Infection control: why now?

The outcome of endodontic treatment depends on the microbiological status of the root canal. In inflamed vital pulps, the infection is commonly limited to the site of exposure causing a localized inflammatory response (1, 2). However, when aseptic technique is used, the effect of endodontic therapy is predictably high as demonstrated by several studies (3–7).

In infected necrotic pulps, microorganisms are present within the root canal system and dentinal tubules, causing an apical inflammatory lesion called apical periodontitis. In these cases, endodontic treatment should be essentially directed toward the prevention and control of pulpal and apical infections, as stated by Kakehashi, Stanley, and Fitzgerald nearly 50 years ago (8). Unfortunately, the success of the therapy for these cases is 10–15% lower when compared to non-contaminated teeth (Table 1.1) (3–7, 9–11). What is more concerning is the fact that this lower outcome has not changed or improved despite all the technological advancements the world of endodontics has seen (12). How come that despite the “art and science” of current endodontic therapy, outcome studies have failed to demonstrate an increase in endodontic success? Why do we fail to predictably control the infection after so many years of research, experiment, and treatment? The answer might be related to the fact that very few advances have ever targeted the real problem, which continues to be microorganisms, especially in the apical third. The success of the endodontic treatment is possible only with an understanding of the molecular biology of the pathogens, their structures, synergies, and weaknesses. No file will ever disinfect a root canal, nor is it designed for that purpose. Recognizing that our
endodontic therapy will end in failure if we do not find a method to completely destroy the microbes within the root canal system, infection control must be our main goal and concern. Therefore, we should focus our research and development on efficient and predictable methods to control the infection and improve the endodontic treatment and healing of apical periodontitis.

**Table 1.1** Outcome of endodontic therapy based on the presence or absence of apical periodontitis.

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Cases/cohort</th>
<th>Recall</th>
<th>Without apical periodontitis (%)</th>
<th>With apical periodontitis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strindberg (1956)</td>
<td>258</td>
<td>6 months to 10 years</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>Kerekes and Tronstad (1979)</td>
<td>491 Norway, Dental school</td>
<td>3–5 years</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>Sjogren et al. (1990)</td>
<td>96 Sweden, Dental school</td>
<td>8–10 years (91%)</td>
<td>96</td>
<td>86</td>
</tr>
<tr>
<td>Friedman et al. (2003)</td>
<td>92</td>
<td>4–6 years (20%)</td>
<td>92</td>
<td>74</td>
</tr>
<tr>
<td>Farzaneh et al. (2004)</td>
<td>94 Toronto, Grad students</td>
<td>4–6 years (48%)</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>Orstravik et al. (2004)</td>
<td>Norway, Dental school</td>
<td>0.5–4 years (83%)</td>
<td>94</td>
<td>79</td>
</tr>
<tr>
<td>Ng et al. (2011)</td>
<td>702 London Grad students</td>
<td>4 years (83%)</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Ricucci et al. (2011)</td>
<td>816 Italy (Ricucci)</td>
<td>5 years (87%)</td>
<td>92.3</td>
<td>82.7</td>
</tr>
</tbody>
</table>

**Terminology and apical definitions**

The term *apical periodontitis* has gained increasing support and is used widely in current literature. The American Association of Endodontists recently published the revised Glossary of Endodontic Terms (13). Some of the terms defined in the glossary are as follows:

- **Normal apical tissues** Teeth with normal apical tissues that are not sensitive to percussion or palpation testing. The lamina dura surrounding the root is intact and the periodontal ligament space is uniform.

- **Symptomatic apical periodontitis** Inflammation, usually of the apical periodontium, producing clinical symptoms including painful response to biting and/or percussion or palpation. It may or may not be associated with an apical radiolucent area.

- **Asymptomatic apical periodontitis** Inflammation and destruction of apical periodontium that is of pulpal origin, appears as an apical radiolucent area and does not produce clinical symptoms.

**Acute apical abscess** An inflammatory reaction to pulpal infection and necrosis characterized by rapid onset, spontaneous pain, tenderness of the tooth to pressure, pus formation, and swelling of associated tissues.

**Chronic apical abscess** An inflammatory reaction to pulpal infection and necrosis characterized by gradual onset, little or no discomfort, and the intermittent discharge of pus through an associated sinus tract.

In biological terms, apical periodontitis means “inflammation of the periodontium.” This is a broad term to describe an inflammatory reaction in the tissues, including lateral and furcal locations of inflammation; it does not distinguish etymologically pulp-induced periodontitis from marginally derived periodontitis. More specific pathologies such as granulomas and cysts were excluded because they do not represent a “clinical or radiographic” diagnostic reality, but rather a diagnosis based on histological findings. The prevalence of apical periodontitis has increased throughout the years (14, 15), even in the low caries-rate adult Danish population (16).

**The evolution of endodontic microbiology**

Miller, in 1890 (17), was the first to demonstrate the presence of bacteria in necrotic human pulp tissue. However, the cause and effect relationship
is attributed to Kakehashi et al. (8) who experimented with gnotobiotic (germ-free) and normal rats. Bacterial contamination in the orally exposed pulp tissue caused necrosis and apical pathoses in normal rats. The study is considered a classic reference as it initiated a new and bright era in endodontic microbiology.

In 1966, Moller (18) established the importance of adequate isolation for microbiological sampling and various culture media for the recovery and identification of anaerobic microorganisms, providing more relevant information regarding the type of bacteria present in root canal systems. Bergenholtz then demonstrated the presence of bacteria in the traumatized teeth. Despite the fact that pulp chambers were not exposed, bacterial growth was observed in 64% of all samples. The flora was dominated by anaerobic microorganisms including Bacteroides, Corynebacterium, Peptostreptococcus, and Fusobacterium (19). Two years later, Sundqvist (20) demonstrated the prevalence of anaerobic bacteria in root canals, supporting the results obtained by Bergenholtz.

In the 1980s, the studies were more focused on understanding the colonization and interactions within the endodontic microflora. Moller investigated the relationship between uncontaminated necrotic pulp and apical tissues. The study maintained uncontaminated necrotic pulp in the root canal during a period of at least 6 months, and evaluated changes in the microbial flora enclosed in the root canal and its capacity to induce apical periodontitis (21). Using 9 monkeys (Macaca fascicularis), the pulp of 78 teeth was aseptically necrotized. Twenty-six of the pulp chambers were sealed and the pulp chamber remained free. Fifty-two teeth were infected with the indigenous flora. Clinical, radiographic and microbiological data was recorded before and after the completion of the study. The root canals were initially uninfected sterile in the final samples. No inflammatory reactions were found on the 26 control teeth. On the experimental teeth inflammatory reactions were observed clinically (12/52 teeth) and radiographically (47/52 teeth). An average of 8 to 15 bacterial strains were identified as facultative anaerobic bacteria including enterococci, coliforms, and anaerobic bacteria such as Bacteroides, Eubacterium, Propionibacterium, and Peptococcus, Peptostreptococcus. Some anaerobic bacteria not present on the initial microbiological test were isolated on the final samples. Histological examination of the apical tissue confirmed the presence an inflammatory reaction to the bacterial contamination (21).

In 1982, Fabricius et al. investigated the pulps of 24 root canals, 8 in each of the 3 monkeys that were experimented on. Teeth were mechanically devitalized and exposed to the oral flora for about 1 week and thereafter sealed. Microbiologic sampling and analysis was performed in 16 teeth (of 2 of the monkeys) after 7 days of closure (initial samples). Afterward, inoculation pulps were sealed for a period of 6 months. Final sampling was taken from the main root canal, the dentin, and the apical region at the same sampling session. All microbiologic analyses were carried out quantitatively. Final root canal samples from the apical region showed a predominance of obligate anaerobic nonsporulating bacteria; in fact 85–98% of the bacterial cells were anaerobic. The most frequently found species were Bacteroides and Gram-positive anaerobic rods. A lower proportion of facultative anaerobic bacteria were found; this was most pronounced for coliform rods in comparison with the strains of Bacteroides melaninogenicus.

Today, electron microscopy has become a great technology in many areas of science. Nair (22) studied the structure of the endodontic flora, its relationship to the dentinal wall, microbial interactions, and dynamics of apical inflammatory response. The study was performed on human teeth with granulomas and cysts. The results showed the presence of microorganisms in all the samples. The flora consisted of cocci, bacilli, and spirochetes filamentous organisms. In most cases, the bacteria were restricted to the canal, but in 4 granulomas and 1 cyst, the bacteria were found in the lesion. There was a distinct bacterial plaque adhering to the dentinal wall at the apical foramen. Nair describes this finding as a group or community of one or more types of microorganisms as well as bacterial condensate, suggesting the formation of plaque in the dentinal wall by the flora of the root canal. This finding is considered to be the subject that currently occupies many researchers: the presence of biofilm on root canal walls.

In 1990, Nair (23) analyzed nine therapy-resistant and asymptomatic human apical lesions (4–10 years) removed during surgery using light and electron microscopy. Six out of nine lesions revealed
microorganisms in the apical root canal. Four contained bacteria and two contained yeasts. Of the three cases with no microorganisms, one revealed a foreign body giant cell granuloma. In the majority of therapy-resistant apical lesions, microorganisms (bacteria, yeast) and foreign body giant cell granulomas play a significant role in treatment failures.

Although endodontic microbiology has evolved significantly, it still lacks an understanding of the ecology of the root canal and requires an analysis of the bond that develops between microorganisms and their surroundings. This relationship is an essential element that provides a glimpse into the understanding of their behavior and ability to invade an area that is rich in nutrients and whose abiotic and biotic factors determine the distribution and quantity of living organisms that may share the root space. In the early 1990s, Sundqvist (24, 25) published a couple of reviews summarizing the available data. Bacterial flora of the root canal is dominated by obligate anaerobes, comprising up to 90% of the total population. Aerobic bacteria are rarely found initially in the infected root canals but could have been introduced during the treatment. During the course of an infection, interrelationships develop between microbial species, based on their nutritional demands and nutritional interactions, and the pathogenicity of the polymicrobial root flora is dependent on bacterial synergy. bacteriocins proteins produced by a microorganism enhance their ability to inhibit growth of some species competing for the same ecological niche. Additionally, they promote bacterial coaggregation and interactions establishing the ecology of the apical tissues.

The identification of the endodontic microbiota in the apical third was reported in 1991 by Baumgartner (26) who employed both aerobic and anaerobic cultures in the same study, in order to isolate and identify the microflora of the apical portion of root canals of teeth with carious pulpal exposures and apical lesions. Ten freshly extracted teeth with carious pulpal exposures and apical lesions contiguous with the root apex were placed inside an anaerobic chamber and the apical 5 mm of the root canals cultured. In addition to anaerobic incubation, duplicate cultures were incubated aerobically. Fifty strains of bacteria from the 10 root canals were isolated and identified. The most prominent bacteria cultured from the 10 root canals were Actinomyces, Lactobacillus, black-pigmented Bacteroides, Peptostreptococcus, nonpigmented Bacteroides, Veillonella, Enterococcus faecalis, Fusobacterium nucleatum, and Streptococcus mutans. Of the 50 bacterial isolates, 34 (68%) were strict anaerobes. Baumgartner’s study demonstrated the presence of predominantly anaerobic bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and apical lesions.

Advancements in the identification of endodontic flora by Molander et al. in 1998 correlated the clinical outcome, refractory lesions, and the presence of certain strains. Molander and coworkers examined the microbiological status of 100 root-filled teeth with radiographically verified apical periodontitis and 20 teeth without signs of apical pathoses. In teeth with apical periodontitis, 117 strains of bacteria were recovered in 68 teeth. Facultative anaerobic species predominated among these isolates (69% of identified strains). Enterococci were the most frequently isolated genera, showing “heavy” or “very heavy” growth in 25 out of 32 cases (78%). In 11 teeth without signs of apical pathoses, no bacteria were recovered while the remaining 9 yielded 13 microbial strains. Eight of these grew “very sparsely.” It was concluded that the microflora of the obturated canal differs from that found normally in the untreated necrotic dental pulp, quantitatively as well as qualitatively.

In 1990, Sha and Collins reclassified the moderately saccharolytic, predominantly oral Bacteroides species, which include B. melaninogenicus, Bacteroides oralis, and related species. These bacteria form a phenotypically and phylogenetically coherent group of species, which differ so significantly from the emended description of the genus Bacteroides that they should not be classified in the same genus and proposed that these species be reclassified in a new genus, Prevotella (27) (Figure 1.1).

By this time the advent of molecular biology techniques advanced in leaps and bounds in the detection and identification of microorganisms, uncovering the intimacy between genetics, biochemistry, and microbiology. Technique sensitive analyses of nucleic acids extracted from microorganisms directly, or from a sample containing the microorganism in question, have identified...
endodontic microorganisms of interest; as was the case with Actinomyces. Polymerase chain reaction (PCR) is a biochemical technology in molecular biology used to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Xia and Baumgartner used PCR with a pair of universal primers for Actinomyces and species-specific primers to evaluate the contents of infected root canals and aspirates from abscesses or cellulitis for the presence of Actinomyces israelii, Actinomyces naeslundii, and Actinomyces viscosus. DNA was extracted from 131 clinical samples (28). DNA reacting with the universal primer for Actinomyces was detected in 72 of 129 (55.8%) clinical samples. Of those, 41 of 51 (80.4%) were from infected root canals, 22 of 48 (45.8%) were from abscesses, and 9 of 30 (30%) were associated with cellulitis.

Since then, Siqueira and Rocas reported hundreds of species obtained from root canals using PCR techniques. In a review article published in 2008, they concluded that bacterial presence in the root canal at the time of filling is a risk factor for posttreatment apical periodontitis (29). About 100 species/phylotypes have already been detected in postinstrumentation and/or postmedication samples, and Gram-positive bacteria are the most dominant. However, it remains to be determined by longitudinal studies if any species/phylotypes persisting after treatment procedures can influence the outcome (29).

Waltimo et al. reported the current trend of recurrent yeast in endodontic infections, and their antimicrobial response confirms that yeast may be isolated in about 5–20% of the infected root canals. Their article identifies multiple virulence factors of Candida that allow it to infect the dentin–pulp complex and penetrate the dental tubules causing an inflammatory reaction and suggesting a pathogenic role of this organism in apical periodontitis (30).

With the discovery and understanding that fungus is present, the complexity of the microbiota became clear. In 2003, Slots et al. (31) were the first to report the presence of cytomegalovirus and Epstein–Barr virus (EBV) in more than 90% of granulomas of symptomatic and large apical lesions (31). Dual infection with cytomegalovirus and EBV is closely associated with symptomatic lesions. Herpes simplex virus’ (HSV) active infection has no apparent relationship to apical disease.

Another study aimed to identify herpes virus, including human cytomegalovirus (HCMV), EBV, HSV-1, and varicella zoster virus (VZV) in-vivo (32, 33). Patients with acute apical abscesses and cellulitis of endodontic origin were used for the study. The identification was carried out by the primary PCR and nested PCR techniques. The results demonstrated the presence of HCMV, EBV, and HSV-1; however, they indicated that the presence of herpes virus that were identified were very low in their genetic copies, and therefore it was concluded that herpes viruses are present but do not have a direct relation to the development or establishment of pathologies such as acute apical abscess or cellulitis of endodontic origin.

The link between endodontic infection and apical disease

The presence of bacteria within the root canal system is essential for the development of apical inflammation (8). Apical periodontitis is a disease characterized by inflammation and destruction of apical tissues caused by microbial agents of endodontic origin. Initially, the tooth pulp becomes infected and necrotic by an autogenous oral microflora. The microenvironment of root canal systems provides excellent conditions for the establishment of a mixed, predominantly anaerobic, flora. Collectively, this polymicrobial
community residing in the root canal has several biological and pathogenic properties, such as antigenicity, mitogenic activity, chemotaxis, enzymatic histolysis, and activation of host cells.

Microorganisms’ growth within the root canal is in the form of biofilm. The microbial community develops as a biofilm and colonizes the environment. It occurs first by the deposition of a conditioning film; then there is adhesion and colonization of planktonic microorganisms in a polymeric matrix. The coadhesion of other organisms and the detachment of biofilm microorganisms into their surroundings happen at a later stage (34). Bacterial biofilm has an open architecture with channels traversing from the biofilm surface. The structure of biofilm affects the movement of molecules, and the gradients are key determinants in its development. Bacteria growing on a surface may display a novel phenotype that, consequently, may allow increased resistance to antimicrobial agents. Resistance can result from restricted inhibitor penetration, slower bacterial growth rates, transfer of resistance genes, suboptimal environmental conditions for inhibitor activity, and the expression of a resistant phenotype (35).

This ecological view on the persisting infection in endodontics suggests that the action of individual species in refractory endodontic infections is secondary when compared to the adaptive changes of a polymicrobial biofilm community undergoing physiological and genetic changes in response to changes in the root canal environment (36). The invasion of root dentinal tubules by root canal bacteria is a multifactorial event in which a limited number of oral bacterial species have the required properties to participate. Current literature has demonstrated that biofilms may remain viable in anatomical areas of the root canal system that remain untouched by either mechanical or chemical disinfection (Figure 1.2) (37–39). Scanning electron microscopy (SEM) examination of root tips associated with refractory apical periodontitis has suggested the presence of bacterial biofilm at the apical portion of the root canal (Figure 1.3) (40–42).

SEM analysis revealed bacterial biofilm surrounding the apical foramen and external radicular surface (Figures 1.4a and 1.2c). Careful observation of these structures under higher magnification revealed clumps of coaggregated bacterial cells in a matrix of extracellular polymeric substance (EPS). *E. faecalis* biofilms displayed a complex three-dimensional structure that demonstrated spatial heterogeneity and a typical architecture showing microcolonies with ramifying water channels (Figure 1.4b). Fibrillar structures appeared to be made up of twisted fibers. Larger structures of wrapped sheets were also present and consisted of small numbers of bacteria cells embedded in a matrix of fibers (Figure 1.4d).

Bacterial endotoxins and by-products egress through portals of exit causing the destruction of the apical tissues. In response, the host has an array of defenses consisting of several classes of cells, intercellular messengers, antibodies, and effector molecules. The microbial factors and host defense forces encounter, clash with, and destroy much of the apical tissue, which result in the formation of various categories of apical periodontitis lesions. In spite of the formidable defense, the body is unable to destroy the microbes well-entrenched in the sanctuary of the necrotic root canal, which is beyond the body’s immune system. Therefore, the major goal of the root canal debridement is to eliminate the biofilm and bacteria-harboring debris.

Contemporary endodontic microbiology has become even more complex with the discovery of fungi and viruses. Waltimo et al. (43) found yeast in 7% of the culture-positive samples from persistent root canal infections. *Candida albicans* was the most frequently isolated yeast (80%). This finding was later confirmed by several studies (44, 45). Attachment to mucosal tissues and to abiotic surfaces and the formation of biofilms are crucial steps for *Candida* survival and proliferation in the oral cavity. *Candida* species possess a wide arsenal of glycoproteins located at the exterior side of the cell wall, many of which play a determining role in these steps. In addition, *C. albicans* secretes signaling molecules that inhibit the yeast-to-hypha transition and biofilm formation. *In-vivo*, *Candida* species are members of mixed biofilms and subject to various antagonistic and synergistic interactions (Figure 1.5) (46). Recently, Gomes et al. (47) confirmed the presence of filamentous fungi, which were isolated in situ from 17 of 60 samples (28.3%). The genus *Aspergillus* was isolated from 7/17 samples (41%).

The presence of HCMV, EBV, and HSV was first reported by Sabeti et al. (48) in symptomatic apical lesions. In a different study, Slots et al. (49) detected
Figure 1.2 Composite figure of photomicrographs representative of microscopic sections of Groups I (EndoVac—ANP), II (Ultrasound—PUI), and III (conventional irrigation—PP) stained by the Brown and Brenn technique, revealing the presence and location of bacteria in the root canal system and apical tissues 180 days after endodontic treatment: (a) Panoramic photomicrograph, showing contamination of the entire extension of the tooth (Zeiss, 1.25×). (b) Detail of panel a (rectangle) in which the cervical root canal third can be visualized with abundant presence of microorganisms inside the dentinal tubules (Zeiss, 20×). (c) Detail of panel a (square) showing the middle root canal third with intense presence of bacteria (Zeiss, 20×). (d) Detail of panel a (circle) revealing the middle root canal third with presence of bacteria in the cemental craters (arrow) (Zeiss, 10×). (e) Detail of panel a (triangle) showing abundant presence of microorganisms in the apical root canal third (Zeiss, 40×). (f) Detail of panel a (asterisk) in which bacteria can be seen in the apical lesion (Zeiss, 20×).

HCMV in 100% of the symptomatic and in 37% of the asymptomatic study lesions (49). EBV was identified only in HCMV-infected apical lesions. The difference of HCMV and EBV between symptomatic and asymptomatic lesions was found to be statistically significant. Current studies confirmed these findings by using primary and nested PCR as well as reverse transcription PCR. (33) EBV DNA and RNA were present in endodontic pathoses in significantly higher percentages (43.9% and 25.6%, respectively) compared with healthy pulp controls.

The DNA of HSV was found in low percentages in endodontic patients (13.4%), and only one patient showed the presence of VZV. In conclusion, the presence of fungi and viruses had been associated with irreversible pulpitis and periodontitis, confirming once again that endodontic micro flora is complex and composed of well-organized biofilms of microorganisms with synergetic interactions.

The host responses to root canal infection have been the subject of much research in recent years. There is great similarity among the pathogenic
Figure 1.3  In-vivo *E. faecalis* biofilm. (a, c) Cocoidal structures attached to mature biofilm (6000x). (b) Extrapolymeric fibers (10,000x). (d) “Mushroom”-shaped structures (10,000x).

Figure 1.4  *E. faecalis* mature biofilm in-vivo. (a, c) Microcolonies wrapped in an extracellular matrix (8000x). (b) Network consisting of fibrillary structures (10,000x). (d) “Mushroom”-shaped structures (10,000x).
processes in marginal and apical periodontitis, and many of the findings in periodontal research have direct relevance to apical periodontitis. A clearer concept of the immunological processes involved in the development of apical periodontitis is emerging (50, 51). Microbiological variability and virulence factors in infected root canals have been demonstrated, and specific data indicate that the bacterial flora varies systematically with the clinical condition of the tooth involved (persistent infection, therapy-resistant infection). Different therapeutic strategies of antimicrobial control may have to be applied, depending on the microbiological diagnosis in a given case.

Apical periodontitis is not a self-healing disease (52). Untreated apical periodontitis may lead to a chronic infection of the oral tissues at locations closer to more vital organs than many other oral infections. Although these infections may remain quiescent for decades, they may also develop and spread with serious consequences for the individual (53, 54). In the face of the risks of such chronic infection from the infected teeth, their extraction and replacement by implants has been put forward and discussed as a viable alternative to endodontic treatment (55, 56). The variable success rates (by strict criteria) of treatment procedures for the cure of apical periodontitis are sometimes used as arguments for the implant “treatment” concept. However, what little evidence there is does not indicate a lower survival rate of endodontically treated teeth, and the superiority of tooth preservation compared to its replacement should be evident as a biological principle of preference. However, the challenge from other treatment concepts, to endodontics as a discipline, should act as a driving force to produce more and scientifically solid evidence for the modalities of cure and prevention applied to our disease of interest, apical periodontitis.

Conclusion

Pulpal and apical inflammation, and 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. Despite recent technological advances in treatment, evidence of improved outcome is still lacking. Alternative treatment involving implants is promoted as being better, but the criteria of evaluation of the outcome of the two forms of treatment are dissimilar; there is no true evidence-based comparison. The advancement, and utilization, of our biologic principles will allow a better understanding of the disease process and add to the fundamental truth that the biologic response to disease continues to be a biologic therapy; those therapies will and must continue to advance with our understanding.

References


