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# Persistent, recurrent, and acquired infection of the root canal system post-treatment

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Apical periodontitis is an inflammatory process in the periradicular tissues caused by microorganisms in the necrotic root canal. Accordingly, to achieve healing of apical periodontitis, the main goal of the treatment must be elimination of the infection and prevention of re-infection. As shown by recent epidemiological studies in several countries around the world, post-treatment endodontic disease is a far too common finding. To understand the reasons for survival of resistant bacteria in the filled root canal, it is important to know in detail the interaction between treatment procedures and the root canal flora in primary apical periodontitis. Therefore, in the first half of this review, the focus is placed on control of infection in primary apical periodontitis. This is followed by a detailed description of the resistant root canal microflora and a discussion about the present and future strategies to eliminate even the most resistant microbes in post-treatment disease.

## Introduction

### Microbial etiology of primary apical periodontitis

The main goal in endodontics is the prevention and treatment of diseases of the dental pulp and periapical tissues. This objective can be best achieved if preventive measures and treatment procedures are based on a thorough and detailed understanding of the etiology and pathogenesis of endodontic diseases. In pulpitis, caused by a deep caries lesion, an inflammatory reaction in the pulp starts long before bacteria invade the pulp tissue. The inflammatory reaction is first initiated by bacterial antigens interacting with the local immune system (1–3). As long as the body of the carious lesion has not entered the pulp, the inflammatory process in the pulp can be reversible, and no endodontic therapy is usually required. With progressing caries, bacterial cells enter the superficial layers of the pulp, which, even though heavily inflamed, is considered to be relatively bacteria-free as long as it remains vital.

Apical periodontitis is an inflammatory process in the periradicular tissues (Fig. 1) caused by microorganisms in the necrotic root canal (4). Several studies have indicated that the prognosis of apical periodontitis after endodontic treatment is poorer if living bacteria are present in the root canal at the time of filling (5–7). Other studies, however, have not been able to show significant differences in healing between teeth filled after obtaining positive or negative cultures from the root canal (8), as well as between treatments performed in one or two appointments (8, 9). Nevertheless, it is generally accepted that healing of primary apical periodontitis depends on effective elimination of the causative agents in the root canal system (10).

Once endodontic therapy has been initiated, several factors may potentially contribute to breakdown of the periapical tissues, resulting in persistence of the disease process. These factors include complications such as perforations, instrument fractures, and extrusion of materials used during the treatment in the periapical area. However, in most of these situations, a mechanical complication is only a secondary factor that

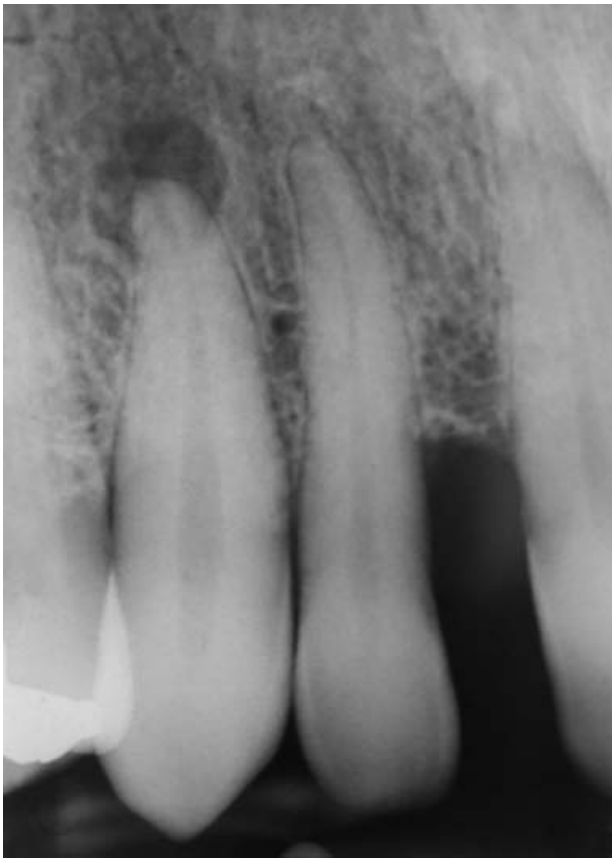


**Fig. 1. Apical periodontitis in right maxillary canine.**

facilitates the microbial infection by impeding or preventing effective disinfection of the root canal system. In addition, development of a radicular cyst or cholesterol crystals may contribute to persistence of disease after endodontic treatment (11, 12).

## Microbial etiology of post-treatment apical periodontitis

The etiology of apical periodontitis in a root-filled tooth (post-treatment disease) is generally the same as in primary apical periodontitis: microbial infection of the root canal (13–16). However, the root-filled tooth and the root canal(s) have already undergone a variety of treatment procedures, including use of mechanical instruments such as burs, and files, local disinfecting agents such as irrigants, and inter-appointment dressings and root filling. Consequently, secondary factors are often highlighted when persistence of disease is analyzed. Nevertheless, as indicated earlier, without the presence of a microbial infection, mechanical complications related to technical procedures and use of materials do not cause more than temporary problems such as short-lasting (aseptic) inflammatory reaction due to physical or chemical trauma and the occasional occurrence of pain.

## The invading infection: from carious dentine to apical periodontitis

The nature of the intracanal infection after the initiation and completion of endodontic therapy can be explained by the microbiological, ecological, and anatomical factors that play a key role in regulating the various phases of the invading endodontic infection before any treatment procedures are started. Bacteria have several possible pathways to invade the pulp. These include caries, enamel and dentine cracks, fractures, exposed and patent dentine tubules in the crown area or in the gingival/periodontal pocket, lateral canals, leaking fillings, and a hematogenous pathway associated with bacteremia. To promote understanding of how root canal infection develops, these pathways are highlighted below.

### *Caries: the major source of infection*

Dental caries is usually the major pathway through which bacteria enter the pulp and root canal system. While mutants streptococci have been the main focus of interest in studies of the initiation of enamel caries, dentine caries is a mixed infection arising from a wide variety of facultative and anaerobic bacteria destroying the dentine structure and advancing towards the pulp. It has been indicated that lactobacilli or other Gram-positive pleomorphic rods may play a special role in the advancing front of the lesion (17). These include genera such as (*Lactobacillus*), *Actinomyces*, *Propionibacterium*, *Bifidobacterium*, *Rothia*, and *Eubacterium* as well as various streptococci. However, Gram-negative anaerobic bacteria are also present in the carious dentine lesion (18).

It is quite possible that bacteria in the front line of the advancing caries lesion are etiologically significant in the development of pulpitis. However, it is important to realize that the first inflammatory reaction in the pulp in response to caries takes place before whole bacterial cells enter the pulp (19–20). Therefore, it cannot be ruled out that the bacterial metabolic end products and microbial components initiating pulpitis also originate from bacteria present in the body of the

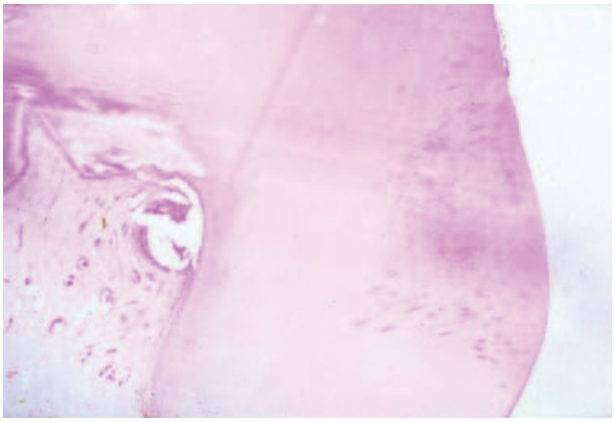


**Fig. 2. A histological section (hematoxylin eosin staining) of a molar with a deep dentinal caries. A microabscess is seen at the area corresponding to the deepest part of the advancing caries lesion. The abscess is surrounded by infiltration of inflammatory cells and hyperemia. However, only 1–2 mm from the abscess, normal pulpal anatomy can be seen.**

caries lesion, not just the front line. Nonetheless, it is mainly the Gram-positive rods in the front line that are the first invaders into the pulp (17, 21).

In pulpitis, the inflammatory reaction often remains surprisingly localized even after the bacteria have invaded the pulp space. A localized necrotic zone is surrounded by a thin zone of hyperemia and accumulation of mainly polymorphonuclear cells. At a distance of 2–3 mm from the necrosis and bacteria, the pulp tissue, as judged from histological sections, usually appears to be healthy (Fig. 2). Eventually, the diseased area grows in size and the bacteria invade deeper into the root canal. Although it is not known in detail, the dynamics of this phase may take a few days/weeks and up to several years. From a clinical point of view, it is important to know that as long as there is vital pulp tissue, there seems to be only a limited number of bacteria in the root canal space, and that the infection has not spread into root dentine. This is the main reason why elimination of infection is not a concern in the treatment of teeth with pulpitis. Therefore, the outcome of endodontic treatment in teeth with pulpitis is excellent (22).

### *Infection in the main root canal and in the lateral canals*

The fate of bacteria that have entered the root canal space is dependent on the following factors: the redox potential (the amount of oxygen in the local environment), access to and availability of nutrients, positive and negative bacterial interactions, and finally, the host's defense system. The redox potential in the necrotic root canal is very low – lower than in a deep periodontal pocket – which favors the dominance of anaerobic bacteria. In a classical series of experiments, Fabricius et al. (23) inoculated necrotic, sterile root canals with a mixture of eight different bacterial species and monitored the short- and long-term changes in the flora by sampling the canals. The eight strains were a complete collection isolated from one root canal of a monkey. These strains were inoculated together, in equal proportions, into 12 root canals. In the same study, 63 canals were inoculated with other combinations of bacteria or with separate strains as pure cultures. At the end of the experimental period, *Bacteroides oralis* (presently *Prevotella oralis*) predominated in most root canals inoculated with mixed infections. However, *B. oralis* did not survive in the root canals when inoculated in a pure culture. In contrast, enterococci survived also as pure cultures. The mixed infections showed the greatest capacity of inducing apical periodontitis, as revealed by radiography, and most pronounced lesions were caused by inoculation with the full eight-strain mix. In another study by the same group (24), the pulps of 24 root canals in three monkeys were mechanically devitalized, exposed to the oral flora for 1 week, and thereafter sealed with a temporary filling. Microbiological sampling and analysis were performed in 16 teeth (in two of the monkeys) after 1 week of temporary closure. The root canals of the infected teeth of the three monkeys were bacteriologically analyzed 90, 180, and 1060 days after inoculation. The final sampling included samples from the main root canal, dentine, and the apical region at the same sampling session. The root canal samples from the apical region showed a predominance of obligately anaerobic non-sporulating bacteria, 85–98% of the bacteria being anaerobic. The most frequently found species were *Bacteroides* spp. and Gram-positive anaerobic rods. Facultatively anaerobic bacteria were also found. The results clearly showed that soon after the start of the experiment, anaerobic bacteria became dominant in the flora, and their dominance became only more prominent over time.

Analysis of several studies on the microflora of primary apical periodontitis indicates that in closed cases (without 'macroscopic' communication between the root canal and the oral cavity), the proportion of

strictly anaerobic organisms varies between 70 and 100%, whereas in cases with carious exposure to the root canal, the relative proportion of microaerophilic and facultative Gram-positive bacteria is higher than in the closed cases (23, 25, 26). The differences in the composition of the flora can be explained by the type and amount of nutrients and oxygen available in the different cases. The possible sources of nutrients are the necrotic pulp tissue, inflammatory exudate entering through the apical foramen, lateral canals, and patent dentinal tubules. An open caries lesion allows access to nutrients rich in carbohydrates as well as increased amounts of oxygen. In summary, despite certain variations, anaerobic dominance is a typical feature of primary apical periodontitis.

The nutrient composition in the necrotic root canal has not been studied in great detail. Because of the lack of defense mechanisms in the necrotic pulp as well as better access to nutrients than in dentinal tubules, the majority of microorganisms in apical periodontitis reside in the main root canal system. The location where the bacteria are found, be that in lateral canals near the apex or the furcation area, or in other parts of the root, has not been studied. However, lateral periodontitis lesions often detected in radiographs indicate the presence of bacteria and bacterial products in lateral canals.

### *Invasion of dentinal tubules*

Various methods have been used in studying bacterial invasion into dentinal tubules. These include histological sectioning and staining (e.g. Brown and Brenn staining, Fig. 3), immunohistochemistry using specific antibodies and a fluorescent detection system, culture studies using dentine powder obtained by burs or endodontic instruments, scanning electron microscopy, transmission electron microscopy, and PCR as well as real-time PCR. The choice of method is partly dependent on the type of material available as well as the kind of information sought. Peters et al. (27) compared the sensitivity of culturing and histology, and found that when the bacterial density in the infected dentine was lower than 10 000 colony-forming units (cfu)/mg dentine, culturing was superior to histology; the latter failed to detect any bacteria below this threshold. A recent study of carious dentine by Martin et al. (28) showed that real-time PCR analysis indicated a greater microbial load than that detected by colony counting. The total number of anaerobes detected was 41 times greater using real-time PCR than colony counting.

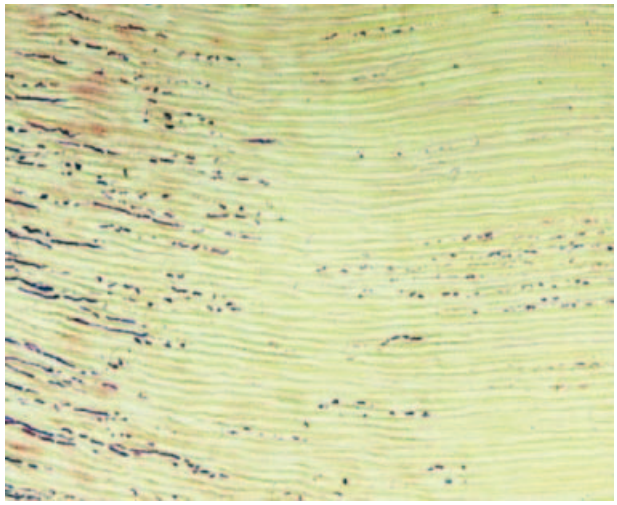


**Fig. 3. Brown and Brenn staining of dentine invaded by *Enterococcus faecalis*.**

Already in 1964, Shovelton (29) examined histologically 97 extracted, previously necrotic teeth, and found bacteria in root dentine in 63% of them. Since then, several *in vivo* and *in vitro* studies have demonstrated invasion of root canal bacteria into surrounding dentine via dentinal tubules. In one of the most recent studies, Peters et al. (27) examined two sets of teeth extracted for apical periodontitis in Holland and in Ireland. The frequency of dentine invasion by bacteria from the root canal was 77% and 87%, respectively. However, the information available on the frequency and quantity of bacterial invasion of dentinal tubules is still far from complete. It seems that there are great differences in ability between different bacterial species to penetrate from the main canal into the dentinal tubules (17, 18, 30–34). Streptococci and some Gram-positive rods such as *Actinomyces* and *Lactobacillus* spp. appear to invade the dentinal tubules better than several Gram-negative species. In another study, Peters et al. (33) demonstrated the role of the smear layer in effectively preventing bacterial invasion into the dentine. However, *E. faecalis* penetrated the dentine even in the presence of the smear layer. *E. faecalis* was also shown to invade the dentinal tubules better than *Actinomyces israelii*. The inhibition of bacterial invasion by the smear layer has also been demonstrated by Love et al. (35).

Variation in bacterial penetration patterns within different portions of root canals has been little studied. In an *in vitro* study with *Streptococcus gordonii*, Love (36) showed that in the coronal and middle portions of the root canals bacterial invasion into tubules was similarly effective and much greater than in the apical portion of the canals. Obviously, in a clinical situation *in vivo*, composition of the flora, patency of the dentinal tubules, and availability of nutrients, in particular, may play a major role in regulating or stimulating bacterial invasion of the dentine in various parts of the root canal. Both *in vitro* studies (33, 37) and *in vivo* observations (38) have clearly shown that removal of root surface cementum by mechanical means or by surface resorption, such as typically seen histologically in the apical area of teeth with apical periodontitis, greatly facilitates invasion of bacteria into dentinal tubules. Stanley et al. (39) reported that dentinal sclerosis in the root was not related to external stimuli such as caries, but rather related to increasing age. Dentinal sclerosis extended with age from the apical towards the cervical area. This could mean that among older people bacterial invasion of dentinal tubules, in particular in the apical portions of canals, is less pronounced than in young patients.

Ando & Hoshino (40) studied the composition of the microflora invading the deep layers of root canal dentin (0.5–2.0 mm from the surface of the root canal wall) of human root canals by sampling the split surfaces of eight freshly extracted teeth in an anaerobic chamber. More bacteria were recovered after anaerobic incubation than after aerobic incubation in air with 30% $CO_2$. Out of 256 predominant bacteria isolated, 80% were obligately anaerobic species. *Lactobacillus* spp. (30%) and *Streptococcus* spp. (13%) were predominant, followed by *Propionibacterium* sp. (9%). In this study, obligately anaerobic Gram-negative rods were not found. The authors concluded that the microflora found in the deep layers of infected root dentine resembled that of the deep layers of carious lesions in coronal dentine. Recently, Matsuo et al. (34) showed bacterial invasion into dentinal tubules in 70% of 40 teeth extracted for apical periodontitis. When the root canals were instrumented, the frequency of bacteria found in the dentinal tubules was almost as high, with 65% of the teeth still showing dentinal invasion by bacteria. Antibodies against 16 selected oral bacteria were used in the same study to identify the species penetrating into the dentine, using strepavidin–biotin labeling. *Fusobacterium nucleatum*, *Eubacterium alactolyticum*, *E. nodatum*, *Lactobacillus casei*, and *Peptostreptococcus micros* were the most frequently detected species. It is noteworthy that *F. nucleatum* is a Gram-negative anaerobic rod, and all others were Gram-positive anaerobic rods and cocci (34).

Although the dominance of Gram-positive bacteria in dentine samples seems quite convincing, there are reports also showing a strong invasion of dentinal tubules by Gram-negative anaerobic bacteria (34). Martin et al. (28) studied bacteria found in the carious dentine of 65 teeth extracted from patients with advanced caries and pulpitis. Analysis of cultured bacteria showed a predominance of lactobacilli and other Gram-positive microorganisms. Gram-negative bacteria were also present in significant numbers, with *Prevotella* spp. being the most numerous anaerobic group cultured. With real-time PCR analysis of the powdered dentine, the relative proportion of Gram-negative anaerobes was somewhat higher than when culturing was used. *Prevotella* spp., *Fusobacterium* spp., *Porphyromonas gingivalis*, and *P. endodontalis* were among the frequently detected species.

Invasion into dentinal tubules by Gram-negative bacteria from periodontal pockets has been reported by Giuliana et al. (41). Microorganisms identified included putative periodontal pathogens such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *F. nucleatum*, and *Bacteroides forsythus* (present name *Tannerella forsythensis*), all frequently found in primary apical periodontitis.

The mechanisms by which the bacteria invade dentinal tubules are not fully understood. However, the ability to penetrate dentinal tubules does not seem to be dependent on the motility of the bacterial cells. In fact, most of the species that best invade the tubules are nonmotile. Love et al. (42) found that the streptococcal antigen I/II family of polypeptides are involved in the attachment of oral streptococci to collagen, and that they also determine the ability of these bacteria to invade dentinal tubules of human teeth. It has also been shown that while serum prevented dentinal invasion by *S. mutans* and *S. gordonii*, the invasion by *E. faecalis* was only reduced but not totally prevented (43). Salivary molecules mucin and immunoglobulin G (IgG), which co-aggregate with bacterial cells, also inhibit dentine invasion (43). In addition, the deposition of dentinal tubule fluid molecules e.g. albumin,

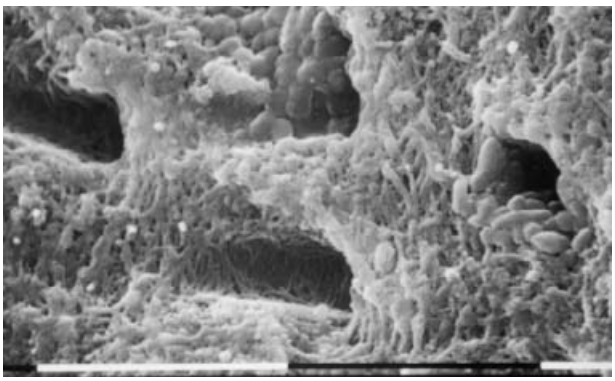


**Fig. 4. A scanning electron micrograph of dentine invaded by *Enterococcus faecalis*. In some areas, the bacteria have started to spread from the tubules into surrounding dentine.**

IgG, or fibrinogen within dentinal tubules also inhibits invasion (43).

When bacteria invade into dentinal tubules, not all the tubules are equally invaded. On the contrary, both *in vitro* and *in vivo* observations show that bacterial penetration into dentinal tubules occurs seemingly at random; while one dentinal tubule is full of invading bacteria, many of the surrounding tubules are totally empty (Fig. 3). Bacteria in dentinal tubules are typically seen as sporadic, dense accumulations of cells, not as a continuously growing rows of cells, extending out from the main canal lumen towards the periphery. In some cases, when the root canal infection has been present for a long time and the availability to nutrients is good, the bacteria break out from the dentinal tubules into surrounding dentine (Fig. 4), which then becomes structurally destroyed (44).

### *Importance of invasion of dentinal tubules in primary apical periodontitis*

The importance of bacterial penetration from the main root canal into dentine in teeth with primary apical periodontitis is not fully understood. From an ecological and microbiological point of view, spreading into new areas is part of a normal 'natural history' of any infection. However, in primary apical periodontitis it may be of relatively little importance. Peters et al. (45) stated that 'In the vast majority of cases, those bacteria (that have invaded radicular dentine) appear not to jeopardize the successful outcome of root canal treatment.' Studies measuring the frequency of dentinal tubules invasion in necrotic teeth present values between 50% and 90%, and instrumentation seems to have little, if any, effect on the number of teeth with infected dentine (34). Yet, after treatment of primary apical periodontitis, disease persists in only 5–20%, when the treatment is carried out with adequate quality. Thus, it is obvious that residual bacteria in root dentine usually do not succeed in interacting with the host in a way that would result in an infection process that would be clinically or radiographically detectable.

### *Extracanal infection: bacteria in the periapical tissues*

Occasionally, bacteria can also be found outside the tooth system in the periradicular tissues. Such situations include, among others, periapical abscesses, periapical actinomycosis, and other similar infections as well as osteomyelitis of the jaw. This article is limited to intracanal infections, while extracanal infections are reviewed in other articles in this issue.

## Elimination of root canal infection

The elimination of endodontic infection is different from elimination and control of most other infections in the human body. Because of the special anatomic environment in the root canal and tooth, host measures that are sufficient to eliminate the infectious organisms in other sites do not suffice for complete elimination of endodontic infections. Therefore, control of an endodontic infection is a concerted effort by several host and treatment factors. Success in all aspects of this cooperation will eventually result in elimination of the infective microorganisms and healing of apical periodontitis (Figs 5A–E). The necessary components in the elimination of endodontic infection are: (i) host defense system, (ii) in some cases, systemic antibiotic therapy, (iii) chemomechanical preparation and irrigation, (iv) local root canal disinfecting medicaments, (v) permanent root filling, and (vi) permanent coronal restoration.

### Host defense

The host's defense system is a key factor in preventing the spreading of the infection from the root canal to the periapical tissues and surrounding bone (Figs 6 and 7). However, a lack of circulation in the necrotic root canal makes it impossible for the phagocytes and the rest of

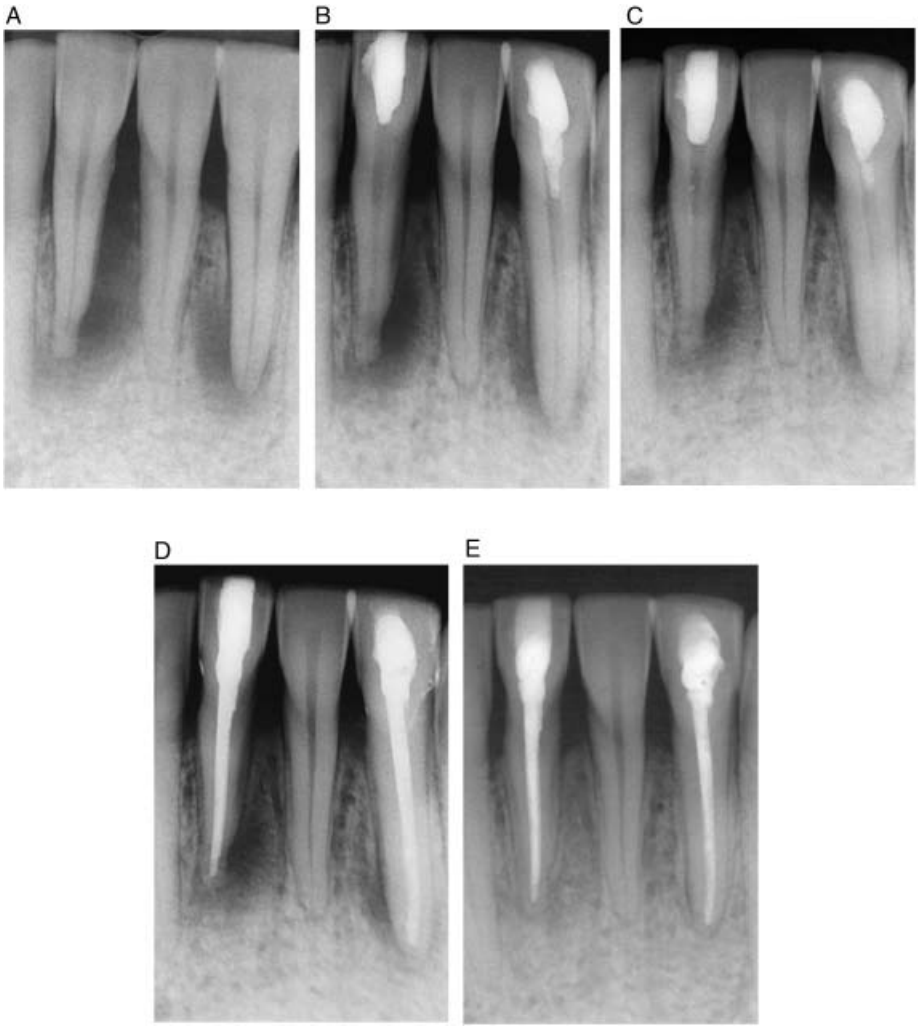



**Fig. 5. (A) Apical periodontitis in two lower incisor teeth. (B) Four months control after the canals were filled with calcium hydroxide. (C) Six months control. Continued healing can be seen (D). The teeth were root filled at 6 months. (E) One-year control radiograph shows complete healing of apical periodontitis lesions.**

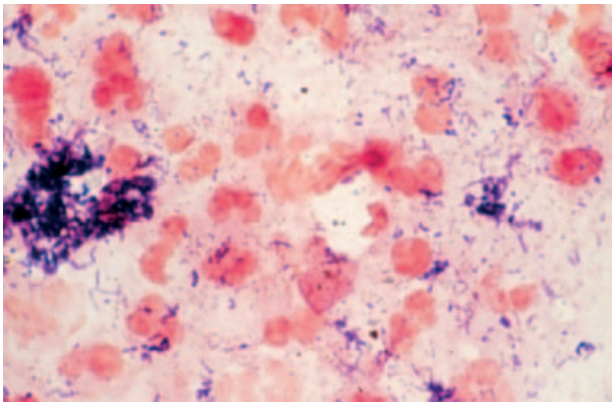


**Fig. 6. Gram-stained smear sample from root canal exudate of a tooth with apical periodontitis. Several polymorphonuclear leukocytes and Gram-positive rod-shaped bacteria can be seen.**

the immune system to penetrate into the root canal space for more than a few hundred micrometers. Therefore, although of crucial importance in maintaining general health, the defense system is limited to achieving a balance between the microbial intruders and the body, but it cannot eliminate the source of the infection in the root canal.

In chronic apical periodontitis, the main mechanism responsible for the destruction of normal bone structure is activation of bone osteoclasts and inhibition of osteoblast activity (46, 47). The sequence of events resulting in osteoclast stimulation is a network of immunological chain reactions where inflammatory cytokines play a major role. Although alternative theories about the major route in osteoclast activation

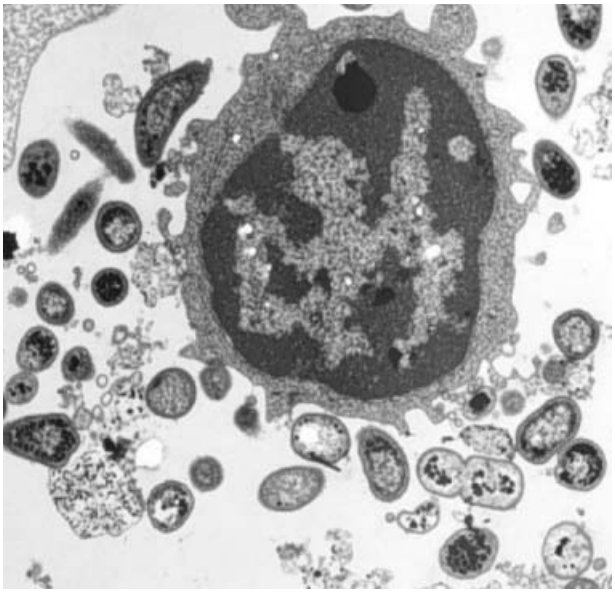


**Fig. 7. A polymorphonuclear cell surrounded by several Gram-negative bacteria.**

have been presented, the key fact remains that it is the host's own osteoclastic cells that remove the bone around the root tip (46, 47). Currently, removal of bone is understood as an important and necessary defense strategy: bone has a poor capability to defend itself against bacterial intruders, and osteomyelitis might ensue if the intracanal infection is allowed to spread. This is the obvious reason why bone is removed by the body's own defense system before the infection reaches the periapical tissues. In apical periodontitis, the lesion is filled with phagocytes and other defense cells, which effectively prevent further spreading of the microbial infection.

## Systemic antibiotics

The use of systemic antibiotics is not a routine part of endodontic treatment of apical periodontitis. On the contrary, antibiotics are only rarely used in endodontics. Minimizing the risk of post-operative symptoms has been one argument often used when prescribing antibiotics to endodontic patients. However, the use of systemic antibiotics has not been helpful in reducing the incidence of flare-ups or other acute problems after the start of the treatment (48). Neither is there scientific evidence that systemic antibiotic therapy has a beneficial effect on the long-term prognosis of the treatment of apical periodontitis. There is presently a consensus in endodontics that systemic antibiotics should be used only when general indications for their use are present (48). Administration of systemic antibiotics should be considered when infection appears to be spreading, indicating failure of local host responses, or when host defense mechanisms are known to be compromised and the patient is at an increased systemic risk (48, 49). Also, when the patient develops fever, antibiotics should be prescribed. The effectiveness of antibiotic therapy is never fully predictable because of a variety of parameters affecting the outcome. Therefore, the focus must always be on local antimicrobial measures, namely chemomechanical preparation and disinfection. Whenever there are general symptoms of spreading infection, the patient must be carefully monitored and hospitalization must be considered.

## Chemomechanical preparation and irrigation

### *Manual instrumentation*

There is a general consensus that high-quality mechanical cleaning and shaping of the root canal is the most important single factor in the prevention or healing of endodontic disease. Together with the use of antibacterial irrigating solutions, the majority, if not all, bacteria in the root canal system can be eliminated. Mechanical instrumentation is a primary means of bacterial reduction in endodontic treatment. Byström & Sundqvist (50) measured the reduction in bacterial counts cultured from the infected root canal when instrumented with hand stainless-steel instruments and saline irrigation. Fifteen root canals with necrotic pulps and periapical lesions were instrumented at five sequential appointments. This procedure greatly reduced the number of cfu, usually 100–1000-fold, but the progression towards achieving bacteria-free root canals was slow. Even after five appointments several canals still showed growth. Corresponding observations were also reported by Ørstavik et al. (51). Since it has become obvious that mechanical preparation with hand instruments and irrigation with saline (lacking any antibacterial activity) are unable to produce bacteria-free root canals predictably, focus has been placed on the combined effect of instrumentation and strongly antibacterial irrigating solutions.

### *Canal irrigation*

The use of irrigating solutions is an important part of effective chemomechanical preparation. It enhances

bacterial elimination and facilitates removal of necrotic tissue and dentine chips from the root canal; thus irrigants prevent packing of infected tissue apically in the root canal and into the periapical area. In addition, many irrigating solutions have other beneficial effects.

EDTA (ethylene–diammine–tetra-acetic acid, 17% disodium salt, pH 7) is a chelating agent widely used in endodontic preparation. It has low or no antibacterial activity, but it effectively removes smear layer by affecting the inorganic component of the dentine. Therefore, by facilitating cleaning and removal of infected tissue, EDTA contributes to the elimination of bacteria in the root canal. It has also been shown that removal of the smear layer by EDTA (or citric acid) improves the antibacterial effect of locally used disinfecting agents in deeper layers of dentine (29, 52).

Sodium hypochlorite (NaOCl), used in concentrations varying from 0.5% to 5.25%, is a strong antimicrobial agent, which plays an important role in dissolving the organic part of pulpal remnants and dentine. Most importantly, it kills bacteria very rapidly even at relatively low concentrations. Pashley et al. (53) demonstrated greater cytotoxicity and caustic effects on healthy tissue with 5.25% NaOCl than with 0.5% and 1% solutions. No *in vivo* studies have clearly shown that the stronger solutions have a better antibacterial effect in the root canal. However, careless use of both NaOCl (in high and low concentrations) as well as EDTA will result in severe pain and extensive tissue damage if they are introduced to the periapical area (54). Niu et al. (55) observed the ultrastructure on canal walls after EDTA and combined EDTA plus NaOCl irrigation by scanning electron microscopy. They reported that more debris was removed by irrigation with EDTA followed by NaOCl than with EDTA alone. Byström & Sundqvist (56, 57) showed that although 0.5% NaOCl, with or without EDTA, improved the antibacterial efficiency of preparation, all canals could not be rendered bacteria-free even after repeated appointments.

NaOCl effectively kills bacteria, but is caustic if accidentally expressed into the periapical area. In addition, the active chlorine in the solution may damage patients' clothing through its strong bleaching effect. Therefore, alternative irrigating solutions have been pursued that could replace NaOCl. Chlorhexidine gluconate (CHX) has been in use for a long time in dentistry because of its antimicrobial properties and its relatively low toxicity, and its use in endodontics has been increasing. Although studies comparing the antibacterial effect of NaOCl and CHX have produced somewhat conflicting results, it seems that when used in identical concentrations, their antibacterial effect in the root canal and in infected dentine is similar (29, 58–60). However, CHX lacks the tissue-dissolving ability, which is one of the obvious benefits of NaOCl. Waltimo et al. (61) studied the antifungal effect of combinations of endodontic irrigants and found that the combinations of disinfectants were equally or less effective than the more effective component when used alone. However, it has been shown that in certain concentrations chlorhexidine and hydrogen peroxide have a strong synergistic effect against *Enterococcus faecalis*, *Streptococcus sobrinus*, and *Staphylococcus aureus* (58, 62).

### *Rotary instrumentation*

The use of rotary preparation with nickel–titanium (NiTi) instruments undoubtedly offers several potential advantages. The most obvious of these are probably the quality of the apical preparation and efficiency. However, rotary instruments have not always compared favorably when the various aspects of preparation have been analyzed (63). Ahlquist et al. (64) showed that hand instrumentation produced cleaner canals than preparation with rotary instruments. Similar results have been reported by Schafer & Lohmann (65). Nevertheless, rotary NiTi instruments appear to maintain the original canal curvature better than hand stainless-steel instruments, particularly in the apical part of the root canal (66).

Dalton et al. (67) compared stainless-steel K-type files and NiTi rotary instruments in removing bacteria from infected root canals with saline as an irrigant. Only approximately one-third of the canals were rendered bacteria-free, and no significant difference could be detected between the two groups. However, larger preparation diameter of the apical canal produced a significant reduction in bacterial counts. Coldero et al. (68) studied the effect of apical preparation on the number of residual bacteria in the root canal. They concluded that additional apical enlargement to size #35 did not further reduce the number of surviving bacteria. However, the size of the original preparation was not given, and it is possible that the size #35 was too small to show differences in bacterial elimination. In fact, Rollison et al. (69) showed that apical

enlargement to size #50 instead of size #35 resulted in a more effective elimination of bacteria in the root canal, although sterility was not obtained.

In a recent study, Card et al. (70) reported sterility in a majority of root canals instrumented by rotary NiTi instruments using large apical sizes and irrigation with 1% NaOCl. The instrumentation and bacterial sampling were carried out in two phases: the first instrumentation utilized 1% NaOCl and 0.04 taper ProFile rotary files. The cuspid and bicuspid canals were instrumented to size #8 and the molar canals to size #7. The second instrumentation utilized LightSpeed files and 1% NaOCl irrigation for further enlargement of the apical third. Typically, canals of molars were instrumented to size #60 and cuspid/bicuspid canals to size #80. All of the cuspid/bicuspid canals and 81.5% of the molar canals were bacteria-free already after the first instrumentation, as shown by negative cultures from samples obtained from the root canals. In the molars, bacteria-free canals increased to 89% after the second instrumentation. When the molar canals were divided into two groups, one with no visible anastomoses between root canals and the other with a complex root canal anatomy, the proportion of sterile canals in the first group was 93% already after the first instrumentation. The results of Card et al. (70) are indirectly supported by earlier observations by Peters et al. (71), who studied rotary preparation of root canals of maxillary first molars. They compared the effects of four preparation techniques on canal volume and surface area using three-dimensionally reconstructed root canals in extracted teeth. Micro CT data were used to describe morphometric parameters related to the four preparation techniques. Specimens were scanned before and after canals were prepared using K-type hand files, LightSpeed instruments, ProFile .04 and GT rotary instruments. Differences in dentine volume removed, canal straightening, the proportion of uninstrumented area, and canal transportation were calculated (71). The results showed that instrumentation of canals increased their volume and surface area. The prepared canals were significantly more rounded, had greater diameters, and were straighter than unprepared canals. However, all instrumentation techniques left at least 35% of the canals' surface area untouched. There were significant differences between the three canal types investigated; however, very few differences were found between instrument types. The relatively large proportion of untouched canal walls in molar root canals offers one explanation as to why in the clinical study (70) it was difficult to eliminate bacteria from such canals totally as compared with canines and premolars.

### *Size of the apical preparation*

The main goals of mechanical preparation are as follows: (i) to remove infected tissue from the root canal, (ii) to facilitate the use and effectiveness of irrigating solutions, (iii) to create sufficient space for effective delivery of intracanal medicaments between appointments, and (iv) to create sufficient space in the root canal to allow placement of permanent root filling of high quality. Despite these clearly defined and widely accepted general goals for preparation, there is no consensus about the recommended size for the apical preparation in various teeth. Theoretically, optimal apical preparation would require an instrument size equal to or bigger than the largest diameter of the apical canal. This would guarantee that all walls in this critically important part of the canal would be engaged by the instruments. Studies by Kerekes & Tronstad (72–74) suggested that the final preparation size should be quite large as compared with the sizes often used in practice: size #50 to #90 in incisors, canines and premolars, and even in molar curved canals sizes #50 to #60. These studies also demonstrated that in oval-shaped roots, such as in maxillary first premolars, it was often impossible to obtain a round apical preparation without perforation of the root, because the narrow external dimension of the root in several teeth was smaller than the larger internal diameter of the root canal. The same was concluded in another study of maxillary first molars by Gani & Visvisian (75).

In clinical practice, there are no available methods that would reliably gauge the size of the apical root canal. Morfis et al. (76) studied the size of apical foramina in various tooth groups and found that the largest foramen was in the distal root of lower molars, the average diameter being almost 0.4 mm (size #40). Wu et al. (77) studied if the first file to bind apically would correspond to the diameter of the canal in the apical region. The canals were prepared three sizes larger than the first binding file, and the quality of the final preparation was then analyzed. The result of this study showed that there was no correlation between the first binding file and the larger diameter of the apical canal. At present, the typical size of the apical

preparation in curved molar canals varies in different parts of the world, from sizes #20 to #60. It is possible that in the treatment of teeth with a vital pulp (pulpectomy), the size of the apical preparation is not critical because of the absence of microorganisms in the apical canal, while in the treatment of apical periodontitis, apical enlargement may be more important (69, 70). However, clear evidence of the importance of apical enlargement for long-term prognosis is still lacking. It is obvious that when canals are apically enlarged to size #25, the apical canal walls often remain relatively untouched; however, it is equally clear that the healing rates of apical periodontitis are still high regardless of the apical enlargement (78).

The quality of apical shaping and cleaning is affected not only by the diameter of the last instrument but also by the taper. A typical 2% taper in manual preparation of a size #30 instrument produces canal diameters corresponding to sizes #32, #34 and #36 in the levels of 1, 2, and 3 mm from the working length, respectively. In contrast, with a size #30 instrument with 9% taper, the diameters at the same levels correspond to sizes #39, #48, and #57 (Figs 8 and 9). It has been speculated that the greater taper may facilitate the effect of antibacterial irrigants in the apical canal (68). However, at present there is no evidence to support the clinical importance of the differences of apical taper.

### *Working length vs. apical foramen*

Anatomic studies about the location of the foramen have shown that it is often found at a distance of 0–3 mm from the anatomic apex (79). Using the radiographic apex of the tooth as the target for working length determination would therefore result in over-instrumentation in a large number of teeth. It is recommended that the working length be determined using electronic apex locators and radiographs (80). The apex locators indicate the location of the apical constriction (81). In pulpitis treatment, the recommended working length is 1–2 mm short of the radiographic apex. In apical periodontitis, elimination of root canal infection, not the least in the apical canal, is the key to successful treatment. In an optimal situation, the root canal should be instrumented, disinfected and filled to the level of the coronal aspect of the apical constriction (Figs 10 and 11), to avoid the possibility of residual microbes surviving in the uninstrumented apical canal (82). This should hold true even in teeth treated for pulpitis, although in these teeth terminating the root canal treatment coronally of the constriction is not expected to influence the outcome adversely.

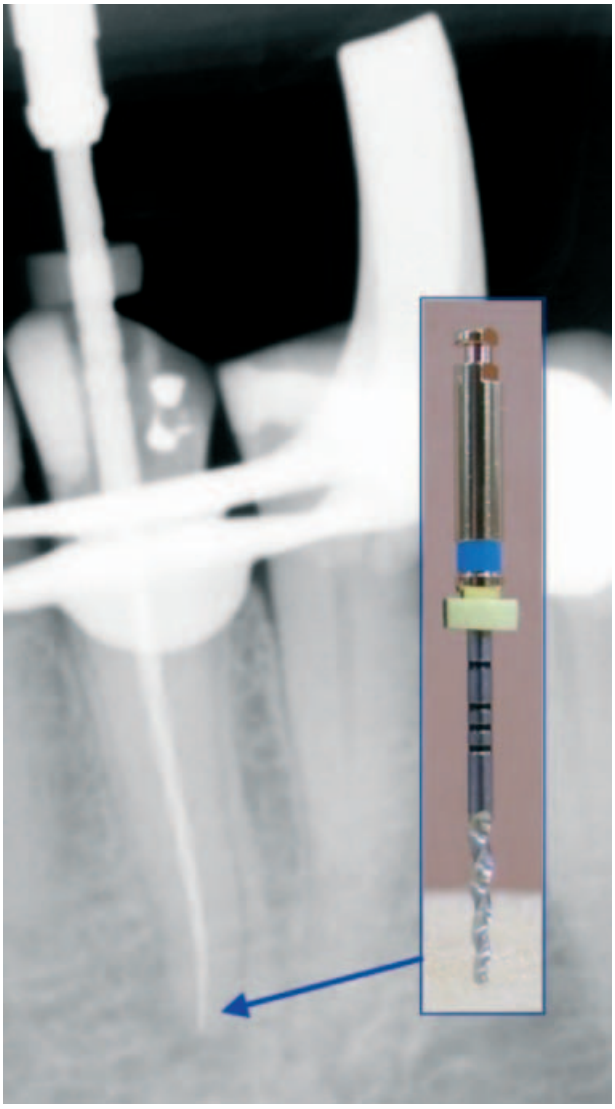


**Fig. 8. A radiograph from a lower canine with a size #30 instrument with 9% taper (ProTaper F3) at working length.**

Overinstrumentation, with the possible exception of the smallest hand files of size #06–#10 in certain situations, should be avoided because of the following reasons: (i) direct physical trauma to periapical tissue, (ii) transportation of necrotic canal contents and dead and living microorganisms into the periapical area that can result in persisting infection, such as periapical actinomycosis, (iii) bleeding into the root canal that provides nutrients to intracanal bacteria, (iv) increase of the foramen size and associated improved possibilities

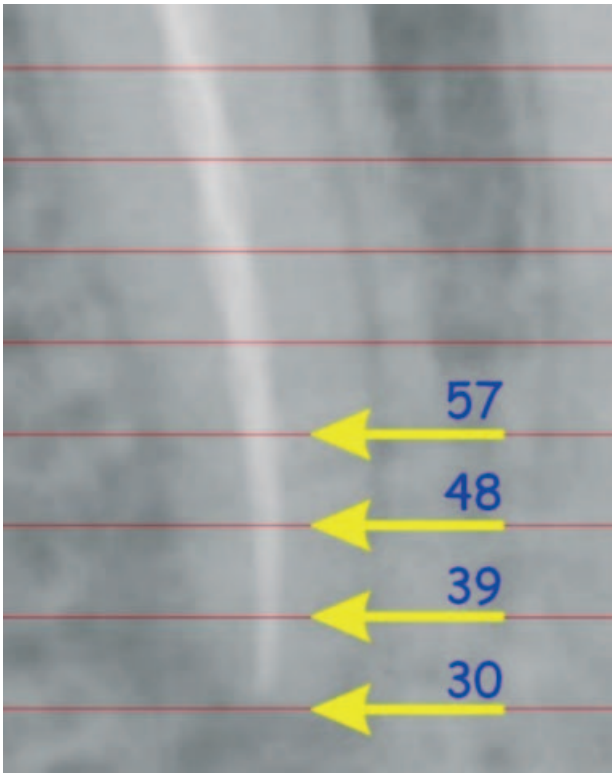



**Fig. 9. A close-up picture of Fig. 8 shows the increase in diameter of the prepared root canal 1, 2, and 3 mm from working length. At 3 mm the diameter is ca. 0.57 mm.**

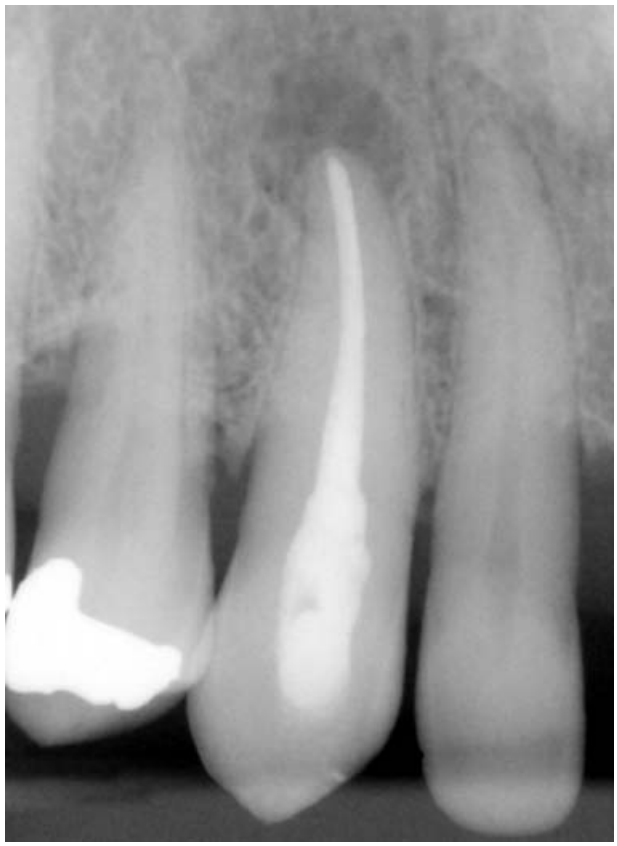


**Fig. 10. The canine shown earlier in Fig. 1 root filled to optimal length.**

for bacteria to receive nutrients from the periapical area (inflammatory exudate), (v) increased risk for extrusion of irrigating solutions and root-filling materials, and (vi) in curved canals ( = most canals), creation of an oval foramen instead of a round one, resulting in poorer apical seal with a round gutta-percha master point (complete compensation with a sealer is theoretical) and therefore hide-out for microbial colonization (Figs 12A and B).

## Disinfection of the root canal by intracanal medication

In pulpectomy, intracanal medication is not an integral part of the treatment because the pulp is bacteria-free or only superficially infected. Only when time limitation has not allowed completion of the treatment in one appointment is the canal space filled e.g. with calcium hydroxide to prevent contamination of the canal in between appointments. In anatomically demanding teeth, interappointment calcium hydroxide may have been selected to facilitate removal of residual pulp tissue or to help to control bleeding.

In the treatment of apical periodontitis, intracanal medication has been recommended to eradicate the microbes that survive instrumentation and irrigation. A variety of medicaments have been used for this purpose. These include calcium hydroxide, phenol compounds eugenol and camphorated parachlorophenol (CMCP), iodine potassium iodide (IPI), glutaraldehyde, formocresol, and pastes containing a mixture of antibiotics with or without corticosteroids. Byström et al. (83) showed that calcium hydroxide was more effective as an intracanal medicament than CMCP or camphorated phenol, rendering 34 out of 35 canals bacteria-free after 4 weeks. The effectiveness of interappointment calcium hydroxide was also demonstrated by Sjögren et al. (84), who showed that the 7-day dressing with calcium hydroxide eliminated bacteria that survived instrumentation and irrigation of the canal, while the 10-min application was ineffective. However, the ability of calcium hydroxide to disinfect the canal has been to some extent challenged by other studies that reported a residual flora in 7–35% of teeth after the use of calcium

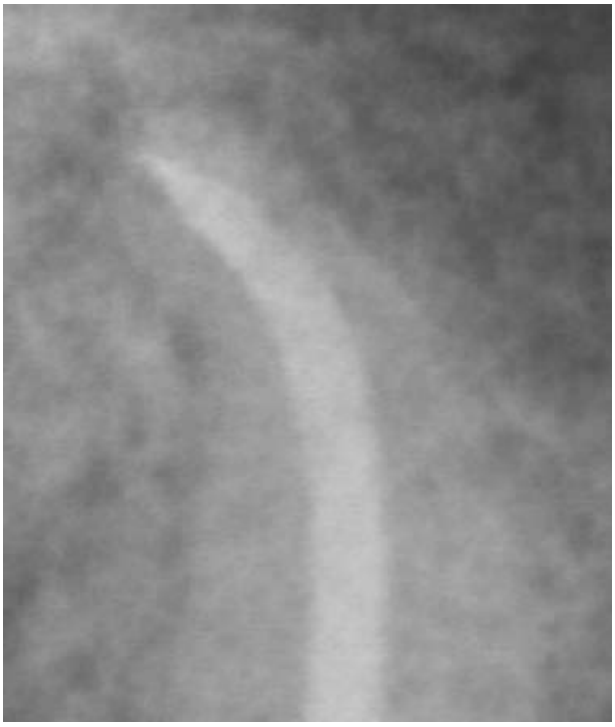
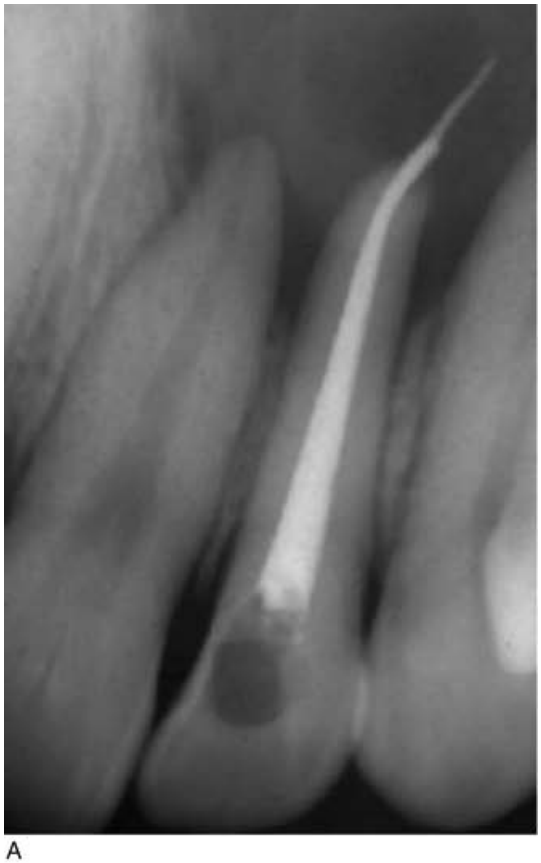
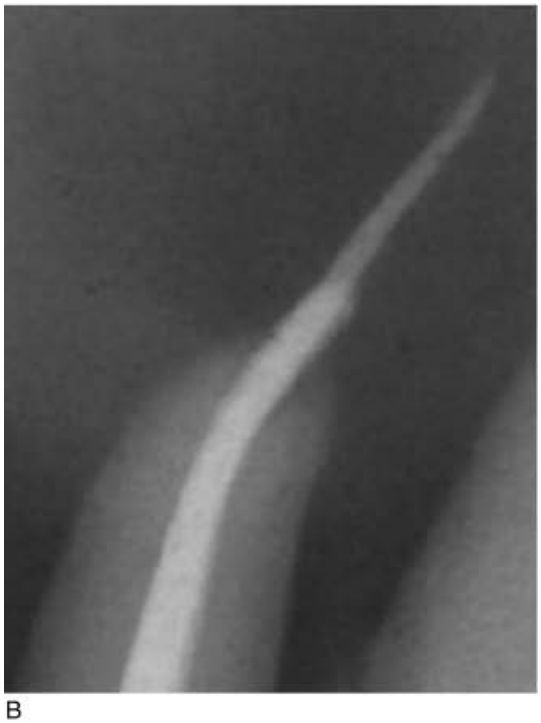


**Fig. 11. A high magnification of a radiograph of a root-filled upper second premolar.**



A



B

**Fig. 12. (A) Upper lateral incisor overfilled after overinstrumentation. (B) A close-up of (A) showing apical transportation and creation of a gap between the root filling and the canal wall as a result of overinstrumentation.**

hydroxide (51, 85, 86). Peters et al. (87) reported that the number of culture-positive canals had increased between appointments even though calcium hydroxide had been used as an intracanal dressing. However, the number of microorganisms had only increased to approximately 1% of their original number. The different results may be partly explained by differences in the clinical cases studied (e.g. intact teeth vs. carious teeth), and in the techniques employed in sampling and culturing the microbes.

Retreatment of root-filled teeth with apical periodontitis has been suggested to have a poorer prognosis than treatment of primary apical periodontitis (22). This may be due to several reasons, such as technical complications, difficult anatomy, unlocated root canals, etc. One possible explanation for the poorer prognosis is the presence in retreatment of a microflora that is more resistant to conventional treatment procedures than the flora in primary apical periodontitis. It is well documented that *E. faecalis* is the dominant microbe in persistent apical periodontitis (retreatment) (13–16, 88, 89). It is ecologically tolerant and can survive in water without nutrients for several months (90). It is also more resistant to most locally used disinfecting

agents than other endodontic microbes (52). *In vitro* and *in vivo* studies have clearly demonstrated that intracanal calcium hydroxide fails to eliminate *E. faecalis* from the infected dentine (52, 91). On the other hand, no other medicament has shown better *in vivo* effectiveness against *E. faecalis* either (91). However, although there is a good agreement about the dominance of *E. faecalis* in retreatment cases of apical periodontitis, the importance of this bacterium for the long-term prognosis of the retreatment has not been demonstrated in clinical studies. Other microbes frequently found in retreatment cases include Gram-positive facultative organisms such as *Streptococcus* spp., *Lactobacillus* spp., *Actinomyces* spp., *Propionibacterium* spp., Gram-negative coliform rods, and the yeast *Candida albicans* (15, 92, 93).

Root canal disinfecting agents are extremely effective against even the resistant microbes when tested in a test-tube environment. The clearly poorer results *in vivo* in the root canal indicate the presence of interfering factors that negatively affect the outcome of the disinfection. Haapasalo et al. (94) and Portenier et al. (95, 96) studied the effect of dentine and other substances present in the root canal milieu on the antibacterial effect of commonly used intracanal medicaments, such as calcium hydroxide, chlorhexidine, and IPI against *E. faecalis*. These studies showed that all three disinfectants were negatively affected by the various substances tested, calcium hydroxide being particularly sensitive to the inhibitory effect of a variety of substances present in the root canal. Earlier, Messer & Chen (97) had reported on the short duration of the vapors from cotton pellets soaked in phenol compounds. The inactivation of locally used disinfecting agents in the root canal may explain the relative resistance of the root canal microflora. Gram-positive facultative bacteria, which best tolerate the harsh ecological conditions created by the chemomechanical preparation, have been shown to increase their relative proportion of the flora after preparation and use of disinfection, even though the total numbers are strongly reduced by the treatment procedures (15, 92).

At present, it seems correct to conclude that no interappointment root canal disinfectant can predictably render canals sterile in the treatment of teeth with apical periodontitis. However, it is clear from most studies that the use of the intracanal medicaments further reduces the number of infecting microorganisms after chemomechanical preparation. Furthermore, calcium hydroxide may help to better remove the residual necrotic pulp tissue at the second appointment, as well as neutralize bacterial antigens remaining in the root canal system (98, 99).

## Root filling and permanent restoration

Eventually, all locally used disinfectants lose their antibacterial effect over time or are washed away from the canal through the apical foramen. Permanent root filling is therefore necessary to prevent bacteria from re-entering the root canal space after chemomechanical preparation and disinfection. It has also been suggested that the root filling may entomb the residual bacteria in the root canal system so that they cannot proliferate and interact with the periapical tissue, which could compromise healing. However, there are very little data available about the effectiveness of such root canal bacteria entombment by the root filling. Sjögren et al. (6) demonstrated the importance of obtaining negative cultures for improving the prognosis of the treatment of apical periodontitis. However, Peters & Wesselink (8) found no difference in healing of teeth with apical periodontitis filled after negative or positive culture at the time of filling. The latter would mean that more bacteria were 'entombed' by the root filling. Katebzadeh et al. (7, 100) simulated such entombment in dog teeth that were experimentally infected and developed apical lesions. Some of the canals were obturated at the first appointment after instrumentation and irrigation with saline, while the rest were disinfected before root canal filling with an interappointment calcium hydroxide dressing. The results showed better healing in teeth where calcium hydroxide had been used. However, the apical anatomy of dog teeth is quite different from that of human teeth, which may have affected the results. Hernandez et al. (101) evaluated the root canal morphology in 72 maxillary fourth premolars and 59 mandibular first molars in dogs. An apical delta was present in all roots ($n = 334$) and represented approximately 12–18% of the total root length for all roots.

In a new study using infected dentine blocks *in vitro*, Saleh et al. (102) showed that root filling with gutta-percha and specific sealers (AH plus or Grossman's sealer) was clearly more effective in eliminating *E. faecalis* from the dentine surrounding the root canal than calcium hydroxide 1 week after filling. Root fillings with gutta-percha and several other sealers proved to be less effective than calcium hydroxide

against *E. faecalis* dentine infection (102). The result supported an earlier observation by Ørstavik (103) that AH 26 was clearly more effective in killing bacteria in dentine around the root canal than other sealers tested. It may be of interest that in the study of Peters & Wesselink (8), AH 26 was used as the sealer. Clearly, in future studies comparing the outcome of single-appointment and two-appointment endodontic treatment of apical periodontitis, the type of the sealer also has to be taken into consideration.

Finally, all root fillings must be protected by a coronal restoration of high quality. Lack of coronal restoration or leaking restorations may result in bacterial contamination of the whole root filling in as little as a couple of weeks (104). Although the clinical relevance of coronal leakage has not yet been convincingly demonstrated, it is obvious that coronal leakage plays an important role in the etiology of post-treatment disease.

## Post-treatment disease (persistent endodontic infection)

Epidemiological studies of root-filled teeth in various countries and different populations have demonstrated the presence of apical periodontitis in a relatively high proportion of these teeth (105–112). In a large study of the quality of endodontic treatment in a Belgian population, De Moor et al. (105) evaluated the periapical conditions in 4617 teeth of 206 adults using panoramic radiographs. Of all the teeth, 6.8% were endodontically treated. Comparison of periapical status showed that apical periodontitis was found in 6.6% of all teeth, and in 40.4% of all root-filled teeth. Inadequate level of the root canal filling was registered in over 50% of these teeth. In another study in Denmark (111), the periapical status of nearly 600 root-filled teeth was compared in 1974–1975 and 1997–1998. Apical periodontitis was observed in approximately 50% of the root-filled teeth in both groups, and in molars the prevalence of disease was as high as 65% in both groups. In a Lithuanian population, the frequency of apical periodontitis in root-filled teeth was 35% (110), while in two selected Canadian populations, the prevalence of apical periodontitis in root-filled teeth was 44% and 51% (113). The lowest prevalence of apical periodontitis in root-filled teeth was reported by Soikkonen (114), who studied 133 dentate old people aged 76, 81, and 86 years living at home in Finland. Only 16% of the 507 root-filled teeth had apical periodontitis.

It should be emphasized that the great majority of root-filled teeth with apical periodontitis (post-treatment disease) are symptom-free, and no clinical, histological, or bacteriological investigation has been included in most of the epidemiological studies. Although some of these teeth may have been treated only recently, and thus may reflect healing in progress, it is likely that most of these lesions will not heal without some kind of intervention. It is possible that (i) the original apical periodontitis lesion has not healed after the primary endodontic therapy, (ii) the lesion has persisted after retreatment, (iii) the original lesion has healed but a new lesion has later emerged, and (iv) there was no lesion at the time of endodontic treatment, but a lesion has developed over time. There is no reliable information available about the proportion of each group, but clinical experience and follow-up studies have shown that they all occur. Therefore, it may be misleading to call categorically all root-filled teeth with a lesion as 'endodontic failures'. In fact, endodontic treatment may have resulted in healing, but subsequent coronal leakage, for example as a consequence of caries, may have caused the recurrence of disease.

### Microbial flora of primary apical periodontitis

As stated earlier in this review, the ecological conditions in the necrotic root canal are the main selective factors for the microbial flora in primary apical periodontitis. Therefore, the flora is characterized by a strong dominance of obligately anaerobic bacteria (Fig. 13) (25, 26, 115–131). The most frequent isolates are *Dialister pneumosintes* (*Bacteroides pneumosintes*), *T. forsythensis* (*B. forsythus*), *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Treponema* spp., *Campylobacter rectus* (*Wolinella recta*), *Micromonas micros* (*P. micros*), *Eubacterium* spp., *Bifidobacterium* spp., *Actinomyces* spp., *Propionibacterium* spp., *Lactobacillus* spp., and *Streptococcus* spp. Most of the above genera are obligately anaerobic; *Actinomyces*, *Propionibacterium* and *Lactobacillus* contain both anaerobic and facultatively anaerobic species and strains, while streptococci are facultative bacteria. *E. faecalis* is usually not found in primary apical periodontitis. However, using checkerboard DNA–DNA hybridization, Siqueira et al.

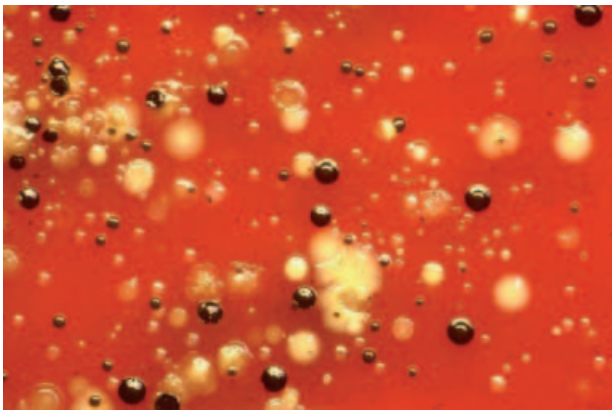


**Fig. 13. Typical mixed anaerobic flora isolated from primary apical periodontitis. Similar flora can sometimes also be isolated from root-filled teeth with apical periodontitis.**

(132) detected *E. faecalis* in 7.5% of primary endodontic infections.

## Intracanal infection post-treatment

Complete disinfection of the root canal system and elimination of viable microbes are the primary short-term goals in endodontic treatment. Thess are believed to secure the most important long-term objective, prevention and/or healing of apical periodontitis. However, as shown earlier in this review, several studies have confirmed the difficulties in predictably obtaining a sterile root canal by chemomechanical preparation combined with the use of local disinfection agents in the root canal (70, 86, 87). The focus, therefore, has recently been on the characterization of the residual flora and the identification of factors related to the resistance of these microbes to endodontic treatment procedures. From a clinical point of view, it is of interest as to whether the persisting microbes in the root canal system negatively impact on the outcome of the treatment.

### *Microbiological sampling and sample processing*

The precautions and preparatory procedures for microbiological sampling in teeth associated with endodontic post-treatment disease are the same as for primary apical periodontitis, including isolation of the tooth with rubber-dam, external cleaning of the tooth with pumice, and disinfection with 30% hydrogen peroxide and 10% iodine tincture (124). Before trepanation into the pulp chamber, the iodine is neutralized by 5% sodium thiosulfate solution. When the superficial layers of dentine (or temporary filling material) have been removed, the disinfection procedures may be repeated when necessary, and new sterile instruments are taken.

If the root canal(s) are filled with a disinfecting agent, this must be removed either by means of paper points or by careful irrigation with sterile water or physiological saline, avoiding contamination of the fluid by contact with the tooth crown. The disinfectants should be neutralized whenever possible. Thiosulfate solution inactivates iodine (124), while Tween 80, cysteine, histidine, and saponine have been assessed as sufficient for neutralizing chlorhexidine (133). The canals are then again dried with paper points and the sample is taken with one of the following methods: (1) apical dentine is collected with a reamer or a K-type file in a rotating motion, using instruments with a diameter larger than the size of the apical canal. The tip of the instrument is then cut using a sterile wire-cutter and collected in a transport medium, or (2) the canal is filled with a transport medium that is then collected with paper points either with or without filing of the canal walls with adequate size files. As paper points may contain chemicals with antimicrobial activity, such as fatty acids, certain precautions have to be taken. Using charcoal-impregnated paper points helps avoiding false-negative samples. The same effect can be obtained by washing the paper points with chloroform before sterilization to remove the free fatty acids.

When the microbiological sample is taken from a previously filled root canal, the root filling must be removed first. This has to be done without the use of chloroform or any other solution with antibacterial activity, to avoid false-negative samples. Canals where this is not possible should be excluded from the study. Most root fillings made of gutta-percha and sealer can be readily removed by using root canal burs, such as Largo (Peeso reamer), Torpan, and Gates-Glidden together with hand reamers and files. Rotary NiTi instruments facilitate the removal procedure. After the removal of the root filling, the sample is taken as described above.

### *Characteristics of the residual microflora in root-filled teeth*

The different microbes present in the necrotic root canal show great variation in their susceptibility to the

treatment procedures and to the various materials used during the treatment and in the root fillings. Ecological changes also play a major role in selecting species that best resist the antibacterial effect of the chemicals and try to adapt to the new ecological milieu. The conclusion based on several studies is that facultative species are more resistant than strictly anaerobic bacteria, and Gram-positive bacteria are stronger survivors than Gram-negative bacteria (13–16, 25, 26, 115–132). In root-filled teeth, the space available for microbes is limited as compared with the necrotic root canals in primary apical periodontitis. Consequently, the cfu counts obtained from retreated teeth are lower on average than those obtained from teeth with primary apical periodontitis. Peciuliene et al. (15) reported a range between 40 and $7 \times 10^7$ cfu in microbiological samples obtained from 40 previously root-filled teeth with asymptomatic apical periodontitis. The number of species and strains per canal is also clearly lower than in teeth with primary apical periodontitis. Typically one to three different species are isolated per canal, the average being close to one strain, whereas in primary apical periodontitis three to 10 different strains are usually found, with the average of six species (13–15, 25, 26).

### *Microflora in root-filled teeth without apical periodontitis*

In an infected, necrotic root canal, the presence of bacteria is invariably associated with the presence of apical periodontitis (4, 26). However, when the root canal space is filled with a root-filling material, bacterial presence in the canal is not always accompanied by the presence of disease. Molander et al. (13) sampled root canals of 20 root-filled teeth that did not have apical periodontitis. Thirteen microbial strains were found in nine of the 20 teeth. The microbes included one strain of *E. faecalis*, streptococci and Gram-positive facultative rods, one strain of *F. nucleatum* (Gram-negative anaerobic rod), and two strains of the yeast *C. albicans*. The cfu counts per canal were lower than in root-filled teeth associated with apical periodontitis included in the same study. The absence of infection and disease in root-filled teeth that harbor bacteria in the root canal space can be explained by the lack of communication between the bacteria in the root canal and the host tissues. The microbial flora in such teeth may be a residual flora from an earlier infection that survived the treatment, but it is more likely to be the consequence of coronal leakage.

### *Microflora in root-filled teeth with apical periodontitis*

Research in endodontic microbiology has clearly been characterized by a greater interest in primary apical periodontitis than in post-treatment apical periodontitis. However, interest in the microbiological profile of post-treatment apical periodontitis has increased considerably during the last few years. This may be mainly because of the perceived poorer prognosis of retreatment as compared with primary treatment of infected root canals (89, 134). It has been suggested that the differences in the outcome of treatment may be related to marked differences in the composition of the microbial flora in the necrotic root canals (13). In post-treatment apical periodontitis anaerobic bacteria constitute the minority, and they are isolated less frequently. *E. faecalis* is the dominant species present in post-treatment apical periodontitis. It is the most frequently isolated species and is usually also the predominant isolate in the canal (13–16, 89, 135). The highest frequencies of isolation of *E. faecalis* have been reported by Peciuliene et al. (15) and Pinheiro et al. (135), at 64% and 53% of the culture-positive teeth, respectively. The corresponding frequency in a North American population reported by Hancock et al. (16) was 30%.

Other bacteria frequently found in root-filled teeth with apical periodontitis are alpha- and non-hemolytic streptococci, *Actinomyces* spp., *Lactobacillus* spp., and *Propionibacterium* spp., all facultative or microaerophilic species (some *Actinomyces* and *Lactobacillus* strains and species are obligate anaerobes). *Staphylococcus* spp. are also found more frequently than in primary apical periodontitis (13, 16, 89, 135, 136). Gram-negative enteric rods and other facultative Gram-negative rods have also been reported from root canal samples of teeth with post-treatment apical periodontitis. The species isolated include *Enterobacter cloacae*, *E. agglomerans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella* sp., *Citrobacter freundii*, *Acinetobacter* sp., *Pseudomonas aeruginosa, Pseudomonas* sp., *Proteus mirabilis*, and *Proteus* sp. (13, 15, 88, 136).

Anaerobic bacteria are clearly in minority, and their frequency of isolation in root-filled teeth is below 50%

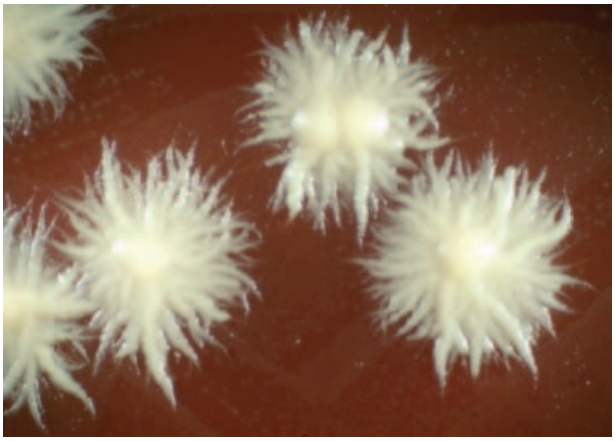


**Fig. 14.** ***Candida albicans*** **(yeast) cells isolated from root-filled teeth with apical periodontitis. On the background a dense growth of** ***Enterococcus faecalis*** **cells can be seen.**

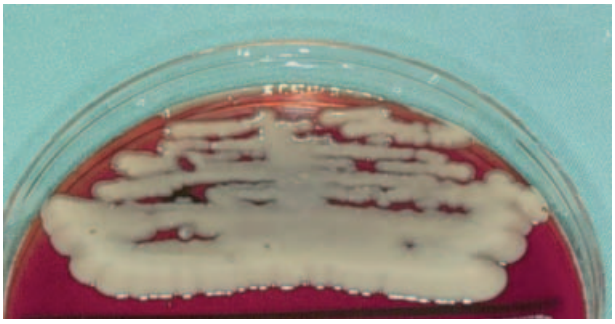


**Fig. 15. A Gram-negative enteric rod,** ***Klebsiella pneumoniae*****, isolated a root-filled tooth with apical periodontitis.**

(13–16, 89, 135). The most common anaerobic bacteria are *Prevotella* spp. and *Fusobacterium* spp., *Peptostreptococcus* spp., *Eubacterium* spp., *Bifidobacterium* spp., and anaerobic strains of *Actinomyces* and *Lactobacillus.* Also, *Porphyromonas* spp. have been isolated from root-filled teeth (16).

The selection of microbes that remains in the root canal after the treatment procedures have been initiated is based on differences in the sensitivity of the species to mechanical and chemical endodontic treatment, and on their ability to survive the ecologically altered root canal milieu, i.e. increased oxygen levels and limited availability of nutrients. In the short-term perspective, the resistance to treatment procedures is obviously more important, but with time, ecological strength becomes the determining factor for microbial survival. *E. faecalis* is probably the most resistant microbial species to chemomechanical preparation, and its proportion in the surviving flora is higher than in the initial root canal flora (137). However, increased numbers of other microbial species that are not usually present in primary apical periodontitis, such as yeasts (Fig. 14) and Gram-negative enteric rods (Fig. 15), have also been reported in root-filled teeth (13, 88, 138, 139). On the other hand, Peciuliene et al. (15) found that while a third of the *E. faecalis* strains survived instrumentation and irrigation with NaOCl and EDTA, none of the yeasts or coliform rods did. Recently, Chavez De Paz et al. (92) reported microbial findings in teeth (mostly primary treatments) after the initiation of the endodontic treatment. Each tooth had been instrumented and medicated one or several times before the sampling. Of the 200 teeth included in the study, 107 showed positive cultures; 85% of the isolates were Gram-positive facultative bacteria. *Lactobacillus* spp. (22%), *Streptococcus* spp. (18%), and *Enterococcus* spp. (12%) were the most common isolates (92). Despite increased knowledge of the persistence of the microbial infection in the root canal, it is obvious that more clinical investigation is needed to better understand the factors related to the susceptibility of the different microbial groups to the various elements of endodontic treatment.

Several studies have demonstrated the relative resistance of *E. faecalis* to calcium hydroxide (52, 57, 88). Therefore, the use of calcium hydroxide as a disinfectant of choice in the retreatment of cases with post-treatment apical periodontitis has been questioned (13). *C. albicans* and some other *Candida* species have been shown to be even more resistant to calcium hydroxide *in vitro* than *E. faecalis* (61). The sensitivity of the *C. albicans* strains to calcium hydroxide was generally low, and incubation of 16 h was required to kill 99.9% of the cells. The level of resistance was the same for *C. guilliermondii*, while 13 h were required to kill *C. krusei*. Strains of *C. tropicalis* were killed between 3 and 6 h of incubation, but all strains of *C. glabrata* survived only between 20 min and 1 h of incubation with calcium hydroxide. Compared with *E. faecalis* (20 min–1 h), however, all *Candida* spp. showed either equally high or higher resistance to saturated calcium hydroxide solution. Interestingly, in the same study, *Candida* strains from root canal infections and from periodontitis were compared, but no differences in susceptibility to calcium hydroxide were found (140). Higher resistance (than *E. faecalis*) to calcium hydroxide *in vitro* was also detected in two strains of *Bacillus cereus* that were isolated from treatment-resistant cases

of apical periodontitis in Finland and Norway (unpublished data).

The clinical factors contributing to the selection of *E. faecalis* or other Gram-positive facultative bacteria in the treated root canal have been only rarely studied. Siren et al. (88) found that if the number of treatment appointments before sampling had been high (over 10 appointments), the probability of isolating *E. faecalis* was very high. Similarly, if the root canal had been unsealed between appointments one or several times, the frequency of isolation of *E. faecalis* was significantly higher (88). In a study of 25 root canals in teeth with post-treatment apical periodontitis, the root-filling materials used did not explain the high frequency of isolation of *E. faecalis* in these teeth (14). Rather, it was concluded that the ecological conditions in the incompletely filled root canal created a selective ecological niche that favored the growth and persistence of *E. faecalis* (14). The quality of root fillings in root-filled teeth with apical periodontitis varies greatly from apparently excellent fillings to almost unfilled canals (Figs. 16 and 17). Although not yet supported by solid scientific evidence, it is likely that the probability to isolate anaerobic bacteria is higher in teeth where much of the (apical) root canal is unfilled.

Little is known about the relationship between symptoms and the composition of the flora in root-filled teeth with persistent infection and apical periodontitis. In most studies, the presence of symptoms has not been reported, but the 'chronic apical periodontitis' given as a diagnosis in some studies is likely to refer to symptom-free teeth. Typically, root-filled teeth with apical periodontitis are symptom-free, and are detected by a radiographical investigation. Peciuliene et al. (15) retreated 40 root-filled teeth with asymptomatic apical periodontitis, and reported a flare-up in two teeth (5%) after initiation of the therapy. In one tooth, *P. mirabilis* was the major isolate (98%) with *E. faecalis* (2%), while in the other *E. faecalis* was the major isolate (98%) with *F. nucleatum* and *Actinomyces viscosus*. *F. nucleatum* is known as a typical member in odontogenic abscesses and spreading infections. No flare-up occurred in teeth where *E. faecalis* was present in monoinfection. Pinheiro et al. (135) correlated the occurrence of symptoms with the microbial findings in 60 root-filled teeth with apical periodontitis. Although *E. faecalis* was the most frequent isolate, it was the anaerobic bacteria, *Peptostreptococcus* spp. and dark pigmenting *Prevotella* species (*P. intermedia/nigrescens*) and *Fusobacterium* spp. that were associated with clinical symptoms. Obviously, although the majority of teeth with post-treatment apical periodontitis are asymptomatic, more research is necessary to better understand the correlation between the infective flora and symptoms in these cases, as well as to identify the microbial 'risk-species' for flare-ups after retreatment is started.

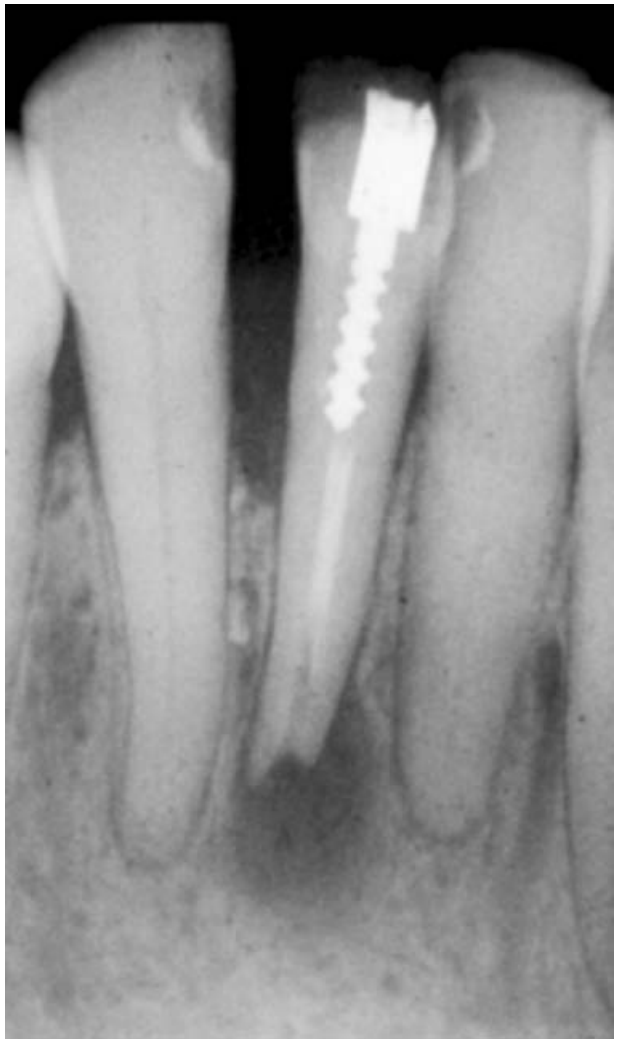


**Fig. 16. A root-filled tooth with apical periodontitis. Coronal and middle root canal are relatively densely filled, whereas the apical canal seems empty.**

## Ingress of bacteria: coronal leakage

In persisting or recurrent endodontic infections, the microbes present in the root canal system may be a residual flora from the original infection, or may have invaded the root canal post-treatment. There are

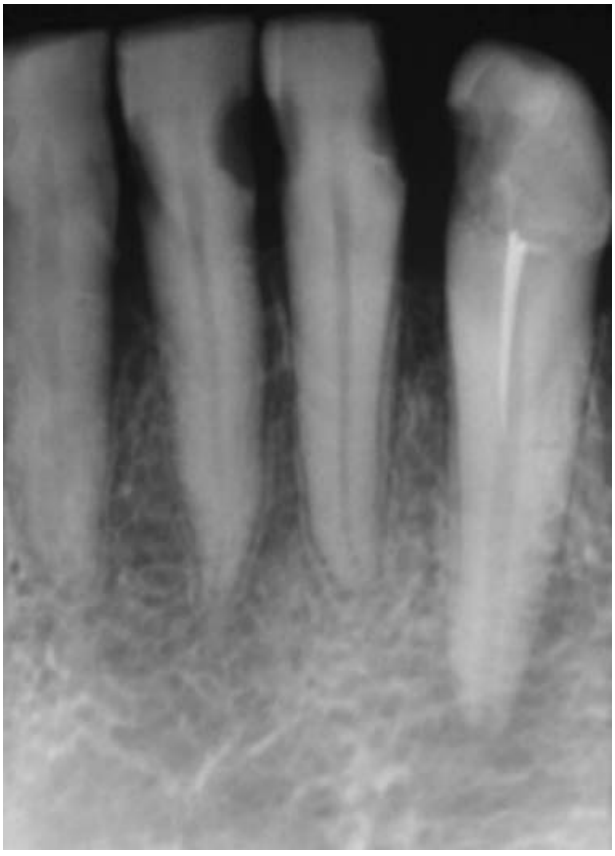


**Fig. 17. A lower canine with apical periodontitis. The root filling leaves most of the canal unfilled, also giving anaerobic bacteria an 'ecological' possibility to establish themselves in the canal microflora.**

several possible pathways for the infecting microbes to invade the filled root canal. These include caries, crown-root cracks and fractures, leaking fillings, lateral canals from the crevice area or from a periodontal pocket, and dentinal tubules exposed by removal of cementum during root planing or abrasion, through the apical foramen from a periodontal pocket extending to the apex, or via bacteremia. Inadequate asepsis during endodontic treatment also gives oral microbes a possibility to invade the root canal. Although impossible to verify by clinical studies because of ethical and practical considerations, it seems reasonable to assume that coronal leakage, in one form or another, is the main mechanism by which oral microorganisms gain access to the root canal during or after endodontic treatment.

After the introduction in the literature of the possibility of coronal leakage, numerous studies have focused on the various aspects of leakage. Wu et al. (141) showed that in many filled roots leakage of fluid occurred, but not passage of bacteria. Alves et al. (142) showed that purified endotoxin penetrated root-filled teeth faster than bacteria, while Carratu et al. (143) observed the opposite and could not show endotoxin penetration through filled root canals. With very few exceptions, bacterial leakage studies have been performed *in vitro*, studying the ability of bacteria from a coronally placed inoculum to penetrate to the apex in canals filled with various materials and techniques. Smear layer has also been studied for its role in coronal leakage. The results so far have shown either no difference (144) or better resistance to leakage in specimens where the smear layer has been removed (144, 145). Both Gram-positive and Gram-negative, motile and non-motile, aerobic, facultative, and anaerobic species have been used in the experiments. The overwhelming majority of these studies demonstrate bacterial penetration through the filled canals in 50–100% of the teeth within 2–12 weeks, regardless of root-filling materials or test bacteria used. So far, no dramatic differences between various sealers have been documented in these leakage studies. Therefore, there is little doubt that the phenomenon of bacterial invasion of the filled root canal space *in vitro*, under the circumstances described in the studies, does occur.

Because of the obvious risk for coronal leakage, the root filling must be coronally covered by a temporary or permanent filling. Khayat et al. (146) showed that when the coronal 3 mm of the root filling were removed and sealed with sticky wax, no leakage of bacteria occurred, whereas all root fillings left intact (performed with either lateral or vertical condensation) were penetrated within 30 days. Also, other studies have used sticky wax in control groups where leakage could not be detected (147); however, sticky wax is not suitable for clinical use because of its physical properties. Cavit, IRM, and zinc-oxide eugenol (ZOE) are considered to be temporary filling materials with good sealing properties. Barthel et al. (148) showed that glass-ionomer cement, alone or combined with IRM, provided better protection against bacterial leakage than Cavit or IRM alone. In another study, Cavit and Dyract resisted bacterial leakage a few days longer than IRM, but all specimens showed leakage at 2 weeks (149). Other studies using Cavit, IRM, TERM and Fermit have shown partly variable results (150, 151). In a recent study, root canals of extracted teeth were filled with either calcium hydroxide or chlorhexidine gel or

both and sealed with an IRM top filling, whereas control teeth were left unsealed. IRM considerably delayed but did not prevent bacterial penetration through the root canal. No statistically significant differences were detected between the medicament groups (152).

A potential limitation in the majority of studies on coronal bacterial leakage is their inability to quantify leakage. However, Barrieshi et al. (153) assessed bacterial leakage of a mixed anaerobic community of organisms by *F. nucleatum*, *P. micros*, and *C. rectus* in filled canals after post-space preparation. Colonization of the apical canal space was observed by scanning electron microscopy. Eighty percent of the teeth demonstrated coronal leakage of *F. nucleatum* and *C. rectus* within 90 days, with bacterial penetration occurring from 48 to 84 days. Scanning electron microscope examination showed a heterogeneous biofilm of various bacterial morphotypes at the apical canal wall.

### *Clinical relevance of coronal leakage*

As opposed to the great number of studies demonstrating coronal leakage *in vitro*, very few studies have focused on its clinical relevance. Friedman et al. (154) observed the degree of periradicular inflammation in root-filled dog teeth, 6 months after inoculating the coronally sealed pulp chamber with plaque, and compared it with teeth where no inoculation was performed. Severe inflammation was detected in histological sections in seven of 48 coronally inoculated teeth (15%) and in one of 23 non-inoculated teeth (4%) without plaque sealed in the pulp chamber. Ray & Trope (155) correlated the quality of both the root filling and the permanent coronal restoration in 1010 teeth with the periapical status as assessed from radiographs. Full-mouth radiographs from randomly selected new patient charts at Temple University Dental School (Philadelphia, PA, USA) were examined. A stronger correlation was found between the presence of a periapical lesion and poor coronal restoration than poor quality of endodontic treatment. The combination of good restoration (GR) and good endodontic (GE) quality had the highest absence of periradicular inflammation (API) at 91.4%. This was significantly higher than poor restoration (PR) combined with poor endodontic (PE) quality, with an API rate of only 18.1%. The impact of GR appeared to be greater than that of GE. Using a similar methodology, Tronstad et al. (156) evaluated the periapical status in 1001 teeth. Full-mouth series of radiographs from randomly selected patient charts at the Dental Faculty, University of Oslo (Norway) were examined. The two groups with technically GEs had the least occurrence of disease. API for the combined GE+GR was 81%, compared with 71% for GE+PR. Groups with technically PEs had significantly lower API rates, regardless of the quality of coronal restoration (PE+GR, 56% and PE+PR, 57%). In a study by Kirkevang et al. (157), a total of 614 randomly selected individuals (20 to over 60 years of age) from Aarhus County (Denmark) had a full-mouth radiographic examination. The quality of endodontic and coronal restorations and the periapical status of root-filled teeth were assessed by radiographic criteria. GR was associated with better periapical status than PR (API 52.0% vs. 36.1%). When both root filling and coronal restoration quality were assessed, API rates ranged from 68.8% (combined optimal quality) to 21.7% (all parameters scored as inadequate). In a recent study, both clinical and radiographic criteria were used to evaluate the periapical, endodontic, and coronal status of 745 root-filled teeth, randomly selected from patients attending Ghent University Dental School (Belgium) (158). Interestingly, when only clinical scoring was used to evaluate the quality of coronal restoration, API rates for GR and PR did not differ significantly (68.9% and 63.2%). However, when evaluation was based on radiographic examination, API rates for GR and PR (76.2% and 50.9%) differed significantly. The significance of the quality of coronal restoration to periapical health of root-filled teeth was also documented in two other recent studies in Canada and India (113, 159). In the former, the coronal restoration had an impact on API in teeth with PE, but not in teeth with GE, corroborating the previous findings by Tronstad et al. (156).

A different approach to the role of coronal bacterial leakage in periapical health was used by Ricucci et al. (160) and Ricucci & Bergenholtz (161), who analyzed histologically 39 roots in 32 extracted teeth, all of which had been lacking coronal restoration for a minimum of 3 months. In some specimens, the root filling had been exposed to the oral environment for several years. As assessed by radiography, apical periodontitis was associated with five roots only (12.8%). Brown and Brenn staining of longitudinal sections of 29 root specimens demonstrated the presence of

bacteria along the main canal wall as well as in the dentinal tubules in the coronal third in 28 specimens. In one specimen bacteria were seen in the apical third of the root canal, but not in the middle or coronal thirds. One of the nine root specimens where the coronal third was destroyed during extraction showed bacteria in the apical third. Although based on a relatively small sample, these two studies demonstrated that despite prolonged exposure to the oral environment and oral bacteria for several months and even years, a large-scale bacterial penetration into the filled root canal occurred only in the coronal portion of the root, while in the apical portion the histological methods used failed to disclose bacteria in the great majority of the roots.

### *Coronal leakage: future*

The evidence indicating the importance of coronal seal for the long-term outcome of endodontic therapy is quite convincing. It is reasonable, therefore, to emphasize the role of adequate, permanent coronal seal as an integral part of endodontic therapy. However, many of the important details of bacterial coronal leakage and its implications for clinical outcomes are yet to be uncovered. One of the central questions not yet answered is when to recommend retreatment of a root-filled tooth, where the root filling has been exposed directly or through leakage to oral bacteria. Our present understanding is based on epidemiological studies and indirect observations and conclusions. Because of many ethical and practical reasons, the study design addressing this research question remains a challenge. Recently, animal models were introduced for testing the effects of leakage on periradicular healing (154, 162). Areas not yet thoroughly studied are the composition of the invading microflora in coronal leakage *in vivo*, the effect of replacing only the coronal seal, without retreatment, on the contaminating microflora, and several quantitative aspects of coronal leakage in relation to apical pathosis. Nevertheless, every effort should be taken to ensure good coronal seal during and after every endodontic treatment.

## Indications for retreatment of post-treatment apical periodontitis

There is overwhelming evidence that both primary and post-treatment apical periodontitis are caused by bacteria and/or yeasts harbored in the root canal system. In periapical actinomycosis, the infective flora, mainly *Actinomyces* species, has managed to establish itself in the periapical area. In some cases of post-treatment apical periodontitis, it is possible that periapical inflammation is caused by a foreign body reaction, or may be connected to the presence of a large accumulation of cholesterol crystals (11, 12). The relative proportion of etiological reasons other than microbes, however, is low. In addition, in order to verify the diagnosis of non-microbial etiology, histopathological examination would be required. Therefore, from a practical point of view, whenever apical periodontitis has been diagnosed to affect a previously root-filled tooth, the treatment should target elimination of microbes. Consequently, the decision on new treatment (retreatment) relies on the correct diagnosis of the periapical status of the tooth. According to the *Consensus report of the European Society of Endodontology on quality guidelines for endodontic treatment* (80), healing of apical periodontitis can be observed for up to 4 years after the treatment (5 years after surgery), before taking a decision on further intervention. The prerequisite for this is that the tooth is symptom-free, and that the apical periodontitis lesion does not become enlarged in the control radiographs. With regard to differential diagnosis, it is important to identify the possibility of healing with scar tissue (163, 164), such as that may occur if both the buccal and palatal cortex have been destroyed. Such may be the case in teeth associated with large lesions or after periapical surgery (165).

In summary, post-treatment apical periodontitis is an indication for endodontic treatment, where elimination of the infection is the key for healing. Retreatment of teeth without apical periodontitis, but with an identified risk for disease development, such as an apparently deficient root filling or coronal leakage, is a more complicated issue. The canal space in many of these teeth is obviously contaminated, however, not to the extent that causes disease. In such situations, too little is known about the risk of developing infection and disease in the future, if coronal restoration is completed without first performing endodontic retreatment.

## Future strategies for eradication of the residual microflora

It should be emphasized that already today, high-quality endodontic therapy of apical periodontitis

provides very good outcomes, and healing can be expected in the great majority of teeth. Recent literature indicates, though, that in many teeth a small number of viable bacteria reside in the root canal system, particularly in the dentinal tubules, at the time of root filling. Although these and other studies suggest that these residual bacteria seldom interfere with healing, there is no doubt that theoretically, residual bacteria pose a potential threat to long-term outcomes. Therefore, in an optimal situation, complete elimination of the residual microflora remains the goal of endodontic treatment. There is presently considerable research activity on new methods and materials used for instrumentation, irrigation, disinfection, and filling of the root canal space to achieve more predictably complete elimination of root canal infection and to prevent reinfection. These include new irrigating solutions (166, 167), combinations of disinfecting agents (168, 169), and new materials added to or replacing existing root-filling materials (170). Inasmuch that some of these innovations may lead to improved outcomes, it is likely that high quality in the various steps of the conventional endodontic treatment remains the key factor in maximizing success in the prevention and healing of apical periodontitis.

## Concluding remarks

In the treatment of apical periodontitis, instrumentation and irrigation with antibacterial solutions dramatically reduce the number of viable bacteria in the infected root canal, but cannot predictably render the canal bacteria-free. Residual infection consists mostly of facultative Gram-positive rods and cocci. They are located in the unprepared parts of the main canal system, as well as in the dentinal tubules. Intracanal medication between treatment appointments further reduces the number of microbes and contributes to the increase of the number of bacteria-free canals. Coronal leakage, either during the treatment (because of poor asepsis) or through a leaking restoration after the treatment, is another possibility for bacteria to enter the root canal. The persisting infection or reinfection can potentially prevent healing or initiate the development of apical periodontitis. The clinical consequences of the presence of bacteria in the filled root canal depend on their possibility to interact with the host's periapical tissues. Although excellent treatment outcomes can already be achieved with today's techniques and materials, future developments in these areas will hopefully further improve our possibilities to eliminate predictably intracanal infection and prevent reinfection, and thus prevent or heal apical periodontitis.

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