JOSÉ F. SIQUEIRA JR
ISABELA N. RÔÇAS

TREATMENT of ENDODONTIC INFECTIONS

2nd EDITION

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Treatment of Endodontic Infections

2nd edition

José F. Siqueira Jr, DDS, MSc, PhD
Isabela N. Rôças, DDS, MSc, PhD
(editors)

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Preface

The ultimate goals of endodontic therapy are to prevent and treat apical periodontitis, which is one of the most common inflammatory diseases that affect humans and is caused by microbial infection of the root canal system. This book provides effective treatment options for achieving both goals. The first section of the book details the microbiologic and pathophysiologic aspects of apical periodontitis. The second section focuses on the principles and practice of predictable endodontic treatment and prevention of apical periodontitis. In this way, a thorough understanding of disease etiology, pathogenesis, and host-pathogen interaction issues sets the groundwork for effective endodontic treatment. By integrating current scientific knowledge with established endodontic techniques, this book is intended to narrow the gap between research and clinical practice and offers an essential, well-informed text for students, clinicians, and researchers alike.

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Dedication

To my wife, Isabela; my children, Esther, Marcus Vinícius, and Thaís; and my parents, José and Léa, for all their love, patience, and support.

José F. Siqueira Jr, DDS, MSc, PhD

To my beloved husband, José, and daughter, Esther; my parents, Wilson and Maria Isabel; and my sisters, Danielle and Patricia, for all their love, patience, and support.

Isabela N. Rôças, DDS, MSc, PhD

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We would like to thank the many people whose support made it possible for us to finish this 2nd edition of Treatment of Endodontic Infections. They include all the dedicated collaborators that gave their time and expertise to contribute state-of-the-art chapters with the highest level of scientific evidence. We are also in debt to our research team, including Flávio Ferreira Alves, José Claudio Provenzano, Alejandro Pérez, and all the others, including our Masters, PhD, and postdoctoral students. We would also like to express our gratitude to the Quintessence team, particularly Johannes Wolters, Christian Haase, Anita Hattenbach, Avril du Plessis, and Sabine Theuring, who believed in this project and worked with us on this journey.

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Foreword

Endodontology as a clinical and scientific discipline has expanded and evolved tremendously alongside medical advances generally over the past decades. Improved insight has led to changing paradigms in endodontic research and new methods in clinical practice. This momentum of progress is dependent on the collective efforts of the scientific and clinical community, but it most of all requires talented and productive individuals, people who challenge conventional concepts and provide new wisdom through high-quality research. The editors and authors of this book are among these pioneers.

One particular line of development in the field can be followed through the terminology used to characterize disease and treatment. The old phrase "the art of endodontics" rightly focuses on the technical finesse necessary for the provision of optimal treatment, and "Endodontics" is mainly associated with the technical treatment procedures. Gradually, a more scientific and comprehensive view has prevailed, with the emphasis on basic science as well as on technical treatment, with "Endodontology" as the overarching name, reflected in many modern textbook titles. From there, prevention and treatment of the two by far most prevalent disease entities, pulpitis and apical periodontitis, took center stage.

Whereas the microbial etiology of these diseases became clear already in the 1960s and 1970s, the clinical impact of this knowledge still needs to be clarified, upheld, and transmitted to practitioners. The initial disease manifestations as such are not a problem but are rather expressions of protective mechanisms by the host. The key is the microbial, largely bacterial, infections of the dental tissues, which have the potential to cause severe illness. Therefore, "Treatment of Endodontic Infections" is both an expression of the current conceptual framework for practice and research and, at the same time, a highly appropriate textbook title. The current volume integrates the cascade of disease development by microbial invasion into tooth substance and the clinical treatment possibilities for the different stages.

As a growing discipline, it follows that Endodontology acquires a rapidly expanding volume of literature of research data and clinical methods. Only active scientists can keep abreast of the literature in the field. José F. Siqueira Jr and Isabela N. Rôças have been at the forefront of the research that has led us to the most detailed insights into the etiology and pathogenesis of pulpal and apical disease for more than two decades. They are therefore ideally suited to provide, in a textbook, an introduction to the essentials of the pulpal and periapical infections that constitute the vast majority of clinical endodontic cases, and between them, they are responsible for the majority of the book's chapters. This secures a unified approach to the subthemes in the book, and with the support of a select few preeminent researchers and clinicians, they give us a complete and up-to-date status on what is indisputably the center and bulk of Endodontology today.

The first edition established this book as an excellent source of information for students of dentistry and endodontics. This revised and upgraded version, with significant added quality by contributing authors, is a most welcome contribution to the discipline that deserves a wide readership.

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Section 1

THE INFECTION

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Introduction to Section 1

Apical periodontitis is essentially an inflammatory disease of microbial origin caused primarily by infection of the root canal system (Fig S1-1).[14] Although chemical and physical factors can induce periradicular inflammation, a large body of scientific evidence indicates that infection is essential to the progression and perpetuation of the different forms of apical periodontitis.[1,4,7,21] Endodontic infection only develops in root canals devoid of host defenses, either as a consequence of pulp necrosis (as a sequel to caries, trauma, periodontal disease, or iatrogenic operative procedures) or pulp removal for previous treatment.

Although fungi, archaea, and viruses have been found in endodontic microbiology studies,[11,17,20,23] bacteria are the primary microorganisms implicated in the pathogenesis of apical periodontitis. More than 450 bacterial species and phylotypes belonging to 100 genera and 9 phyla have been detected in the different types of endodontic infections.[18] High-throughput sequencing technology has revealed that these numbers can be even higher.[3,5,12,13,15,16,22,24] Therefore, apical periodontitis is regarded as a disease of bacterial infection.

In the advanced stages of the endodontic infectious process, bacteria are observed primarily organized in biofilm structures.[10] A strong association of bacterial biofilms located in the apical portion of the root canal system as well as both primary and post-treatment apical periodontitis have been demonstrated. Consequently, apical periodontitis has been included in the group of biofilm-induced oral diseases.[10]

Bacteria colonizing the root canal system gain access to the periradicular tissues via apical and lateral foramina as well as via iatrogenic root perforations. As a consequence of the encounter between bacteria and host defenses, inflammatory and immunologic reactions take place in the periradicular tissues (Fig S1-2). Although protective, these defense mechanisms can also be destructive and induce the development of apical periodontitis. Depending on several bacterial and host-related factors, endodontic infections can lead to acute or chronic forms of apical periodontitis. As histopathologic conditions do not always correlate with clinical symptoms, apical

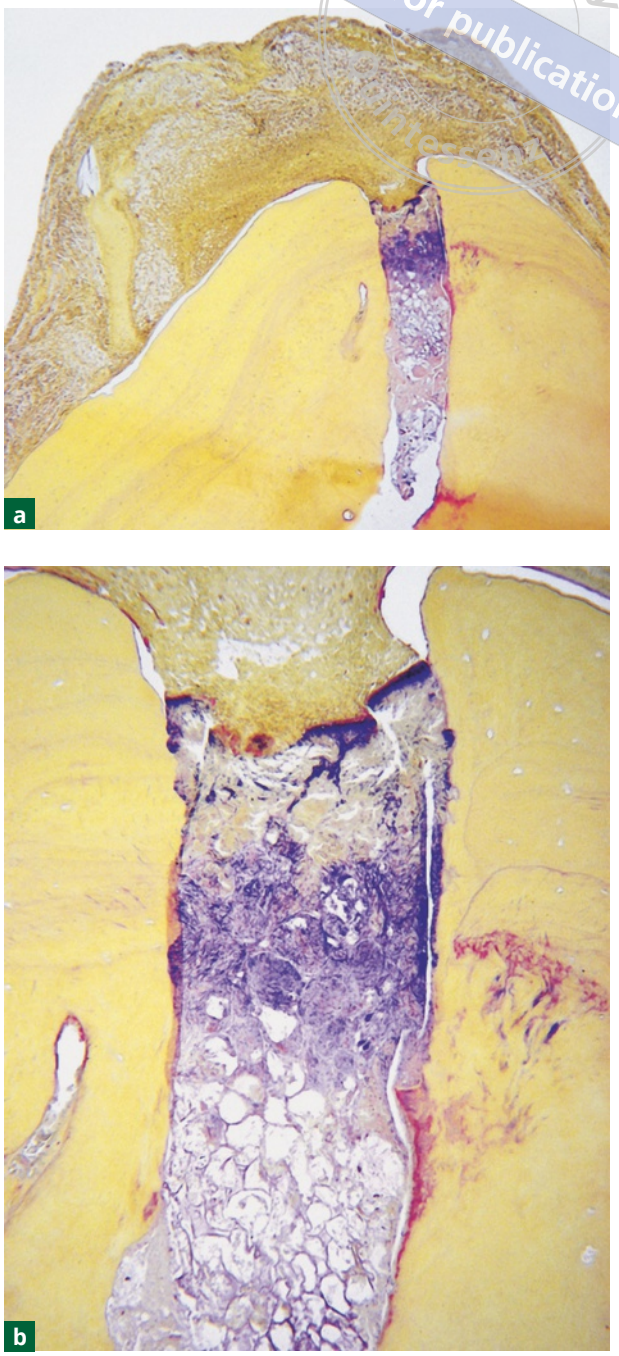

Fig S1-1 **(a)** Apical periodontitis is an inflammatory disease primarily caused by bacteria infecting the root canal system. **(b)** Note the border line between infection and defense near the apical foramen (courtesy Domenico Ricucci).

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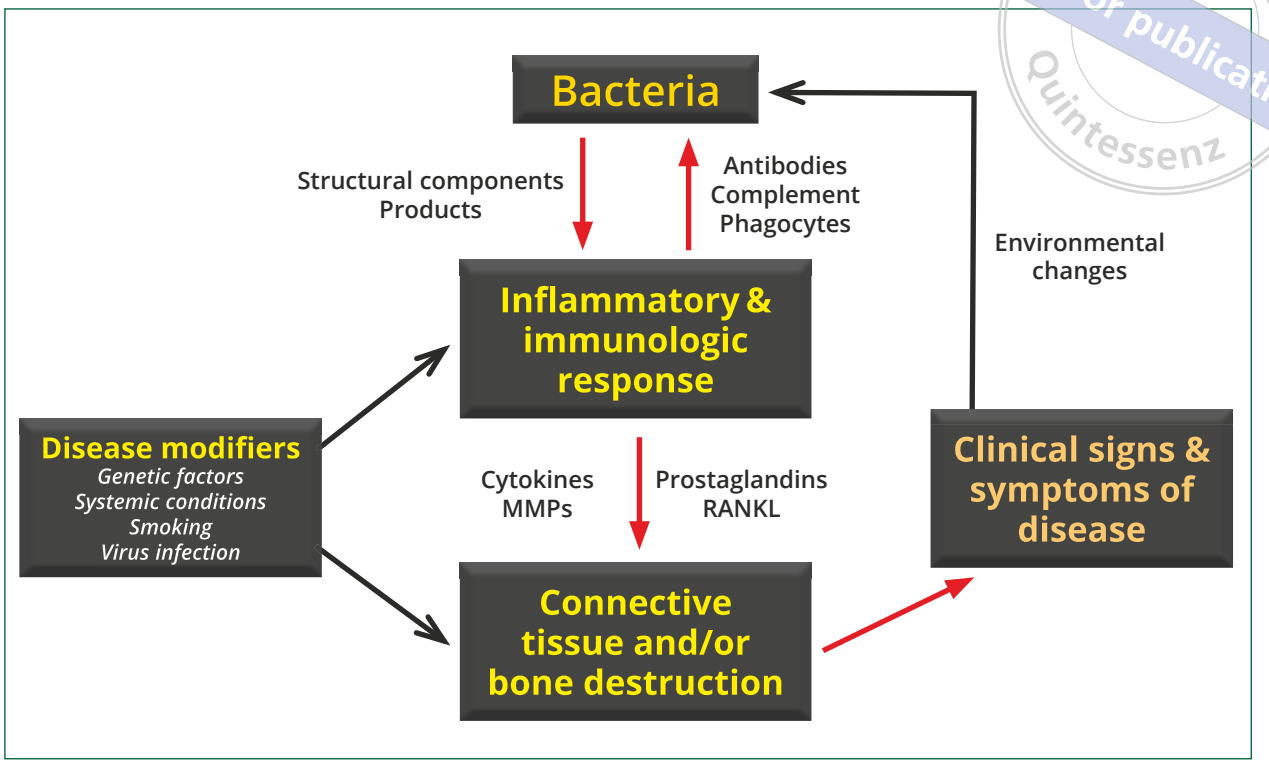

Fig S1-2 Bacteria infecting the root canal system evoke inflammatory and immunologic responses at the periradicular tissue level. These responses can be protective or destructive. Apical periodontitis develops as a result of connective tissue and bone destruction. Environmental changes induced by the pathologic condition may influence the composition and virulence of the intraradicular bacterial community, starting a vicious cycle. The host response to bacterial infection may be modified by genetic or acquired conditions. These factors may influence the progression and severity of the disease as well as its response to treatment.

periodontitis may be clinically symptomatic or asymptomatic. Once established, the disease process alters the environment and influences aspects of bacterial ecology, including selection of the dominant species. Although bacterial infection is the cause of apical periodontitis, the progression and severity of the disease and its response to treatment can be influenced by host-related disease modifiers that interfere with host resistance to infection (see Fig S1-2).[2,6,8,9,19]

Apical periodontitis alone seldom poses a medical problem of significant magnitude, particularly if asymptomatic (or chronic). However, there is mounting evidence that it may contribute to the total oral infectious burden and thus influence systemic health. Even if asymptomatic, the apical periodontitis lesion may increase in size and directly affect nearby anatomical structures such as the maxillary sinus and the mandibular canal, causing sinusitis and paresthesia, respectively. In turn, the acute apical abscess, which represents the most severe form of symptomatic apical periodontitis, can spread from the original site of infection and cause serious complications at relatively distant body sites.

The ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or to create adequate conditions for periradicular tissue healing. As apical periodontitis is an infectious disease, the rationale for endodontic treatment is to eradicate the infection and/or to prevent microorganisms from infecting or reinfecting the root canal or the periradicular tissues. A thorough understanding of disease etiology and pathogenesis is cardinal to any healthcare profession and provides the framework for effective treatment. Consequently, a thorough understanding of the micro-

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biologic aspects of apical periodontitis is essential for high-quality endodontic practice based on a solid scientific foundation. The first section of this book deals with microbiologic and pathophysiologic aspects of apical periodontitis, while the second section describes the best evidence for predictable treatment and prevention of the disease.

References

1. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy 1974;25:347–358.
2. Fouad AF. Diabetes mellitus as a modulating factor of endodontic infections. J Dent Educ 2003;67:459–467.
3. Hong BY, Lee TK, Lim SM, et al. Microbial analysis in primary and persistent endodontic infections by using pyrosequencing. J Endod 2013;39:1136–1140.
4. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340–349.
5. Keskin C, Demiryurek EO, Onuk EE. Pyrosequencing analysis of cryogenically ground samples from primary and secondary/persistent endodontic infections. J Endod 2017;43:1309–1316.
6. Menezes-Silva R, Khaliq S, Deeley K, Letra A, Vieira AR. Genetic susceptibility to periapical disease: conditional contribution of MMP2 and MMP3 genes to the development of periapical lesions and healing response. J Endod 2012;38:604–607.
7. Möller AJ, Fabricius L, Dahlén G, Öhman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475–484.
8. Morsani JM, Aminoshariae A, Han YW, Montagnese TA, Mickel A. Genetic predisposition to persistent apical periodontitis. J Endod 2011;37:455–459.
9. Petean IBF, Kuchler EC, Soares IMV, et al. Genetic polymorphisms in RANK and RANKL are associated with persistent apical periodontitis. J Endod 2019;45:526–531.
10. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277–1288.
11. Saboia-Dantas CJ, Coutrin de Toledo LF, Sampaio-Filho HR, Siqueira JF Jr. Herpesviruses in asymptomatic apical periodontitis lesions: an immunohistochemical approach. Oral Microbiol Immunol 2007;22:320–325.
12. Santos AL, Siqueira JF Jr, Rôças IN, et al. Comparing the bacterial diversity of acute and chronic dental root canal infections. PLoS One 2011;6:e28088.
13. Shin JM, Luo T, Lee KH, et al. Deciphering endodontic microbial communities by next-generation sequencing. J Endod 2018;44:1080–1087.
14. Siqueira JF Jr. Microbiology of apical periodontitis. In: Ørstavik D, Pitt Ford T (eds). Essential Endodontology, ed 2. Oxford, UK: Blackwell Munksgaard, 2008:135–196.
15. Siqueira JF Jr, Alves FR, Rôças IN. Pyrosequencing analysis of the apical root canal microbiota. J Endod 2011;37:1499–1503.
16. Siqueira JF Jr, Antunes HS, Rôças IN, Rachid CT, Alves FR. Microbiome in the apical root canal system of teeth with post-treatment apical periodontitis. PLoS One 2016;11:e0162887.
17. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632–641.
18. Siqueira JF Jr, Rôças IN. Diversity of endodontic microbiota revisited. J Dent Res 2009;88:969–981.
19. Siqueira JF Jr, Rôças IN, Provenzano JC, et al. Relationship between Fcgamma receptor and interleukin-1 gene polymorphisms and post-treatment apical periodontitis. J Endod 2009;35:1186–1192.
20. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:327–331.
21. Sundqvist G. Bacteriological studies of necrotic dental pulps [Odontological Dissertation no. 7]. Umea, Sweden: University of Umea, 1976.
22. Tzanetakis GN, Azcarate-Peril MA, Zachaki S, et al. Comparison of bacterial community composition of primary and persistent endodontic infections using pyrosequencing. J Endod 2015;41:1226–1233.
23. Vianna ME, Conrads G, Gomes BPFA, Horz HP. Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol 2006;44:1274–1282.
24. Zandi H, Kristoffersen AK, Ørstavik D, et al. Microbial analysis of endodontic infections in root-filled teeth with apical periodontitis before and after irrigation using pyrosequencing. J Endod 2018;44:372–378.

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Endodontic infections and the etiology of apical periodontitis – an overview

José F. Siqueira Jr
Isabela N. Rôças

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Apical periodontitis: an infectious disease

The occurrence of bacteria in a necrotic root canal was first observed in the 17th century by Dutch amateur microscope builder Antonie van Leeuwenhoek (1632–1723), who reported that the root canals of a decayed tooth *"were stuffed with a soft matter"* that seemed to him to be alive.[7] He called these organisms *"animalcules."* However, the role played by bacteria in disease causation was unsuspected at that time, and it took almost 200 years for this to be established, especially based on the efforts of Robert Koch in Germany, and Louis Pasteur in France.

Indeed, a cause-and-effect relationship between bacteria and apical periodontitis was first suggested in 1894 by Willoughby Dayton Miller, an American dentist working at Koch's laboratory in Berlin, Germany.[20] By bacterioscopic examination of root canal samples, Miller identified the basic bacterial morphotypes (cocci, bacilli, and spirilla or spirochetes) (Fig 1-1) and reported differences in the composition of the endodontic microbiota in the coronal, middle, and apical parts of the root canal. Spirochetes observed in high frequencies in apical abscesses were suspected to play an etiologic role in this disease. Most of the bacteria that Miller saw under the light microscope could not be cultivated using the technology available at that time. They were presumably anaerobic bacteria, which were only successfully cultivated about 50 to 100 years later with the advent of anaerobic culture techniques. Incidentally, a large number of bacterial species living in diverse environments, including the root canal, remain to be cultivated today.[2,31] Based on his findings, Miller hypothesized that bacteria could be the cause of apical periodontitis.

Approximately 70 years after this classic study, Miller's assumptions were definitively confirmed in 1965 in an elegant study by Kakehashi et al,[12] who investigated the response of the dental pulps of conventional and germ-free rats to exposure to the oral cavity. Histologically, they determined that pulp necrosis and apical periodontitis lesions developed in

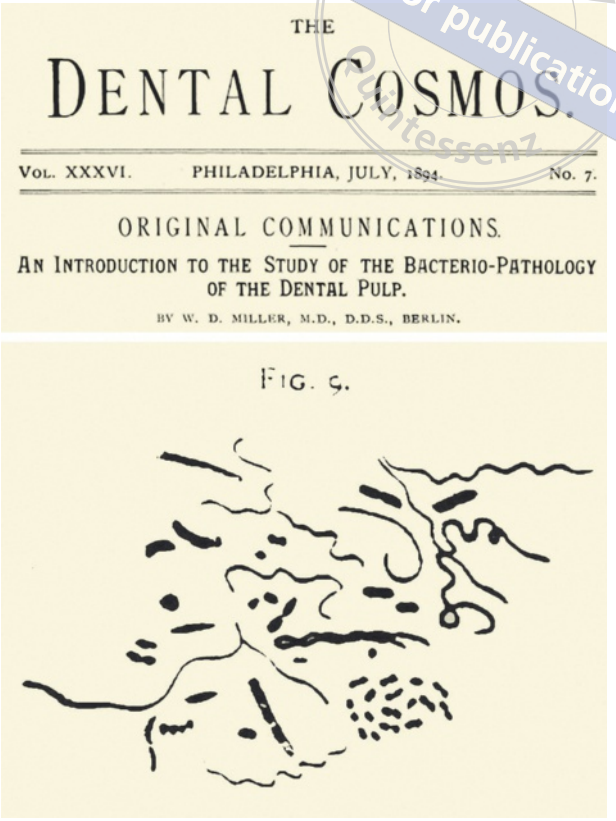
THE
DENTAL COSMOS.
VOL. XXXVI. PHILADELPHIA, JULY, 1894. No. 7.
ORIGINAL COMMUNICATIONS.
AN INTRODUCTION TO THE STUDY OF THE BACTERIO-PATHOLOGY OF THE DENTAL PULP.
BY W. D. MILLER, M.D., D.D.S., BERLIN.
FIG. 5.

Fig 1-1 Miller's classic study on endodontic infection. Drawings from his study showing different bacterial forms in a root canal sample observed by microscopy.

all conventional rats, whereas the pulps of germ-free rats not only remained vital but also repaired themselves by hard tissue formation. In the absence of bacteria, dentin-like tissue sealed the exposure area and re-isolated the vital pulps from the oral cavity.

The essential role of bacteria in the etiology of apical periodontitis was further confirmed in another classic study by Sundqvist (1976),[43] who applied anaerobic culturing techniques to evaluate bacteria occurring in the root canals of teeth whose pulps had become necrotic after trauma. The fact that the bacteria were present only in the root canals of teeth exhibiting radiographic evidence of apical periodontitis confirmed the infectious etiology of this disease. Anaerobic bacteria accounted for more than 90% of the isolated strains. Sundqvist's study also demonstrated that the necrotic pulp tissue and stagnant tissue fluid in the root canal could not induce and per-

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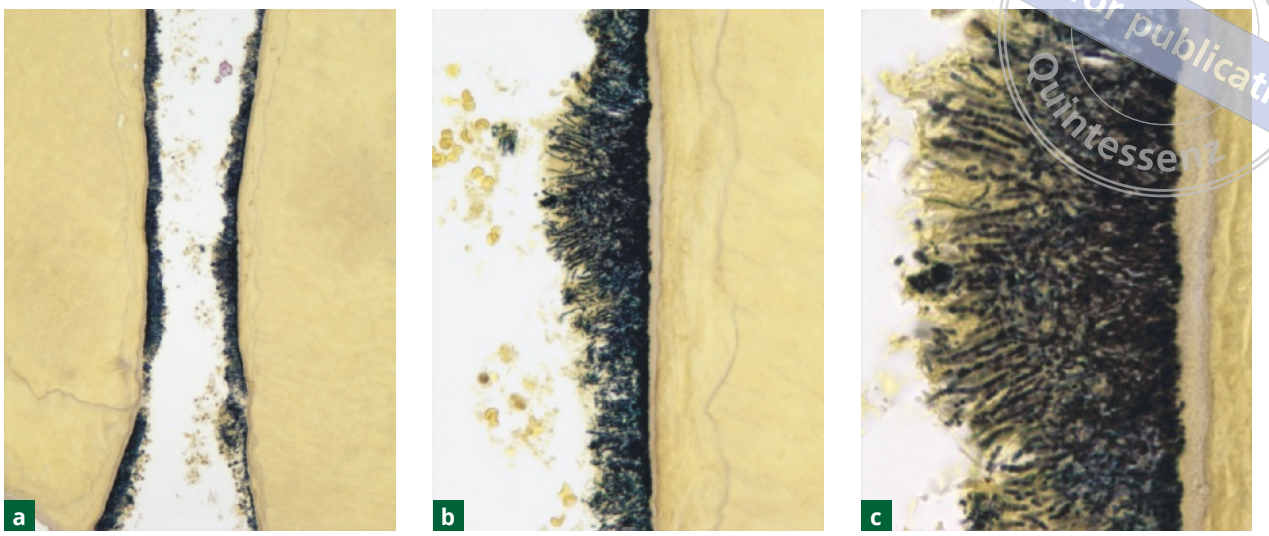

Fig 1-2 **(a)** Intraradicular bacterial biofilm attached to the root canal walls. This is the main form in which bacteria are found colonizing the root canal. Higher magnifications are seen in **(b)** and **(c)** (courtesy Domenico Ricucci).

petuate apical periodontitis lesions in the absence of infection.

In 1981, Möller et al[21] provided further strong evidence for the microbial causation of apical periodontitis. Their study in monkeys demonstrated that apical periodontitis lesions developed only in teeth that had devitalized and infected pulps, whereas teeth with devitalized but noninfected pulps showed no significant pathologic changes in the periradicular tissues. In addition to corroborating the importance of microorganisms for the development of apical periodontitis, this study also confirmed that the necrotic pulp tissue per se is not able to induce and maintain apical periodontitis lesions.

It has been a long-held desire of endodontic microbiology researchers to find a species or a group of species that is the major causative agent of apical periodontitis. However, as information brought about by culturing and molecular identification methods as well as morphologic studies has accumulated, evidence has mounted that apical periodontitis is primarily caused by root canal bacterial infections, mostly organized in mixed bacterial communities adhered to the root canal walls. This prompted Siqueira and Rôças to apply the community-as-pathogen concept to the etiology of apical periodontitis.[40] Based on this concept, the disease outcome is related to the collective pathogenicity of the intracanal bacterial communities, which depends on the composition, abundance, and interactions between the species that form the community.

Morphologic studies of the patterns of microbial colonization of root canals had reported bacterial condensations or agglomerations that are nowadays recognized as biofilm-like structures.[22,36,41] However, the study by Ricucci and Siqueira[34] was the first to look for the prevalence of intraradicular and extraradicular biofilms, and tried to establish their association with primary and posttreatment apical periodontitis. Bacterial infection occurred in all teeth with apical periodontitis, confirming the disease's infectious etiology. Bacteria were organized in biofilms in the apical segment of untreated and treated teeth in a high prevalence (Fig 1-2). This study used criteria previously published in the literature to include apical periodontitis in the list of biofilm-induced diseases.

Routes of endodontic infection

Under normal conditions, the pulpodentin complex is sterile and isolated from the oral microbiota by overlying enamel and cementum. The sterility of the

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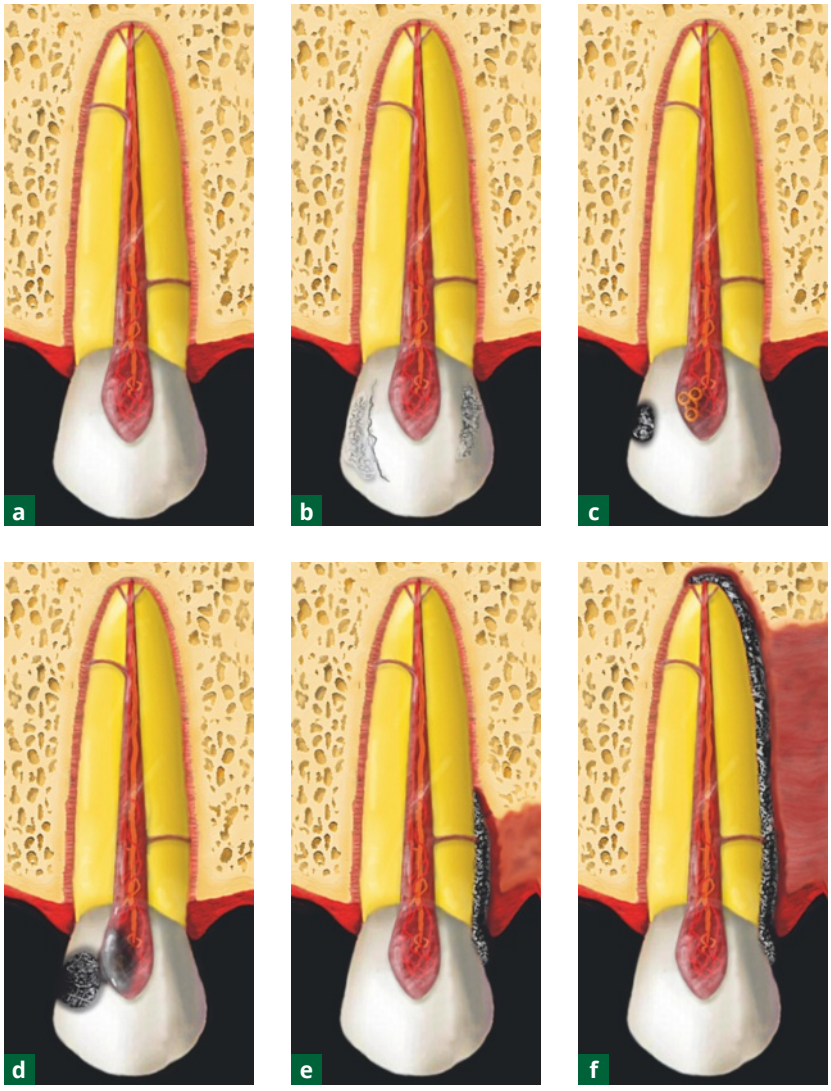

Fig 1-3 Routes of pulpal infection. **(a)** Normal pulp. **(b)** Cracks in the enamel reaching dentin and exposing tubules. **(c)** Caries lesion exposing dentinal tubules. **(d)** Direct pulp exposure. **(e)** Periodontal disease exposing cervical dentin or lateral canals. **(f)** Periodontal disease reaching the root apex.

healthy pulp tissue was questioned by a molecular study that reported the detection of bacterial DNA in samples from pristine healthy teeth.[46] However, the negative controls used in that study were inadequate, and the detected bacterial DNA was very likely to be contaminants from the analytical reagents. Molecular methods are very effective in detecting and identifying bacteria, but when the sample has few or no DNA, some artifacts are generated such as amplification of contaminant DNA, which may occur in reagents used in DNA extraction, PCR, and sequencing library preparation.[37] This statement is reinforced by the fact that the most frequent and abundant taxa found in the healthy pulp samples were nonoral bacteria,[46] including representatives of the genera *Ralstonia, Burkholderia, Staphylococcus, Micrococcus*, and *Acinetobacter*, which are, not surprisingly, the main contaminants present in water and reagents used for molecular methods.[4,14,15,37,44] Therefore, there is so far no evidence to contradict the long-held concept that the healthy pulp tissue is sterile.

In the event that the integrity of the natural layers that isolate the pulp from the oral cavity has been breached (e.g. as a result of caries, trauma-induced fractures and cracks, restorative procedures, scaling and root planing, attrition, or abrasion, etc) or is naturally absent (e.g. due to gaps in the cemental coating at the cervical root surface), the pulpodentin complex will be exposed to the oral environment. Consequently, it is challenged by bacteria present in

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caries lesions, the saliva bathing the exposed surfaces, or the dental plaque accumulated on the exposed surfaces (Fig 1-3). Bacteria from subgingival biofilms associated with periodontal disease may invade the pulp via the dentinal tubules in the cervical region of the tooth or via the lateral and apical foramina (see Fig 1-3). Microorganisms may also invade the root canal any time during the endodontic intervention, usually by a breach in the aseptic chain, or even after treatment, usually by coronal leakage of saliva.

As the permeability of normal dentin is dictated by its tubular structure, the pulp is put at risk of infection whenever dentin is exposed (Fig 1-4). Dentinal tubules traverse the entire width of dentin and have a conical shape. They are widest near the pulp (mean 2.5 µm-diameter) and narrowest in the periphery, near enamel or cementum (mean 0.9 µm-diameter).[8] The smallest tubule diameter is compatible with the cell diameter of most oral bacterial species (range 0.2 to 0.7 µm). Thus, one might well assume that, once exposed, dentin offers bacteria unrestricted access to the pulp via the tubules. Nevertheless, this is not necessarily the case. Bacterial invasion of dentinal tubules was found to occur more rapidly in nonvital than in vital teeth.[23] In vital teeth, the outward movement of dentinal fluid and tubular contents (including odontoblast processes, collagen fibrils, and the sheath-like lamina limitans lining the tubules) influences dentinal permeability and can conceivably delay intratubular invasion by bacteria.

Due to the tubular contents, the functional or physiologic diameter of the tubules is only 5% to 10% of the anatomical diameter seen by microscopy.[18] Thus, although the microscopic diameter of dentinal tubules at the dentinoenamel junction is reported to be about 1 µm, they function as if they were only 0.1 µm in diameter.[27] Moreover, other factors such as dentinal sclerosis beneath a caries lesion, tertiary dentin, smear layer, and intratubular deposition of fibrinogen also reduce dentin permeability and thereby limit or impede bacterial progression to the pulp via dentinal tubules.[29] Host defense molecules such as antibodies and components of the complement system may also be present in the dentinal

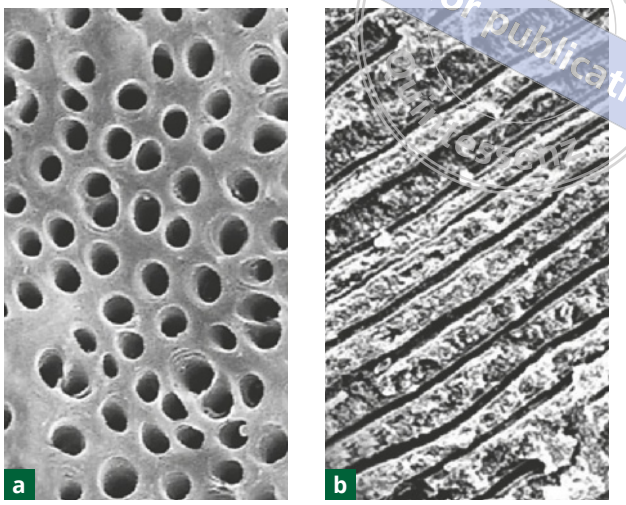

Fig 1-4 Scanning electron micrographs of dentin showing tubules in cross-sectional **(a)** and longitudinal **(b)** views.

fluid of vital teeth, and assist in protecting the dentin against deep bacterial invasion.[1,24,25] Therefore, as long as the pulp is vital, dentinal exposure does not represent a significant route of pulpal infection, unless dentin thickness is considerably reduced and dentin permeability significantly increased.

Although bacteria have no frank access to the vital pulp via tubules, they shed products that dissolve in dentinal fluid and reach the pulp long before the bacterial cells themselves. These products may exert a direct effect on the pulp tissue even before direct pulp exposure.[3,30,45] Depending on the amount and virulence potency of bacterial products, the thickness of the remaining dentin, the area of dentin involved, and the state of the pulpal circulation, this shedding of bacterial products may or may not cause significant pulpal inflammation.[26] As for dentin permeability, it has been reported that the diffusion of toxic material into the peripheral ends of exposed dentinal tubules is unlikely to significantly irritate the pulp if the dentin is at least 3-mm thick, but may induce substantial irritation if the remaining dentin thickness is only 0.3 mm.[28] In the latter case, the dentin is extremely permeable and no longer able to function as a reliable diffusion barrier.

As the caries process progressively destroys dentin and approaches the pulp, bacterial aggression toward the pulp increases. Reeves and Stanley[32]

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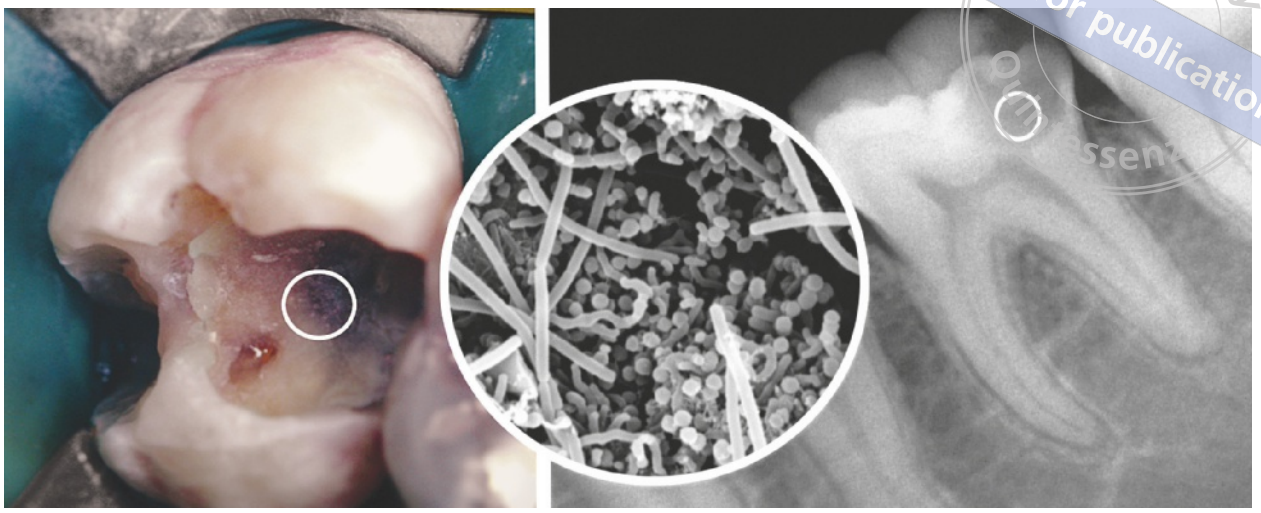

Fig 1-5 Direct pulp exposure by caries is the most common route of root canal infection.

observed that when the distance between the invading bacteria and the pulp (including the thickness of reparative dentin) averaged 1.1 mm or more, the inflammatory response was negligible, and when the lesions came within 0.5 mm of the pulp, the extent of inflammation increased significantly. However, acute pulp inflammation did not occur until the reparative dentin that had formed beneath the lesion was invaded by bacteria.

Most of the bacteria involved in the caries process are nonmotile organisms that invade dentin by repeated cell division, which pushes cells into tubules. Bacterial cells may also be forced into tubules by the hydrostatic pressure exerted on dentin during mastication.[19] Bacteria inside the tubules under a deep caries lesion may reach the pulp even before frank pulp exposure occurs.[11] However, it is assumed that the pulp will not be infected as long as it is vital. If only a few bacteria reach the pulp, this will be of no importance since the vital pulp can eliminate such a transient infection and rapidly clear or remove bacterial products. This efficient clearance mechanism tends to prevent injurious agents from reaching concentrations high enough to induce significant inflammatory reactions.[26] On the other hand, if the vitality of the pulp is compromised and the defense mechanisms are impaired, very few bacteria are needed to initiate infection.

Bacteria have been isolated from necrotic pulps of traumatized teeth with apparently intact crowns.[43,47] This inevitably raises the question as to how the bacteria reached the root canal. In the past, it was believed that bacteria from the gingival sulcus or periodontal pockets could enter the root canals of teeth whose pulps became necrotic after trauma through severed blood vessels of the periodontium in a process called anachoresis.[10] However, this theory has never been supported by scientific evidence. Actually, trauma can expose dentin by inducing crown fracture or enamel cracks. Macro- and microcracks in enamel may occur in most teeth (not only traumatized teeth) and do not necessarily end at the dentinoenamel junction, but deep in the dentin.[17] A single crack can expose a large number of dentinal tubules to the oral environment. If clogged with dental bacterial biofilm, the crack can serve as a portal of entry for bacteria. If the pulp remains vital after trauma, the dentinal fluid and tubular contents counteract bacterial penetration into the tubules, and pulpal health is not usually jeopardized. On the other hand, when the pulp becomes necrotic as a consequence of trauma, it loses the ability to protect itself against bacterial penetration and, regardless of dentin thickness, the dentinal tubules will become true avenues for bacterial invasion of the necrotic pulp.

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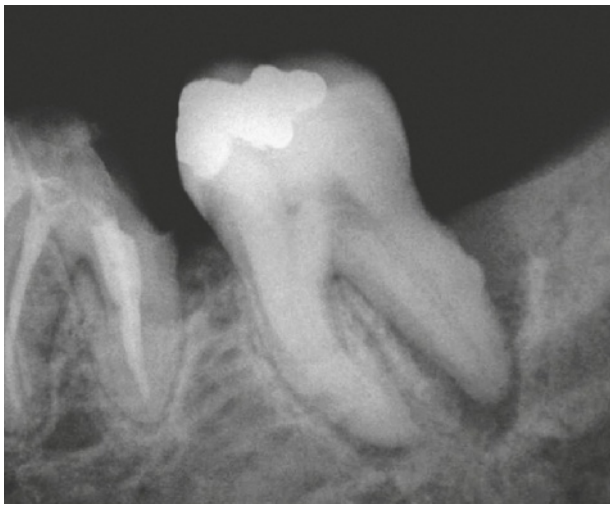

Fig 1-6 Periodontal disease affects pulp vitality when the subgingival bacterial biofilm reaches the apical foramen (courtesy Wilson Rosalém).

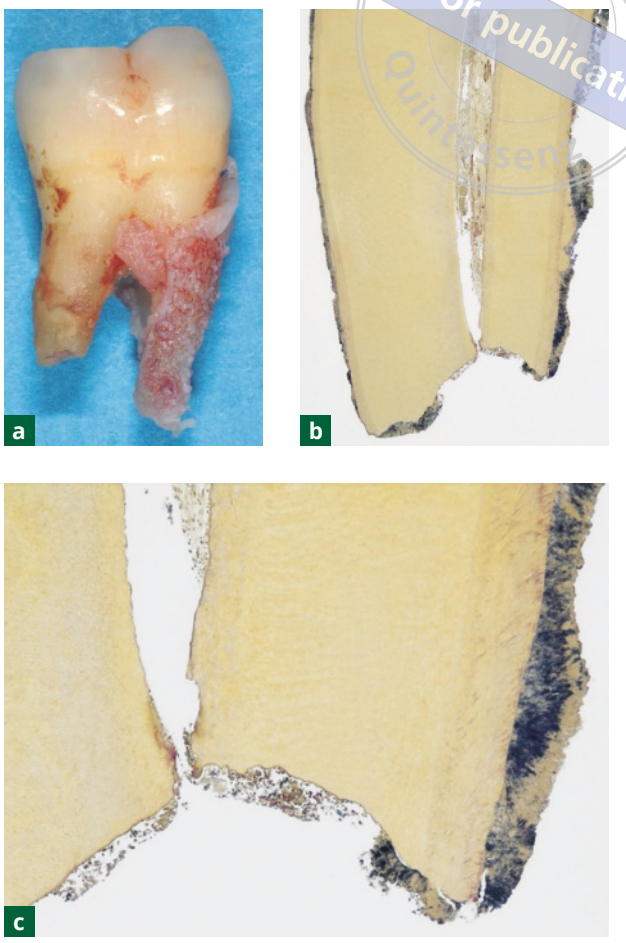

Fig 1-7 **(a)** Mandibular molar extracted because of extensive periodontal disease reaching the apex. **(b)** Histologic section showing the biofilm reaching the apical portion of the root. The pulp shows necrotic and degenerated areas. **(c)** Higher magnification of the periodontal biofilm attached to the apical part of the root (courtesy Domenico Ricucci).

Direct exposure of the dental pulp to the oral cavity is the most obvious route of endodontic infection (Fig 1-5). Caries is the most common cause of pulp exposure, but direct pulp exposure as a result of iatrogenic restorative procedures or trauma may also allow bacteria to reach the pulp. The exposed pulp tissue comes into direct contact with oral bacteria in caries lesions, saliva, and/or plaque biofilm accumulations on the exposed surfaces. Almost invariably, the exposed pulp will undergo inflammation, necrosis, and infection. The time lapse between pulp exposure and infection of the entire canal is unpredictable but generally slow.[5]

Bacteria and their products egressing from infected root canals through apical, lateral or furcation foramina, dentinal tubules without an external cementum covering, and iatrogenic root perforations directly affect the surrounding periodontal tissues and induce pathologic changes in these tissues. However, there is no consensus as to whether the opposite is true, i.e. whether subgingival biofilms associated with periodontal disease can directly cause pulpal disease.

Conceptually, bacteria in subgingival plaque biofilms associated with periodontal disease could reach the pulp by the same pathways as those by which intracanal bacteria reach the periodontium, and could thereby exert harmful effects on the pulp. However, it has been demonstrated that although degenerative and inflammatory changes of different degrees may occur in the pulp of teeth with associated marginal periodontitis, pulpal necrosis secondary to periodontal disease develops only if the periodontal pocket reaches the apical foramen, leading to irreversible damage to the main blood vessels that penetrate through this foramen to irrigate the pulp (Figs 1-6 and 1-7).[13] Once the pulp becomes necrotic, periodontal bacteria can reach the root canal system via exposed dentinal tubules at the cervical area of the root or via lateral and apical foramina to establish an endodontic infectious process.

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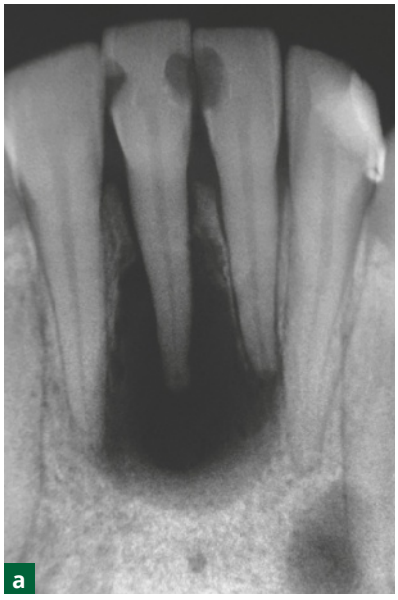

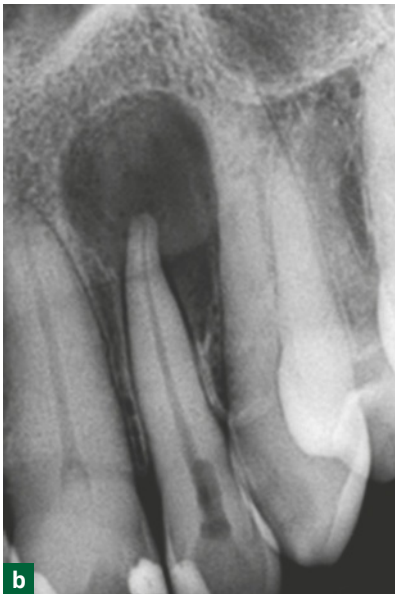

Fig 1-8 Primary intraradicular infection is the main cause of primary apical periodontitis. **(a and b)** Large apical periodontitis lesions as revealed by periapical radiographs.

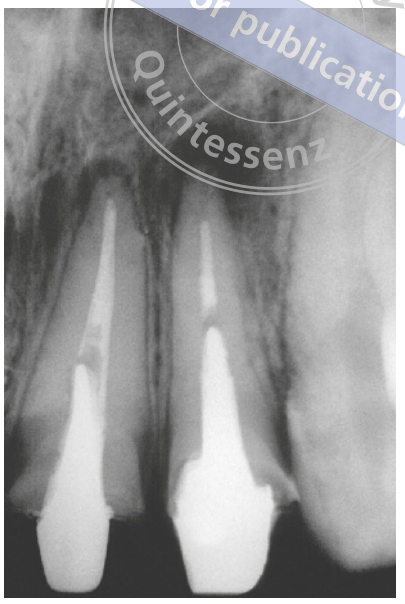

Fig 1-9 Persistent/secondary intraradicular infection is the main cause of posttreatment apical periodontitis.

As reported above, it has been claimed that bacteria can reach the pulp by anachoresis. According to this theory, bacteria are transported by blood or lymph to an area of tissue damage, where they leave the vessel, enter the damaged tissue, and establish an infection.[9,35] However, there is no clear evidence showing that this process is a route for root canal infection. In fact, it was revealed that, when the blood stream was experimentally infected, bacteria could not be recovered from unfilled root canals unless they were overinstrumented during bacteremia, resulting in injury to periodontal blood vessels and seepage of blood into the canal.[6] Another argument against anachoresis as a route of pulpal infection comes from the study by Möller et al,[21] who induced pulpal necrosis in monkey teeth and reported that all teeth with aseptic necrosis remained bacteria-free after 6 to 7 months of observation. Although anachoresis has been suggested to be the mechanism through which traumatized teeth with seemingly intact crowns become infected,[10] current evidence indicates that the main pathway of pulpal infection in these cases is dentinal exposure due to enamel cracks.[16,17]

Whatever the route of bacterial access to the root canal, necrosis of pulp tissue is a prerequisite for the establishment of primary endodontic infections. As long as the pulp is vital, it can protect itself against bacterial invasion and colonization. However, if the pulp becomes necrotic as a result of caries, trauma, operative procedures, or periodontal disease, the necrotic tissue can be very easily infected. This is because host defenses no longer function in the necrotic pulp tissues, and those in the periradicular tissues do not reach deep into the root canal space.

The root canal system is also devoid of host defenses in cases where the pulp has been removed for treatment. For instance, microorganisms can gain entry into the root canal space during treatment, between appointments, or after root canal filling and cause a secondary infection.[39]

The main causes of microbial invasion of the canal during treatment include residual dental plaque biofilm, calculus or caries on the tooth crown, a leaking rubber dam, contamination of endodontic instruments (e.g. by touching them with the fingers), and contamination of irrigants or other intracanal solutions (saline solution, distilled water, citric acid, etc).

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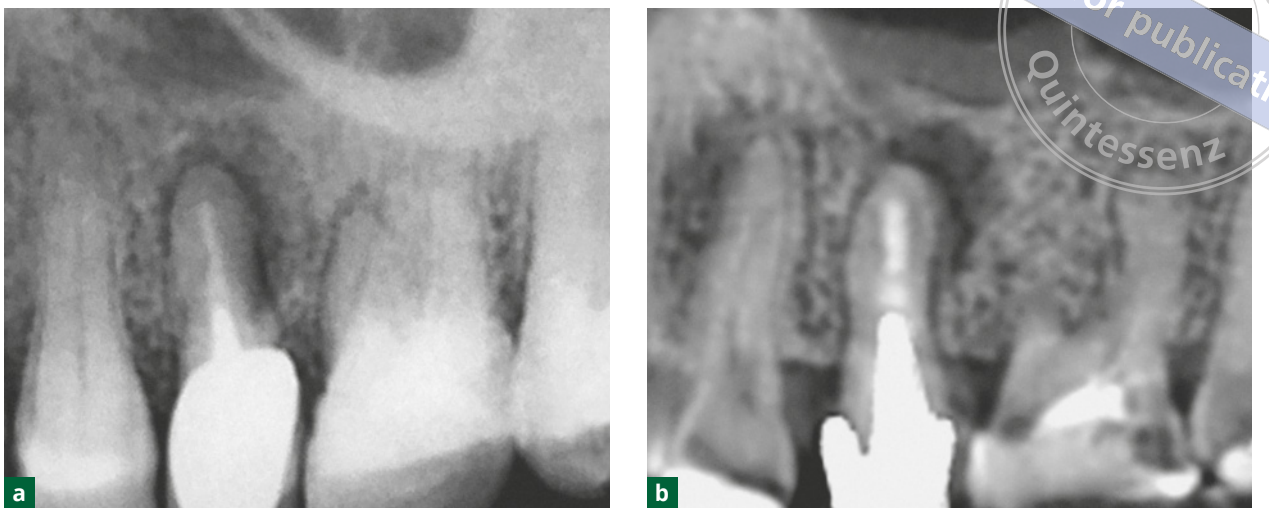

Fig 1-10 Maxillary second premolar with posttreatment apical periodontitis. **(a)** Periapical radiograph. **(b)** Cone beam computed tomography (courtesy Fátima G. Bueno-Camilo).

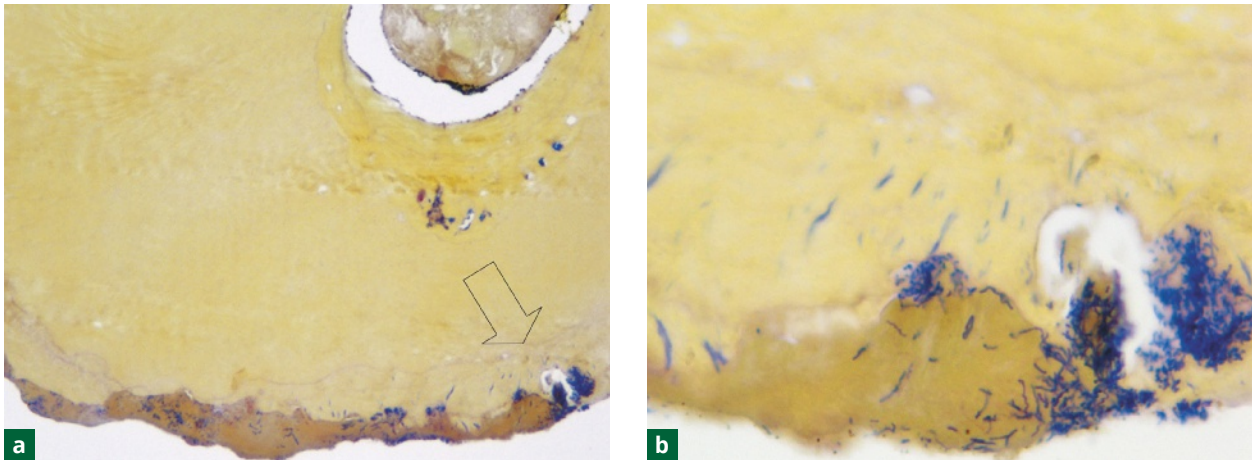

Fig 1-11 **(a)** Extraradicular bacterial biofilm of endodontic origin. **(b)** Higher magnification (courtesy Domenico Ricucci).

Microorganisms can enter the root canal system *between appointments* by the following mechanisms: leakage through temporary restorative material, breakdown, fracture or loss of a temporary restoration, fracture of tooth structure, and teeth with the canal left open for drainage.

Microorganisms can still penetrate the root canal system *after completion of a root canal filling* if there is leakage through temporary or permanent restorative material, breakdown, fracture or loss of a temporary or permanent restoration, fracture of tooth structure, recurrent decay exposing root canal filling material, or delayed placement of permanent restorations.

Types of endodontic infections

According to the definitions proposed by Siqueira in 2002,[38] endodontic infections can be classified as follows:

Intraradicular infection

Intraradicular infection occurs when microorganisms colonize the root canal system and can be subclassified as primary, secondary, or persistent according to the time at which the microorganisms entered the root canal system.

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a) *Primary intraradicular infection* is caused by the microorganisms that initially invade and colonize the necrotic pulp tissue (initial or "virgin" infection). It is the cause of primary apical periodontitis (Fig 1-8).
b) *Secondary intraradicular infection* is caused by microorganisms that were not part of the primary infection but which gained entry into the root canal some time after professional intervention. For this reason, it is called a secondary infection (secondary to treatment).
c) *Persistent intraradicular infection* occurs when microorganisms involved in the primary or secondary infection somehow manage to resist intracanal antimicrobial procedures and endure periods of nutrient deprivation in treated canals.

Persistent and secondary infections are responsible for several clinical problems, including persistent exudation ("wet canal"), persistent symptoms, interappointment exacerbations (flare-ups), and post-treatment apical periodontitis, which characterizes the failure of the endodontic treatment (Figs 1-9 and 1-10). For the most part, persistent and secondary infections are clinically indistinguishable. Exceptions include infectious complications (e.g. apical abscesses) arising after the treatment of noninfected vital pulps or cases in which apical periodontitis was absent at the time of treatment but present on follow-up radiographs. Both are typical examples of secondary infections.

Extraradicular infection

Extraradicular infection is characterized by microbial invasion of the inflamed periradicular tissues as a sequel to intraradicular infection. Bacteria in extraradicular infections may be located as aggregations within the body of the lesion and surrounded by defense cells, within the cyst lumen, or attached to the outer root surface as biofilms (Fig 1-11). Extraradicular infections are usually associated with symptoms and sinus tracts, showing a high prevalence in teeth with chronic and especially acute apical abscesses.[33,42] When present, these infections are in the large majority of cases dependent on the intraradicular infection. However, on some occasions they can be independent of the intraradicular infection, in the sense that they are no longer fostered by the latter and, as such, are not responsive to nonsurgical root canal treatment.

References

1. Ackermans F, Klein JP, Frank RM. Ultrastructural localization of immunoglobulins in carious human dentine. Arch Oral Biol 1981;26:879–886.
2. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 1995;59:143–169.
3. Bergenholtz G. Effect of bacterial products on inflammatory reactions in the dental pulp. Scand J Dent Res 1977;85: 122–129.
4. Biesbroek G, Sanders EA, Roeselers G, et al. Deep sequencing analyses of low density microbial communities: working at the boundary of accurate microbiota detection. PLoS One 2012;7:e32942.
5. Cvek M, Cleaton-Jones PE, Austin JC, Andreasen JO. Pulp reactions to exposure after experimental crown fractures or grinding in adult monkeys. J Endod 1982;8:391–397.
6. Delivanis PD, Fan VS. The localization of blood-borne bacteria in instrumented unfilled and overinstrumented canals. J Endod 1984;10:521–524.
7. Dobell C. Antony van Leeuwenhoek and his "little animals". London: Staples Press Limited, 1932.
8. Garberoglio R, Brännström M. Scanning electron microscopic investigation of human dentinal tubules. Arch Oral Biol 1976;21:355–358.
9. Gier RE, Mitchell DF. Anachoretic effect of pulpitis. J Dent Res 1968;47:564–570.
10. Grossman LI. Origin of microorganisms in traumatized, pulpless, sound teeth. J Dent Res 1967;46:551–553.
11. Hoshino E, Ando N, Sato M, Kota K. Bacterial invasion of non-exposed dental pulp. Int Endod J 1992;25:2–5.
12. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340–349.
13. Langeland K, Rodrigues H, Dowden W. Periodontal disease, bacteria, and pulpal histopathology. Oral Surg Oral Med Oral Pathol 1974;37:257.
14. Lazarevic V, Gaia N, Emonet S, et al. Challenges in the culture-independent analysis of oral and respiratory samples from intubated patients. Front Cell Infect Microbiol 2014;4:65.

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15. Lazarevic V, Gaia N, Girard M, Schrenzel J. Decontamination of 16S rRNA gene amplicon sequence datasets based on bacterial load assessment by qPCR. BMC Microbiol 2016;16:73.
16. Love RM. Bacterial penetration of the root canal of intact incisor teeth after a simulated traumatic injury. Endod Dent Traumatol 1996;12:289–293.
17. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002;13:171–183.
18. Michelich V, Pashley DH, Whitford GM. Dentin permeability: a comparison of functional versus anatomical tubular radii. J Dent Res 1978;57:1019–1024.
19. Michelich VJ, Schuster GS, Pashley DH. Bacterial penetration of human dentin in vitro. J Dent Res 1980;59:1398–1403.
20. Miller WD. An introduction to the study of the bacteriopathology of the dental pulp. Dent Cosmos 1894;36: 505–528.
21. Möller AJ, Fabricius L, Dahlén G, Öhman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475–484.
22. Molven O, Olsen I, Kerekes K. Scanning electron microscopy of bacteria in the apical part of root canals in permanent teeth with periapical lesions. Endod Dent Traumatol 1991;7:226–229.
23. Nagaoka S, Miyazaki Y, Liu HJ, et al. Bacterial invasion into dentinal tubules of human vital and nonvital teeth. J Endod 1995;21:70–73.
24. Okamura K, Maeda M, Nishikawa T, Tsutsui M. Dentinal response against carious invasion: localization of antibodies in odontoblastic body and process. J Dent Res 1980;59:1368–1373.
25. Okamura K, Tsubakimoto K, Uobe K, Nishida K, Tsutsui M. Serum proteins and secretory component in human carious dentin. J Dent Res 1979;58:1127–1123.
26. Pashley DH. Clinical considerations of microleakage. J Endod 1990;16:70–77.
27. Pashley DH. Clinical correlations of dentin structure and function. J Prosthet Dent 1991;66:777–781.
28. Pashley DH. Dentin-predentin complex and its permeability: physiologic overview. J Dent Res 1985;64(spec no): 613–620.
29. Pashley DH. Dynamics of the pulpo-dentin complex. Crit Rev Oral Biol Med 1996;7:104–133.
30. Pissiotis E, Spångberg LS. Dentin permeability to bacterial proteins in vitro. J Endod 1994;20:118–122.
31. Rappe MS, Giovannoni SJ. The uncultured microbial majority. Annual Rev Microbiol 2003;57:369–394.
32. Reeves R, Stanley HR. The relationship of bacterial penetration and pulpal pathosis in carious teeth. Oral Surg Oral Med Oral Pathol 1966;22:59–65.
33. Ricucci D, Loghin S, Gonçalves LS, Rôças IN, Siqueira JF Jr. Histobacteriologic conditions of the apical root canal system and periapical tissues in teeth associated with sinus tracts. J Endod 2018;44:405–413.
34. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277–1288.
35. Robinson HB, Boling LR. The anachoretic effect in pulpitis. Bacteriologic studies. J Am Dent Assoc 1941;28:268–282.
36. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol 1995;11:6–9.
37. Sheik CS, Reese BK, Twing KI, et al. Identification and removal of contaminant sequences from ribosomal gene databases: lessons from the census of deep life. Front Microbiol 2018;9:840.
38. Siqueira JF Jr. Endodontic infections: concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:281–293.
39. Siqueira JF Jr, Lima KC. *Staphylococcus epidermidis* and *Staphylococcus xylosus* in a secondary root canal infection with persistent symptoms: a case report. Aust Endod J 2002;28:61–63.
40. Siqueira JF Jr, Rôças IN. Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:870–878.
41. Siqueira JF Jr, Rôças IN, Lopes HP. Patterns of microbial colonization in primary root canal infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:174–178.
42. Siqueira JF Jr., Rôças IN. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev 2013;26:255–273.
43. Sundqvist G. Bacteriological studies of necrotic dental pulps [Odontological Dissertation no. 7]. Umea, Sweden: University of Umea, 1976.
44. Tanner MA, Goebel BM, Dojka MA, Pace NR. Specific ribosomal DNA sequences from diverse environmental settings correlate with experimental contaminants. Appl Environ Microbiol 1998;64:3110–3113.
45. Trowbridge HO. Pathogenesis of pulpitis resulting from dental caries. J Endod 1981;7:52–60.
46. Widmer C, Skutas J, Easson C, et al. Culture-independent characterization of the microbiome of healthy pulp. J Endod 2018;44:1132–1139.
47. Wittgow WC Jr, Sabiston CB Jr. Microorganisms from pulpal chambers of intact teeth with necrotic pulps. J Endod 1975;1:168–171.

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The invaders: bacterial biofilm communities and pathogenicity

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Teamwork is what counts – community as pathogen

Some classic diseases caused by exogenous pathogens have a "single-species etiology." Apical periodontitis, on the other hand, is similar to most human endogenous infections in that no single pathogen but rather a set of pathogenic species usually organized in mixed biofilm communities is involved in its etiology.[10,86,120,143,144]

In ecological hierarchy, individual microorganisms group to form *populations*, which in turn form microcolonies that interact with one another to form *communities*. A community is thus an integrated assemblage of populations that coexist and interact in a given environment. Following this concept, an infected root canal harbors an endodontic microbial community composed of several populations (microcolonies). Individual populations sometimes consist of a single species but more frequently are composed of different bacterial species that collectively give rise to a mixed community.[136,137] Each population has a functional role (*niche*) that contributes to the overall community and helps to maintain the ecological balance of the ecosystem.

There is a recent trend to move away from the paradigm that a single pathogen causes a given human endogenous infection toward a more holistic view of the pathogenic community as the underlying unit of pathogenicity.[131] According to this holistic approach, the whole is often greater than the simple sum of its parts, and no component can be thoroughly understood except in relation to the whole.[65] Like caries and marginal periodontitis, apical periodontitis is the result of the collaborative activities of a biofilm community.[131]

Bacterial communities and apical periodontitis

The concept of endodontic infections as bacterial biofilm communities brings a more holistic view of the etiology of apical periodontitis.[131] Mounting evidence indicates that there is little specificity in the involvement of single species in the etiology of apical periodontitis, but more specificity when bacterial community profiles are taken into account. In other words, while association of a single bacterial species with a specific form of apical periodontitis is seldom, if ever, observed, bacterial community profiles seem to follow some patterns related to the different presentations of apical periodontitis.[131]

Community profiles are essentially determined by species richness (number of different species) and abundance (proportion of each species). Community profile analyses of endodontic microbiota have revealed some interesting findings, for example, that:

a) Different types of endodontic infections, including persistent or secondary infections associated with treated teeth,[14,113,114,121,132] are composed of mixed bacterial communities;[71,134]
b) Some underrepresented uncultivated bacteria are commonly found in infected root canals;[70,133]
c) Endodontic bacterial communities associated with the same clinical disease exhibit great interindividual variability,[134] i.e. each individual harbors a unique endodontic microbiota in terms of species richness and abundance. The fact that individuals suffering from the same disease have endodontic microbiota compositions that differ consistently[71,114,134] suggests a heterogeneous etiology of apical periodontitis in which multiple species combinations lead to similar disease outcomes;
d) Bacterial communities seem to follow a specific pattern according to the clinical condition (asymptomatic apical periodontitis, acute apical abscesses or posttreatment apical periodontitis).[114,134] Therefore, it is reasonable to assume that the severity of disease (intensity of signs and symptoms) or response to treatment may be related to bacterial community composition. In other words, from the perspective of the single-pathogen concept, apical periodontitis can be considered to have no specific microbial etiology. However, based on the community-as-pathogen concept, it is possible to infer some specificity;[120,134]

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e) Interindividual variability is even more pronounced in individuals from different geographic locations;[71,114,134,135]
f) The composition of the microbiota in the apical portion of the root canal is significantly different from that in the more coronal aspects of the root canal.[3] Bacterial communities in the apical portion of the canal are as diverse as those in the middle and coronal third of the root canal. A high level of interindividual (samples from the same region but from different patients) and intraindividual (samples from different regions of the same tooth) variability is observed.[3]

The biofilm lifestyle

Biofilm can be defined as a sessile multicellular microbial community characterized by cells that are firmly attached to a surface and enmeshed in a self-produced matrix of extracellular polymeric substance (EPS) (Fig 2-1).[20,30] The vast majority of microorganisms in nature invariably grow and function as members of metabolically integrated communities or biofilms.[20,77] In medical microbiology, the ability to form biofilms has been regarded as a virulence factor.[45]

Biofilms are not merely passive assemblages of bacterial cells stuck to surfaces, but are structurally and dynamically organized complex biologic systems. Bacterial cells in biofilms form microcolonies (about 15% by volume) that are embedded and non-randomly distributed in the EPS matrix (about 85% by volume) and separated by water channels.[23,30,136,142] Depending on the time they have to accumulate and remain undisturbed, dental biofilms can reach up to 300 or more cell layers in thickness.[136]

The EPS matrix confers unique features to the biofilm community and plays an essential role in biofilm physiology, output, and protection. EPS can be regarded as hydrated biopolymers, mostly composed of polysaccharides, but also proteins, nucleic acids, and lipids.[20] The main functions of the EPS matrix include:[34]

a) adhesion to surfaces, usually serving as a "biologic glue;"
b) mechanical stability of the biofilm;
c) accumulation of extracellular enzymes that may have important activities for the community, including nutrient acquisition and cooperative degradation of complex macromolecules;
d) maintenance of the biofilm cells in close proximity, favoring interactions;
e) nutrient source in periods of nutrient deprivation, even though some components of the matrix cannot be completely degraded;
f) retention of water to maintain a highly hydrated microenvironment;
g) protection against phagocytes and host defense molecules;
h) protection against antimicrobial agents used during treatment.

The structure of biofilms differs significantly according to the overall physical, chemical, and biologic features of the environment.[20,109,141] Among other mechanisms, the shapes of microcolonies in biofilms are governed by shear forces related to the flow of fluid or air over the biofilm. Microcolonies usually take the shape of "towers" or "mushrooms" when subjected to low shear forces and may appear elongated and able to oscillate at high shear forces.[141] Such shear forces do not normally occur in root canals. The main structural and physiologic features of endodontic biofilms remain to be determined.

Biofilm bacteria form populations that are not randomly distributed but are spatially and functionally organized throughout the mixed community. Indeed, the populations are strategically positioned for optimal metabolic interaction, and the resultant architecture favors the ecological role of the community. The properties displayed by a mixed biofilm community are mostly dictated by interactions between populations, which creates novel physiologic functions.

The community lifestyle affords the following advantages to biofilm bacteria:[21,22,30,45,76,78,136,142]

a) broader habitat range for growth of a more diverse microbiota;

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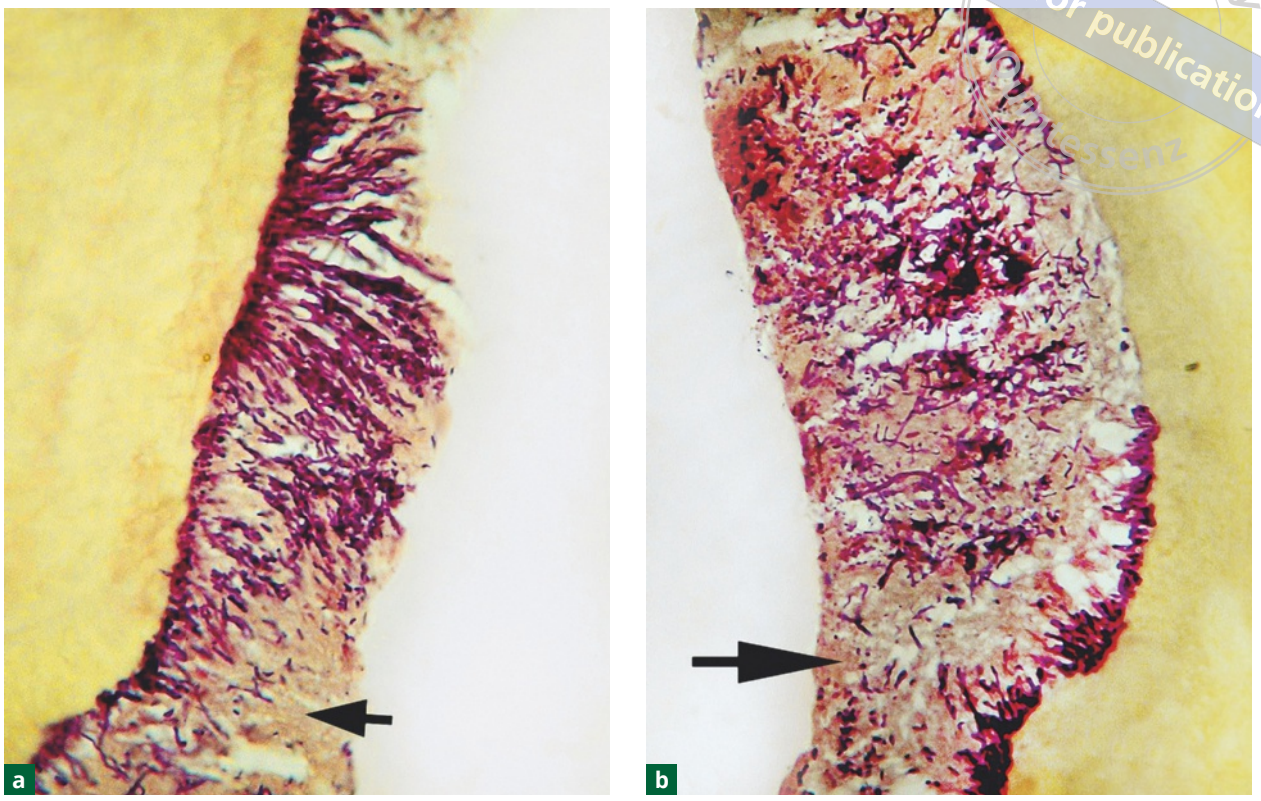

Fig 2-1 (a and b) Bacterial biofilms adhered to the tooth surface. Note the several cell layers composed of different morphotypes (cocci, rods, and filaments) and enmeshed in an extracellular matrix (arrows).

b) increased metabolic diversity and efficiency due to food webs;
c) protection from competing microorganisms, host defenses, antimicrobial agents, and environmental stress (Fig 2-2);
d) facilitated genetic exchanges, which may involve genes encoding antibiotic resistance and virulence factors;
e) enhanced pathogenicity.

Community-based microbial pathogenesis

The concept of pathogenic communities is based on the principle that teamwork is what eventually counts. The behavior of a bacterial community and the outcome of host/bacterial community interactions depend on which species compose the community and how the myriad associations occurring within the community affect and modulate the virulence of its members. The virulence of a given species allegedly differs when the species acts in pure culture, in pairs, or as part of a larger bacterial "society" (community).

The development of apical periodontitis requires the concerted action of bacteria in a community. Bacterial virulence factors involved in the pathogenesis of apical periodontitis represent the summation of substances produced by the bacterial endodontic community. Thus, the biologic effects of endodontic bacteria are the result of the collective pathogenicity of the community, which depends on the overall population density and species composition as well as the synergistic interactions between them.

It is possible that a certain species might have more than one role in the community while another species performs similar functions. This is termed functional redundancy and helps explain why communities with different bacterial compositions

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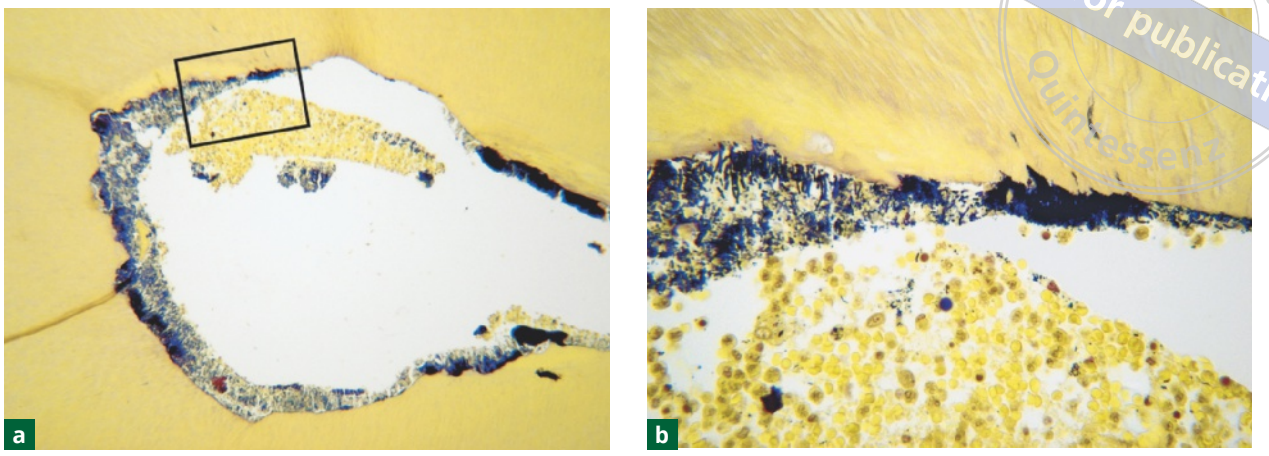

Fig 2-2 **(a)** Biofilm on the walls of a mesial root canal from a mandibular first molar associated with apical periodontitis. **(b)** Higher magnification of the inset in a. Note the accumulation of polymorphonuclear neutrophils in the canal near the biofilm (courtesy Domenico Ricucci).

can be found in different individuals with similar disease.[131]

In mixed communities, a broad spectrum of relationships may arise between the component species, ranging from no effect (rare) or reduced pathogenicity to additive or synergistic pathogenic effects. Endodontic abscesses are polymicrobial infections in which bacterial species that are usually low-virulent and unable to cause disease individually can cause disease in association with other species as part of a mixed consortium (pathogenic synergism).[17,146]

Acute (planktonic) and chronic (biofilm) infections

Mechanisms by which bacteria forming biofilm communities survive and induce tissue damage are a result of collective and cooperative activities and are quite different from those used by planktonic cells of specialized pathogens.[20] Consequently, the outcome of biofilm community infections usually differs from that of infections caused by pathogens occurring in a planktonic state.

Apical periodontitis may be chronic or acute depending on a number of factors. Acute infection is usually caused by a highly virulent bacterial community. Such high virulence may be due to the presence of virulent species or strains and/or the occurrence of synergism between species. Acute infections are usually related to bacterial cells in a planktonic state, high cell counts, and some tissue invasion ability, counteracted by diminished host resistance.[130] In endodontic infection, planktonic cells are often observed in the lumen of the main root canal and may have been detached and released from the biofilm, or they may have been carried by saliva in cases with large exposure to the oral cavity. It has been demonstrated that, in some pathogens, genes coding for many virulence factors are expressed in planktonic cells much more frequently than in sessile (biofilm) cells, suggesting that planktonic cells are more likely to participate in acute infections.[38] The phenotype of cells in a planktonic state is fundamentally different from the much more diverse biofilm phenotype.[12,96,123] The shift in gene expression toward a planktonic phenotype is usually conducive to rapid growth and mobility. Many enzyme and toxin genes are turned off when bacteria grow in the biofilm phenotype, but production of these factors can be reinitiated and amplified when individual cells are released from biofilms and assume the planktonic phenotype.[20] However, it must be recalled that transition to a planktonic state renders cells more susceptible to antimicrobial agents and phagocytosis.[24,45,140]

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Table 2-1 Definition of terms related to the mechanisms of bacterial pathogenicity and virulence

Term	Definition
Pathogenesis	the chain of events leading to the development of a disease
Pathogenicity	the ability of a microorganism to cause disease
Virulence	quantitative measure of the pathogenicity of a microorganism
Virulence factors	microbial products, structural components or strategies that contribute to pathogenicity
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
Endogenous infection	infection caused by members of the normal human microbiota
Exogenous infection	infection caused by microorganisms not belonging to the normal microbiota but introduced in the host
Pathogen	a microorganism that causes disease
Primary or true pathogen	a microorganism that often causes disease within a given host
Opportunistic pathogen	a microorganism that causes disease only when host defenses are impaired
Putative, suspected or candidate pathogen	a microorganism suspected of being associated with a disease based on cross-sectional study findings not yet confirmed in longitudinal studies

Chronic infection, on the other hand, is usually associated with low virulence of the bacterial community, which however generally represents a persistent source of aggression to the tissues. Persistence of chronic infections usually occurs because bacterial communities are organized in biofilms and are inaccessible to host defenses as a result of their anatomical location.[16,23,24] The juxtaposition of bacterial biofilms to tissues not accustomed and adapted to their presence triggers protective and, at the same time, destructive inflammatory and immunologic responses. In chronic apical periodontitis, bacteria in the necrotic root canal cause chronic infection by forming protected biofilms on the canal walls and maintaining close contact with the apical periodontal ligament, which reacts by persistent inflammation. Disease is usually mediated by host-derived factors in an attempt to eliminate these sessile communities.

To summarize, biofilms are generally less aggressive in causing immediate tissue damage but are potentially dangerous because they can stimulate persistent inflammation associated with collateral tissue damage. Also, biofilms serve as potential foci for acute exacerbations by releasing sufficient planktonic cells to initiate an acute infection. Tissue damage and resultant inflammation are generally proportional to the cellular density and species composition of the biofilm.

Mechanisms of bacterial pathogenicity

Key terms that are important for further discussion of mechanisms of bacterial pathogenicity and virulence are defined in Table 2-1.

Most bacteria involved in endodontic infections are normal inhabitants of the oral microbiota that take advantage of changes in the dental pulp that reduce local defenses and make the root canal amenable to colonization. These bacteria are regarded as opportunistic pathogens, and endodontic infections are classified as endogenous infections. Apical periodontitis is diagnosed based on the development of signs (bone radiolucency, swelling, sinus tract, etc) and symptoms (pain) of bacterial infection of the canal and damage to the periradicular tissues.

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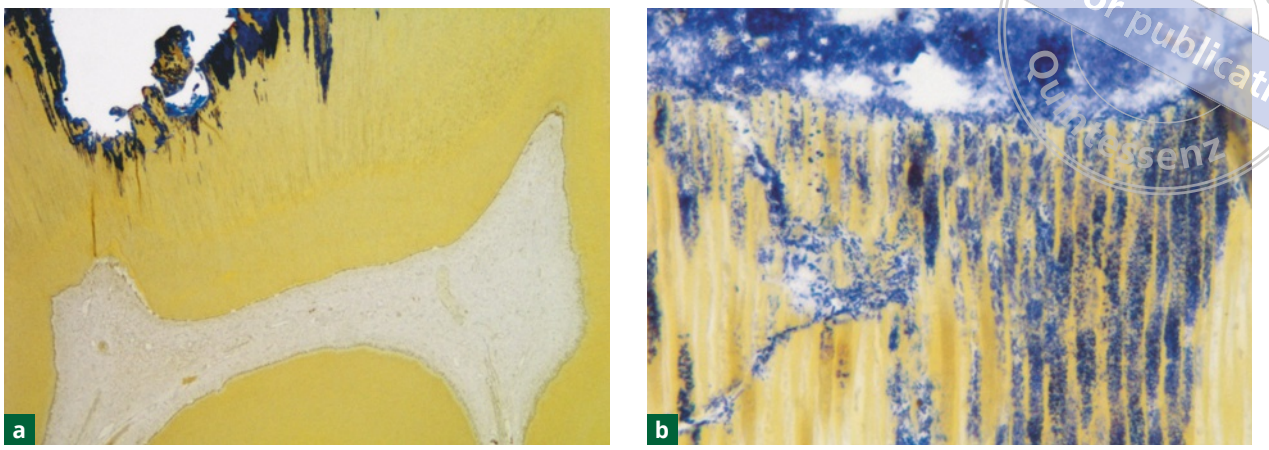

Fig 2-3 Caries affecting dentin. **(a)** Bacteria and their products penetrate the dentinal tubules. Diffusion of bacterial products through the tubules can cause pulp inflammatory changes long before the pulp is exposed. **(b)** Heavy intratubular infection from caries bacteria (courtesy Domenico Ricucci).

Bacteria involved in the pathogenesis of apical periodontitis may have participated in the early stages of pulp inflammation and necrosis or they may have gained entry into the canal space after the occurrence of pulp necrosis. In the former situation, involved bacteria are usually those present in the advanced front of caries lesions and in saliva bathing the affected area. Bacteria implicated in the early stages of pulp disease form authentic biofilms on dentin affected by caries. Diffusion of bacterial products through dentinal tubules induces pulpal inflammation long before the pulp tissue is exposed (Fig 2-3). After pulp exposure, the tissue surface can be colonized and covered by bacteria present in the caries biofilm. The exposed pulp tissue is in direct contact with the causative bacteria and their products, and responds with severe inflammation (Fig 2-4). Tissue invasion by some bacteria may also occur. Bacteria at the battlefront have to survive the counterattack from the host defenses and, at the same time, must acquire nutrients to stay alive. In this bacteria–pulp clash, the latter invariably "loses the war" and becomes necrotic. The bacteria then move forward and "occupy" (i.e. colonize) the necrotic pulp tissues (see Fig 2-4). These events occur in individual tissue compartments, which coalesce and move toward the apical part of the canal until virtually the entire root canal is necrotic and infected. At this stage, involved bacteria can be regarded as the early root canal colonizers or pioneer species.

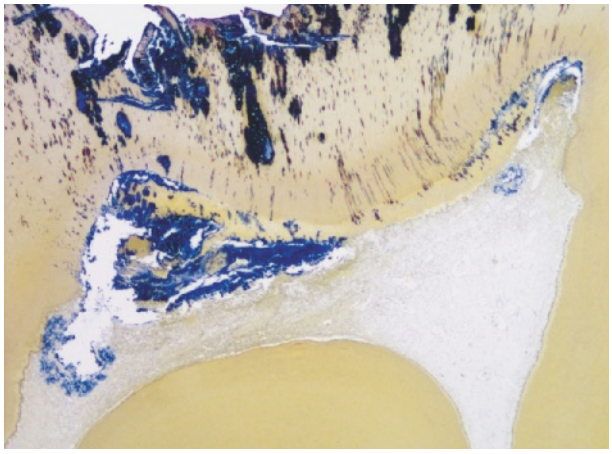

Fig 2-4 When caries exposes the pulp, there is an intense combat between bacteria from the caries biofilm and the host defenses in the pulp tissue. In the event of a compartment of inflamed pulp tissue becoming necrotic, it is invaded by bacteria that colonize this area, and the infectious process advances in an apical direction (courtesy Domenico Ricucci).

As the infection front approaches the portals of exit of the root canal system (apical or lateral foramina), inflammation reaches the periradicular tissues and damage occurs. Periradicular inflammation can be observed even before bacteria reach the apical or lateral foramina (Figs 2-5 and 2-6).[4,138,158] A study

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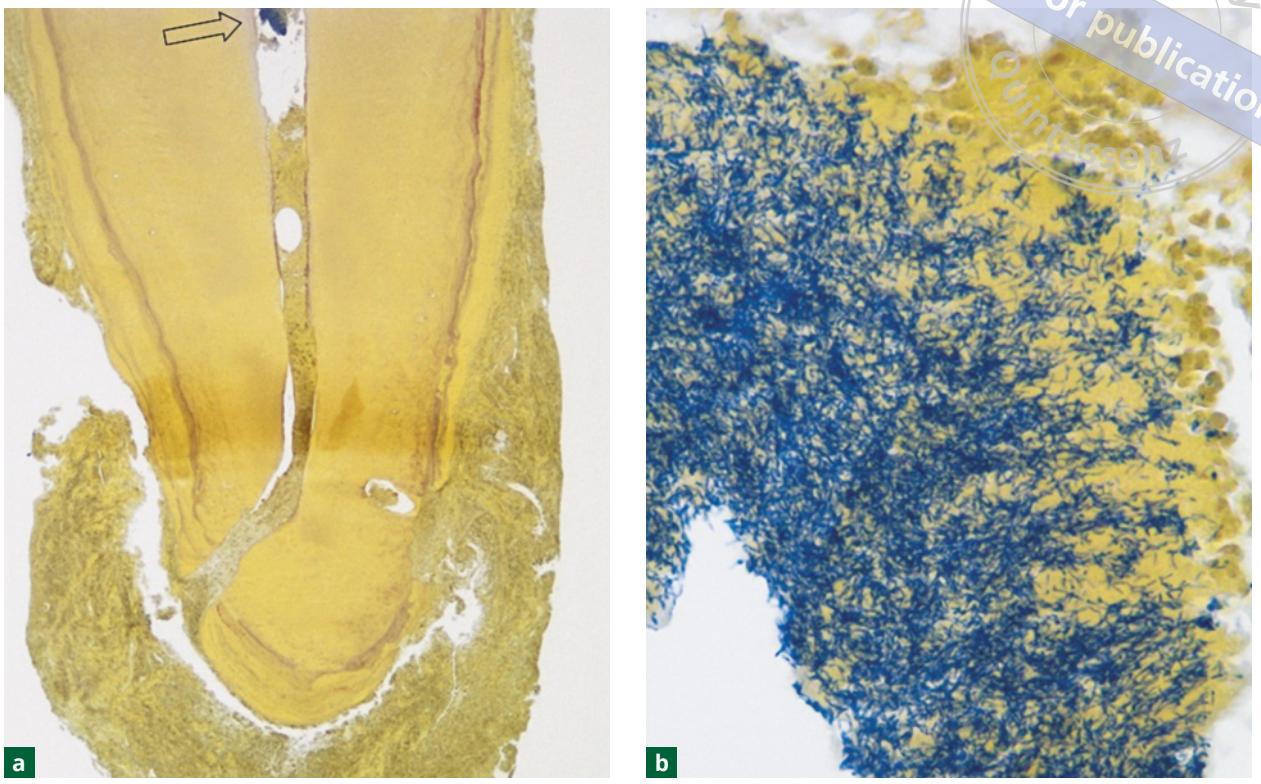

Fig 2-5 **(a)** Frontline of pulp infection located between the middle and apical third of the canal (arrow). The apical pulp tissue is vital, but inflamed, and apical periodontitis is already present before the most advanced front of bacterial infection reaches the apical foramen. **(b)** Higher magnification of the bacterial organizations in the frontline of infection (courtesy Domenico Ricucci).

showed that the apical part of the pulp tissue was still vital, though inflamed, in about one third of the teeth with apical periodontitis.[110]

The early colonizers play an important role in the initiation of the apical periodontitis disease process. Environmental conditions in the canal are modified by the pioneer species and the disease process; at this point, they may be conducive to the establishment of bacterial groups different from the early colonizers. Once the pulp is necrotic, species other than those that participated in the initial infectious process may also have access to the canal via coronal exposure or exposed dentinal tubules. In fact, shifts in the microbiota can be observed due to changes in the proportions of pioneer species and latecomers. In all probability, some early colonizers will no longer participate in the consortium in advanced disease.

With the passage of time, the endodontic microbiota becomes more and more structurally and spatially organized. Some virulence attributes required for pathogens to thrive in other sites (e.g. the ability to evade the host defenses) may be of no value to bacteria that reach the root canal after necrosis. This is because latecomers face no significant opposition from host defenses, which are drastically decreased or no longer active in the canal after necrosis. Although colonization may appear an easy task for late colonizers, other environmental factors such as interaction with pioneer species, oxygen tension, and nutrient availability will determine whether the new species entering the canal will succeed in establishing themselves therein. Thus, latecomers will join the early colonizers to make up a dynamic mixed community in the root canal. Eventually, the root canals of teeth evincing radiographically detectable apical periodontitis lesions harbor early colonizers that managed to stay in the canals and late colonizers that managed to adapt to the new but propitious environmental conditions.

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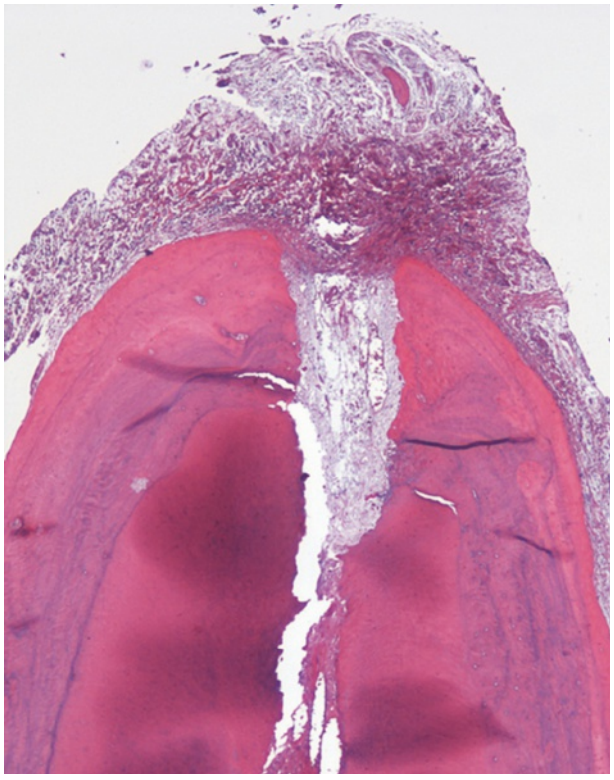

Fig 2-6 Apical periodontitis can develop even before the bacterial infection front reaches the apical foramen. Note the presence of vital inflamed tissue in the apical root canal (courtesy Domenico Ricucci).

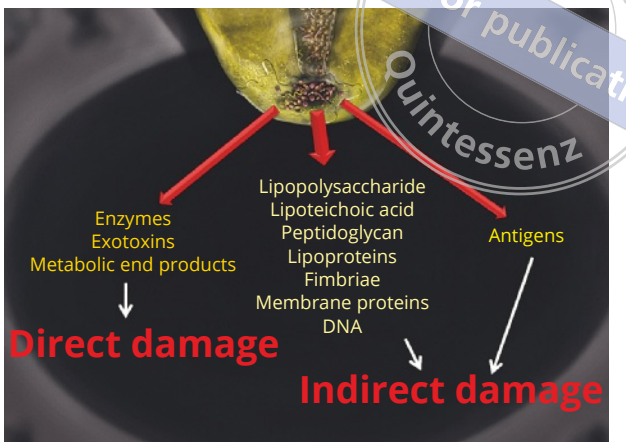

Fig 2-7 Bacteria exert their pathogenicity by causing direct or indirect tissue damage. While secreted bacterial products are expected to cause direct damage, structural components of the bacterial cell are more related to indirect damage by stimulating the host defenses, with resultant protective and destructive effects.

The mechanisms of bacterial pathogenicity include damage to the host tissues through direct and/or indirect effects (Fig 2-7). Bacterial factors that cause direct tissue harm include those that damage host cells and/or the intercellular matrix of the connective tissue. These factors usually involve secreted products, including enzymes, exotoxins, and metabolic end-products.[129] Furthermore, bacterial structural components, including lipopolysaccharide (LPS or endotoxin), peptidoglycan, lipoteichoic acid (LTA), fimbriae, flagella, outer membrane proteins and vesicles, DNA, and exopolysaccharides, are shed into the periradicular tissues and act as modulins by stimulating the development of host immune reactions capable not only of defending the host against infection but also of causing severe tissue destruction.[53,149] The bacterial products that stimulate the immune response are also referred to as pathogen associated molecular patterns (PAMPs) and their effects are related to their binding to pattern recognition receptors (PRRs) on the host cells, which will activate antimicrobial and/or inflammatory functions, depending on the cell involved.

Periradicular inflammation is a result of an interaction of host cells with live or dead bacteria present in the root canal system. As endodontic infections are usually characterized by a mixed community with several different species, the host immune cells are expected to encounter multiple PAMPs that will interact with their specific PRRs to stimulate a multitude of reactions involved with the pathogenesis of apical periodontitis.

Bone resorption is a clear example of indirect damage caused by bacterial infection of the root canal. Inflammatory and noninflammatory host cells are stimulated by bacterial components to release cytokines that are involved in the induction of bone resorption typically observed in chronic apical periodontitis lesions.[122] Proinflammatory cytokines stimulate osteoclastic bone resorption by enhancing the proliferation and differentiation of osteoclast precursors, promoting the activation of mature osteoclasts, or both.[115]

Pus formation in the acute apical abscess is another example of indirect tissue damage induced by

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bacteria. Host defense mechanisms against bacteria emanating from the root canal appear to be the most important factor involved in pus formation associated with abscesses. Hyperactive, supernumerary or dysregulated polymorphonuclear neutrophils (PMNs) cause tissue damage and liquefaction through the release of toxic substances such as oxygen-derived free radicals or tissue-degrading lysosomal enzymes. Therefore, bacteria can exert indirect destructive effects, which seem to be more significant in the tissue damage associated with acute and chronic apical periodontitis lesions.

The isolated location of root canal microbiota indicates that, to exert their pathogenicity, bacteria must either invade the periradicular tissues or their products, and/or structural components must penetrate the tissue and be able to evoke a defense response in the host. Bacteria can invade tissues by means of motility or growth. Motile bacteria can escape phagocytes by rapid movement. Invasion by growth requires that the rate of reproduction overcomes the host defense mechanisms. Only a few oral bacteria have motility; consequently, invasion by growth is the main form used by endodontic pathogens. Frank invasion of the periradicular tissues is rather uncommon and, when it occurs, bacteria are usually eliminated quickly. Depending upon several factors, massive invasion of the periradicular tissues by bacteria may sometimes result in abscess formation. The presence of more virulent species or strains or of a more virulent mixed consortium are important factors influencing abscess formation.

Most members of the oral microbiota are not highly pathogenic and have low virulence. This is consistent with the chronic, slowly progressive nature of the most common form of apical periodontitis. Therefore, as bacterial infection (invasion, survival, and proliferation) of the periradicular tissues rarely occurs (except in abscess cases), direct or indirect aggression toward the tissues must be caused by secreted bacterial products or structural components that diffuse out from the canal or are released by bacterial cells that reached the periradicular tissues but were rapidly eliminated therein by the host defenses. In acute cases or in the rare cases where an asymptomatic lesion is infected (e.g. sinus tracts, actinomycosis, infected cysts), tissue damage also results from factors released by viable bacterial cells directly in the tissues.

More than likely, few, if any, of the putative endodontic pathogens are individually capable of inducing all of the events involved in the pathogenesis of the different forms of apical periodontitis. The process requires integrated and orchestrated interaction of the selected members of the mixed endodontic communities and their respective virulence attributes (Tables 2-2 and 2-3).

"Quorum sensing" systems – a bacterial talk

Bacteria living in communities can communicate with one another, capacitating them to behave collectively as a group. This intercellular communication phenomenon, referred to as *quorum sensing*, can occur in both Gram-positive and Gram-negative bacteria.[8,31,83,98,157] Quorum sensing involves the production, release, and subsequent detection of chemical signaling molecules called autoinducers. As the bacterial population producing and releasing autoinducers multiplies, the extracellular concentration of autoinducers also increases. As the autoinducer concentration reflects the number of bacterial cells, perception of a threshold level of such a signal molecule indicates that the population has reached a quorum and is ready to change its behavior according to alterations in gene expression patterns.[98]

Quorum sensing systems are known to regulate virulence, secondary metabolite production, and biofilm formation.[9,28,99,147] Some opportunistic pathogens express virulence factors in response to sensing their own cell density. It has been presumed that, in an attempt to avoid alerting the host defenses to their presence, quorum sensing bacteria delay virulence factor production until their cell numbers are high enough to ensure that secretion of virulence factors will result in productive infection.[8]

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Table 2-2 Bacterial virulence factors involved with different stages of the infectious process

Function	Virulence factors
Attachment	Adhesins (fimbriae, afimbrial surface proteins) Exopolysaccharides Lipoteichoic acid Outer membrane proteins Outer membrane vesicles
Invasion	Flagella Enzymes (collagenase, hyaluronidase, chondroitin sulfatase, fibrinolysin, acid phosphatase, and DNase)
Survival (evasion of host defenses or acquisition of nutrients)	Exopolysaccharides (capsule) IgM, IgG, IgA, C3, and C5 proteinases Lipopolysaccharide (antigen-O portion) Flagella Exotoxins Heat-shock proteins Metabolic end-products
Direct damage	Exotoxins Enzymes (collagenase, hyaluronidase, chondroitin sulfatase, gingipains, aminopeptidases, phospholipase, neuraminidase, and acid phosphatase) Metabolic end-products (short-chain fatty acids, polyamines, volatile sulfur compounds, indole, and ammonia)
Indirect damage	Lipopolysaccharide (mainly lipid A portion) Peptidoglycan Lipoteichoic acid Fimbriae Exopolysaccharides Outer membrane proteins (porins) Lipoproteins DNA Heat-shock proteins

Some candidate endodontic pathogens have been shown to produce quorum sensing signal molecules.[37,102,153,160] Quorum sensing systems are likely to be involved in bacterial adaptability to the root canal environment and to coordinate community activity resulting in enhanced pathogenicity.

Virulence factors

Bacterial virulence factors are represented by structural components, many of which are located at the cell surface, and products that are secreted into the immediate environment. Bacterial strategies that contribute to pathogenicity, including the ability to co-aggregate and form biofilms, are also regarded as virulence factors. Other than causing host tissue damage, the primary function of the so-called virulence factors is structural or physiologic, and that of doing harm is merely coincidental and consequential. In most cases, different virulence factors act in combination at various stages of infection, and a single factor may have several functions at different stages.

Virulence factors may be involved in attachment to host surfaces, tissue and host cell invasion, proliferation in the host, direct and indirect tissue damage, and survival strategies, including evasion of host defense responses (see Table 2-2). It is highly unlikely that a single virulence factor is responsible for tissue damage associated with apical periodontitis lesions. A given factor may play a major role in some cases, but its independent effects are probably not sufficient for disease pathogenesis. Finally, endodontic

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Table 2-3 Main virulence factors of some candidate endodontic pathogens

Microorganism	Main features	Putative virulence factor
Treponema denticola	Anaerobic Gram-negative spirillum	Major surface protein; chymotrypsin-like protease complex; extracellular or membrane-associated proteolytic and hydrolytic enzymes; lipooligosaccharide; lipoprotein; phospholipases; metabolites (acetic and lactic acids, H_2S); flagella; heat-shock proteins
Tannerella forsythia	Anaerobic Gram-negative rod	Lipopolysaccharide; trypsin-like enzyme; acid phosphatase; metabolites (acetic, propionic, butyric, isovaleric and phenylacetic acids); apoptosis-inducing factor; heat-shock proteins
Porphyromonas endodontalis	Anaerobic Gram-negative rod	Lipopolysaccharide; capsule; outer membrane proteins; proteinases; acid phosphatase; metabolites (butyric and propionic acids, indole, H_2S)
Porphyromonas gingivalis	Anaerobic Gram-negative rod	Lipopolysaccharide; fimbriae; capsule; lipoproteins; outer membrane vesicles; proteinases; fibrinolysin; phospholipase; acid phosphatase; DNase; hyaluronidase; chondroitin sulfatase; hemolysins; metabolites (H_2S, methylmercaptan, dimethyl disulfide, butyric and propionic acids, indole, ammonia); heat-shock proteins
Fusobacterium nucleatum	Anaerobic Gram-negative rod	Lipopolysaccharide; outer membrane proteins; capsule; metabolites (butyric and propionic acids, ammonia, indole); heat-shock proteins
Prevotella intermedia/ nigrescens	Anaerobic Gram-negative rod	Lipopolysaccharide; fimbriae; metabolites (indole, H_2S, ammonia, acetic and succinic acids); proteinases; hemolysins; acid phosphatase; phospholipase; heat-shock proteins
Campylobacter rectus	Anaerobic Gram-negative rod	Extracellular cytotoxin; lipopolysaccharide; S-layer; arylsulfatase; H_2S; heat-shock proteins
Parvimonas micra	Anaerobic Gram-positive coccus	Peptidases; hyaluronidase; capsule; H_2S
Streptococcus anginosus group	Anaerobic or microaerophilic Gram-positive coccus	Peptidoglycan; lipoteichoic acid; enzymes; metabolites
Enterococcus faecalis	Facultative Gram-positive coccus	Lipoteichoic acid; gelatinase; hyaluronidase; cytolysin; aggregation substance; pheromones; heat-shock proteins
Candida albicans	Yeast	Mannose-containing proteins; mannan; phospholipase; proteinases; hyaluronidase; acid phosphatase; chondroitin sulfatase; phospholipase; heat-shock proteins

infections are mixed, and certainly a large plethora of virulence factors are available to cause disease.

Structural components

Lipopolysaccharide

Lipopolysaccharide (LPS), an amphipathic molecule, is a major constituent of the outer leaflet of the outer membrane of most Gram-negative bacteria (Fig 2-8). A single bacterial cell can contain approximately 3.5 x 10^6 LPS molecules. It has been estimated that about three-quarters of an *Escherichia coli* cell surface consists of LPS, and the remaining portion is composed of proteins.[112]

Chemically, LPS is subdivided into three domains: an O-polysaccharide-specific chain (O-antigen), a core oligosaccharide component, and a hydrophobic glycolipid component (lipid A).[111] Lipopolysaccharides lacking the O-antigen are named lipooligosaccharides. Lipid A is embedded in the outer membrane, whereas the core and the O-antigen portions extend outward from the bacterial surface. Although antigenic, the LPS molecule has reduced toxicity

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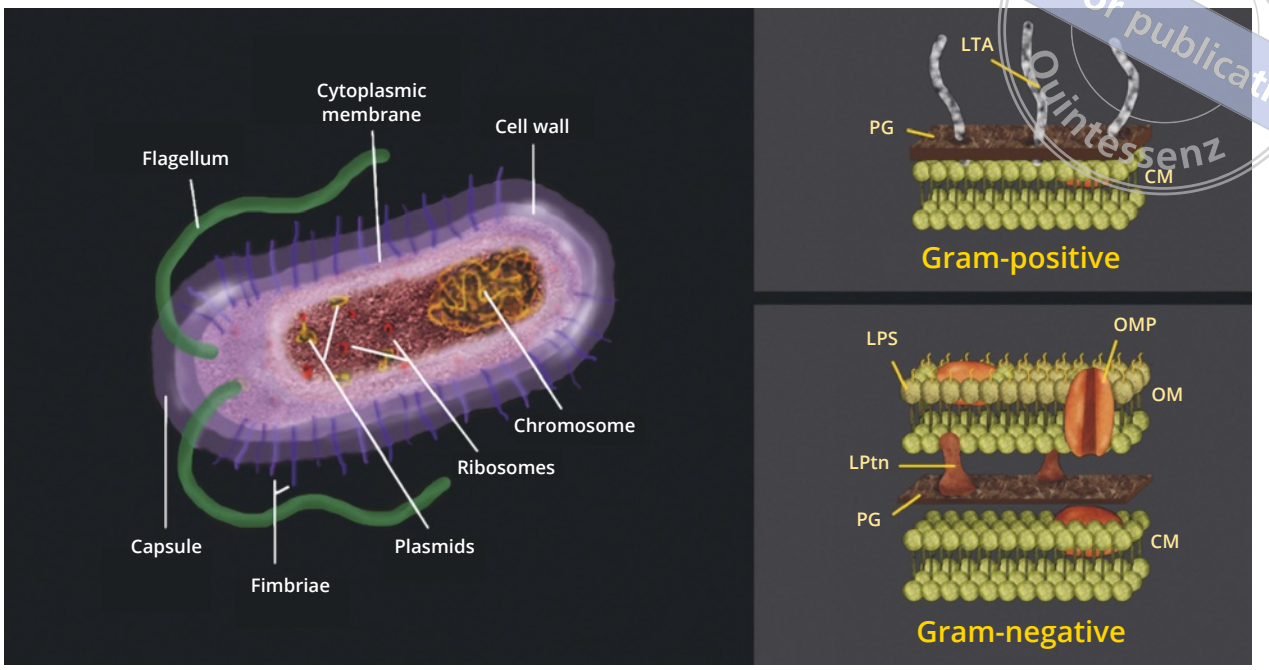

Fig 2-8 Bacterial cell and its structural components that can act as virulence factors. On the right is a detailed scheme of the bacterial cell walls of Gram-positive and Gram-negative bacteria. PG, peptidoglycan; LTA, lipoteichoic acid; CM, cytoplasmic membrane; LPS, lipopolysaccharide (endotoxin); OMP, outer membrane protein; OM, outer membrane; LPtn, lipoprotein.

when still incorporated in the bacterial outer membrane. However, when released from the cell wall, its toxic moiety (lipid A) is exposed to host defense cells and can evoke an inflammatory response. Lipid A is released from the outer membrane during bacterial multiplication or after death, in which case LPS is released either in free form or in a complex with bacterial surface proteins (endotoxin). LPS can also be removed from the membrane directly by the action of the soluble host plasma LPS-binding protein (LBP), a lipid transferase.

The major inflammatory effects ascribed to LPS depend upon its interaction with host cells, and macrophages are the key cells involved in host responses to LPS. When released from bacteria, LPS is initially bound to a LBP and then delivered to CD14, an LPS receptor on the surface of macrophages (Fig 2-9).[128] Subsequent macrophage activation is the result of signals triggered by Toll-like receptors (TLRs). The Toll family of signal-transducing receptors encompasses transmembrane molecules linking the extracellular compartment, where contact and recognition of pathogens occur, and the intracellular compartment, where signaling cascades leading to cellular responses are initiated. TLRs are responsible for cell signaling to a variety of bacterial components (Fig 2-10). Toll-like receptor 4 (TLR-4) is involved in cellular activation by LPS from most bacteria. While the LBP component acts as the carrier of LPS, and CD14 is the recognizing receptor, TLR-4 functions as the signal-transducing component of the macrophage response to LPS.[1] TLR-2 may be involved in cell signaling to some types of LPS. Engagement of the receptor activates transcription factors, which induce activation of genes encoding several proinflammatory cytokines.

Recognition of LPS can stimulate inflammation, but it is essential for the host immune system to initiate the clearance of infection by Gram-negative bacteria. However, uncontrolled bacterial overgrowth leads to the release of large amounts of LPS, which can in turn stimulate an exaggerated immune response. The most severe example is septic shock, with a high risk of death.

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Apical periodontitis is one of the most common inflammatory diseases that affect humans and is caused by microbial infection of the dental root canal system. A thorough understanding of the etiology and pathogenesis of apical periodontitis is essential for high-quality endodontic practice based on a solid scientific foundation. The first section of this book deals with microbiologic and pathophysiologic aspects of the different manifestations of apical periodontitis, while the second section describes the best evidence for predictable treatment and prevention of the disease. Clinical techniques and protocols to treat endodontic infections are described in detail. This new edition boasts a team of renowned authorities in the field that contribute state-of-the-art evidence about the biology and practice of the endodontic treatment of teeth with infected root canals. The content is supplemented with numerous full-color illustrations and radiographs. This book is a definitive guide for those involved with the prevention and treatment of endodontic infections.

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