 CBCT Evaluation

Cone beam computed tomography (CBCT) has become a widely accepted tool for evaluation in endodontics [213, 218] (see Chapter 6), but it is limited by the relatively high radiation exposure. In surgical treatment planning, CBCT is helpful for assessment of the extent and location of apical periodontitis, of the bone thickness over pathologic defects, and of the proximity to anatomical structures such as the infra-alveolar and mental nerve, nasal and sinus cavities and adjacent root structures [74, 218]. A vertical root fractures cannot be accurately detected by CBCT due to limited resolution and beam hardening effects of root-filling materials, but a narrow vertical pattern of bone destruction can indicate its presence [44, 156]. The CBCT software will also allow the clinician to make accurate measurements of, e.g., the distance from the buccal bone surface to a root tip and the length of a root [28]. Guided microsurgical

techniques have been developed that employ a preoperative CBCT together with a conventional or digital impression to prefabricate a stent that allows the surgical access bur to directly target the root tip to be resected. This may be especially useful for the resection of roots in very close proximity to important anatomical details [4, 83] (Figure 12.1a–f).

12.5.2 Armamentarium

Modern endodontic surgery is a microsurgical technique requiring specialized instruments [125]. A typical microsurgical instrument kit contains miniaturized versions of standard surgical instruments, some particularly designed for work under a dental microscope. Necessary instruments include:

- dental mirror
- periodontal probe
- endodontic explorer, and microexplorer for examination
- surgical blades, blade holder, and tissue elevators for incision and flap elevation
- periodontal curettes, surgical curettes, and mini-endodontic curettes for removal of pathologic tissues
- micromirrors and handle for inspection
- carriers and pluggers for root-end filling.

In addition to these hand instruments, the following armamentarium will be required, and are described in detail in the procedure section of this chapter:

- surgical handpiece and burs for osteotomy and root resection
- tissue retractors
- ultrasonic unit with corresponding tips for root-end preparation
- microsurgical tissue forceps, needle holder, and scissors
- miscellaneous instruments such as anesthesia syringe, college pliers, air water syringe and a micro irrigator.

Disposable items include:

- anesthetic solution,
- gauze and cotton-pellets
- hemostatic agents, dyes, saline
- root-end filling and perforation repair materials
- bone grafting and membrane materials
- sutures.

A piezoelectric bone cutting device for specific osteotomy techniques may also be useful.

12.5.3 Patient Positioning

Endodontic surgery is an option for the majority of teeth, except most maxillary and mandibular second and third molars if they require root-end preparation and filling. Correct positioning of the patient, practitioner, and assistant is key to the procedure [119, 134]. The patient's comfort and the practitioner's ability to perform the procedure adequately both have to be kept in mind. For endodontic microsurgery, the dental microscope will provide high magnification, co-axial illumination, ergonomic seating of the clinician, and direct vision of the resected root surface without the need for a bevel [125, 220]. Throughout soft tissue elevation, osteotomy, and root resection, the patient should remain in a position in which the long axis of the tooth being worked on is in a horizontal position. As soon as the resection is completed and verified, and ultrasonic root-end preparation begins, direct vision of the resected root surface must be obtained to avoid misalignment of the ultrasonic tip and limit the osteotomy to an acceptable size. This will be achieved by uprighting the chair when working on maxillary teeth, and further reclining for working on mandibular teeth. The original position may resume after root-end filling is completed.

12.6 Anesthesia

Local anesthesia in endodontic surgery is used for both profound analgesia and hemostasis [127]. Identification of a resected root surface requires examination under high

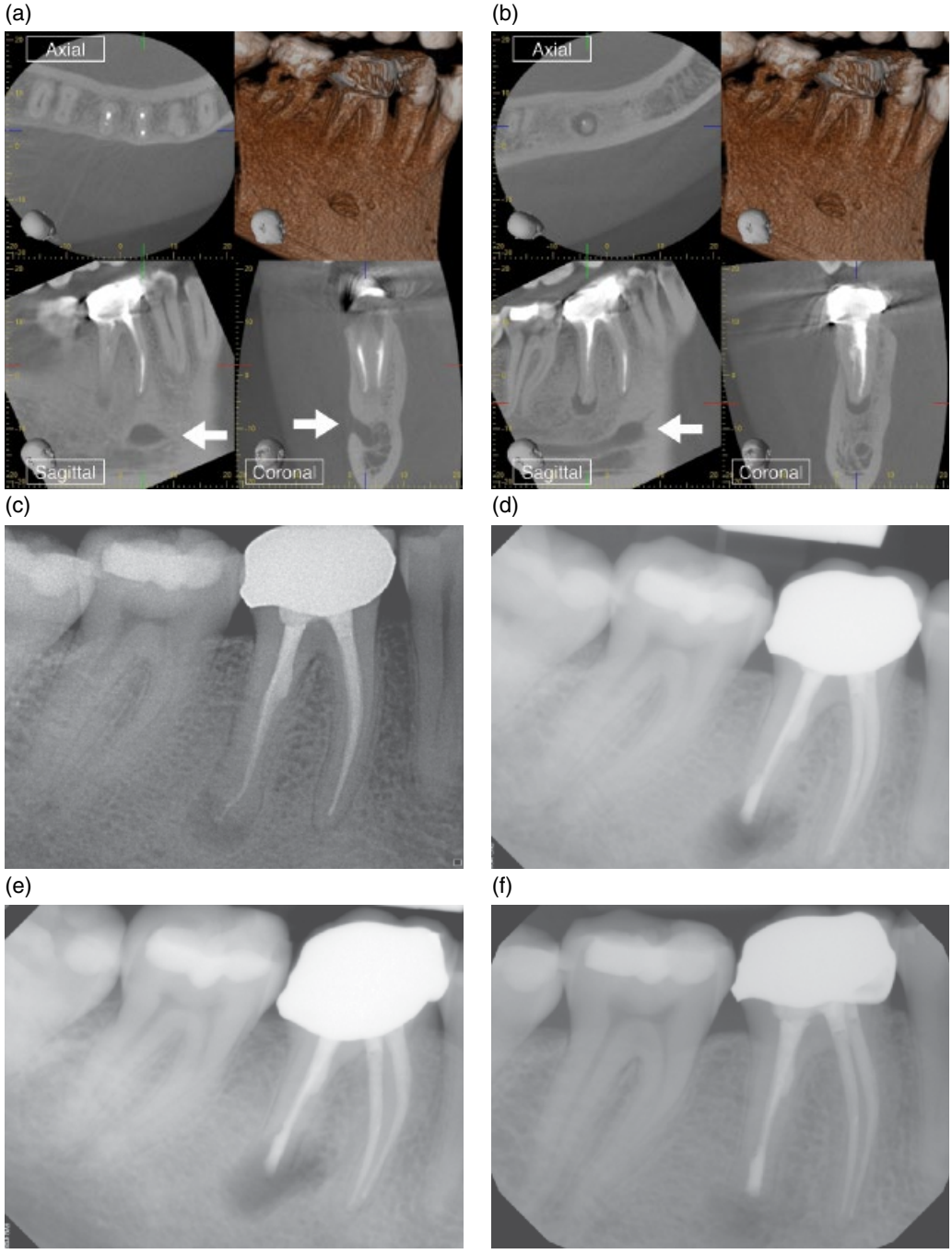

Figure 12.1 Pre-surgical treatment planning using CBCT for endodontic surgery on distal root of a first right mandibular molar. (a, b) Assessment of location of infra-alveolar nerve canal and mental foramen (arrows), allowing for distance measurements in sagittal and coronal planes. (c) Preoperative radiograph. (d, e) Postoperative radiographs, root-end filling in situ. (f) 12-months follow-up, radiographic healing observed, no clinical symptoms. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

magnification with the microscope. The hemostasis provided by the local anesthetic is achieved by adding a vasoconstrictor, typically 1:50,000 epinephrine (depending on availability, 1:80, 000 as alternative) [35, 93, 126]. The higher concentration of the vasoconstrictor reduces bleeding [93, 113, 280] if it is injected in the submucosa.

Concerns whether higher concentration of epinephrine has effects on the systemic circulation had been raised [258, 280]. Local anesthetics containing epinephrine 1:50,000 should only be placed in the buccal submucosa (or palatal tissues, respectively), 1–2 teeth mesial and distal from the tooth undergoing surgery. These higher epinephrine concentrations are not necessary for the infra-alveolar nerve block, as it does not contribute to hemostasis in the surgical field. If direct injection in blood vessels is avoided, any cardiovascular effects should be minimal and short-lived and well tolerated by most patients. For patients suffering from severe cardiovascular disorders or who have had cardiovascular surgery higher epinephrine concentrations may be contraindicated, requiring a consultation with the patient's physician to establish a different anesthetic regimen, if advised [23, 128, 283].

12.7 Surgical Anatomy

12.7.1 Soft Tissue Anatomy

The general surgical access to the roots is from buccal, with the exception of the palatal roots of the maxillary molars. On the buccal aspect, three types of soft tissues are present, the alveolar mucosa, the attached gingiva, and the marginal gingiva. The alveolar mucosa is a thin, nonkeratinized mucosal layer covering the alveolar processes of both maxilla and mandible. It is loosely attached to the underlying bone and can be stretched and pulled by movement of the cheeks or lips. The attached gingiva is the portion of gingiva that extends from the base of the gingival crevice to the mucogingival junction. It is firmly joined to the underlying bone and cementum and is thus immovable. The marginal gingiva is the crest of free gingiva surrounding the tooth like a collar and forming the soft tissue portion of the gingival sulcus. During endodontic surgery great care must be taken to minimize any potential scaring from the operative procedure.

12.7.2 Flap Designs

The primary purposes of proper flap design and tissue elevation are to allow for adequate surgical access to the underlining bone, root structure, and pathologic lesion, as well as to provide uncomplicated and scar-free soft tissue healing [147, 160].

A variety of flap designs had been proposed over the years [189, 263, 264, 271]. Now obsolete is the semilunar flap, a design that involves a curved incision entirely placed in the mucosa [32, 96]. Disadvantages of this flap include limited surgical access to larger periapical lesions, difficult reapproximation, and often secondary healing by granulation tissue, more postoperative swelling and pain, flap shrinkage, and, due to the incision across blood vessels and fiber lines, possible compromised blood supply to the flap and more scarring than any other designs [132]. Another design is the single vertical incision above the root, with lateral retraction of tissues to expose bone over the apex [36, 277]. While it benefits the surgical access to very long roots, and does not cut across blood vessels, its disadvantages include reduced access to larger lesion, and an increased risk of postoperative infection, as the incision is often placed above blood clot filling the osteotomy site after the surgical procedure.

The two most widely used contemporary incisions are the intra-sulcular and the submarginal flap designs.

Intra-sulcular Incision

The intra-sulcular incision dates back to 1930s [105]. It is a full-thickness flap design, allowing for healing by primary intention, and keeping the blood supply intact [96] (Figure 12.2).

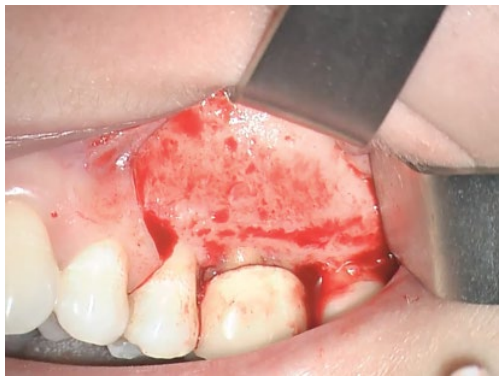

Figure 12.2 Triangular sulcular flap for posterior surgery, allowing access to buccal roots of a first left maxillary molar. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

Depending on whether one or two vertical release incisions are used, both triangular and rectangular designs are a possibility. A triangular intra-sulcular flap is mostly sufficient for access to the surgical site, however, a rectangular flap design may be necessary if a large periradicular lesion needs to be addressed, or more than one tooth is receiving surgery. Intra-sulcular flap designs allow for good re-attachment of the flap, and have demonstrated minimal postoperative pain and swelling, as infection of the blood clot in the osteotomy site is unlikely [96]. Disadvantages include a slight gingival recession, which can be an esthetic problem when artificial crown margins are present, particularly in the maxillary anterior area [132]. There has been also reported damage to the dental papilla [91, 262], in particular when the tissue is poorly keratinized, papilla is very thin, or when the soft tissues are not carefully managed. Adequate blood supply to the papilla with this flap design is best assured if the vertical incision joins the horizontal incision lateral to the papilla at a 90 degree angle. Particular attention needs to be paid to patients that present with a high smile line, or a thin-scalloped periodontal biotype. A variation of the intra-sulcular incision that aims avoiding these problems is the papilla base incision, where the intra-sulcular component of the incision only occurs in the buccal-cervical aspect of the tooth, but the papilla remains intact by continuing the incision line through the base of the papilla without detaching it with the flap [261]. There may be significantly less recession of the papilla if this incision is used [262].

Submarginal Incision

The submarginal incision was introduced in the 1920s [167], and further mentioned by Ochsenbein-Luebke [148]. In contrast to the intra-sulcular incision, the free gingiva surrounding the teeth will not be detached by this flap design. A horizontal, submarginal incision will be placed in the middle of the attached gingiva following the coronal margin of the teeth (Figure 12.3). Both triangular and rectangular versions of the flap are possible, with the same indications as outlined above. This flap design is better suited for areas of wider attached gingiva, in particular in the maxillary anterior area or in the presence of artificial crown margins, or other esthetically challenging situations where it is beneficial to leave gingival tissues undisturbed [91]. This flap design may be disadvantageous where the surgical access is limited, or if a large periradicular lesion is present, particularly in the coronal-apical dimension. While the flap design generally allows good preadaptation and wound healing, postoperative scarring has been observed in areas where the soft tissues are more likely to tear [132].

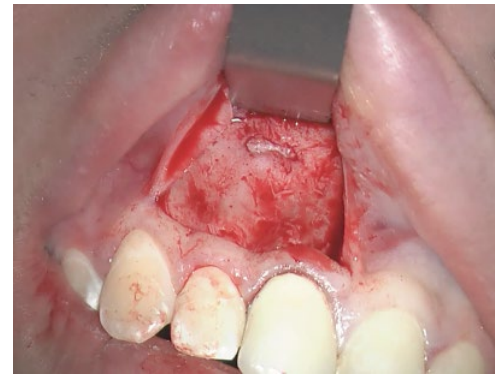

Figure 12.3 Triangular submarginal flap for anterior surgery, allowing access to periapical lesion associated with a right lateral maxillary incisor. Note buccal plate defect. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

12.7.3 General Osseous Anatomy

In some scenarios, the cortical plate has been perforated by the periradicular lesion, so that simple soft tissue curettage will help to identify the root tip. If the root tip is covered, the clinician should have a strong indication of the location of the root tip from the pre-surgical treatment planning, including clinical observation and preoperative measurements gained from periapical radiographs or a CBCT. The proximity of the apices to apices of adjacent teeth, the mental foramen, the infra-alveolar nerve, or the sinus space need to be taken into consideration [112, 136, 174]. In general, the size of the osteotomy should be kept as small as possible, but as large as necessary to allow for removal of inflammatory tissues and proper execution of root resection, inspection of the resected root surface, root-end preparation, and root-end filling.

Alternative osteotomy techniques have been described. In situations with no detectable cortical plate fenestration, or where a thick cortical plate is expected a bone window technique may be employed [123]. This may be undertaken with the use of a surgical saw, or a piezo surgery device with fine-toothed saw tips. A rectangular-shaped bone window will be created, the root tips uncovered, the bone stored in a suitable salt solution), and placed back into its original position at the end of the surgical procedure. Recently, guided surgery techniques using 3D-printed stents created from CBCT scans and impressions have allowed easier access to surgical sites in intimate proximity of sensitive anatomical structures [4, 83].

12.7.4 Specific Anatomy

Maxillary Sinus

If a periapical lesion reaches or perforates the sinus, an opening to the oral cavity may be created by excision of the inflammatory tissues [104]. When a perforation has occurred or appears likely to happen, precaution needs to be taken not to displace any tissue remnants or foreign body materials into the sinus cavity. There has been debate if it is advisable to shave off the root tip and thus close attention must be paid not to get dentinal shavings into the sinus [142], or if carefully resecting the root tip as a whole while securing the apex is the preferable technique [92]. A sterile cotton gauze pad secured by a suture can be placed as a temporary barrier to block any shavings or foreign body materials from entering the sinus [125] (Figure 12.4a–b). The outcome of endodontic surgery will not

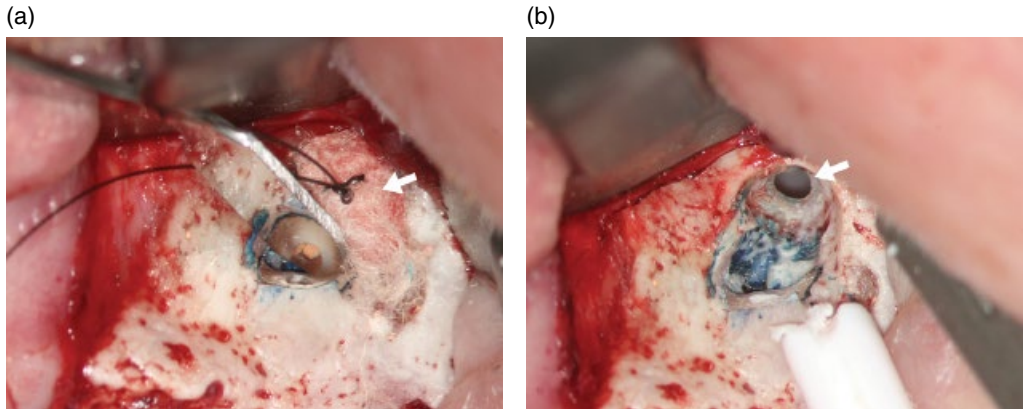

Figure 12.4 Intra-operative protection of sinus opening after enucleation of a periapical lesion associated with a second left maxillary premolar. (a) Micro-mirror view of root-end preparation. Cotton pellet secured by a surgical thread blocking the sinus perforation in situ. (b) Situation after placement root-end filling and removal of cotton pellet. Note sinus perforation (arrow). Courtesy of Dr Karla Sermeño de Castillo, Austin, TX, USA.

be compromised by a sinus opening itself [276], as the repositioned flap will provide protection after the surgical procedure For larger openings, a bone grafting material such as collagen or a membrane covering the buccal defect may be advised. In healthy patients, antibiotics may not be necessary, but a decongestant may be advisable to prevent any pressure issues from within the sinus to disturb the wound healing.

Palatine Neurovascular Bundle

Palatal surgery is mostly limited to first molars. Maxillary premolars can generally undergo surgery from a buccal approach. Second molars are in almost all situations inaccessible from palatal due to anatomical risks. The greater palatine foramen is located approximately 3–4 mm anterior to the posterior border of the hard palate, with nerve and blood vessels running in an anterior direction in the submucosa approximately midway between midline of palate and gingival margin. Soft tissue elevation for surgery on the palatal root of a first molar may include the neurovascular bundle within the flap, but any posterior access to attempt surgery on a second molar may involve the foramen with danger to the bundle and greatly increased risk of severe hemorrhage or nerve injury. As a precaution, a relieving incision for a palatal flap should be placed between the canine and the first premolar [95, 96]. Transantral surgery to reach palatal roots has been described [8, 275], but is practically often limited to root fusions of distobuccal and palatal roots, as correct root-end preparation and root-end filling is greatly complicated by the distance to the palatal apex from the buccal approach and the risk of leaving dentinal shavings and foreign body materials in the sinus.

Mental and Inferior Alveolar Neurovascular Bundles

Radiographic assessment and/or CBCT imaging and careful treatment planning are prerequisites for endodontic surgery in the mandibular premolar and molar area [108, 109, 162, 223, 267]. The mental foramen is most frequently located near the apex of the second mandibular premolar, less frequently near the apices of the first molar or the first premolar. The distance of the mental foramen from the closest root may range from 0.3–9.8 mm [10]. Less frequently, its location was reported to be at the same level or even coronal to the apices of adjacent teeth, or present with a secondary foramen [1, 174]. The mandibular canal is located inferior and lingual to the root apices of mandibular molars. In most situations the canal is at a safe distance from the root tips of first molars. However, its proximity to the apices of the second molars, together with their posterior location and the thickness of the bone in the area of the ramus commonly prohibits surgery on these teeth.

To protect the mental foramen from damage during mandibular posterior surgery, an intra-sulcular incision should be chosen for better overview, with a vertical incision mesial of the first premolar for surgical procedures of both second premolar and first molar. To protect the mental nerve from damage, the placement of a bony groove using a surgical bur or a piezoelectric device for safe anchorage of the surgical retractors has been advocated to prevent tissue damage by accidental slippage [1] (Figure 12.5a–c).

12.8 Clinical Steps in Root-end Surgery

12.8.1 Apical Curettage

Apical curettage involves the removal of the soft tissue lesion of the apical periodontitis around the root tip without root-end resection or root-end filling [144]. While the excision of the inflammatory tissues is an integral part of root-end surgery, there are limited indications for traditional apical curettage as a self-sufficient procedure. Procedures where no resection or root-end filling will be rendered may include exploratory procedures that identify vertical root fractures or other

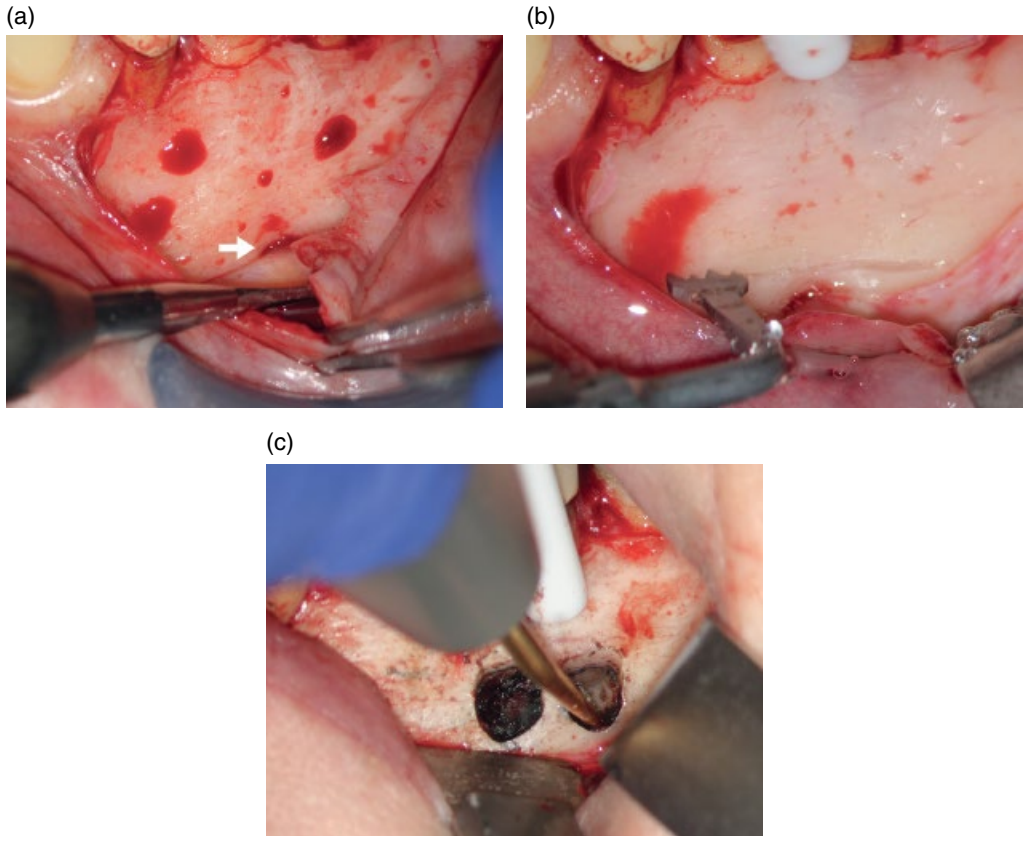

Figure 12.5 Intra-operative protection of mental neurovascular bundle. (a) Clinical view of mental foramen and neurovascular bundle inserting into buccal soft tissues. (b) Preparation of a groove to anchor a surgical retractor using a piezo-electric device. (c) Safe placement of retractors during surgical procedure on first left mandibular molar in groove protecting the mental nerve. Note epinephrine containing cotton pellet improving hemostasis in mesial osteotomy during ultrasonic root-end preparation of distal root. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

reasons why a tooth is deemed non-restorable. Failed endodontic cases, particularly after non-surgical retreatment, that are treatment planned for a surgical procedure, should receive treatment that addresses potential intraradicular, as well as extraradicular causes for failure. Thus, other than for crown or root resection procedures, root-end resection and root-end filling should be included in the procedure to maximize the prognosis. While granulomas or abscesses should heal after the etiology of a failed endodontic treatment has been addressed, it cannot be determined during the surgical procedure whether an extraradicular infection is present. Thus, as part of a surgical procedure, it should be attempted to remove the entire periradicular lesion [32, 144, 171, 206, 207], to include any epithelial remnants that might continue proliferation of a cystic lesion, or any extraradicular infection.

12.8.2 Root-end Resection

After inflammatory tissues have been removed from the periradicular area, the root apex should be clearly identifiable. Root-end resection will remove the anatomical complexities that may harbor intraradicular infection: apical ramifications, accessory canals, or severe apical curvatures; iatrogenic mishaps that prevented access to the entire root canal

system throughout non-surgical retreatment, including perforations, ledges, transportations, or foreign body materials; apical root fractures or cracks; or apical resorptions that prevented an adequate seal during the conventional procedure. The root-end resection will also aid in removing etiological factors in the tissues, such as foreign bodies and extraradicular infections. Resection of the root apex should be performed with a fissure bur or a Lindemann bone cutting bur, to achieve a smooth resected root surface that permits the inspection of the internal root anatomy, thereby allowing for the identification of potential reasons for the failure of the previous treatment. No consensus exists in the literature how much apical root structure should be resected. However, resection to the base of the lesion [37] is regarded unnecessary [95, 96]. As much buccal bone as possible should be preserved. The apical foramen, as well as accessory and lateral canals are the major physiological pathways for intraradicular infection to reach the periradicular tissues. Bacteria will be able to penetrate dentinal tubules, but may not reach periodontal tissues across an intact cementum layer. Microorganisms in dentinal tubules exposed to the periradicular tissues by the root resection were discussed as a potential source of infection in the surrounding tissues after surgical endodontics. Nevertheless, a correlation between the presence of microorganisms in the dentinal tubules and the degree of periradicular inflammation could not be demonstrated [206]. A minimally beveled resection angle aids in keeping the number of open dentinal tubules after resection at a minimum. In addition, root-end preparation will also greatly reduce the numbers of bacteria in the dentinal tubules, as the majority of bacteria in the apical third have been shown to be located immediately adjacent to the root canal system [117, 225].

Based on an anatomical study, three mm of root-end resection eliminated 98% of apical ramifications and 93% of lateral canals [125], and can aid as a clinical guideline. This amount may vary depending on individual situations, for example, it may be more in the proximity of anatomical structures such as the mental nerve or to remove a fracture line, or less, if only limited root structure is available apical of a post. The root should be a resected at a shallow 0–10° bevel, rather than a traditional steep 45° bevel [42, 125, 206], in order to preserve apical root structure; decrease the chance of missing lingual and accessory canals; guarantee complete root resection; expose less dentinal tubules that may aid in spreading intraradicular infection [51, 85, 97, 245]; and allow for an easier co-axial root-end preparation with ultrasonic tools.

The inspection of the resected root surface is done after complete hemostasis is achieved. After rinsing the osteotomy site with saline, still existing bleeding spots may be stopped by using cotton-pellets soaked with epinephrine or hemostatic agents such as ferric sulfate or aluminum chloride. All hemostatic agents will have to be removed by the end of the surgical procedure, to clear away any toxic components that might have potentially adverse effects on healing [98, 110, 114, 141], and allow the osteotomy site to fill in with blood. Methylene blue or other suitable dyes is used to stain the resected root surface [125] after carefully drying it with a micro irrigator, such as the Stropko device [237]. This procedure will not only outline the circumference of the periodontal ligament to ensure complete resection, but aid in identifying missed canals, microfractures, isthmuses – narrow connections between main canals, often harboring tissue remnants and infection – other anatomical details, aberrations, or iatrogenic errors (Figure 12.6a). The inspection of the resected root surface should be undertaken at high magnification (16–24×) [272]. At a 3-mm resection level from the original apex, isthmuses were found at 90% of the mesio-buccal roots of maxillary first molars, 30% of the maxillary and mandibular premolars, and over 80% of the mesial roots of the mandibular first molars [107, 279] (Figure 12.7).

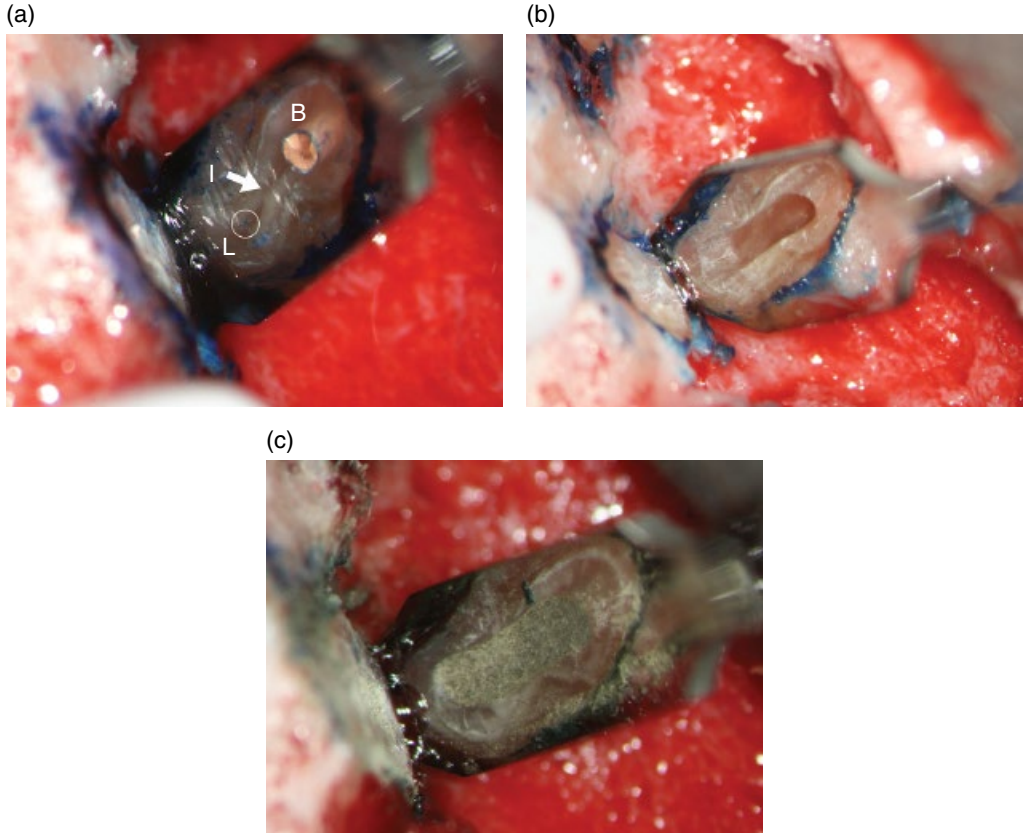

Figure 12.6 Clinical steps of resected root inspection, root-end preparation and root-end filling of a first left mandibular premolar. (a) Micro-mirror view of resected root surface after staining with methylene blue. Note buccal canal with existing root filling [B], missed lingual canal [L] (circle), and isthmus [I] (arrow) connecting buccal and lingual canals. (b) Root-end cavity, including isthmus preparation. (c) Root-end filling in situ (MTA). Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

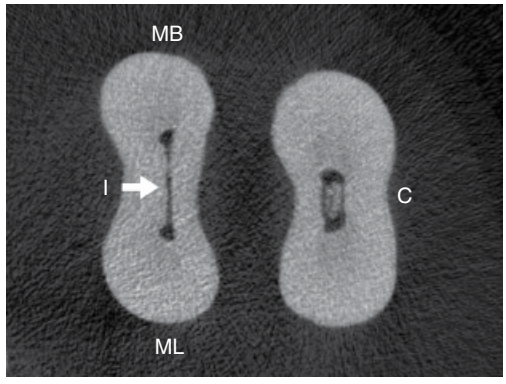

Figure 12.7 Axialμ-CT view of mesial and distal roots of a first mandibular molar. Isthmus [I] (arrow) connecting mesiobuccal canal [MB] and mesiolingual canals [ML]. Note calcification [C] in distal canal . Courtesy of Dr Vanessa Cabrera Saez, Philadelphia, PA, USA.

12.8.3 Root-end Preparation

Root-end preparation aims at cleaning portions of the root canal system that have been left untouched by previous conventional root canal therapy. This may include previously non-negotiated canals and situations where the quality of the existing root filling is unsatisfactory, either due to insufficiency of the sealer to fill all gaps between the core filling material and the root canal wall or to anastomoses between canals. Even fine isthmuses between canals should be instrumented during root-end preparation [153] (Figure 12.6b).

Root-end preparation is done with ultrasonic tips. Prior to cavity preparation, the operator should align the ultrasonic tip in the

direction of the long axis of the root at low magnification (4×–8×) (Figure 12.5b). Special tips with variations in angulations exist for different areas of the jaw. While there have been concerns about cracks in the root after ultrasonic root-end preparation [75, 140], these seem not to be relevant for the clinical outcome [19, 122]. Smaller or larger tips may be used dependent on the original shape and size of the root canal. Prior to root-end filling, the cavity should be dried and inspected at high magnification using a micro-mirror to verify that all filling remnants in the cavity have been removed completely, particularly in the buccal aspect, as this part of the root canal wall cannot be directly observed during ultrasonic preparation [237].

12.8.4 Need for Root-end Preparation

A number of properties may be attributed to an ideal root-end filling material. Biocompatibility and sealing ability are of primary importance; also. bactericidal or bacteriostatic activity is conducive to the overall purpose of treatment. In addition, technical properties support these basic functions: adhesion to the root canal surface; dimensional stability; non-corrosiveness; resistance to dissolution; ease of manipulation with an adequate working time; non-staining of teeth or tissues; osteo- and cementogenic properties; and radiopacity.

A wide range of root-end filing materials have been proposed, investigated, and reviewed [50, 118, 253]. Materials that have been widely used, also in clinical studies, include amalgam, gutta-percha [173, 186], zinc oxide/calcium sulfate cements (Cavit) [73, 172, 187], polyvinyl resin (Diaket) [244], glass-ionomer cements [116], composite resin (Geristore, Retroplast) [9], zinc oxide/eugenol cement (IRM, SuperEBA) [101], and calcium silicate cements (mineral trioxide aggregate (MTA), biodentine, bioceramic root repair material (BC, RRM). With the exception of amalgam, which was included due to its historical importance and that it is still being used by some practitioners [33], only the most contemporary root-end filling materials are discussed in detail.

12.8.5 Root-end Filling Amalgam

Amalgam was the most popular and widely used root-end filling material for many years [26, 77, 273]. Amalgam has been criticized for its lack of biocompatibility, corrosion, risk of crack formation in the root apex, hard tissue staining and soft tissue tattoos due to silver salts [100], and poor performance in regard to outcome [7, 63, 73, 116, 178, 221] (Figure 12.8a–e). Amalgam has been demonstrated to leak under in vitro conditions based on dye penetration tests [77, 177], or the the fluid filtration model [234, 282]. While it was criticized that this were not in vivo conditions [188, 201], amalgam also showed bacterial leakage [53, 255], and consistently allowed for greater leakage than any other material under review, independent from the methods [53]. Hence, it is likely that amalgam allows for leakage as a root-end filling material [13, 53, 96]. Amalgam's lack of biocompatibility derives from its mercury content, which has been described as an environmental hazard, and attached great scrutiny for its use as a restorative filling material [66]. Histologically, amalgam or its corrosion products have been considered responsible for unfavorable inflammatory tissue responses [16, 51, 188, 252, 256], with traces of amalgam still detectable from the root end [188]. Periapical tissue inflammation was shown to be most severe and extensive among all materials compared [51, 188, 192, 252, 254].

Composite Resin

Good sealing ability of composite resin in a root-end cavity has been demonstrated in in vitro studies [3, 150, 151], and Geristore, a dual-curing hydrophilic modified composite resin, has been used as a material for conventional root-end filling, and for the repair of subgingival or subosseous defects, and as a barrier material for guided tissue regeneration (GTR).

A different technique to seal the resected root apex by composite resin materials was

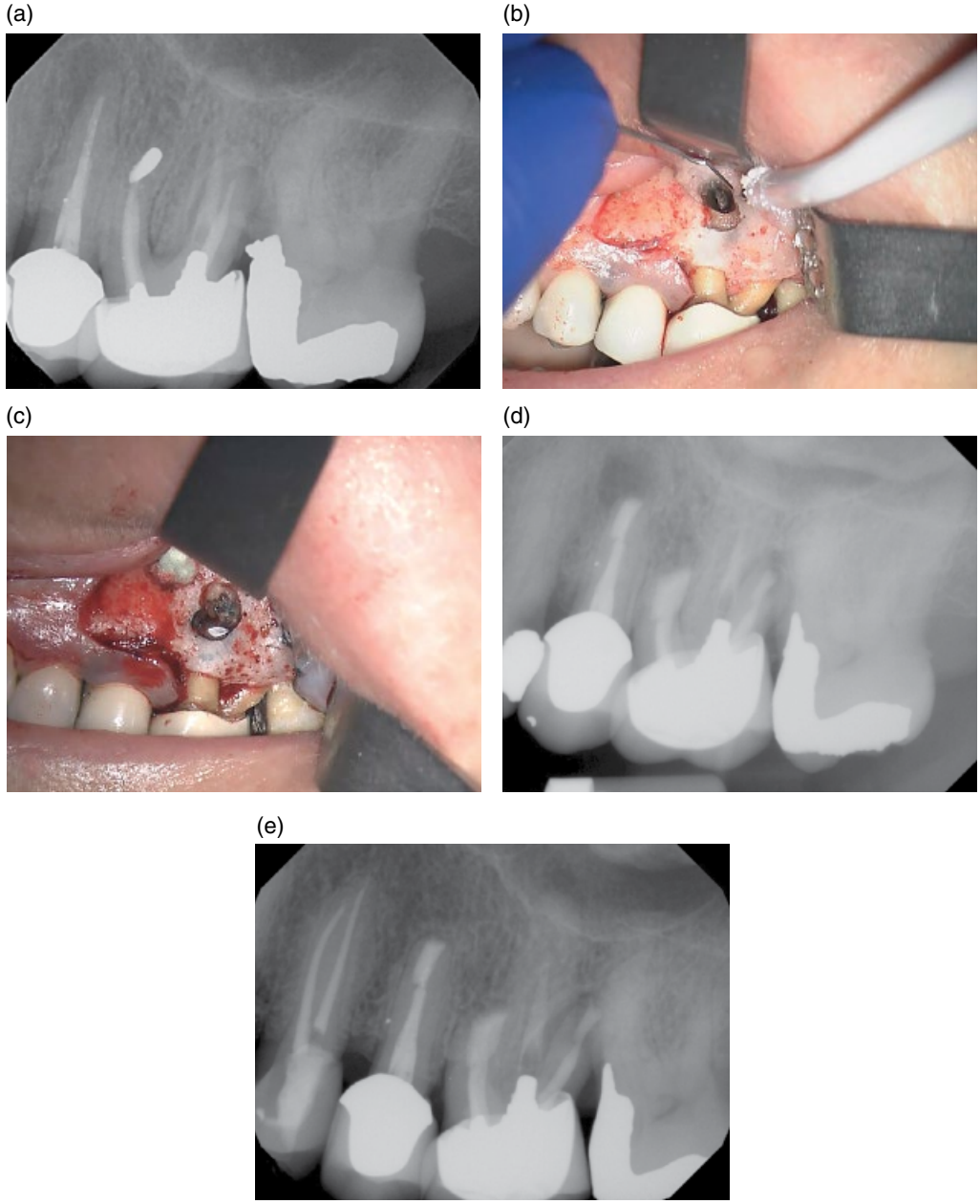

Figure 12.8 Surgical treatment in left maxillary quadrant. Apical periodontitis on second premolar and buccal roots of first molar (verified by CBCT). History of traditional root-end surgery using amalgam retrograde filling in mesiobuccal root of first molar. Buccal swelling and symptoms originating from overextended amalgam filling mesial of second molar. (a) Preoperative radiograph. (b) Clinical view of existing amalgam retrograde filling. (c) New root-end filling in situ (RRM). (d) Postoperative radiograph with root-end fillings on premolar and molar in situ. Situation after re-contouring of existing amalgam filling on second molar. (e) 12-months follow-up radiograph. Radiographic healing observed, no clinical symptoms. Note endodontic treatment and new coronal restoration on first premolar. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

first introduced by Rud et al. in 1991 [209], and more recently used by von Arx et al [268]. While using high magnification of a microscope or an endoscope, the technique differs significantly from other traditional or contemporary approaches, as no conventional root-end preparation and root-end filling is done. Instead, a round bur is used to create a concave cavity over the entire resected root surface, which is then etched with EDTA, and a bonded resin material is placed in a domelike fashion. The materials used with this technique is Retroplast, a dentine-bonded dual-curing composite resin [209, 268]. While the technique has demonstrated favorable results compared to traditional root-end surgery techniques [115, 209, 210, 211], it is less effective compared to contemporary techniques [130], and indications restricted to situations where an ultrasonically prepared root-end cavity cannot be prepared. The technique's major drawback is its dependency on excellent moisture control, as otherwise the filling material will not stay connected to the resected root surface [210, 211].

Zinc Oxide/Eugenol

Zinc oxide/eugenol (ZOE) cements were recommended for many decades [81, 171] as materials to be used in root-end surgery. The two most commonly used materials are intermediate restorative material (IRM), a ZOE cement reinforced by the addition of polymethacrylate to the powder, and super ethoxybenzoic acid (SuperEBA), modified by the partial substitution of eugenol liquid for orthoethoxybenzoic acid and the addition of fused quartz or aluminum oxide (alumina) to the powder. Both materials demonstrated a significantly better outcome than amalgam [63, 204, 205], and histologically proved to be more biocompatible than unmodified ZOE, although there was still presence of some inflammatory cells on the root surface [188, 191, 192]. Both IRM and SuperEBA exhibit low solubility [48], good antibacterial action [49, 251], and little leakage in dye penetration tests [47, 175]. SuperEBA allows for significantly less leakage and better root wall adaptation compared to amalgam. Studies that used either IRM or SuperEBA as root-end filling materials, use ultrasonics for root-end preparation and employ the use of high magnification commonly qualify for inclusion in meta-analysis evaluating the outcome of Endodontics microsurgery [130, 220, 221].

Calcium Silicate Cements

Originally deriving from Portland cement, a silica, alumina and calcium compound construction material, a variety of dental filling and repair materials have been developed, including mineral trioxide aggregate (MTA), BioDentine, or Bioceramic root repair material (BC, RRM). All materials are hydrophilic, but vary in setting time and methods of preparation. The calcium silicate cements offer significant improvement over zinc oxide/eugenol cements, showing reduced cytotoxicity [29, 55], increased biocompatibility [84, 158], increased cell attachment [55], cemento-, and osteoinductive properties [46, 84], as well as increased pH values [88].

MTA was the first material introduced [252] and has been comprehensively investigated. Several histological studies of root-end fillings in animals [16, 197, 252, 254] have shown that MTA demonstrate considerably less inflammation than amalgam, and allows new cementum to form over the resected root surface (Figure 12.9) and the root-end

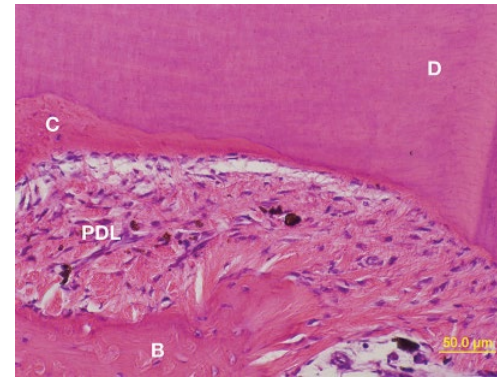

Figure 12.9 Deposition of new cementum [C] layer on resected dentin [D]. Note absence of inflammation, proximity of bone [B], and periodontal ligament structures [PDL]. Modified from [45].

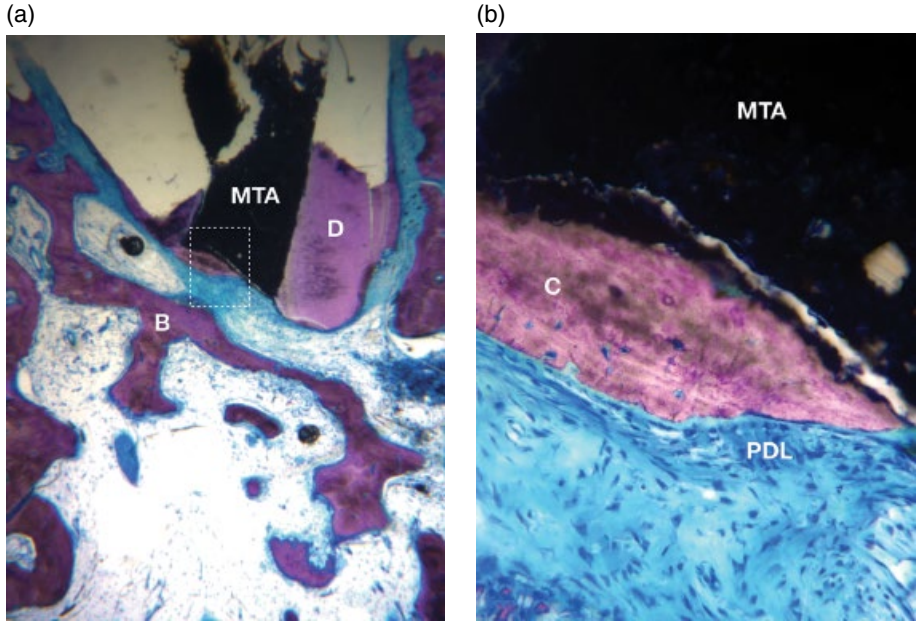

Figure 12.10 Histologic representation of root and periapical area after placement of MTA root-end filling. (a) Section of root with root-end filling [MTA]. (b) Close-up of marked area in 12.10a. Newly deposited, mineralized cellular cementum [C] growing over MTA. Modified from Baek et al. [16].

filling itself [252, 254] (Figure 12.10a–b). Recent investigations have demonstrated the formation of a hydoxyapatite (HA) layer on the MTA surface in contact with tissue fluid during setting of the material, described as "biomineralization" [25]. It was suggested that this layer creates a biologic seal between MTA and the dentin interface and thus enhances the long-term sealing ability of MTA. Disadvantages of MTA included its handling properties and a potential for tooth discoloration, for both its gray and white formulations [131]. Prepared MTA has the consistency of a moist, granular paste, and it may be difficult to place it in a root-end cavity. MTA may also be prone to washout effects in the presence or excessive bleeding or other tissue fluids compromising its sealing ability.

The newer formulations of calcium silicate cements, BioDentine, a capsule-based material to be mixed in a triturator, and the premixed RRM alleviated some of the problems encountered with MTA. Both materials exhibited less tooth discoloration effects than MTA [131], RRM has received more attention as a root-end filling material than BioDentine, which is more often used for perforation repair. RRM is dimensionally stable, demonstrates a high pH, and needs as short as 2 hours of setting time. No significant differences were demonstrated between RRM and MTA in regard to their antimicrobial efficacy [146], biocompatibility [5], and sealing ability [166]. In an in vivo study comparing RRM and MTA as a root-end filling materials [45], no or minimal inflammation was evident at the surgical site upon healing, with cementum-like tissues observed adjacent to RRM, comparable to previous findings for MTA (Figure 12.11a–b). Two randomized controlled trials investigating endodontic microsurgery have demonstrated highly favorable outcomes [224, 285].

12.8.6 Wound Closure and Postoperative Care

Once a surgical procedure has been completed, wound closure and postoperative care will determine a great part of the biologic and

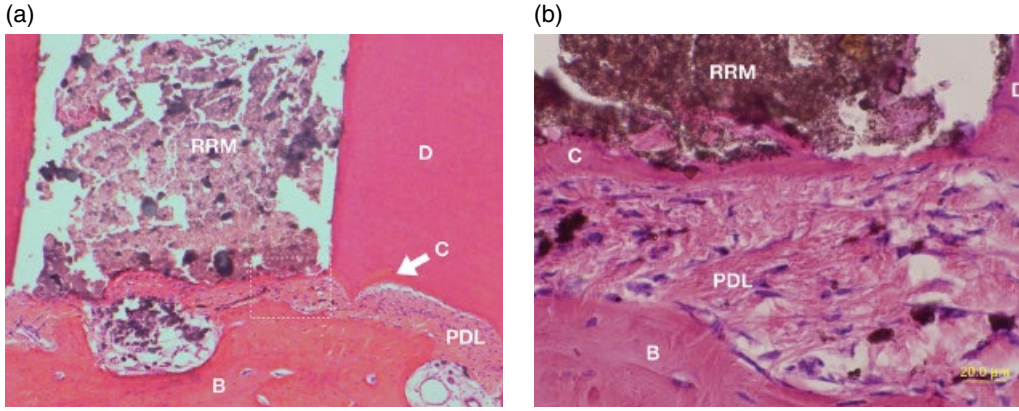

Figure 12.11 Histologic representation of root and periapical area after placement of RRM root-end filling. (a) Section of root with root-end filling [RRM]. (b) Close-up of marked area in (a). Mineralized tissue formation on surface of RRM. Note periodontal ligament structure [PDL] and proximity of bone. Modified from [45].

esthetic healing process. The osteotomy site should be inspected and cleaned of any excess materials and hemostatic agents to avoid foreign body reactions and to remove any toxic byproducts from materials such as ferric sulfate. Blood should be allowed to fill back into the osteotomy site, to allow for the formation of a blood clot and the subsequent osseous healing. Any grafting material or membrane should be placed at this stage of the procedure. The soft tissue is moistened with a wet gauze containing saline before flap repositioning, as the soft tissue may have become dehydrated during the surgical procedure and rehydration will aid in regaining the tissues' natural elasticity. Soft tissue management may have a great impact on the esthetic results. Sutures will be necessary to place the mucoperiostal flap back into its original position. Accuracy will be increased by using a microscope or loupes, in particular for smaller suture diameters. Common sizes are 5-0 or 6-0 for standard interventions, in esthetically demanding areas, such as the anterior maxilla, or if a papilla base flap was chosen, 7-0 sutures may be advised for repositioning of the papillae. For any flap design, close contact to the underlying bone needs to be established to minimize the thickness of the subperiostal blood clot and allow for healing by primary intention. Nylon, polypropylene, or polytetrafluoroethylene (PTFE) monofilament or coated monofilament sutures have become the material of choice [265], as traditional silk sutures may promote bacterial colonization and impede wound healing. Resorbable sutures, such as gut, reduce the number of office visits, but add an inflammatory component to the wound healing process by their resorption. The need for suture removal will also allow the clinician to check on the patient 48 hrs [96] to 4 days [95, 263] days after the surgical intervention and have a close control of the healing process. It was reported that if sutures were left too long some flap margins may be compromised by infection [91, 96]. Needle shape, size, and curvature are important for the particular procedure, but its selection may vary with the periodontal biotype, access to the area, and preference of the operator [265]. Single interrupted sutures are generally preferred over continuous sutures as they allow for a more controlled readaptation. A sling suture may be used for interproximal readaptation in the posterior areas. The patient should be instructed on the first 1–2 days to avoid exercise and not to pull up his or her lips, which could precipitate bleeding or open up flap margins, which may pose a risk of infection to the underlying blood clot with associated pain and swelling,

and delayed healing. The patient should refrain from smoking as long as possible, and for several days only have food that can easily be removed from the surgical site by regular dental cleaning. Interval cooling with an ice-pack will prevent excessive swelling [94, 96]. The patient should also be informed that besides swelling and pain, temporary discoloration and bruising may occur in a small number of patients. If the patient can tolerate NSAIDs, this type of analgesics should be the first choice, as it is anti-inflammatory and analgesic. Antibiotic coverage is not generally advised [94], but may be indicated based on the patient's medical or dental history. Careful rinsing with a chlorhexidine solution after the first day will be beneficial to the wound healing process, as it reduces bacterial content in the oral cavity and thus minimizes the risk of postoperative infection of the surgical site. The patient should receive contact information for after hour emergency assistance. Lastly, the patient should be instructed that he/she should return in the event of any recurrent symptoms after the initial healing phase. The patient should return after 4 weeks for a follow-up to ensure successful soft tissue healing, and the absence of any clinical symptoms, and after 1, 2, and 4 years to radiographically monitor the healing process and ensure that the long-term success is guaranteed after several years.

12.9 Perforation Repair

A perforation is an abnormal communication between the pulpal space and the periradicular tissues [198]. Perforations usually occur iatrogenically through access perforation, incorrect instrumentation, post preparations, or aggressive canal enlargement. Less frequently, perforations occur when correct treatment creates communication with resorptive or carious defects. Locations for perforations may vary from the gingival sulcus to subcrestal, mid-root or apical parts of the root. Successful perforation repair depends primarily on whether the perforation site harbors infection, and on the size of the defect, access and visualization to facilitate repair. Immediate perforation repair has a better prognosis than the repair of a perforation that has allowed microorganisms to invade the perforation area, with inflammation and severe bleeding compromising repair [170, 216]. Perforation within the gingival sulcus can be repaired by a conventional restoration, or by raising a small flap to have good control over the margins of the restoration. Depending on moisture control, a bonded composite resin or a modified composite resin are the materials of choice. Subcrestal repairs may be subject to non-surgical repair from inside the root canal system [198] or to surgical repair if they are out of reach of application syringes or micro-pluggers; if they cannot be visualized because they are located beyond a curvature; or if bleeding from periradicular tissues cannot be controlled. The ideal properties of a subcrestal repair material are essentially identical with those put forward for root-end filling materials. The formation of cementum or cement-like tissues over materials such as MTA [193, 254] or RRM [45] make calcium silicate cements suitable materials [198, 248]. Most perforations do not require surgical repair [20, 24, 137]. If a surgical repair is indicated, protocols will generally follow those described for root-end surgery, with the modification that the resection needs to include the perforation area. In situations where the crown-to-root ratio may be compromised, a deviation from a near perpendicular resection angle may be indicated to allow for more periodontal support from the remaining root structure.

12.10 Replantation

Intentional replantation is the purposeful removal of a tooth and its almost immediate replacement after root-end preparation and root-end filling extraorally prior to reimplantation [90]. It is not considered the first choice of retreatment [278], however, where

a non-surgical retreatment option cannot be performed for restorative or endodontic reasons, or if this option was already exhausted, and a regular surgical retreatment approach cannot be facilitated due to anatomical limitations, intentional replantation may be performed to avoid tooth extraction [2, 68, 89, 133]. An updated review and meta-analysis including studies from 1966–2014 demonstrated an 88% survival rate for teeth following intentional replantation, and an 11% incidence rate for root resorption following treatment [247]. A protocol will include a slow and careful extraction of the tooth to avoid fractures; loss of the coronal restoration; and damage to the alveolar bone, the periodontal ligament in the socket, and the cementum layer (Figure 12.12a–f). Curettage of the socket walls should be avoided in order to allow for re-establishment of the periodontal ligament. After the tooth has been extracted, great care must be taken not to touch the root surface to prevent periodontal tissue damage and root resorption [12, 121]. For the same reason, scaling and root planing of the replanted tooth is contraindicated. Some surface resorption inevitably takes place shortly after replantation peaking after 2–4 weeks, but this is transient and diminishes after approximately 2 months. During the extraoral phase, the tooth must be carefully inspected for fractures or any other iatrogenic damage during the extraction, and to identify possible reasons for failure of the prior endodontic treatment. In deviation from the standard protocol, root-end preparation may be performed with a thin fissure bur, since there are no space restrictions that would necessitate the use of an ultrasonic tip. The extraoral time should not exceed 15 minutes [129] in order to avoid degeneration of the periodontal ligament structures on the root surface. Irrigation of the tooth with an isotonic salt solution should be carried out frequently, so that drying out does not occur [86]. Upon reimplantation, care must be taken to place the tooth in the correct orientation, preferably in slight infraocclusion, since the tooth has been resected. If the tooth is slightly out of occlusion during the healing phase, a better re-attachment of the PDL may occur, as occlusal forces are minimized. The mobility of a replanted tooth should be kept at a minimum; however, splinting is only needed in situations with increased mobility. The patient should be advised to avoid chewing on the extracted tooth for at least two weeks postoperatively. Regular follow-up according to the protocol for endodontic surgery is indicated.

12.11 Root Amputation, Hemisection

Root amputation procedures were first mentioned in the 1880s for treatment of multi-rooted teeth with furcation involvement [71]. Root amputation is considered a "root resection" technique as it only involves the removal of root structure below or at the level of the cemento-enamel junction without removal of portions of the crown [222]. In contrast "crown resection" includes hemisection, trisection, and premolarization (bicuspidization), i.e., all procedures where a dissection transverses through the furcation and the crown of a multi-rooted tooth so that a root and the associated portion of the crown may be removed (hemisection, trisection) or all root/crown section are being retained (premolarization, bicuspidization) [222]. Indications and contraindications for crown and root resection include the following [159, 222, 235]:

Indications:

- severe bone loss affecting one root, not amenable to other forms of therapy
- moderate to advanced furcation involvement with divergent roots
- unfavorable root proximity between adjacent teeth
- root fracture, perforation, root caries, or external root resorption involving one root or the furcation area
- endodontic treatment of a particular root canal cannot be performed, and root-end surgery is contraindicated

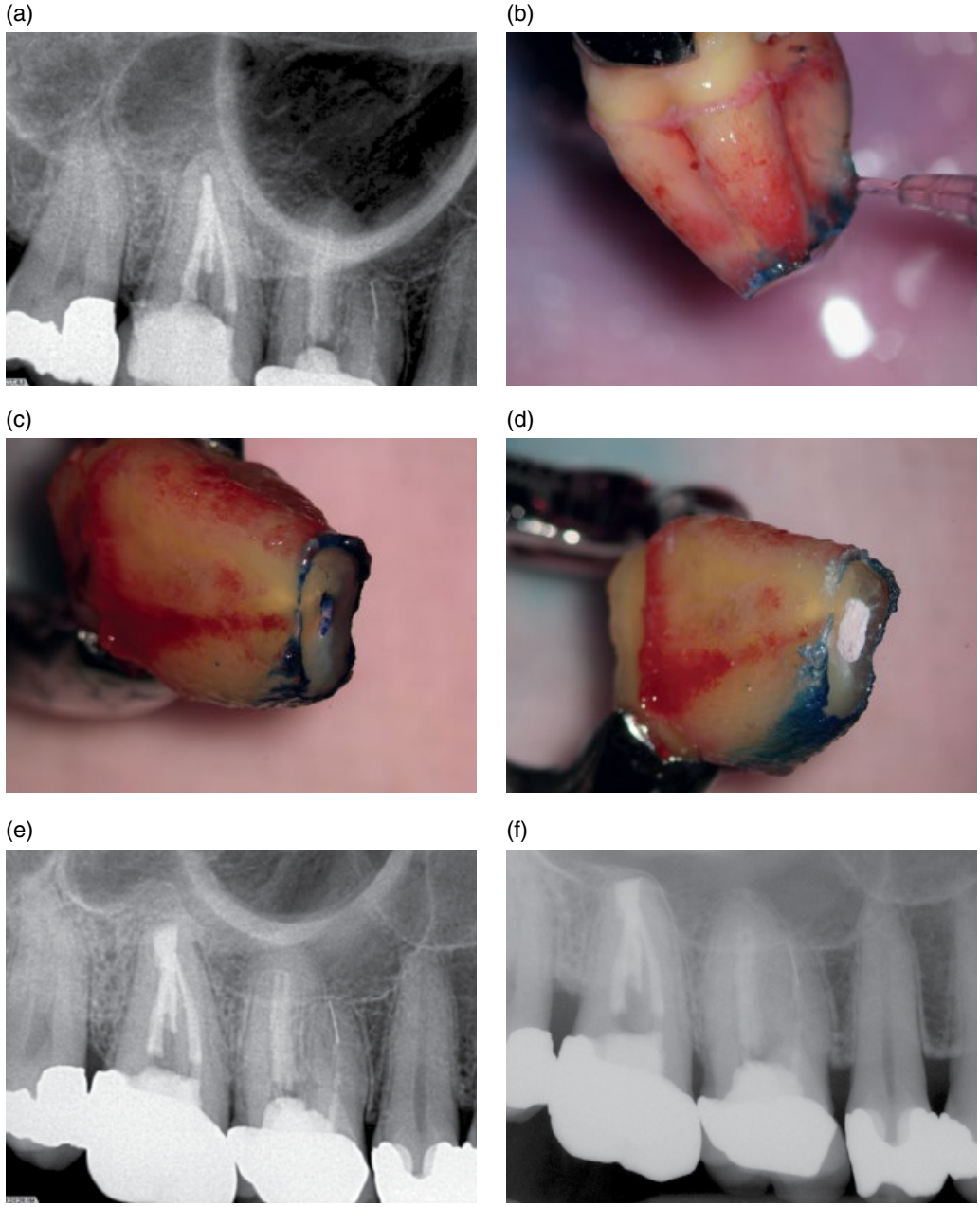

Figure 12.12 Treatment of second right maxillary molar by intentional replantation. History of unsuccessful non-surgical retreatment with presence of clinical symptoms. (a) Preoperative radiograph. (b) Irrigation of the extracted tooth with an isotonic salt solution. (c) Resected root surface after staining with methylene blue. Note long oval canal cross-section with unfilled canal portions. (d) Root-end filling with RRM. (e) Postoperative radiograph. (f) 18-months follow-up, radiographic healing observed, no clinical symptoms. Courtesy of Dr Bekir Karabucak, Philadelphia, PA, USA.

- a tooth, which is an abutment of a bridge, can be retained after removal of a particular root
- when anatomical situations preclude implant placement.

Contraindications:

- insufficient bone support around the remaining roots or in the furcation area
- the furcation is too close to the apex, not sufficiently separated, or roots are fused
- it is impossible to perform endodontic treatment in the remaining roots, unfavorable anatomy of the remaining roots
- extensive caries or root resorption in the furcation area
- minimal strategic value of the remaining root structures.

Proper endodontic treatment should be done prior to the surgical procedure [159]. If the resection requires cutting through metal restorations or involves the removal of considerable amounts of coronal tooth structure, this should be carried out prior to incision and reflection of the flap to prevent tooth particles and metallic fragments to be left behind in the soft tissues. Surgical access should be provided by buccal and lingual full-thickness muco-periosteal flaps to allow for sufficient access and visualization for the completion of the resection and proper wound closure. Inflammatory tissue must be removed, and the root to be removed dissected from the main trunk of the tooth by a long fissure bur or Lindemann bone-cutting bur. Full separation should be checked by using a periodontal probe carefully probing through the furcation area and by testing the mobility of each root individually. Once a root is cleanly separated, it can be carefully removed avoiding damage to the remaining tooth structure. The remaining coronal structure is contoured to ensure that no overhanging tooth structure remains, which could be plaque-retentive. The root surfaces should be cleaned, all granulation tissue removed, and and osteoplasty performed for the remaining bone, to eliminate any irregular contours and provide a biologic width for the dentogingival complex after healing. The flap should then be repositioned and sutured. Follow-up instructions follow the same guidelines as for root-end surgery. The permanent restoration should allow the patient good hygiene access. There have been a number of clinical studies [21, 38, 40, 41, 61, 69, 80, 99, 106, 139, 180, 241, 284], and a systematic review and meta-analysis on the outcome of root resection, discussed in detail below [222].

12.12 Guided Tissue Regeneration

Guided tissue regeneration (GTR), or the use of bone grafting materials and/or membranes, has been used extensively in periodontology and implant dentistry, but much less so in endodontics [259]. Membranes to separate bone healing from connective tissue healing were introduced in the 1950s, yet much later for a dentoalveolar application 1960s [30]. For endodontic surgery, membranes have been investigated in animal studies [58, 59, 149], as well as in patients [67, 185]. The placement of any grafting materials or membranes occurs immediately prior to wound closure at the end of the surgical procedure. Common materials include polytetrafluoroethylene (ePTFE, Goretex), collagen or polylactide for membranes; freeze-dried bone allografts, demineralized freeze-dried bone allograft, hydroxyapatite, tricalcium phosphate, bioglass or calcium sulfate for grafts. GTR effectively excluded epithelial ingrowth into lesions and permitted bone regeneration compared to control lesions with no barrier usage [14, 82, 266].

The application of GTR for endodontic surgery has been reviewed [143, 259]. In endodontic surgery, a distinction can be made between uncomplicated defects, complicated defects, and periodontally involved defects.

Uncomplicated defects are endodontic lesions without any periodontal component,

such as deep probings on the tooth, or even a connection between periodontal and endodontic defects. No differences were seen in the healing rate with or without membrane placement [83, 149] or the placement of membrane combined with a graft at a 1 year recall follow-up [243]. Similarly, no differences were observed in periapical bone healing and bone density using CT scans after a 6-month follow-up [212].

Complicated defects are also endodontic lesions without any periodontal component; however the defect exceeds 10 mm in diameter, presents with a bucco-lingual "through and through" defect, and/or perforation to the nasal cavity, or a large perforation to the maxillary sinus. These lesions may benefit from GTR techniques. Buccal and lingual membrane placement in "through and through" rat calvaria defects showed complete healing versus no healing without membranes in the control group [66]. In similar defects, membrane placement showed complete healing after endodontic surgery of cases where a barrier was utilized versus controls that demonstrated only fibrous connective tissue [57, 58]. In humans, healing of through and through lesions was 88% with GTR placement versus 57% without [242]. These findings indicate that through and through lesions should have buccal and lingual barriers to heal effectively [27]. Lesions sizes that exceeded 10 mm in diameter demonstrated faster healing and better outcome when GTR techniques were utilized versus without [184, 185], including clinical, radiographic, and histologic results [185, 196, 246].

Periodontally involved defects present with bone loss in the furcation, apico-marginal or perio-endo communication defects, or a loss of the buccal plate due to a dehiscence or completely denuded root. The overall success rate of endodontic surgery has been shown to be significantly decreased for periodontally involved defects compared to endodontic lesions alone [124, 233]. The usefulness of GTR to improve the periodontal status of a tooth has been demonstrated by histologic and clinical outcome studies. Membrane placement in situations with buccal plate loss in dogs significantly increased the amount of alveolar bone regenerated [65], and membrane placement plus a graft may significantly increase cementum deposition [31, 60].

In conclusion, GTR techniques appear beneficial in situations of complicated and periodontally involved defects.

12.13 Retreatment of Failed Surgical Cases

The most common cause of failure of the initial surgical procedure is the absence or incorrect placement of a root-end filling [133]. A long-term follow-up of cases that were deemed successful at short-term follow-up identified long-term failures to be mostly related to operator mistakes [232]. If the cause of failure is an insufficient root filling, coronal leakage is detected, and the root canal system is accessible and negotiable, non-surgical retreatment should be the first choice of treatment [231]. Non-surgical retreatment after failed endodontic surgery was evaluated by Mente et al. [157]. In this prospective case series, orthograde retreatment and filling with an apical MTA plug was performed in 25 cases, with an overall success rate of 87%. However, the success for anterior teeth was 100% compared to 80% for posterior teeth, highlighting the increased difficulties and challenges associated with this procedure on multi-rooted teeth [157]. If non-surgical retreatment is not the preferred or most predictable option, re-surgery, or extraction should be considered [207]. If failure is associated with one particular root, crown or root resection may be an option to save the tooth [222]. Some studies reported that re-surgery had a very poor prognosis, and might often be contraindicated [215, 270], and a systematic review of endodontic re-surgery, reported a weighted pooled success rate of only 36%, albeit including traditional techniques for the secondary surgical procedure [190]. If microsurgical techniques and biocompatible root-end filling materials

such as MTA and Super-EBA were used, a much higher success rate for endodontic re-surgery may be achieved, approaching those of first-time surgery [231].

12.14 Modes of Healing

Healing of the periapical tissues requires recruitment of stem/progenitor cells from the bone marrow, endosteum, periosteum, and the periodontal ligament to differentiate into osteoblasts, PDL cells, and cementoblasts. Healing of the excisional wound after surgery takes place faster than the regression of a granuloma or cyst after non-surgical treatment, where the inflamed tissues must first be degraded by phagocytic debridement [143]. After endodontic surgery, the bony defect is filled with blood, and the blood clot will be protected from infection from the oral cavity by the repositioned flap. Epithelial cells will proliferate to close the wound from the surgical incision. Antiseptic solutions are recommended for the patient during the first week after the surgery to reduce the risk of infection of the surgical site. Below the soft tissues, healing takes place as two separate processes, osseous healing and dentoalveolar healing.

12.14.1 Osseous Healing

Osseous healing after surgical intervention will require hemostasis, by vasoconstriction and platelet aggregation [143]. There are three basic phases of wound healing, with considerable overlap between the stages: inflammation, proliferation, and remodeling [54, 60, 135, 152, 281]. Within these three phases a complex and coordinated series of events occurs. The inflammatory phase includes chemotaxis and phagocytosis [135, 152]. In the proliferation phase neocollagenesis, epithelialization, and angiogenesis result in the formation of granulation tissue originating from the PDL and endosteum [52, 102, 135, 152]. Lastly, in the remodeling phase, active collagen remodeling and tissue maturation take place, which either results in repair or regeneration [135]. For the osseous wound, this translates into the revascularization of the initial blood clot and the formation of a mineralizing matrix, which from woven bone eventually matures into cancellous bone. New bone formation starts in the internal area and progresses externally towards the level of the former cortical plate. As newly laid woven bone reaches the lamina propria, the overlying membrane becomes functional periodontium, which is part of the osseous healing process. This is separate from dentoalveolar healing, as it occurs as a response to the surgical excision.

12.14.2 Dentoalveolar Healing

During the development of apical periodontitis, periodontal ligament, cementum and dentin were resorbed at the root apex. Viable periodontal ligament (PDL) cells from the adjacent root surface proliferate during the dentoalveolar healing process and cover the denuded root surface [143, 145]. From these tissues PDL stem cells will differentiate into cementoblast-like cells, allowing cementum to regenerate [143, 145]. Root resorption that involved both cementum and dentin can only be repaired by cementoid tissue, because PDL stem cells are unable to differentiate into dentin producing osteoblasts [217]. In the absence of infection or severe inflammatory reactions, cementum has been shown to grow back to cover the resected dentin surface [11, 13, 102, 202]. MTA and RRM both allow cementum apposition directly on the material surfaces [45, 252, 254]. In addition, MTA may support the re-establishment of a PDL width comparable to its natural thickness, whereas the bone-to-material distance was twice that for SuperEBA and four times as large for amalgam [15].

12.15 Outcome of Surgical Endodontics

Just as for non-surgical endodontics, the purpose of endodontic surgery is the elimination of apical periodontitis and prevention of its

recurrence. The most widely used classification to assess the outcome of endodontic surgery is based on the criteria by Rud and Molven [161, 208]. Success is defined radiographically as complete healing or incomplete (scar tissue formation) healing and clinically by the absence of pain, swelling, percussion sensitivity, or a sinus tract (Figure 12.13a–d). Failure included the radiographic categories of uncertain healing (reduced lesion size) or unsatisfactory healing (unchanged or increased lesion size) and clinically the presence of any of the symptoms mentioned above. According to Molven et al. [161] postoperative healing-related changes mostly occur within the first year after the surgical intervention. Asymptomatic cases with complete or incomplete healing are then considered success, whereas situations with uncertain healing should be re-evaluated for up to 4 years and then designated as success or failure. Survival of the tooth has been validated as the positive outcome measure for resective therapies [222]. Criteria for 3-dimensional assessment of periapical healing after endodontic surgery have recently been introduced [45, 214] and validated [269] (Figure 12.14a–f). The majority of studies evaluating the outcome of surgical procedures have, however, relied on

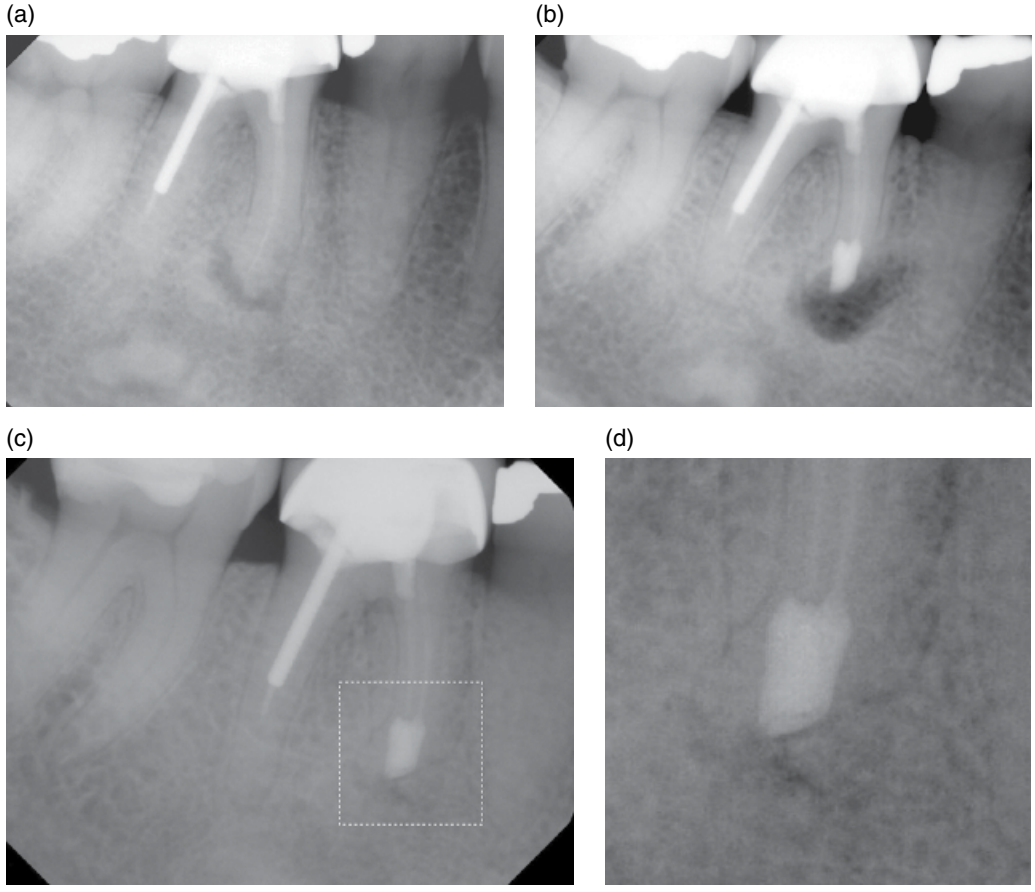

Figure 12.13 Outcome evaluation using conventional 2-dimensional radiography after surgery on mesial root of a first right mandibular molar. (a) Preoperative radiograph. (b) Postoperative radiograph. Note isthmus preparation and root-end filling in situ (RRM). (c) 12-months follow-up, radiographic healing observed, no clinical symptoms. (d) Close-up of marked area in (c). Note radiographic re-establishment of periodontal ligament outline and osseous healing in the periradicular area. Courtesy of Dr Frank Setzer, Philadelphia, PA, USA.

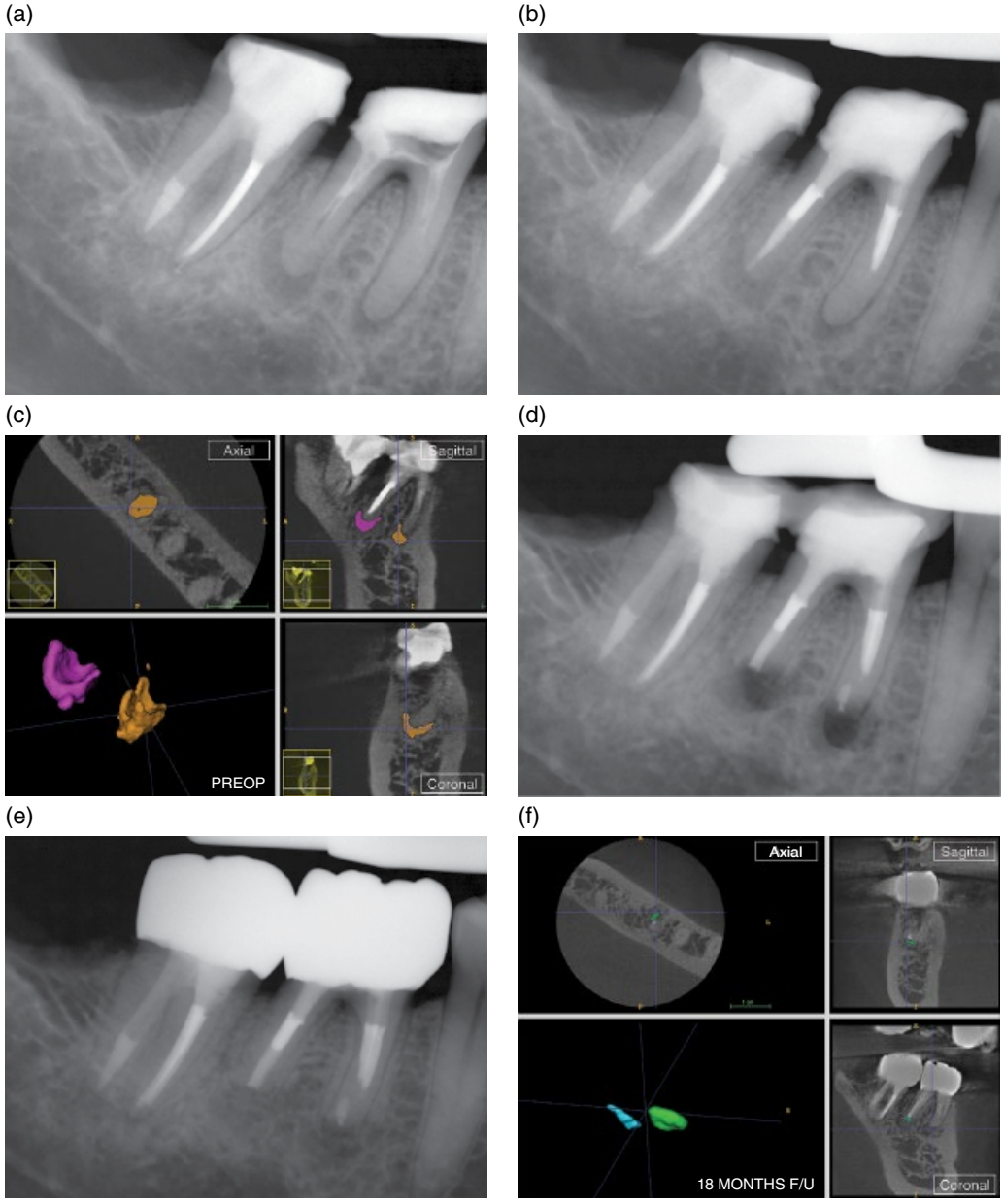

Figure 12.14 Outcome evaluation comparing conventional 2-dimensional radiography with 3-dimensional CBCT imaging after surgery on mesial root of a first right mandibular molar. (a) Preoperative radiograph prior to non-surgical retreatment. (b) Postoperative radiograph after retreatment. Note that the mesial root canal system could not be negotiated. Presence of clinical symptoms. (c) Preoperative 3-dimensional segmentation of periapical lesions. (d) Postoperative radiograph after surgical procedure with mesial root-end filling (MTA). (e) 18-months follow-up radiograph. Radiographic healing observed, no clinical symptoms. (f) Three-dimensional segmentation of remnant low density areas identified in CBCT image, allowing for volumetric comparison and more precise assessment of periapical areas (follow-up CBCT imaging approved by institutional review board). Courtesy of Dr Tom Schloss, Nuremberg, Germany.

clinical evaluation and 2-dimensional radiographic interpretation.

A systematic review of studies investigating the outcome of surgical endodontics found success rates ranging from 37% to 91%, varying with the operator and the specific techniques used for the surgical procedures, and including all historic techniques [78]. The evaluation of outcome has to be undertaken cautiously, however, since there are many variables in treatment protocol and methodology between different studies, including study design, sample sizes, inclusion and exclusion criteria, follow-up periods, lack of standardized clinical and radiographic parameters for healing, the periodontal condition of the teeth prior to surgery, variations in the quality of previous endodontic treatment, the coronal restoration, and the surgical materials and techniques itself. Moreover, many studies reporting the success and failure of periradicular surgery were case series or other studies with a low level of evidence [154]. However, as it has been demonstrated that results of well-designed observational studies, cohort or case-control designs do not systematically overestimate the magnitude of the effects of treatment as compared with those in randomized controlled trials on the same topic [56], results from systematic reviews and meta-analyses assessing the best available evidence should be considered valid.

Few dental techniques have undergone such a substantial evolution as root-end surgery. A number of systematic reviews and meta-analyses have documented the differences in outcome for periradicular surgery depending on the techniques used for the procedures. Traditional, now obsolete techniques using a straight surgical handpiece, a beveled root resection, and often a retrograde amalgam filling demonstrated weighted pooled success rates of 59.0% [221]. The use of loupes, ultrasonic root-end preparation, and more biocompatible filling materials increased this outcome to 86% of periapical healing [220]. Endodontic microsurgery, using the same tools and techniques, but replacing loupes with a dental operating microscope capable of providing high magnification demonstrated even higher success rates ranging from 91.4% to 94.4% for true endodontic lesions [130, 221, 250, 260]. On the other hand, a recent systematic review and meta-analysis assessed the outcome of resin-based endodontic surgery, using high magnification to preparation a shallow and concave root-end cavity and fill it with bonded resin-based root-end filling material – such as the Retroplast technique, to be successful in 82.2% of the cases, with failures likely to be attributed to bonding failure in the moist periradicular environment [130]. Bioceramic root repair material has been successfully used as an alternative to mineral trioxide aggregate with success rates ranging between 92.0–94.4% [224, 285]

A reversal of healing after periradicular surgery has often been debated. Del Fabbro et al. 2007, assessed randomized clinical trials comparing non-surgical versus surgical retreatment, and described faster healing after the surgical procedure compared to non-surgical retreatment after 1 year, but a regression of successful cases for endodontic surgery over a follow-up period of 4 years [62]. Similar assertions were stated by Friedman [79]. However, majority of healing reversals described were related to obsolete techniques, such as retrograde gutta-percha or amalgam fillings, and there is abundant data now confirming that surgical procedures with modern techniques and proper case selection do not undergo excessive failure rates, but rather minimal attrition of the population of the cases successful at follow-up. According to Song et al. in 2012 teeth that had undergone surgery using microsurgical techniques, a success rate of 93.3% was maintained for more than 6 years. Cases in the unsuccessful group were analyzed during re-surgery, demonstrating that failure was predominantly associated with root fractures and operator mistakes, including missing root-end fillings, incorrect root-end preparation, missed canals, or unaddressed and leaking isthmuses, during the initial surgery [232].

Lower outcome rates are expected, however, for periodontally compromised teeth undergoing periradicular surgery, and for resective therapies. Comparing periodontally healthy teeth with teeth that suffered from moderate to severe bone loss undergoing surgery, the difference in success between healthy teeth of 95.2% versus a success rate of 77.5% for the periodontally compromised teeth was statistically significant [124]. Based on this study, the lowest success rates were seen for cases with probing to the apex of the root with or without complete loss of the buccal cortical plate, suggesting that careful treatment planning is indicated in these situations. A recent systematic review and meta-analysis evaluating the outcome of crown and root resection demonstrated a cumulative survival rate of 85.6%, with no statistical difference between crown resection and root resection techniques [222].

In conclusion, while endodontic surgery should be reserved for teeth with failed non-surgical retreatment, or situations where non-surgical retreatment cannot be performed for technical reasons [249], the outcome of studies utilizing modern techniques justifies its use with proper case selection.

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