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Eradication of endodontic infection by instrumentation and irrigation solutions

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Debridement of the root canal by instrumentation and irrigation is considered the most important single factor in the prevention and treatment of endodontic diseases. In clinical practice, instrumentation of the root canal(s) within the affected tooth is usually the most time consuming and technically demanding element of the treatment. The technical success of the treatment, as judged by the post-operative radiograph after the root filling, is based on optimized root canal instrumentation. Mounting evidence from epidemiological research is also indicating that the combination of high-quality coronal restoration and technically satisfactory root canal treatment is associated with the greatest long-term prognosis. Therefore, it is not surprising that for several decades of endodontic research, a substantial number of articles on instruments and instrumentation have been published in the scientific literature. Although interest in the effects of instrumentation on intracanal infection is not new, it is obvious that during the last few years a renewed focus of interest has appeared on the relationship between instrumentation and infection control in the root canal. The ongoing discussion in international endodontics about one-appointment therapy in the treatment of apical periodontitis has naturally further motivated the newly emerged research activities. The goal of this review is to gather the relevant and most recent literature and provide an updated analysis of the effect of preparation (instrumentation and irrigation) on the microbial infection in the necrotic root canal.

Microbial etiology of pulpitis and apical periodontitis

While various chemical and physical irritants can cause irritation and even necrosis of the pulp, the most common causes for pulpal inflammation (pulpitis) are bacteria and/or their products entering the pulp through a deep caries lesion or a leaking filling, e.g. 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 carious lesion has not entered the pulp, the pulpal inflammation is likely to be reversible. However, when the carious lesion does reach the pulp and the hard tissue barrier is breached, bacteria can invade the pulp. Even after this point, the infection may remain relatively superficial and most of the pulp tissue is vital and bacteria free. For this reason, endodontic treatment of pulpitis should be considered to be treatment of an inflammation and prevention of an infection.

In apical periodontitis, bacteria invade further and colonize the entire root canal system. Apical periodontitis is an inflammatory process in the periradicular tissues caused by microorganisms in the necrotic root canal (4–6). Accordingly, to promote healing of apical periodontitis, microorganisms within the root canal system must be eliminated. Several studies have indicated that the prognosis of apical periodontitis after root canal treatment is poorer if viable microorganisms are present in the canal at the time of the root filling (7–9). However, some other studies have failed to show significant differences in healing between teeth filled after obtaining positive or negative

cultures from the root canal (10), as well as between treatments finished in one or two appointments (10, 11). Nevertheless, there is a general agreement that successful elimination of the causative agents in the root canal system is the key to health (12).

General strategy of infection control

In most parts of the human body, elimination of opportunistic infections is accomplished by the action of the host defense system alone, sometimes helped by a systemic antibiotic therapy. In this regard, elimination of endodontic infection is quite different from most other sites in the human body. Host measures that are sufficient to eliminate the infectious organisms in other sites do not suffice for complete elimination of endodontic infections, mainly because of the special anatomy, and physiology, of the tooth and the root canal.

Therefore, control of an endodontic infection is based on a joint effort by several host and treatment factors (13). Success in all aspects of this cooperation will best guarantee elimination of the infection and healing of the apical lesion. The necessary elements in the control of endodontic infection are: host defense system, systemic antibiotic therapy (only occasionally and with special indications), instrumentation and irrigation, locally used intracanal medicaments between appointments, root canal filling, and coronal restoration (13). Thus, it is important to bear in mind that although instrumentation together with irrigation is the focus of this review, they are part of a concerted effort to control infection. The role of the other factors in infection control has been reviewed recently in detail (13): in this article, the focus will be on instrumentation and irrigation.

Composition of flora and localization of bacteria in endodontic infections

The composition of the flora as well as the localization of microorganisms in the necrotic root canal are affected by several local factors: the amount of oxygen in the root canal (redox potential), access to and availability of nutrients, bacterial synergism and competition, and the host's defense system. In primary apical periodontitis, the ecological selection in the canal favors strictly anaerobic bacteria, which clearly constitute the majority in these infections (5, 6, 14, 15). The infection may be purely anaerobic, but the anaerobes are, in many cases, accompanied by microaerophilic and facultative bacteria, such as *Actinomyces* spp., *Lactobacillus* spp., and streptococci (5, 6, 14, 15). In previously root-filled teeth with apical periodontitis, the ecology may be quite different, and in many cases the environment no longer supports the dominance of anaerobic bacteria. The most frequently isolated species by far in previously root-filled teeth with apical periodontitis is *Enterococcus faecalis*, but several other facultative and even anaerobic bacteria are often isolated (16–23). While monoinfections are not detected in primary apical periodontitis, *E. faecalis* is often found in pure culture in previously root-filled teeth with apical periodontitis. However, *E. faecalis* is often found together with streptococci, lactobacilli, other facultative bacteria, and also with anaerobic bacteria. Gram-negative enteric rods (e.g. coliforms and *Pseudomonas* spp.) and yeasts are found almost entirely only in previously root-filled teeth with apical periodontitis (16–24).

The ecological niches in the necrotic root canal have not been thoroughly studied. Because of the absence of a cell-mediated defense, such as phagocytosis and a functioning immune defense system in the necrotic pulp, the localization of the residing microorganisms is mainly affected by the redox potential and availability of nutrients in the various parts of the root canal (25, 26).

Although no exact data are available, it is likely that the majority of bacteria in most primary root canal infections are located in the main root canal, while a minority of the cells would have invaded further into the dentinal tubules and lateral canals. In previously root-filled teeth with apical periodontitis, the situation may be somewhat different, depending on the quality and length of the root filling.

As long as the infective microorganisms are residing in the main canal, they can be directly targeted by instrumentation and irrigation. However, in many instances, bacteria have penetrated from the main root canal into dentinal tubules, lateral canals, and other canal irregularities. The diameter of dentinal tubules is large enough to allow bacterial penetration. Numerous studies have shown that dentine invasion occurs in ca. 50–80% of the teeth with apical periodontitis (27–32). The invading bacteria are dominantly Gram-positive facultative and anaerobic cocci and rods, but Gram-negative species have also been reported (33–35). The

invasion seems to occur at random, i.e. a dentinal tubule filled with invading bacteria is typically surrounded by several empty tubules (30, 36). Invasion does not seem to be dependent on bacterial mobility; on the contrary, the best invaders, enterococci, streptococci, *Actinomyces* spp., and most lactobacilli, are non-motile species. It has also been indicated that the invasion is more effective at the coronal and middle portion of the root canal (37). However, root surface resorption and thus loss of cementum, which is often present at the root apex in chronic apical periodontitis, facilitate bacterial penetration into dentine, and invasion through the whole thickness of the root can be seen (36). A more detailed review of the bacterial invasion into dentinal tubules can be found in a previous issue of this journal (13). Bacteria that have penetrated deeper into the tooth structure, lateral canals, and dentinal tubules are obviously more difficult to reach directly by instrumentation (38). Recently, Matsuo et al. (35) showed bacterial invasion into dentinal tubules in 70% of 40 teeth extracted after a diagnosis of apical periodontitis. After instrumentation of the root canals, the frequency of bacteria found in the dentinal tubules was almost equally high, as dentinal invasion by bacteria could still be shown in 65% of the teeth. Nevertheless, adequate instrumentation does play a key role in facilitating the elimination or control of dentine infection.

Finally, there are reports of a biofilm formation by root canal bacteria on the external root surface (39–41). From the point of view of chemomechanical preparation, a biofilm certainly creates a great challenge for effective infection control.

The goal of endodontic treatment

In the great majority of teeth requiring root canal treatment, the goal is either prevention or treatment of apical periodontitis (42), or more precisely, prevention or elimination of a microbial infection in the root canal system. It is clear that in some special situations, such as resorptions and endodontic complications, there may be a variety of intermediate goals, but even then the final success is usually dependent on successful infection control. There is a widely accepted view that cleaning and shaping of the root canal system is the most important step toward sterility of the canal. In this review, the microbiological success and failure of cleaning and shaping will be critically evaluated in light of the relevant literature.

Instrumentation of the root canal

Technical and biological goals of instrumentation

Technically, the goal of instrumentation and irrigation is to remove all necrotic and vital organic tissue as well as some hard tissue from the root canal system, and give the canal system a shape that allows easy debridement and predictable placement of locally used medicaments and a permanent root filling of high technical quality. (Micro)biologically, the goal of instrumentation and irrigation is to remove and/or kill all microorganisms in the root canal system, and neutralize any antigenic/biological potential of the microbial components remaining in the canal. If this goal could be predictably achieved at the first appointment, most treatments could be finished in one visit, if only the time available would allow it. In cases where this (complete eradication of root canal microorganisms) cannot be achieved, instrumentation and irrigation are aimed at creating optimal conditions for the placement of an antibacterial interappointment dressing to enhance disinfection of the canal.

Effect of manual instrumentation on root canal bacteria

Mechanical instrumentation is the core method for bacterial reduction in the infected root canal. Byström & Sundqvist (43) measured the reduction in bacterial counts cultured from infected canals by instrumentation with hand stainless-steel instruments under irrigation with physiological saline solution. Fifteen root canals with necrotic pulps and periapical lesions were instrumented at five sequential appointments. The access cavity was sealed between the appointments with a bacteria-tight temporary filling, but the canals were left empty with no antibacterial dressing. This procedure caused a substantial reduction in bacterial numbers, usually 100–1000-fold, but achieving bacteria-free root canals proved difficult. After five appointments, seven of the 15 root canals still contained cultivable bacteria (43). The relatively limited antibacterial efficiency of mechanical prepara-

tion was also reported by Ørstavik et al. (44). In fact, Cvek et al. (45) had already compared the antibacterial effect of biochemical root canal cleansing in permanent non-vital maxillary incisors with immature apices with those with mature roots. The material comprised three groups (34, 46, and 28 teeth), in which mechanical cleansing was accompanied by irrigation with sterile saline and 0.5% or 5.0% sodium hypochlorite (NaOCl) solutions. Samples were taken in root canals initially after removal of necrotic tissue and after completed cleansing. The antibacterial effect of mechanical cleansing with sterile saline was reported to be very low and limited to the teeth with mature root. NaOCl increased the antibacterial effect as compared with saline irrigation. Interestingly, no statistical difference was found in the antibacterial effect between 0.5% and 5.0% NaOCl solutions. The authors concluded that mechanical cleansing of root canals in teeth with immature root with the instruments then available was inadequate. This inadequacy could not be compensated for by use of even a concentrated solution of NaOCl.

Dalton et al. (46) compared intracanal bacterial reduction in 48 patients on teeth instrumented either with 0.04 tapered nickel–titanium (NiTi) rotary instrumentation or with a stainless-steel K-file stepback technique with saline irrigation. The canals were sampled before, during, and after instrumentation. Teeth with apical periodontitis all harbored cultivable bacteria at the beginning, whereas vital control teeth, diagnosed with irreversible pulpitis, were sterile.

A similar reduction in bacterial counts was observed with progressive enlargement with both techniques. At the end of the preparation, only 28% of the teeth were bacteria free, and viable bacteria could be cultured from 72%. Siqueira et al. (47) also demonstrated a poor antibacterial effect of instrumentation combined with saline irrigation in a group of teeth enlarged manually with NiTi flex K-files to apical size #40. However, the results indicated that increasing the size of apical preparation from #30 to #40 resulted in a significant reduction in the numbers of cultivable bacteria. In a study by Pataky et al. (48), the antimicrobial efficacy of various root canal hand preparation techniques and instruments was compared in 40 human first maxillary premolars extracted for orthodontic reasons. Teeth were sterilized, and the root canals were then infected with *E. faecalis* for 24 h. Teeth were then instrumented using saline irrigation. Samples were taken for culture before and after the root canal preparation. A considerable reduction in bacterial counts was measured after each type of preparation; however, none of the teeth was sterile at the end of the preparation and saline irrigation. It may be noteworthy, although, that a stepback technique was used in the preparation, the master apical file size being #25, and the largest instrument used for preparation was #40 (48).

Taken together, these studies demonstrate that mechanical preparation with hand instruments and irrigation with saline cannot predictably eliminate the bacteria from the infected root canals. Keeping in mind the present knowledge about the frequency of bacterial invasion into dentinal tubules and the lateral canals from the main root canal, the complexity of the root canal system in most teeth, the physical limitations of metal (steel or NiTi) instruments, and the insignificant antibacterial activity of saline, it would in fact be quite surprising if these studies showed high numbers of sterile root canals. Moreover, with regard to the limitations of sampling from the root canal, it is possible that the true frequency of canals with viable microorganisms is actually higher than that reported (see also the paper by Siqueira, this issue). Therefore, the focus of interest concerning the antibacterial efficiency of instrumentation and irrigation has been on the use of irrigating solutions with strong antibacterial activity as the necessary supplement to mechanical preparation.

Antibacterial irrigating solutions

NaOCl

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. Irrigants can prevent packing of the infected hard and soft tissue apically in the root canal and into the periapical area. NaOCl is the most widely used irrigating solution. In water, NaOCl ionizes to produce Na^+ and the hypochlorite ion, OCl^-, which establishes an equilibrium with hypochlorous acid, HOCl. Between pH 4 and 7, chlorine exists predominantly as HClO, the active moiety, whereas above pH 9, OCl^- predominates (49). Hypochlorous acid has long been considered the active moiety responsible for bacterial

inactivation by chlorine-releasing agents, the OCl^- ion having a minute effect compared with undissolved HOCl. This correlates with the observation that the activity of NaOCl is greatest when the percentage of undissolved HOCl is highest (49). Hypochloric acid has been found to disrupt oxidative phosphorylation and other membrane-associated activities (50). It has also been indicated that DNA synthesis is sensitive to HOCl (51).

NaOCl is used in concentrations varying from 0.5% to 5.25%; it is a potent antimicrobial agent, and effectively dissolves pulpal remnants and organic components of dentine. It is used both as an unbuffered solution at pH 11 in concentration 0.5–5.25%, and buffered with bicarbonate buffer (pH 9.0) usually as a 0.5% solution (Dakin's solution) (49). Contradicting earlier statements, Zehnder et al. (52) reported that buffering had little effect on tissue dissolution, and Dakin's solution was equally effective on decayed (necrotic) and fresh tissues. In addition, no differences were recorded for the antibacterial properties of Dakin's solution and an equivalent unbuffered hypochlorite solution.

NaOCl is best known for its strong antibacterial activity; it kills bacteria very rapidly even at low concentrations. Waltimo et al. (53) showed that the resistant microorganism, *Candida albicans*, was killed *in vitro* in 30 s by both 5% and 0.5% NaOCl, whereas concentrations 0.05% and 0.005% were too weak to kill the yeast even after 24 h of incubation. The high susceptibility of *C. albicans* to NaOCl was recently also verified by Radcliffe et al. (54). However, Vianna et al. (55) contrasted these results partly, as 0.5% NaOCl required 30 min to kill *C. albicans*, whereas 5.25% solution killed all yeast cells in 15 s. Gomes et al. (56) tested *in vitro* the effect of various concentrations against *E. faecalis*. The microbe was killed in less than 30 s by the 5.25% solution, while it took 10 and 30 min for complete killing of the bacteria by 2.5% and 0.5% solutions, respectively. The clearly higher resistance to hypochlorite by *E. faecalis* as compared with the yeast *C. albicans* was confirmed by Radcliffe et al. (54). Recent laboratory experiments using three Gram-negative anaerobic rods typically isolated from primary apical periodontitis, *Porphyromonas gingivalis*, *P. endodontalis*, and *Prevotella intermedia* demonstrated high susceptibility to NaOCl, and all three species were killed within 15 s with all concentrations tested (0.5–5%) (55).

The three main differences between the conditions of *in vitro* and *in vivo* studies are the high volume of the medicament available for killing, direct access to all microbes, and absence of other materials in the *in vitro* experiments that potentially protect bacteria *in vivo*. *In vivo* studies have failed to show a better antibacterial effect in the root canal by highly concentrated hypochlorite solutions as compared with low concentrations. Byström & Sundqvist (57, 58) showed that although 0.5% NaOCl, with or without ethylene-diammine-tetra-acetic acid (EDTA), improved the antibacterial efficiency of preparation compared with saline irrigation, all canals could not be rendered bacteria free even after several appointments. The same authors could not show any significant difference in antibacterial efficiency *in vivo* between 0.5% and 5% NaOCl solutions. Siqueira et al. (59) also demonstrated the superior antibacterial affect against root canal bacteria of hypochlorite in comparison with physiological saline. However, similar to Byström & Sundqvist (58), the latter study showed no difference among 1%, 2.5%, and 5% NaOCl solutions. It should be noted that in the study by Byström & Sundqvist (58), the root canal flora was mixed anaerobic, which may partly explain why no difference was found between different NaOCl concentrations. However, in the study by Siqueira et al. (59), the test organism used to infect dentine (*in vitro*) was *E. faecalis*.

The literature about the antibacterial effect of NaOCl against root canal bacteria describes mostly *in vitro* studies performed in a test tube, in the root canals of extracted teeth, or in prepared dentine blocks infected with a pure culture of one organism at a time. The *in vivo* studies, on the other hand, have focused on the elimination of microorganisms from the root canal system in teeth with primary apical periodontitis. However, Peciuliene et al. (19) studied the effect of instrumentation and NaOCl irrigation in previously root-filled teeth with apical periodontitis. Existing root fillings were removed with endodontic hand instruments and chloroform was not used to avoid a negative effect on microbial viability. After the first microbiological sample, the canal was cleaned and shaped with reamers and Hedström files, using 2.5% NaOCl (10 mL per canal) and 17% buffered EDTA (pH 7, 5 mL) as irrigating solutions. All canals were prepared to size #40 or larger. Chemomechanical instrumentation was completed at the same appointment in all cases. The canals were dried with paper points and a second

microbiological sample was taken from all teeth. Bacteria were isolated in 33 of the 40 teeth examined before the instrumentation: *E. faecalis* was found in 21 teeth (in 11 teeth as a pure culture), yeast *C. albicans* in six teeth, Gram-negative enteric rods in three teeth, and other microbes in 17 teeth (19). While no enteric Gram-negative rods or yeasts were found in the second sample after the preparation and irrigation, *E. faecalis* still persisted in six root canals. Other microbes were found in five canals after preparation. Although not known with certainty, the disappearance of yeasts and the persistence of *E. faecalis* in the root canals in this study may reflect the results of the above-mentioned *in vitro* studies (53, 54, 56), which indicated that *E. faecalis* is much more resistant to killing by NaOCl than *C. albicans* and Gram-negative rods.

NaOCl has been criticized for its unpleasant taste, relative toxicity, and its inability to remove smear layer (60, 61). It is also clear that the *in vivo* effectiveness of NaOCl in the root canal against the infecting microflora is somewhat disappointing in light of the more promising *in vitro* results, which show killing of practically all microorganisms in a few seconds, when concentrated solutions are used. One natural explanation to poorer *in vivo* performance is root canal anatomy, in particular, the difficulty in it reaching the most apical region of the canal with large volumes of fresh irrigant. However, it should not be forgotten that the chemical milieu in the canal is quite different from a simplified test tube environment. Marcinkiewicz et al. (62) showed that nitrite prevented HOCl-mediated bacterial killing. Haapasalo et al. (63), using dentine powder, showed that the presence of dentine caused marked delays in the killing of the test organism, *E. faecalis*, by 1% NaOCl.

Pashley et al. (64) compared the biological effects of mild and strong NaOCl solutions and demonstrated greater cytotoxicity and caustic effects on healthy tissue with 5.25% NaOCl than with 0.5% and 1% solutions. Chang et al. (65) also showed the relationship between the concentration and cytotoxicity of NaOCl. Therefore, it might be recommended to use 0.5–1% NaOCl for canal irrigation instead of the 5.25% solution. However, evidently, more *in vivo* research on persistent endodontic infections and retreatment is required to obtain a better understanding of the relationship between NaOCl concentration and its antimicrobial activity against specific microorganisms, before final conclusions can be drawn.

Chlorhexidine (CHX)

While NaOCl kills bacteria quite effectively, it is caustic if accidentally expressed into the periapical area or adjacent structures such as the maxillary sinus (66). In addition, the active chlorine in the solution may damage patients' clothing through its strong bleaching effect. Therefore, there has been an ongoing search for alternative irrigating solutions that could replace NaOCl.

CHX is probably the most widely used biocide in antiseptic products in general. It is able to permeate the cell wall or outer membrane and attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. High concentrations of CHX cause coagulation of intracellular constituents (49).

CHX gluconate has been in use for a long time in dentistry because of its antimicrobial properties, its substantivity, and its relatively low toxicity. Despite the advantages of CHX, its activity is pH dependent and is greatly reduced in the presence of organic matter (67). It has a wide antimicrobial spectrum and is effective against both Gram-positive and Gram-negative bacteria as well as yeasts, while mycobacteria and bacterial spores are resistant to CHX (68, 69).

CHX is not considered to be an effective antiviral agent, and its activity is limited to lipid-enveloped viruses (70). In direct contact with human cells, CHX is cytotoxic; a comparative study using fluorescence assay on human PDL cells showed corresponding cytotoxicity with 0.4% NaOCl and 0.1% CHX (65). Its potential and use in endodontics have been under active research over the last few years. 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 (71–73).

However, an *in vitro* study by Gomes et al. (56) demonstrated marked differences in the killing of enterococci by CHX and NaOCl. Only the highest concentration of 5.25% of NaOCl killed *E. faecalis* rapidly in 30 s, while with a lower concentration, (4–0.5%) 5–30 min were required for complete killing to occur. CHX digluconate, on the other hand, killed *E. faecalis* cells in 30 s or less in concentrations of 0.2–2%. The result was later supported by Oncag et al. (74) and Vianna et al. (55), who also showed *in vitro* CHX to be superior to NaOCl in killing of *E. faecalis* and *Staphylococcus aureus*. The same study revealed that

CHX in a gel form required a much longer time to kill *E. faecalis* than the corresponding concentration in a liquid.

Waltimo et al. (53) studied the antifungal effect of combinations of endodontic irrigants including CHX. CHX effectively killed *C. albicans*, which is in accordance with previous studies that have shown that CHX is an effective antifungal agent *in vitro* (75–77). Waltimo et al. (53) also found that the combinations of disinfectants were equally or less effective than the more effective component when used alone.

Heling & Chandler (71) studied the antimicrobial effect of irrigant combinations within dentinal tubules *in vitro* against *E. faecalis* and found that a specific combination of 3% hydrogen peroxide (H_2O_2) and CHX was superior in its antibacterial activity in dentine compared with other regimens such as CHX alone and NaOCl. These studies were continued in a series of *in vitro* experiments by Steinberg et al. (78), who challenged *E. faecalis* suspensions in trypticase soy broth (a culture medium rich in peptides) with various combinations of CHX and H_2O_2. The experiments demonstrated that the combination of the two substances totally killed *E. faecalis* in concentrations much lower than each component alone. According to that study, the bactericidal effect of CHX derives from its ability to denaturate the bacterial cell wall while forming pores in the membrane, while H_2O_2 is effective against intracellular organelles such as DNA. Although the exact synergistic mechanism of CHX and H_2O_2 is not known, it can be postulated that the exposure of bacteria to CHX leads to a more permeable cell wall that H_2O_2 can penetrate easily and hence damage the intracellular organelles (78). Corresponding synergistic effects were not detected between H_2O_2 and NaOCl in the dentine block model (71).

Dona et al. (79) showed that the combination of CHX and H_2O_2 was a more effective antiplaque mouth rinse than either component alone. There are no reports of clinical studies where the combinations of CHX and H_2O_2 have been used to disinfect the root canal system in cases of primary apical periodontitis or persistent endodontic infections. However, cytotoxicity of the medicament combinations should first be investigated. Interestingly, combinations of CHX and carbamide peroxide have been shown to be additive in their cytotoxicity (80).

A potential weakness of CHX in the root canal may be its susceptibility to the presence of organic matter (67). In an *in vitro* study, Haapasalo et al. (63) showed that the effect of CHX is reduced, although not prevented, by the presence of dentine. Portenier et al. (81) demonstrated total loss of activity of CHX by bovine serum albumin. This might indicate the possibility that inflammatory exudate, rich in proteins such as albumin, entering the root canal through the apical foramen, may weaken the antibacterial effect of CHX. In a separate study, Portenier et al. (82) showed further that CHX was strongly inhibited by dentine matrix (the organic component of dentine) as well as heat-killed cells of *E. faecalis* and *C. albicans*. It is quite possible that inhibitions like the ones described in these studies can partly explain the poorer *in vivo* performance of CHX in the root canal as compared with killing experiments *in vitro* in a test tube environment.

CHX lacks the tissue-dissolving ability, which is one of the obvious benefits of NaOCl. While the *in vitro* studies have demonstrated the antibacterial effect of CHX against *E. faecalis* to be superior to that of NaOCl, there are no *in vivo* studies yet available that would confirm the better activity of CHX against this resistant species also in the infected root canal. Nevertheless, there is no doubt that CHX gluconate, in concentrations between 0.2% and 2%, offers a good alternative for root canal irrigation with potent antimicrobial activity. Future studies of CHX combinations are needed to establish whether these could give additional advantage in the fight against resistant root canal microbes.

Irrigation with iodine compounds

Iodine compounds have been used for decades for disinfection of surfaces, skin, and operation fields. Although iodine is less reactive than chlorine (e.g. NaOCl), it is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal (83). Aqueous iodine solutions are rather unstable; in solution, several iodine species are present, with molecular iodine (I_2) being mostly responsible for the antimicrobial activity (83). This was the reason for the development of iodophors ('iodine carriers'): povidone–iodine and poloxamer–iodine. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active 'free' iodine (83). Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures (alcoholic solutions of iodine). The antimicrobial

action of iodine is rapid, even at low concentrations, but the exact mode of action is not fully known. Iodine penetrates into microorganisms and attacks key groups of cell molecules, such as proteins, nucleotides, and fatty acids, resulting in cell death (83, 84).

In endodontics, iodine potassium iodide (IPI) has been the final component of the classical tooth surface disinfection sequence, as described by Möller (85). Potassium iodide is needed to dissolve iodine in water, but it is the iodine that accounts for the antimicrobial activity of the mixture. Recently, Ng et al. (86) compared the effectiveness of 2.5% NaOCl or 10% iodine for decontamination of the operation field (tooth, rubber dam, and retainer) by using bacterial cultivation and polymerase chain reaction (PCR). The operation field was disinfected with 30% H_2O_2, followed by 10% iodine or 2.5% NaOCl, before and after access cavity preparation. The authors reported that there was no significant difference in the recovery of cultivable bacteria from various sites in either group. In contrast, PCR detected bacterial DNA significantly more frequently from the tooth surfaces after iodine (45%) than after NaOCl (13%) decontamination. Molander et al. (87) investigated the effect of pretreatment of the root canal with 5% IPI before filling the canals with calcium hydroxide in teeth with apical periodontitis. The authors suggested that pretreatment irrigation with IPI from a quantitative point of view did not seem to add antimicrobial power, but it might reduce the frequency of persisting strains of *E. faecalis*. Peciuliene et al. (19) studied the effect of iodine irrigation in 20 teeth with previously root-filled canals and apical periodontitis. Existing root fillings were removed with endodontic hand instruments without using chloroform. The canals were prepared to size #40 or larger with reamers and Hedström files, irrigating with 2.5% NaOCl (10 mL per canal) and 17% neutral EDTA (5 mL). Bacteria were isolated in 16 of the 20 teeth before the instrumentation, and in five teeth after the instrumentation and irrigation, three of the five canals contained *E. faecalis* in pure culture and one in mixed culture. After 5 min irrigation with IPI (2% iodine in 4% potassium iodide), the third sample taken after neutralizing the antibacterial activity of IPI with sodium thiosulphate revealed growth in only one canal. The only persisting case was *E. faecalis* in pure culture.

Similar to other root canal irrigants with disinfecting activity, iodine compounds in the root canal face a complex chemical milieu, which can potentially affect their antimicrobial potential. Haapasalo et al. (63) demonstrated that dentine powder effectively abolished the effect of 0.2/0.4% IPI against *E. faecalis*. This was later confirmed by Portenier et al. (81), who also showed that unlike dentine, corresponding amounts of hydroxyl apatite and bovine serum albumin had little or no effect on the antibacterial activity of IPI. In another study, Portenier et al. (82) showed the inhibitory effect on 0.2/0.4% IPI by dentine, (organic) dentine matrix, and heat-killed cells of *E. faecalis* and *C. albicans*. Taken together with the difficulty to effectively irrigate apical canal segments, inhibition of iodine by substances present in the root canal makes it easier to understand the failure to predictably disinfect the root canal completely by iodine compounds.

EDTA and citric acid

EDTA (17%, disodium salt, pH 7) has little if any antibacterial activity. On direct exposure for extended time, EDTA extracts bacterial surface proteins by combining with metal ions from the cell envelope, which can eventually lead to bacterial death. EDTA is an effective chelating agent, which is widely used in endodontic preparation (88). It effectively removes smear layer by chelating 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 (30, 89). Niu et al. (90) studied the ultrastructure on canal walls after EDTA and combined EDTA plus NaOCl irrigation by scanning electron microscopy: more debris was removed by irrigation with EDTA followed by NaOCl than with EDTA alone.

In addition to EDTA, citric acid can also be used for irrigation of the root canal to remove the smear layer (88, 91, 92). Concentrations ranging from 1% to 50% have been used (91). Gutmann et al. (93) showed that 10% citric acid was more effective in removing the smear layer from apical root-end cavities than ultrasound. Yamaguchi et al. (94) compared the chelating and antibacterial properties of citric acid and EDTA. Powdered dentine–resin mixture was found to be more soluble in a 0.5, 1, and 2 M citric acid solutions than in a 0.5 M EDTA solution. Citric acid solution showed antibacterial effects on all 12 root canal bacteria tested.

However, Liolios et al. (95) reported better removal of smear layer by commercial EDTA preparations than with 50% citric acid. Di Lenarda et al. (96) and Scelza et al. (97) reported a minor or no difference in smear layer removal with citric acid and 15% EDTA. In a recent study, Machado-Silveiro et al. (98) measured the demineralization capability of 1% and 10% citric acid, 10% sodium citrate, and 17% EDTA during immersions of 5, 10, and 15 min on root canal dentine. Ten percent citric acid was more effective than 1% citric acid, which was more effective than EDTA. Takeda et al. (99) studied the effects of three endodontic irrigants and two types of laser on a smear layer created by hand instrumentation *ex vivo* in the middle and apical thirds of root canals. Irrigation with 17% EDTA, 6% phosphoric acid and 6% citric acid did not remove the entire smear layer from the root canal system. In addition, these acidic solutions demineralized the intertubular dentine around tubular openings, which became enlarged. The CO_2 laser was useful in removing and melting the smear layer on the instrumented root canal walls, and the Er : YAG laser was the most effective in removing the smear layer from the root canal wall.

Removal of the smear layer is an important step to facilitate disinfection of the root canal. Both EDTA and citric acid can effectively remove the smear layer created during canal instrumentation. Although citric acid may also have an antibacterial effect, this has not been compared with other root canal disinfecting agents in *in vitro* or *in vivo* studies.

H_2O_2

H_2O_2 is a widely used biocide for disinfection and sterilization (49). It is a clear, colorless liquid that is used in a variety of concentrations in dentistry, ranging from 1% to 30%. H_2O_2 is environmentally non-problematic, as it degrades into water and oxygen. H_2O_2 solutions are quite stable, but they may contain stabilizers to prevent decomposition. H_2O_2 is active against viruses, bacteria, yeasts, and even bacterial spores (100). It has greater activity against Gram-positive than Gram-negative bacteria. Production of catalase or superoxide dismutase by several bacteria can afford those species some protection against H_2O_2. H_2O_2 produces hydroxyl free radicals (• OH), which attack several cell components such as proteins and DNA (49).

In endodontics, H_2O_2 has long been used because of its antimicrobial and cleansing properties. Möller (85) recommended 30% H_2O_2 as the first step (after mechanical cleaning) in tooth surface disinfection. Potent H_2O_2 solution will affect the organic matter on the tooth in such a way that the disinfectants, such as iodine, will more effectively kill the microbes. It has been particularly popular in cleaning the pulp chamber from blood and tissue remnants, but it has also been used in canal irrigation. However, there are much less research reports about the effectiveness of H_2O_2 in the root canal than of other disinfectants. Siqueira et al. (101) showed that a combination of NaOCl and H_2O_2 was no more effective against *E. faecalis* in contaminated root canals *ex vivo* than NaOCl alone. Heling & Chandler (71) compared the antibacterial effect of CHX and H_2O_2 in various concentrations against *E. faecalis*-infected dentine. CHX proved to be superior in its antibacterial effect; however, a combination of the two medicaments at low concentration was far more antibacterial than any other tested medicament alone. A similar synergistic effect was not measured with a combination of H_2O_2 and NaOCl (71). The synergism between H_2O_2 and CHX was subsequently verified by Steinberg et al. (78).

In a recent study by Möller et al. (102) in monkey teeth, 10% H_2O_2 was used as part of the irrigating protocol. A total of 186 root canals in 176 teeth were inoculated with preselected combinations of bacteria for several months: group 1, anaerobes and streptococci; group 2, *E. faecalis*+group 1. The first bacteriological sample was taken before preparation taking great care to avoid contamination. The root canals were treated according to a standardized protocol: mechanical instrumentation by hand files to size #40–#60, with irrigation with buffered 1% NaOCl solution, followed by 10% H_2O_2. The procedure was completed by rinsing with NaOCl solution. This solution was subsequently inactivated with 5% sodium thiosulpfate solution in the root canal. A second bacteriological sample was then taken. In group 1 (160 canals), bacteria were found in 98% and 68% of the canals in samples 1 and 2, respectively. In group two (24 canals), the corresponding frequencies were 100% and 88%. Although the bacterial counts were greatly reduced, it is correct to conclude that the protocol used could not predictably produce sterile root canals in monkey teeth (102).

Although H_2O_2 has long been used in disinfection and canal irrigation in endodontics, the available

literature does not support its use over that of other irrigating solutions. However, it has a role in tooth surface disinfection, and the potential usefulness of the synergistic effect with CHX has not yet been fully evaluated.

MTAD

MTAD (a *m*ixture of *t*etracycline isomer, *a*cid, and *d*etergent, Biopure, Tulsa Dentsply, Tulsa OK, USA) is a new product in the quest for a better root canal irrigant, with a pH as low as 2.15 (103, 104). Although many of the existing root canal-irrigating solutions have a number of positive effects in the canal, all of them also have weaknesses. Therefore, in order to maximize the benefits of irrigation, several different solutions must be used during the preparation, in varying volumes and time. In addition, although poorly studied, there is a general uncertainty about the efficiency of irrigation in the narrow, most apical part of the canal.

MTAD consists of doxycycline, citric acid, and the detergent Tween-80 (103). In that study with this new irrigant, focusing on the removal of smear layer, 48 extracted single-rooted teeth were prepared by using passive stepback and rotary 0.04 taper NiTi files. Distilled water or 5.25% NaOCl was used for irrigation followed by a 5 mL irrigation with one of the following: sterile distilled water, 5.25% NaOCl, 17% EDTA, or MTAD. The effect on the smear layer and the amount of erosion on the root canal walls at the coronal, middle, and apical portion were examined using a scanning electron microscope. The results indicated that MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules, when canals are first irrigated with NaOCl, followed by a final rinse of MTAD (103). EDTA caused more erosion of dentine in the coronal and middle parts of the canal than MTAD. In the apical third, canals irrigated with MTAD (final irrigation) were cleaner, as judged from scanning electron micrographs, compared with final irrigation with EDTA (103). In another study, the same group investigated the effect of various concentrations of sodium NaOCl as an intracanal irrigant before irrigation with MTAD as a final rinse on the smear layer. The results showed that MTAD removed most of the smear layer when used alone; however, remnants of the organic component of the smear layer could be detected on the root canal walls. There were no significant differences between the ability of 1.3%, 2.6%, and 5.25% NaOCl as root canal irrigants and MTAD as a final rinse to remove the smear layer. All combinations removed both the smear layer as well as the organic remnants. Therefore, it seems to be reasonable to use 1.3% NaOCl during instrumentation, followed by MTAD to remove the smear layer (104).

Beltz et al. (105) compared the tissue-solubilizing action of MTAD, NaOCl, and EDTA. MTAD solubilized dentine well, whereas organic pulp tissue was clearly more unaffected by it. Zhang et al. (106) evaluated the cytotoxicity of MTAD on fibroblasts by comparing the 50% inhibitory dose with other irrigating regimens. The results showed that MTAD is less cytotoxic than eugenol, 3% H_2O_2, $Ca(OH)_2$ paste, 5.25% NaOCl, Peridex (a CHX mouth rinse with additives), and EDTA, but more cytotoxic than 2.63%, 1.31%, and 0.66% NaOCl (106).

One of the key points of interest with MTAD is its antibacterial activity, as it contains tetracycline, detergent, and has a low pH (103, 104). In an *in vitro* study, the antibacterial effects of MTAD, NaOCl, and EDTA were compared using a disk-diffusion test on agar plates. The results showed that even highly diluted MTAD produced clear zones of inhibition of the test bacterium, *E. faecalis* (107). However, it is important to bear in mind that the agar diffusion test only shows inhibition of growth, which may not be the same as bacterial killing. With regard to the high concentration of tetracycline in MTAD, the result is as expected with the agar diffusion test. Shabahang et al. (108) and Shabahang & Torabinejad (109) investigated the effect of MTAD on root canals contaminated with either whole saliva or *E. faecalis* of extracted human teeth and reported good antibacterial activity.

Rotary instrumentation

Following the development of rotary NiTi instruments for root canal preparation during the last 10 years, there has been a growing shift from manual to rotary, engine-driven preparation. Although manual instrumentation is still the most popular way of preparing the root canals, many specialists and an increasing number of general practitioners are using rotary NiTi instruments. Although one of the main reasons to start the use of rotary NiTi instruments may have been the desire to

complete the canal preparation in a shorter time than before, rotary preparation with these instruments may offer other potential advantages. One of these is the quality of the apical preparation. However, rotary instruments have not always been found to be superior to hand instruments, when the various aspects of preparation have been compared (110). Ahlquist et al. (111) and Schäfer & Lohmann (112) showed that hand instrumentation produced cleaner canals than preparation with rotary instruments. On the other hand, 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 (113). Sonntag et al. (114) compared the quality of root canal shaping with manual and rotary NiTi files performed by dental students. The preparations were performed in standardized simulated canals. Zips and elbows occurred significantly less frequently with rotary than with manual preparation. Moreover, the correct preparation length was achieved significantly more often with rotary instruments. Instrument fractures were recorded in 1.3% of the cases with both rotary and manual preparation. The time required for the preparation was also significantly longer with the manual than with the rotary preparation (114). Although a cause-and-effect relationship is difficult to prove by research, it is quite obvious that a symmetrical preparation following the original path of the root canal is an advantage in the elimination of intracanal infection (115, 116). However, in another study, Deplazes et al. (110) found no significant differences in displacement of canal centers or between the mean cross-sectional areas of the instrumented root canals between the Lightspeed and NiTi K-file groups.

Dalton et al. (46) compared the ability of stainless-steel K-type files and NiTi rotary instruments to remove bacteria from infected root canals using saline as the irrigating solution. The canals were sampled for microbes before, during, and after instrumentation. In this study, only about one-third of the canals were rendered bacteria free, and no significant difference was detected between canals instrumented with hand files and rotary instruments. Interestingly, with larger apical preparation, a significant reduction in bacterial counts was achieved. Coldero et al. (117) studied the effect of the size of apical preparation on the number of bacteria remaining in the root canal. The conclusion from this study was that additional apical enlargement to size #35 did not further reduce the number of surviving bacteria. However, in light of our knowledge about the natural size of the apical root canal, the possibility exists that the sizes #25/#35 are too small to show differences in bacterial elimination. In fact, the study by Rollison et al. (118) showed that apical enlargement from size #35 to size #50 resulted in a greater reduction of bacteria in the root canal. However, this study also demonstrated the difficulty in obtaining a sterile root canal. Contrary to these results, Card et al. (119) 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. Canals in cuspids and bicuspids were instrumented to size #8 and the molar canals to size #7. After bacteriological sampling, the canals were further instrumented in the apical third by LightSpeed files and 1% NaOCl irrigation and sampled again. Molar canals were instrumented to size #60 and cuspid/bicuspid canals to size #80. No growth was detected from any of the cuspid/bicuspid canals (11 teeth), and 81.5% of the molar canals after the first instrumentation. In the molars, the proportion of bacteria-free canals increased to 89% after the second instrumentation. Interestingly, 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 clearly greater difficulty in eliminating bacteria from molar canals than from premolars and canines (119) may be partly explained by a greater variation in morphology in molar canals than in other teeth: (i) molar roots often have two (or even more) canals in one root, and these canals often communicate through a complex network of anastomoses, (ii) the cross-section of the molar canals is often oval, with long and narrow extensions at one end of the canal, and (iii) most molar canals curve, some severely, which makes them a challenge for instrumentation. Peters et al. (120) studied rotary preparation of root canals of maxillary first molars by comparing 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 and three rotary instruments (120). The prepared canals were significantly more rounded and had greater diameters. However, the canals were also straighter than unprepared specimens, and all instrumentation techniques left at least 35% of the surface area of the dentine surface untouched. While there were significant differences between the three canal types investigated, very few differences were found between the four instrument types.

Size of the apical preparation

The goal of instrumentation and irrigation is to (i) remove tissue debris and infected tissue from the canal, (ii) facilitate effective canal irrigation, and (iii) create sufficient space for the placement of intracanal medicaments between appointments, as well as for permanent root filling. Although the technical goals of instrumentation are quite clearly defined and agreed upon, it has not been possible to agree on recommendation on the size for the apical preparation in various groups of teeth. In order to secure apical cleaning of good quality, the instruments should be in contact with every part of the canal wall. Generally, the instruments work either in a filing or reaming action. In the apical canal of most teeth, it is technically not possible to have a proper control over the files and systematically press the instruments against the walls in every direction ('circumferential filing'). A recent study with mandibular incisors with oval canals showed that both the balanced force and circumferential filing techniques left large portions of the canal wall uninstrumented (121). Shaping of the apical canal can best be accomplished by a reaming action (reaming, balanced force, rotary preparation). For the instruments to create an optimal apical preparation would, in theory, require an instrument size equal to or larger than the largest diameter of the apical canal. Kerekes & Tronstad (122–124) measured the short and long diameters of apical canals and suggested that the final preparation size should be quite large: size #50 to #90 in incisors, canines, and premolars, and even in molar curved canals sizes #50 to #60. The authors also demonstrated that it was impossible on occasions 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 (122–124). This conclusion was later supported by a study of maxillary first molars by Gani & Visvisian (125).

Today, there are no methods available to reliably measure the size of the apical root canal. Morfis et al. (126) studied the size of apical foramina in various tooth groups and found that the largest foramen was in the distal root of mandibular molars, the average diameter being almost 0.4 mm (size #40). Wu et al. (127) studied whether the first file binding apically would predict the diameter of the canal in this 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. The size of the apical preparation in curved molar canals shows great variation in different parts of the world, ranging from #20 to #60. While in vital treatments (pulpectomy), the size of the apical preparation may not be critical for success, because the canal should be free of bacteria, in the treatment of apical periodontitis, the quality and size of the apical preparation may be more important (118, 119). However, there are no controlled clinical studies comparing the effect of apical preparation with long-term prognosis of the treatment. Nevertheless, there is mounting evidence that wider apical preparation to sizes #50–#80 results in a greater reduction in bacterial numbers in the root canal, and should therefore be the goal whenever possible (e.g. remaining dentine thickness) without compromising the quality of the preparation.

Studies of the frequency and depth of penetration of bacteria into dentine surrounding the main root canal (128–130) indicate that even with the largest recommended sizes for enlargement of the canals, one would fail to remove the infected dentine in all canals (35). Because of technical and anatomical reasons, it will not be possible to remove all infected dentine by instrumentation. However, wider apical preparation is likely to promote the action of antibacterial irrigating solutions and local disinfecting medicaments.

The quality of apical shaping and cleaning is supposed to be affected both by the diameter and the taper of the last instrument used. While the canal diameter in a #30/0.02 taper apical preparation at the levels of 1, 2, and 3 mm from the working length is #32, #34 and #36, respectively, the corresponding diameter in a #30/0.10 taper canal is #40, #50, and #60. It has been speculated that the greater taper (GT) may facilitate the

effect of antibacterial irrigants in the apical canal (117). Usman et al. (131) studied the influence of instrument size on root canal debridement using GT rotary NiTi files of three different tapers (0.06, 0.08, and 0.10) with file tip sizes of 20, 30, and 40. The efficacy of root canal debridement in the apical 3 mm was compared after instrumenting with an apical size of #20 or #40 with the instruments. Twenty matched human cadaver teeth with 32 canals were decoronated and instrumented with rotary GT files to either size #20 or size #40. NaOCl, EDTA, and RC Prep were for irrigation and as chemical aids for debridement. After finishing the preparation, the teeth were extracted, decalcified, and sectioned at 0.5, 1.5, and 2.5 mm from the apex. The sections were then prepared for histological examination and quantification of remaining debris in the canal. The authors found no differences between each level within each apex size group; however, the GT size 20 group left significantly more debris in the apical third compared with the GT size 40 group. A regression analysis showed that the apical third cleanliness could be predicted mainly by instrument size and to a lesser extent by the canal length. Irrigant volume, number of instrument changes, and depth of penetration of the irrigation needle explained the differences in debridement poorly. Lumley (132) assessed canal cleaning in 30 mesial and 30 distal canals in mandibular molars following shaping with hand files of GT. Hand files with taper 0.08 and 0.10 were used for the preparation. Canals stepped back through to size 60 were significantly cleaner than those instrumented to size 35 only.

Working length vs. apical foramen

The apical foramen is usually found at a distance of 0–3 mm from the anatomic apex (133). If the radiographic apex of the tooth is used as the measure for working length determination, overinstrumentation and transportation of the foramen would likely occur in the majority of teeth. It is therefore recommended that the working length should be determined by the combined use of an electronic apex locator and a radiograph (134). The multifrequency apex locators detect the apical constriction with high accuracy (135–137). Ideally, the whole root canal should be instrumented, disinfected, and filled so that there are neither overfilling nor residual empty, unfilled root canal areas. It has been suggested that in the treatment of teeth with pulpitis, the working length should be 2–3 mm short of the radiographical apex, while in apical periodontitis the recommended length is 0–2 mm short of the radiographic apex (138). Arguably, aiming to prepare and fill the canal to the level of the coronal aspect of the apical constriction would most likely lead to the desired result. However, clinically the determination of apical canal anatomy remains a challenge, and apical constriction is often absent (138). In apical periodontitis, elimination of root canal infection is the key to successful treatment. It is therefore important to prevent residual microbes from surviving in the apical root canal (139). However, according to several studies, overfilling (extrusion of filling materials beyond the apical foramen) in cases with apical periodontitis results in a lower prognosis (140–142). It should be noted, although, that as many of these cases show complete healing, it is likely that overfilling reflects the problems in infection control caused by apical transportation, destruction of the shape of the apical foramen, extrusion of infected debris, and poor filling quality in the apical canal rather than being the direct cause of failure itself. I think we can present the last sentence a bit more clearly. Sjögren et al. (141) also reported that teeth with canals filled 2 mm or more short of the correct length have a lower long-term prognosis. A logical explanation would be a safe haven for residual bacteria in the apical canal, beyond the reach of effective host defense apparatus.

Sonic and ultrasonic preparation: more effective cleaning?

The use of ultrasonics to disinfect the root canal was first investigated by Martin (143). Using four microorganisms, he demonstrated that ultrasonics combined with a biocidal agent produced an efficient bactericidal synergism. This finding led to further studies, in which root canal debridement and antimicrobial effectiveness of endosonic and hand instrumentation techniques were evaluated (144–146). Taken together, these studies suggest that the ultrasonic energizing of the irrigant and the files contributed to a better cleaning of the root canal system then hand instrumentation alone. Both cavitation and acoustic streaming of the irrigant may contribute to the biological–chemical effects (147). In a study investigating the physical mechanisms governing the hydrodynamic response of an oscillating ultrasonic file, the authors concluded that properties

such as stable and transient cavitation of a file, steady streaming, and cavitation microstreaming, all contributed to enhanced cleaning effects during root canal debridement (148).

The effectiveness of ultrasonics has been investigated using bacteriological, histological, and microscopic techniques. Investigators have focused on the comparison between hand instrumentation and ultrasonics in the biomechanical preparation of the root canal system.

Hand vs. ultrasonic instrumentation was evaluated histologically in small curved canals (149). Although there was no significant difference in the remaining debris when the canal was divided into coronal, middle, and apical regions, these investigators concluded that overall in the root canal system, stepback hand instrumentation was more effective than sonic and ultrasonic instrumentation for removing predentine and debris, and for planing canal walls. However, a scanning electron microscopic study later reported contradictory results (150): overall and at each level of the canal, there was no difference for removal of debris and smear layer between these techniques. These authors concluded that efficacy was similar for hand and ultrasonic instrumentation, and that neither technique completely removed the smear layer but left some debris in the canal. Other investigations corroborated the finding that the smear layer remained after instrumentation using sonics or ultrasonics (151–153). Precurving endosonic files resulted in a decreased amount of debris when compared with straight files, but did not affect smear layer removal (154). When EDTA preparations were used with ultrasonics after NaOCl irrigation during root canal preparation, the smear layer was much reduced (155, 156).

In combining these two different instrumentation techniques, Goodman et al. (157) found that stepback instrumentation coupled with ultrasonic preparation resulted in better debridement of the root canals and isthmuses than with the hand instrumentation technique alone. This finding was corroborated in a later study (156). In a histological study, Archer et al. (158) compared *in vivo* debridement of mesial root canals of mandibular molars using a stepback technique or a combined hand–ultrasonic instrumentation technique. It was shown that canal and isthmus cleanliness was significantly higher at all 11 apical levels evaluated when utilizing the ultrasonic technique. In a bacteriologic study of ultrasonic root canal instrumentation, it was determined that the ultrasonic technique eliminated bacteria from canals more effectively than hand instrumentation (159). However, another investigation comparing the effectiveness of hand and ultrasonic instrumentation for removing an inoculum of bacteria from the root canal revealed that there was no significant difference between the two instrumentation groups (160). Other studies of the effectiveness of ultrasonics indicated that it had little bactericidal effect during root canal instrumentation and failed to disrupt bacteria and resulted in increases in viable counts (161, 162). Furthermore, a histological assessment of the hand vs. ultrasonic instrumentation techniques revealed that the presence of debris inside the root depended more upon the anatomic variation of the canal rather than on the technique used (163). It seems apparent from the differing results of these studies that the conditions under which ultrasonics are used during root canal preparation, and the irrigation used, are important factors in the development of a protocol for effective elimination or reduction in intracanal bacteria.

Other relevant questions related to the use of ultrasonics during root canal cleaning and shaping are (i) the effect of ultrasound on the root canal shape, (ii) remaining dentine thickness after using ultrasonics, (iii) effect of ultrasonics on the amount of extruded debris, and (iv) the occurrence of symptoms following ultrasonics. In a microscopic study, canal shape was examined after hand and ultrasonic instrumentation (164). It was observed that transportation of the original canal occurred to a greater degree when ultrasonics was used. This transportation of the canal was found to be more severe in canals having curvatures of 30° or greater, resulting in severe straightening, zipping, and strip perforations (165). In curved canals, it was found that curved files oscillate more freely than straight files in the canals, and this suggested that it was advantageous to precurve ultrasonic files before use (166). The total amount of dentine removed by instrumentation was less with the stepback technique than with the ultrasonic technique (166). McCann et al. (167) examined the remaining dentine thickness after hand or ultrasonic instrumentation. They found that although there was encroachment upon the furcal aspect of the mesial roots of mandibular molars, there was no statistical difference between instrumentation types. In comparing endosonic with balanced force and stepback filing techniques for the amount of extruded apical debris, it was shown that the balanced force technique extruded significantly less debris than the

stepback or ultrasonic technique (168). There was no significant difference between endosonic and stepback filing techniques with respect to apical debris extrusion. In a histobacteriological study of teeth with non-vital pulps after ultrasonic root canal instrumentation, compacted debris and microorganisms were frequently observed in the apical region and in the dentinal tubules (169). Overinstrumentation led to contamination of the periapical region with microorganisms and root canal debris, which has the potential to cause symptoms via stimulation of inflammatory mediators. In a comparison of four different canal instrumentation techniques, the step preparation technique resulted in a greater amount of extrusion than the standard technique, which it turn caused a larger amount of extrusion than the crowndown and ultrasound techniques (170). All instrumentation techniques caused extrusion of debris beyond the apical foramen.

Interestingly, the suggestion that the use of ultrasonics during instrumentation resulted in a cleaner canal was overshadowed by the development and widespread use and interest in rotary NiTi instrumentation in the 1990s. During this time, research focused on the cleanliness of canals instrumented with rotary NiTi instruments, and the effect of different irrigants and techniques on the smear layer. A histological evaluation of five instrumentation techniques for cleaning the apical third of the root canal showed that there was no significant difference between hand and ultrasonic instrumentation techniques (171). A study comparing passive sonic and passive ultrasonic-activated instrumentation with hand instrumentation revealed that there was significantly less debris for passive sonics and passive ultrasonic than for hand instrumentation alone (172). Utilizing passive ultrasonic irrigation, following step-down instrumentation of single canal roots, with either 2% CHX or 5.25% NaOCl, it was revealed that the CHX had a residual antimicrobial activity lasting up to 168 h (173). In a bacteriological study examining the effect of passive ultrasonic activation, it was shown that bacterial counts were higher when ultrasonics was not used (174). A comparison of the cleaning efficacy of passive sonic and ultrasonic irrigation after hand instrumentation revealed that ultrasonic irrigation produced significantly cleaner canals than sonic irrigation, and that both sonic and ultrasonic passive irrigations resulted in significantly cleaner canals than hand filing alone (175). After rotary instrumentation using two different instruments, one cutting and one non-cutting, manufactured from two different alloys, canals were irrigated with 5.25% NaOCl and 17% EDTA. It was found that ultrasonically activated irrigants did not reduce debris or smear layer scores (176). Another study utilizing EDTAC and NaOCl with ultrasonic agitation revealed that EDTAC removed the smear layer from the canal walls, but the smear layer persisted on canals irrigated with water or 1.0% NaOCl (177).

It appears from these investigations on the utilization of ultrasonics during and after root canal preparations that ultrasound would have little direct effect on intracanal bacteria. Furthermore, ultrasonics seems to exert its antimicrobial effect in conjunction with irrigants, perhaps via the physical mechanisms of cavitation and acoustic streaming. However, under the influence of ultrasonics, it is possible that in cases with complex canal anatomy the irrigants are more efficiently directed to areas that are not easily reached by normal irrigation.

Lasers in root canal disinfection

The main areas of use for lasers in dentistry are surgery, periodontics, and operative dentistry. However, there has also been considerable interest in the potential of lasers in endodontics. Fegan & Steiman (178) reported that Nd : YAG laser was effective in inhibiting the growth of *Bacillus stearothermophilus* in artificially infected root canals *in vitro*. Moshonov et al. (179) assessed the efficacy of Nd : YAG laser irradiation in disinfecting the root canal system infected for 60 min with an overnight culture of *E. faecalis*. While Nd : YAG laser irradiation significantly reduced the number of bacteria, it was inferior to NaOCl irrigation, which effectively disinfected the canals. Similar results were obtained by Blum et al. (180) with Nd : YAP laser on root canals infected with *Streptococcus mitis*. Excellent antibacterial efficiency against *E. faecalis* was reported by Gutknecht et al. (181), who determined the bactericidal effect of a holmium : yttrium–aluminum–garnet (Ho : YAG) laser on root canals infected with this species *in vitro*: On average, 99.98% of the bacteria injected in the root canal could be eliminated. Le Goff et al. (182) evaluated the effectiveness of a CO_2 laser in root canal disinfection and reported an average 85% decrease in the colony-forming units in the laser-treated group. However, irrigation with 3% NaOCl was

superior to the CO_2 laser treatment. Contrary to this result, Kesler et al. (183) had indicated that complete sterility of the root canal can be obtained with a CO_2 laser microprobe coupled onto a special hand piece attached to the delivery fiber.

Schoop et al. (184) studied the effect of an Er : YAG laser in 220 extracted human teeth and reported a good antibacterial effect, and that the bactericidal effect was dependent on the applied output power and specific for the different species of bacteria investigated. However, sterility could not be obtained predictably. Piccolomini et al. (185) evaluated the efficacy of the pumped diodium–Nd : YAG laser in sterilizing contaminated root canals: after hand instrumentation, 30 teeth were inoculated with *Actinomyces naeslundii* and 30 teeth with *Pseudomonas aeruginosa* and incubated for 24 h. The results indicated an average of a 34.0% decrease in colony-forming units for *A. naeslundii* and 15.7% for *P. aeruginosa* ATCC 27853 with the 5 Hz/15 s laser treatment, and a decrease of 77.4% for *A. naeslundii* CH-12 and 85.8% for *P. aeruginosa* ATCC 27853 with the 10 Hz laser frequency. However, both results were inferior to NaOCl, as no bacteria were detected in the canals treated with 5.25% NaOCl, used as a control (185). Mehl et al. (186) also investigated the antimicrobial properties of Er : YAG-laser radiation in root canals. The canals of 90 freshly extracted anterior teeth were enlarged mechanically, sterilized, and randomly divided into subgroups. The root canals were superficially contaminated by inoculating them with *Escherichia coli* or *S. aureus* for 2 h. Bacterial counts were reduced to 0.034–0.130% from the original inoculum with the time and energy parameters used. A corresponding reduction (0.020–0.033%) was obtained with 1.25% NaOCl solution (186).

Another important aspect of laser radiation in endodontics is the effect of lasers on the smear layer, which may be important for effective disinfection of the canal. Liesenhoff et al. (187) reported that a secure and effective root canal preparation is possible by Excimer Laser radiation. SEM investigations on roots split axially showed root canal walls free of smear layer with open dentinal tubuli. Goodis et al. (188) compared the ability of pulsed and continuous wave 1.06 μm wavelength Nd : YAG lasers to clean and shape root canals with conventional methods. The results demonstrated that the laser was capable of removing the smear layer entirely. Machida et al. (189) used a KTP : YAG laser on 30 extracted single-rooted human teeth and reported removal of smear layer and debris from the root canal surface at temperatures below the thermal injury threshold for periodontal tissue. Blum & Abadie (190) evaluated canal cleanliness achieved by five different preparation techniques and found that sonic preparation and laser together showed the cleanest preparation with opened tubules and very little debris. The relationship of removal of the smear layer and the energy setting of the laser was demonstrated by Harashima et al. (191) and Takeda et al. (192), who also showed melting of dentine when high laser energy was used. Similar observations were reported by Arrastia-Jitosho et al. (193). In another study, Takeda et al. (99) evaluated the effects of three endodontic irrigants and two types of lasers on smear layer *in vitro* in the middle and apical thirds of root canals: irrigation with 17% EDTA, 6% phosphoric acid, and 6% citric acid did not completely remove smear the layer from the root canal system. In addition, the three solutions caused some erosion of the intertubular dentine. The CO_2 laser removed and melted the smear layer on the instrumented canal walls, while the Er : YAG laser was the most effective in removing the smear layer. Effective removal of smear layer was also observed by Kesler et al. (194), using Er : YAG laser with special microprobes. However, contradictory results were reported by Barbakow et al. (195), who could not detect any difference in the ability to remove the smear layer between canals prepared with and without laser, while indicating a potential for heat damage to periradicular structures. Similarly, Kaitsas et al. (196) reported that despite effective smear layer removal, cleaning all root canal walls with laser is difficult and, a certain degree of thermal damage and morphological changes in dentine structure may occur.

In conclusion, the antibacterial effects of CO_2 and X : YAG lasers have been convincingly demonstrated. However, comparative studies in simulated root canal infections *in vitro* have shown that the effect is at best equal to or weaker than that of irrigation with concentrated NaOCl. In addition, complex root canal systems and apical curvatures further reduce the effectiveness of laser in these areas. As the same anatomical restrictions obviously apply to the ability to remove the smear layer, it can be postulated that the full potential of laser in endodontics will not be available until further technological developments are developed and introduced.

New developments in root canal disinfection

The clearly documented difficulties to predictably sterilize the infected root canal by the currently available treatment protocols have stimulated research in new areas in order to achieve complete killing of the microorganisms in the root canal. The new techniques include use of ozone, photoactivated disinfection with low-energy laser, electrochemically activated water, and electric current (197–200). Gulabivala et al. (198) compared electrochemically activated water and 3% NaOCl for their ability to eradicate *E. faecalis* in an infected tooth model. The results indicated that NaOCl was superior to electrochemically activated water in its antibacterial effect. Nagayoshi et al. (200) recorded almost comparable killing of *E. faecalis* with ozonated water and 2.5% NaOCl when the specimen was irrigated with sonication. However, in another study, NaOCl was superior to ozonated water in killing *E. faecalis* in broth culture and in biofilms (201). Ongoing research in several laboratories will, in the near future, give us a better understanding of the value of these new methods for root canal disinfection.

Special considerations

Overinstrumentation: local damage and systemic complications?

During instrumentation, dentinal and pulpal debris can block access to the most apical region of the canal, increasing the possibility of complications such as ledge formation, transportation, or perforation (202). Therefore, apical patency is advocated by some authorities as an important precaution to avoid such complications, and a survey carried out in 1997 showed that 50% of the United States dental schools teach apical patency in their curriculum (203). Goldberg & Massone (204) studied the effect of patency files on transportation of the apical foramen using files of sizes #10–#25. The authors reported transportation in 18 of the 30 specimens studied, and concluded that if a patency file is used, one should use the smallest file size possible. No difference was observed between steel and NiTi files (204).

Overfilling is connected to reduced prognosis of endodontic treatment (138, 141). It is likely that the risk of overfilling is increased after overinstrumentation of the canal. A study with freshly extracted human teeth that were overinstrumented and overfilled revealed bacteria at the root apices around the main foramen, remaining firmly attached to resorptive lacunae despite the fact that the apices had undergone great changes, including fracture or zipping (205). Although direct evidence of the potentially negative consequences of overinstrumentation is lacking, it can be speculated that overinstrumentation, with the possible exception of the smallest hand files of size #06–#10 for apical patency (and in certain special situations such as drainage through the canal), should be avoided because of the following reasons: (i) direct physical trauma to periapical tissues, (ii) extrusion of necrotic canal contents including dead and living microorganisms into the periapical area that could cause a flare-up, bacteremia, or even a persisting infection, such as periapical actinomycosis, (iii) overinstrumentation may stimulate bleeding into the root canal that provides nutrients for intracanal bacteria, (iv) increase of the foramen size and consequently improved possibilities for bacteria to receive nutrients from the periapical area, e.g. via inflammatory exudate, (v) increased risk for extrusion of irrigating solutions with the possibility of postoperative pain and discomfort, (vi) extrusion of sealer and/or gutta-percha or other root-filling materials, and (vii) creation of an oval apical foramen (transportation) that would reduce possibilities for proper apical seal with a round gutta-percha master point.

Instrument fracture: effect on prognosis

Fracture of an endodontic instrument during preparation of the root canal is a most undesirable complication. However, very little data are available on the true long-term effects of fractured instruments on the prognosis of root canal treatment. Strindberg (140) reported a statistically significant 19% higher failure frequency in cases with an instrument fracture compared with those without fracture. Crump & Natkin (206) studied 8500 root-filled teeth and found 178 cases (2.1%) with a broken instrument in the final radiograph. However, a higher failure rate was not found when these cases were compared at follow-up with matched cases without a fracture.

In the treatment of pulpitis, the pulp tissue in the canals is likely to be bacteria free and, consequently, the impact of a fractured instrument on treatment outcome

is negligible, if the canal is not contaminated during the treatment or later through coronal leakage. Saunders et al. (207) studied the effect of a fractured instrument on the time required for bacterial penetration of obturated root canals. The average time required for bacterial penetration in teeth with and without file fracture was 44 and 43 days, respectively. The results showed no significant difference between the two experimental groups. However, if the instrument fracture occurs at the beginning of the root canal treatment, the situation may be different. Although there are no epidemiological studies reporting such situations, the following treatment alternatives seem most realistic: (i) removal of the fractured instrument through the canal using ultrasonically activated small files and operating microscope, (ii) removal of the fractured instrument during apical surgery, and (iii) long-term (3 months) calcium hydroxide therapy to control the residual infection in cases where alternatives 1 and 2 have a poor prognosis. It is important to emphasize that in the absence of evidence-based data on treatment alternatives when instruments fracture in apical periodontitis, the decision is based largely on the operator's own analysis of each case.

Effect of instrumentation in primary vs. retreatment

Earlier literature on the effects of instrumentation and irrigation on root canal infection has focused largely on primary apical periodontitis. However, it would be of equal or even greater interest to understand the effect of treatment procedures on microorganisms in the root canal system within root-filled teeth. However, there are very few *in vivo* studies available on the effects on infection control by preparation in retreatment cases (19). On the other hand, in laboratory studies, *E. faecalis* has been the most widely used test organism, which may allow some speculation on the effects of instrumentation and irrigation on *E. faecalis* in root-filled teeth with an apical lesion. Based on such fragmentary information, it seems likely that elimination of infection in root-filled teeth with apical periodontitis by chemomechanical preparation is even more difficult than in primary cases. This suggestion is based on the following facts: (i) *E. faecalis* is relatively tolerant to NaOCl when compared with other bacteria, (ii) dentine and other canal components weaken the effect of the antibacterial solutions, and (iii) remnants of old root-filling materials may obstruct the penetration of disinfecting agents to parts of the root canal system.

Treatment planning may also be hampered by the fact that in previously root-filled teeth, it is usually difficult to obtain a proper bacterial sample from the canal before removing the old root filling. Therefore, the initial sample in retreatment cases is not comparable with the initial sample before preparation in primary treatment. Keeping such limitations in mind, Peciuliene et al. (19) reported that, in retreatment cases, *E. faecalis* was difficult to eliminate by instrumentation and irrigation with EDTA and NaOCl. However, the results indicated that iodine irrigation at the end of the preparation helped to eradicate *E. faecalis* (19).

Is an intracanal interappointment medicament required to complete the disinfection of the root canal?

In the treatment of teeth with a vital pulp, there is no need for intracanal medication. However, if time does not allow completion of the treatment in one appointment, it is generally recommended that the root canal should be filled with an antibacterial dressing, e.g. calcium hydroxide, between appointments to secure the sterility of the canal space, until it is filled at the next appointment. However, there are no studies comparing the bacteriological status of the root canals some time after pulpectomy, when the canals have been left empty or filled with an antibacterial dressing. The question of the role of intracanal medicaments becomes more relevant, and complex, in the treatment of apical periodontitis. There is overwhelming evidence in the literature that many if not most root canals contain viable microorganisms after the completion of the chemomechanical preparation at the end of the first appointment (10–12, 19, 57, 58, 117–119). Therefore, a variety of intracanal medicaments have been used between appointments to complete disinfection of the root canal. Byström et al. (208) reported that calcium hydroxide was an effective intracanal medicament rendering 34 out of 35 canals bacteria free after 4 weeks. The effectiveness of interappointment calcium hydroxide was also reported by Sjögren et al. (209), who demonstrated that a 7-day dressing with calcium hydroxide eliminated all bacteria in the root canal. However, other studies have challenged the ability of

calcium hydroxide to disinfect the canal, and reported a residual flora in 7–35% of teeth after one or more weeks with calcium hydroxide in the canal (44, 210–212). Recently, Kvist et al. (213) compared the antimicrobial efficacy of endodontic procedures performed in a single visit (with 10 min iodine irrigation) with a two-visit procedure, including an interappointment dressing with a calcium hydroxide paste. The authors reported residual microorganisms in 29% of the one-visit teeth and in 36% of the two-visit-treated teeth, with no statistically significant differences between the groups.

In addition to killing bacteria, intracanal medicaments may have other beneficial functions. Calcium hydroxide neutralizes the biological activity of bacterial lipopolysaccharide (214, 215), and makes necrotic tissue more susceptible to the solubilizing action of NaOCl at the next appointment. Another psychological aspect in using intracanal medicaments in practice may be that a more thorough instrumentation is achieved because of the longer overall time used for the treatment. On the other hand, several appointments can also increase the risk for aseptic complications, for instance, through a leaking temporary filling (16).

Several studies have indicated a poorer prognosis for the treatment of apical periodontitis if viable bacteria are residing in the root canal system at the time of filling (7–9). Other studies, however, have contradicted these results and reported no significant differences in healing between teeth filled after positive or negative cultures from the root canal (10), as well as between treatments performed in one or two appointments (10, 11). It can be speculated that a permanent root filling of high quality using endodontic cements having some residual antibacterial activity (216) can effectively seal and entomb residual microorganisms in the canal, thus preventing them from communicating with periradicular tissues. Moreover, further killing of the microorganisms could continue due to the antibacterial activity of the root-filling materials and unavailability of nutrients, which is particularly harmful to many bacteria, including anaerobes.

Future strategies for canal preparation: microbiological point of view

The complex anatomy of teeth and root canals creates an environment that is a challenge to instrument and clean. In addition, the complex chemical environment of the root canal prevents antimicrobial irrigating solutions and medicaments from exerting their full potential against all microorganisms found in endodontic infections (63, 81, 82). While our knowledge of persistent bacteria, disinfecting agents, and the chemical milieu of the necrotic root canal has greatly increased, there is no doubt that more innovative basic and clinical research is needed to optimize the use of existing methods and materials, and to find new techniques and materials, or combination of materials, to achieve the goal of predictable, complete disinfection of the root canal system in apical periodontitis.

Cleaning and shaping of the root canal is the single most important factor in the prevention and treatment of endodontic diseases, and the effects of instrumentation and irrigation on intracanal infection have been a focus of increased activity in endodontic research. Although sterility of the root canal can occasionally be achieved by instrumentation and irrigation with antibacterial solutions, the protocols used today cannot predictably provide sterile canals. As none of the elements of endodontic therapy (host defense system, systemic antibiotic therapy, instrumentation and irrigation, intracanal medicaments, permanent root filling, and coronal restoration) can alone guarantee complete disinfection, it is of utmost importance to aim at the highest possible quality at every phase of the treatment.

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