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What We Leave Behind In Root Canals After Endodontic Treatment: Some Issues and Concerns

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Abstract

The benefits of using sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) as endodontic irrigants, and calcium hydroxide as an inter-appointment medicament, are well known to dentists. Many steps undertaken during endodontic treatment and retreatment are rather mechanical in nature, and less attention is committed to understanding the biological issues underlying endodontic treatment and retreatment. It should be noted that dentine is the fundamental substrate in endodontic treatment, and its properties and characteristics are the key determinant of nearly all disease and post-disease processes in the teeth. In this article the effects and counter-effects of NaOCl and EDTA on root canal dentine, and some other related issues are reviewed. This information will enable clinicians to use the beneficial effects of these chemicals, while necessary steps are considered to reduce their harmful effects on dentine substrate.

Introduction

It is now widely accepted that apical periodontitis is an inflammatory destruction of periapical tissues caused by the presence of aetiological agents from the root canal system (1). It is also widely accepted that the main cause of apical periodontitis is the presence of bacteria in the root canal system since Kakehashi *et al.* showed in 1965 that no apical periodontitis developed in germ-free rats when their molars were kept open to the oral cavity (2). Traditionally, root canal treatment is carried out to retain a tooth with apical periodontitis in a disease-free state in the oral cavity, and this objective of endodontic treatment is achieved by (i) cleaning and shaping the root canal system, and (ii) by root filling the prepared root canal lumen.

The cleaning and shaping step is intended to disinfect and remove dead tissue, bacteria and bacterial products from the root canal system. Shaping of root canals facilitates irrigation of the root canal system and subsequent root filling. The filling of the root canal system is meant to seal the periapical region from the root canal and to "bury" any remnant bacteria in the dentinal tubules. The purpose of the root filling is to deprive remnant bacteria of any nutrients and to prevent the entry of any body fluids into the root canal space. In doing so, most teeth with apical periodontitis heal (3). However, according to some investigators bacteria remaining in the dentinal tubules, though they may be buried by a root filling, would constitute an important reservoir from which reinfection may occur (4). Furthermore, the importance of a well-sealed coronal restoration has also been emphasised for a better treatment outcome (5).

Past studies have shown that instrumented cleaning of the root canal system with files will not be sufficient to remove tissues and bacteria. This is mainly because of the complex anatomy and varied configurations of root canal systems. Geometrically symmetrical instruments will not be able to reach many naturally occurring depressions, cul-de-sacs, isthmuses and other anatomical variations, which are asymmetrically disposed, within the root canal systems. In addition, the root canal diameter is usually larger than the instrument calibre used and this may further contribute to inadequate cleaning by instrumentation only (6). Consequently, bactericidal root canal irrigation and application on inter-visit medicament have become very important steps in the disinfection of root canal systems (1). The main purpose of root canal irrigation is to eliminate bacteria, and in the past, different concentrations of various chemicals were used to achieve this goal.

Studies of the microbial flora of the root canals of teeth with persistent apical lesions subsequent to root canal therapy have revealed that their bacterial flora differs markedly from that of the untreated, necrotic pulp (primary endodontic infection). While primary endodontic infection typically has a polymicrobial flora with approximately equal proportions of Gram-positive and Gram-negative bacteria, dominated by anaerobes, the microbial flora of the teeth with failing endodontic treatment (post-treatment endodontic infection) are characterised by monoinfection, predominantly Gram-positive microorganisms. These have been demonstrated to be of approximately equal proportion of facultative or obligate anaerobes (7). Further, *Enterococcus faecalis* has been found to be one of the commonest bacteria in the teeth with failed root canal therapy (8). This distinct difference in the endodontic bacterial flora is attributed to the "endodontic environmental transition (EET)".

This variation in the EET and selection of specific microbial flora can be attributed to the difference in selective pressures (hydrodynamic pressure or oxygen tension that exist in the infected untreated root canals versus those in the treated root canals) and the nature of intracanal irrigants and medicaments used during treatment (7, 9). Moreover, the deterioration of the mechanical integrity of the post-treatment dentine structure has been an unrelenting issue in dentistry. It is vital to realise that dentine is the fundamental substrate in an endodontic treatment and its properties and characteristics are the key determinant of nearly all disease and post-disease processes in teeth. In this article, the effects and counter-effects of commonly used intracanal irrigation on root canal dentine and some of the related issues are reviewed (Fig. 1).

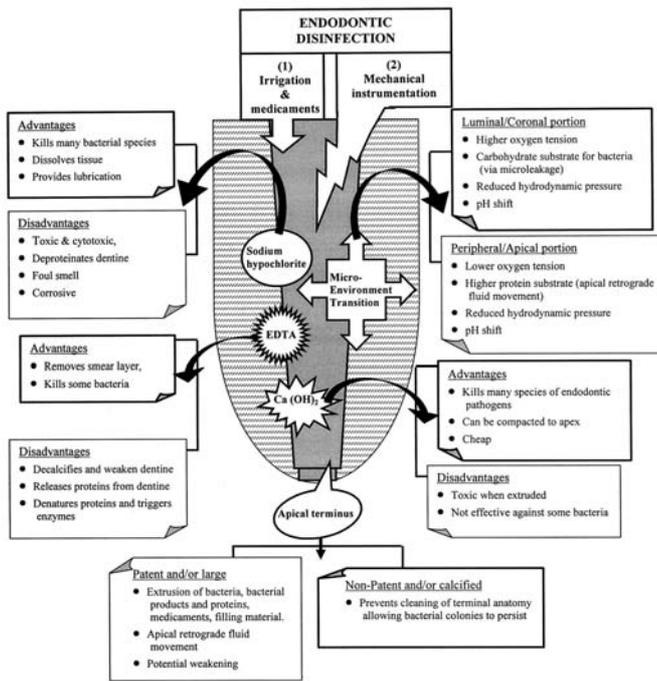


Figure 1: Schematic representation of different factors that affect the root canal system.

Sodium Hypochlorite And Disinfection: Its Effects And Counter-effects

Sodium hypochlorite is a commonly used irrigant when performing endodontics and it is taught in many schools in North America and Europe (10). Sodium hypochlorite irrigation is also widely practised in Asia, Australia and other countries. Sodium hypochlorite is used in endodontics for two main purposes: (i) to dissolve pulp tissue, and (ii) to destroy bacteria (11, 12). Sodium hypochlorite is a very reactive and toxic compound. Even a 0.5% concentration is considered by some to be too toxic for wound care (13). Dakin's solution, a buffered sodium hypochlorite solution used historically for wound cleaning has also recently been reported to be too toxic for open wound care (14). In endodontics, concentrations of 0.5% to 5.25% are regularly used. In a survey in Australia, the most commonly used concentration was a 1% solution of sodium hypochlorite (15). The paper did not discuss why this is the most commonly used concentration nor the rationale for its selection, but observed that specialists were more likely to select a higher concentration than general practitioners practising endodontics. It was suggested that the reason might be that specialists wanted a more thorough therapeutic effect or a shorter treatment time.

Often, we ignore the fact that this very reactive chemical can bring along with its desired therapeutic effects, undesired effects on

the root canals, especially on the dentine substrate. Recently, there have been several reports of the adverse effects of sodium hypochlorite on strength and other physical properties such as flexural strength, elastic modulus and microhardness of dentine (16, 17, 18). These changes in physical properties of dentine come not only from changes in the inorganic phase but also from the organic phase of dentine (19, 20). Moreover, in their zeal to ensure "complete" disinfection, dentists vary not only the concentration, but also the volume, duration, flow rate and temperature in their attempts to eliminate all bacteria.

Does Use Of Higher Concentration Really Kill Bacteria More Effectively?

Despite our best intentions, one-third to a half of all root canals treated and irrigated with 5.25% sodium hypochlorite remain infected (21, 22). In addition, Siqueira *et al.* (23) observed that there was no significant difference in the ability of the three different concentrations to kill bacteria. They found large zones of inhibition with all three concentrations even when the test bacterium was *E. faecalis*. It was concluded from that study that large volumes of the solution would compensate for the loss of efficacy by a lower concentration. Moreover, it was reported that even the use of 13% sodium hypochlorite together with ultrasonics might not remove all bacteria trapped in dentine behind fins of the root canal (24). It has been shown, however, that higher concentrations of sodium hypochlorite leave behind a cleaner canal when three concentrations, 0.5%, 1% and 5% sodium hypochlorite, were compared in irrigating canals instrumented by rotary NiTi files. Whilst there was significant difference between 0.5% and 5%, the 1% group showed no significant differences in debris remaining on canal wall to the other two groups in this morphometric analysis (25).

Although a higher concentration of sodium hypochlorite was hypothesised to be effective in eliminating all bacteria from the entire root canal system, the observation from *in vitro* investigations were not very supportive of this hypothesis. Besides, clinicians should be mindful of the higher toxicity of the higher concentrations on biological tissues (26). In addition to the higher toxicity of higher concentrations, it is quite clear from the literature that the higher the concentration of sodium hypochlorite, the greater would be the deleterious effects on dentine (27). These deleterious effects include reduction of the elastic modulus and the flexural strength. Given this knowledge, and the fact that 1% sodium hypochlorite cleaned root canals just as well as those irrigated with 5.25% (25), why then do some clinicians choose to use 5.25% sodium hypochlorite? Perhaps it is because these clinicians are concerned with killing bacteria within the dentinal tubules and they believe that a higher concentration would diffuse through to kill bacteria more effectively. How far does sodium hypochlorite penetrate into dentinal tubules?

Sodium Hypochlorite Penetration Of Dentinal Tubules

Berutti *et al.* (28), published a study that controlled total volume irrigant used and duration of irrigation. In the absence of a substance to lower surface tension (Triton), there was no appreciable penetration of dentinal tubules by sodium hypochlorite and EDTA as represented by the presence of bacteria that extended from the lumen to a depth of 300 μm after irrigation. With the use of Triton, there was a bacteria-free zone of about 130 μm from the lumen after irrigation. We do not know from this experiment if teeth when

irrigated for longer than the 11 minutes, without use of Triton, would have any zone free of bacteria. Why then do we soak the root canals with sodium hypochlorite?

Increasing The Temperature Of Sodium Hypochlorite

Cunningham and Joseph reported that collagen-dissolving ability of 2.6% sodium hypochlorite was comparable to that of 5.25% at both 21°C and 37°C (29). Comparison of ability to kill bacteria at different temperatures was also made. They tested the ability of 2.6% and 5.25% sodium hypochlorite in reducing a planktonic culture of *E. coli* to below culturable level at 22°C and 37°C. They found that it took a less time to kill *E. coli* in both concentrations at 37°C (30). Interestingly, it was also reported that increasing the temperature of sodium hypochlorite to 50°C did not help in making the root canal cleaner. However, at the higher temperature (50°C), Berutti *et al.* observed a thin, less organised and less adherent smear layer on the root canal wall. This thinner layer was not evident on root canals irrigated with sodium hypochlorite at 21°C (31).

From the above studies it is apparent that raising the temperature may have some benefit in killing bacteria more quickly; however, raising the temperature to 37°C did not help dissolve tissues more effectively. Though we may think of raising the temperature of irrigants to kill bacteria more effectively, we should not raise the temperature more than few degrees above the body temperature as this may have harmful effects on the cells of the periodontal ligament (32).

EDTA And Smear Layer Removal: Effects And Counter-effects

Ethylenediaminetetraacetic acid (EDTA) is an irrigant commonly used by dentists to remove the smear layer at the end of root canal preparation. The common concentrations used are 15–17% disodium EDTA (33). Whilst smear layer removal continues to be controversial, there can be many benefits in removing this layer or organo-mineral, which is not sufficiently adherent to dentine surfaces to prevent leakage (34). Removing the smear layer not only helps to improve the seal of root fillings; it also removes bacteria, toxins and remnant pulpal tissues that may be in the smear layer.

EDTA was studied in relation to endodontic treatment by Nygaard-Ostby in 1957 (35). He reported that with use of 15% EDTA at pH 7.3, with an added detergent, there was 20–30 μm penetration by EDTA as shown by a zone of demineralisation using polarised light microscopy, after only five minutes. This zone of demineralisation did not increase beyond 50 μm , even when used over a long period. There was a clear demarcation line. This shows that EDTA did not diffusely penetrate into tubules but rather there was a self-limiting reaction. A 5-min. exposure of the root canal to EDTA would remove the smear layer and open the dentinal tubules to a depth of 20–30 μm .

A recent study showed that 3 min. of 8% Salvizol-EDTA (a di-hemi-potassium salt solution) irrigation is as effective as 1 min. 15% EDTA irrigation (36). The authors were looking at the ability of a new EDTA solution in cleaning the root canal after instrumentation. The need for a new EDTA solution was because 17% EDTA irrigation following sodium hypochlorite irrigation resulted in opening the dentine tubular orifices, destruction of intertubular dentine and in reduction of dentine microhardness (37–39).

Whilst it may be important to remove smear layer for a better seal of the root filling, we have to be mindful not to cause harm to the substrate we leave behind after treatment. Calt and Serper (40) showed that duration of application was a crucial factor in avoiding erosion of root canal dentine. In their study, dentine was irrigated with 10 ml 17% EDTA for 1 and 10 mins. This was followed by 10 ml of 5% sodium hypochlorite irrigation. Whilst the 1-min. EDTA irrigation proved to be effective in removing the smear layer, the 10-min. EDTA irrigation group had excessive peritubular and intertubular dentinal erosion. Accordingly, they advised that EDTA irrigation should not be for more than a minute.

In another study, the Vickers microhardness of the root canal dentine irrigated with 5.25% NaOCl, 2.5% NaOCl, 3% H₂O₂, 17% EDTA and 0.2% chlorhexidine gluconate for 15 min. each was studied. Except for chlorhexidine, all irrigants were found to reduce dentine surface hardness. Further, analysis for roughness showed that only H₂O₂ and chlorhexidine did not alter the roughness of dentine (41). Although most clinicians are aware of the effects of acid on dentine, the effects of EDTA on the dentine surface is less commonly understood. Atomic force microscopy has shown that the intertubular surface dentine etched by phosphoric (3mM and 5mM) and citric acids (5mM) was smooth, whereas 0.5M (17%) EDTA treatment gave rise to intertubular dentine surfaces that were significantly rough. The acid-treated groups had an average root mean square roughness of 15 nm, whereas the EDTA treated groups had an average value of 32 nm (42). All these investigations vividly highlight that EDTA is a liquid that can have profound effects on dentine substrate.

EDTA is most often used as a liquid for final flush at the end of root canal preparation to remove the smear layer. Recently, many paste-form chelator preparations have become available for use by practitioners. Some nickel-titanium alloy (NiTi) file manufacturers actively promote the use of these chelators with the use of their files. Whilst there may be some benefit from the lubricating properties of these pastes as the NiTi files are rotating during canal preparation, their role on the smear layer removal while the canal is being prepared is less well researched. Nevertheless, it has been shown that these paste-form chelators do not remove the smear layer as effectively as liquid EDTA (43, 44).

Sodium Hypochlorite And EDTA In Concert: Effects And Counter-effects

Whilst EDTA and sodium hypochlorite when used alone can each affect the physical and mechanical properties of dentine adversely, not surprisingly, many studies have shown that the combination of removal of the inorganic phase as well as the organic phase of dentine give rise to dire effects. It was shown that after the removal of the inorganic phase, applying sodium hypochlorite to it would also remove the organic phase, resulting in a porous dentine surface with many channels. This porous dentine surface was observed 40 sec. after exposure to sodium hypochlorite. It was unique and was not seen after acid-etching alone. Furthermore, there was a concomitant loss of mechanical strength to 75% of the untreated samples (45). Although this experiment was carried out using acid to etch dentine, we do not think that a very different result would be the outcome had EDTA been used. This was because EDTA has also displayed similar if not more drastic effects on dentine (46). An increase in porosities and channels would probably contribute to improved micro-mechanical retention of posts if a resin cement was used (47); it could be argued that this increase could create a weak interface between the root filling and

the root canal dentine and have an increased propensity for bacterial leakage.

Niu *et al.* (39) conducted a scanning electron microscopic study of the root canal dentine surface subsequent to final irrigation with EDTA and sodium hypochlorite solutions. They evaluated different irrigation regimes such as initial irrigation with 3 ml of 15% EDTA for 1 or 3 mins, followed by 3 ml of 6% sodium hypochlorite for 2 min. Whether 1 or 3 min. irrigation with EDTA, as long as the two irrigants, EDTA and sodium hypochlorite were used, there was erosion of dentine. In another study carried out to observe the effects of irrigation time on smear layer removal, they found that canal irrigation with EDTA and sodium hypochlorite for 1, 3 and 5 min. were equally effective in removing the smear layer from the canal walls of straight roots. Fifteen per cent EDTA and 1% sodium hypochlorite were used in this study (48). From their published photomicrograph, showing a clean smear-free dentine, a hint of dentine erosion is observable. Furthermore, in our own study (unpublished data) we saw severe erosion of dentinal tubules when dentine was irrigated with 25 ml of 5.25% sodium hypochlorite for 30 min. followed by 17% EDTA (pH 7.4) for 5 min.

From these foregoing studies, we can infer that it is probably realistic to use a lower concentration of sodium hypochlorite, such as 1%, for irrigation. For removing smear layer, EDTA use for a short duration of about one minute would be sufficient. Bergenholtz *et al.* (49) felt that since there is an efficient microbial effect for sodium hypochlorite at low concentration, but high toxicity at high concentration, 0.5–1% sodium hypochlorite should ideally be used for root canal irrigation. Using a higher concentration does not mean a better therapeutic value (50).

Which Should Be The Last Irrigant?

Whilst removal of smear layer may be desirable, we have to consider which irrigant should be the last. If the last irrigant is EDTA, it is reasonable to believe that a layer of collagen is on the surface of the root canal lumen (39). Collagen can be important for the binding of bacteria including *E. faecalis* (50, 8). It may therefore not be prudent to use EDTA as the final irrigant. Many dentists use the regime of irrigating with sodium hypochlorite during treatment and then use a final flush of EDTA (51). We should perhaps end with a low concentration of sodium hypochlorite since a high concentration caused dentine erosion (39). Any collagen, and/or other proteins left exposed by EDTA would be removed by a short exposure to sodium hypochlorite (52). Besides, using EDTA as a final rinse may also expose other dentine extracellular matrix proteins. An example of one such protein is the MMP2 (53). If these proteins are exposed during root canal preparation, some of these macromolecules could be extruded, together with other debris and dentine mud. Canal debris extrusion is a common occurrence during root canal preparation, especially with techniques involving a filing (linear) motion (54). Extruded macromolecules such as MMPs have many biological activities including degradation of extracellular matrix proteins, alteration of cellular behaviour and modulation of the activity of many other biologically active molecules. MMPs are suggested to even interfere with the local homeostasis and subsequently delay healing of periapical lesions (55).

Endodontic Retreatment: How Do We Deal With It?

Currently, clinicians deal with retreatment of failed cases in much the same way as primary endodontic treatment after crown, post and root filling disassembly. No other recommendations have been

made. As many retreatment cases are referred to endodontists, those performing retreatment are unlikely to be aware of what strategies were adopted during primary treatment. The new strategies of those providing retreatment, however, need not necessarily work! Bacteria, when exposed to low concentrations of hypochlorite have been found to be resistant to the same concentrations upon subsequent exposure. In particular, *E. faecalis* in retreatment cases are likely to be in a nutrient-deficient environment, and nutrient-starved *E. faecalis* have been shown to have a high level of resistance to sodium hypochlorite (58). Should we then use stronger concentrations and bear the consequences of dentine weakening, or should we be considering another method of irrigating and disinfecting the root canal?

Recently much emphasis has been given to the biofilm mode of bacterial growth in infected root canals. The nature of the infection in retreatment is very likely that of the presence of a biofilm in the root canal (59, 60). Biofilm mode of bacterial growth is an adaptive mechanism, where a community of microbes is adsorbed to a solid surface and is embedded within a common matrix. The altered genetic and metabolic processes, along with the complex matrix of the biofilm structure, prevent the entry and action of antimicrobial agents (61, 62). Subsequently the colonising organism gains protection against unfavourable environmental and nutritional conditions (63). The ability of *E. faecalis* to form distinct biofilms under different environmental and/or growth conditions has been shown recently (64). Bacterial biofilms in root canals will throw more challenges to the disinfection methods currently used in endodontics.

Size Of Apical Preparation: Effects And Counter-effects

There is disagreement as to what the apical preparation size should be. Some workers believe that with a larger apical size preparation, more of the anatomical variations that are commonly found in the apical region can be reduced. This, they believe, would aid in pushing up the success rate (56). The extent to which this can be done, however, depends on the size, curvature, length and technique used during preparation. It is easy to understand that removing too much dentine from the root canal by over-rigorous mechanical debridement can lead to deleterious effects. An apical terminus of large diameter can cause large volume of retrograde fluid movement into the apical portion of the root canal, especially when the canal is fluid-filled. This issue is further amplified in root canals that have poor apical seal (57).

Other Perplexing Problems

Despite our best intentions, modern disinfection techniques do not bring about a better outcome when compared to older techniques (65). The main prognostic factor was the presence of a periapical lesion at commencement of treatment. The authors in this work lamented, "the current strategies applied in this study have been commonly perceived to improve the outcome of treatment relative to older techniques". In this paper, no information was given as to if EDTA was used, and it was not possible to determine the importance of EDTA in the overall outcome of treatment. It was suggested that vertical compaction of heated gutta-percha root fillings did not have a better outcome when compared to lateral condensation. This study, however, was plagued by a drop-out rate of 49%. Interestingly though, there was an 18% differential when comparing cases treated in one visit versus those treated over two visits. Fifty-eight per cent of those treated in one visit healed whilst

76% of those treated in two visits had healed.

Another perplexing problem is the increasing reports of *E. faecalis* isolated as a single species infection in failed cases (66). Curiously, in another study, only 1% of oral rinse isolates of 100 dental students with no history of endodontic treatment had *E. faecalis*, but this increased to 11% in 100 patients undergoing endodontic treatment (67). What caused this increase? Recently, Kayaoglu *et al.* reported that a high prevailing pH in the treated root canal gave rise to increased bacteria adhesion to collagen (68). Are we selecting for *E. faecalis* when we use calcium hydroxide in our treatment? Are we creating a bed of collagen if we use EDTA in a first visit, and then fail to put in calcium hydroxide sufficiently into the canal so that the pH does not rise above that which can kill bacteria, but somehow manage to increase the pH that helps more *E. faecalis* to adhere, dooming these teeth to failures? Even if there were no *E. faecalis* to grow on this bed of collagen, collagen so exposed have been shown to denature and degrade over time leading to post failures (69).

Calcium hydroxide is a frequently used medicament, and in retreatment, it is likely to be the inter-visit medicament of choice. Calcium hydroxide used on dentine irrigated by either sodium hypochlorite or EDTA alone did not give rise to any topographical changes, yet calcium hydroxide is not without any effects on dentine (70). Saturated calcium hydroxide is found to reduce the flexural strength of dentine (16). Furthermore, *E. faecalis* resists the bactericidal effects of calcium hydroxide (71), while tooth specimens experimentally infected with *Candida albicans* showed that specimens treated with calcium hydroxide/camphorated paramonochlorophenol/glycerin paste, or with chlorhexidine/zinc oxide paste were completely disinfected after 1 h of exposure. Only calcium hydroxide/glycerin paste consistently eliminated *C. albicans* infection after 7 days of exposure. Calcium hydroxide mixed with chlorhexidine was ineffective in disinfecting dentine even after one week of medication exposure (72).

Conclusion

Many biological questions about endodontics remain unanswered to date, and further research is crucial to answer these questions. Meanwhile, it is important for clinicians to bear in mind the mechanical and biological characteristics of the dentine and the host response at the periapical region. Having an efficient system to mechanically shape the root canal, to receive a root filling that appears to be sufficiently radiopaque and extends to the full length of the root canal, by itself, may not guarantee success. In the process of achieving a well-condensed root filling, it is crucial to respect all biological principles before aiding in the healing of the periapical diseases. Sound reasoning, of not only which chemicals and at what concentrations to eliminate the microbes, but also their effects on the biological substrate and their consequences, are important to strike a predictable success in endodontic treatment.

References

1. Nair PNR. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med* 2005; 15:348–81.
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg* 1965; 20:340–49.
3. Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. *J Endod* 2004; 30:846–50.
4. Oguntebi BR. Dentine tubule infection and endodontic therapy implications. *Int Endod J* 1994; 27:218–22.
5. Iqbal MK, Johansson AA, Akeel RF, Bergenholtz A, Omar R. A retrospective analysis of factors associated with the periapical status of restored, endodontically treated teeth. *Int J Prosthodont* 2003; 6:1–8.
6. Jou Y-T, Karabucak B, Levin J, Liu D. Endodontic working width: current concepts and techniques. *Dent Clin North Am* 2004; 48:323–35.
7. Hancock HH, Asgeir S, Martin T, Julian M. Bacteria isolated after unsuccessful endodontic treatment in a North American population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; 91:579–86.
8. Love RM. *Enterococcus faecalis* – a mechanism for its role in endodontic failure. *Int Endod J* 2001; 34:399–405.
9. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J* 1998; 31:1–7.
10. Qualtrough AJE, Whitworth JM, Dummer PMH. Preclinical endodontology: an international comparison. *Int Endod J* 1999; 32:406–14.
11. Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. *Oral Surg Oral Med Oral Pathol* 1971; 31:96–103.
12. Shih M, Marshall FJ, Rosen S. The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1970; 29:613–19.
13. Lineaweaver W, Howard R, Soucy D, McMorris S, Freeman J, Crain C, Robertson J, Rumley T. Topical antimicrobial toxicity. *Arch Surg* 1985; 120:267–70.
14. Smith RG. A critical discussion of the use of antiseptics in acute traumatic wounds. *J Am Podiatr Med Assoc* 2005; 95:148–53.
15. Clarkson RM, Podlich HM, Savage NW, Moule AJ. A survey of sodium hypochlorite use by general dental practitioners and endodontists in Australia. *Aust Dent J* 2003; 48:20–6.
16. Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001; 34:113–19.
17. Goldsmith M, Gulabivala K, Knowles JC. The effect of sodium hypochlorite irrigant concentration on tooth surface strain. *J Endod* 2002; 28:575–79.
18. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. *J Dent* 1999; 27:43–6.
19. Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: I. Demineralisation. *Biomaterials* 2001; 22:787–92.
20. Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteinisation. *Biomaterials* 2001; 22:793–97.
21. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985; 18:35–40.
22. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5% sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol* 1983; 55:307–12.
23. Siqueira JF Jr, Rocas IN, Faviera A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5% and 5.25% sodium hypochlorite. *J Endod* 2000; 26:331–34.

24. Huque J, Kota K, Yamaga M, Iwaku M, Hoshino E. Bacterial eradication from artificial smear layer by ultrasonic irrigation. *Int Endod J* 1998; 31:242–50.
25. Baratto-Filho F, de Carvalho JR Jr, Fariniuk LF, Sousa-Neto MD, Pecora JD, da Cruz-Filho AM. Morphometric analysis of the effectiveness of different concentrations of sodium hypochlorite associated with rotary instrumentation for root canal cleaning. *Braz Dent J* 2004; 15:36–40.
26. Spangberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surg Oral Med Oral Pathol* 1973; 36:856–71.
27. Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J* 2001; 34:120–32.
28. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. *J Endod* 1997; 23:725–27.
29. Cunningham WT, Joseph SW. Effect of temperature on the bactericidal action of sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980; 50: 569–71.
30. Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980; 49:175–77.
31. Berutti E, Marini R. A scanning electron microscopic evaluation of the debridement capability of sodium hypochlorite at different temperatures. *J Endod* 1996; 22:467–70.
32. Saunders EM. *In vivo* findings associated with heat generation during thermomechanical compaction of gutta-percha 2. Histological response to temperature elevation on the external root surface. *Int Endod J* 1990; 23:268–74.
33. Perez F, Rouqueyrol-Pourcel N. Effect of a low-concentration EDTA solution on root canal walls: a scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005, Mar; 99:383–87.
34. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: A review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; 94:658–66.
35. Nygaard-Ostby B. Chelation in root canal therapy: ethylenediaminetetraacetic acid for cleansing and widening of root canals. *Odontologisk Tidskrift* 1957; 65:3–11.
36. Perez F, Rouqueyrol-Pourcel N. Effect of a low-concentration EDTA solution on root canal walls: a scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; 99:383–87.
37. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. *J Dent* 1999; 27:43–6.
38. Calt S, Serper A. Smear layer removal by EDTA. *J Endod* 2000; 26:459–61.
39. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. *Int Endod J* 2002; 35:934–39.
40. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. *J Endod* 2002; 28:17–19.
41. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. *J Endod* 2004; 30:792–95.
42. Marshall GW, Balooch M, Kinney JH, Marshall SJ. Atomic force microscopy of conditioning agents on dentine. *J Biomed Mater Res* 1995; 29:1381–87.
43. Peters O, Barbakow F. Effects of irrigation on debris and smear layer on canal walls prepared by two rotary techniques: a scanning electron microscopic study. *J Endod* 2000; 26:6–10.
44. Hulsmann M, Schade MJ, Schafers F. A comparison of root canal preparation with HERO 642 and Quantec SC rotary NiTi instruments. *Int Endod J* 2001; 34:538–46.
45. Marshall GW, Yucel N, Balooch M, Kinney JH, Habelitz S, Marshall SJ. Sodium hypochlorite alterations of dentine and dentine collagen. *Surface Science* 2001; 491:444–55.
46. Marshall GW, Balooch M, Kinney JH, Marshall SJ. Atomic force microscopy of conditioning agents on dentine. *J Biomed Mater Res* 1995; 29:1381–87.
47. Kostka E, Roulet JF. The root filled tooth in prosthodontic reconstruction. In: Bergenholtz G, ed. *Endodontology*. Oxford: Blackwell; 2003. Ch. 12.
48. Teixeira CS, Felipe MC, Felipe WT. The effects of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. *Int Endod J* 2005; 38:285–90.
49. Bergenholtz G. Controversies in Endodontics. *Crit Rev Oral Biol Med* 2004; 15:99–114.
50. Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of *Enterococcus faecalis* proteases and the collagen-binding protein, Ace, on adhesion to dentin. *Oral Microbiol Immunol* 2003; 18:121–26.
51. Regan J, Gutmann JL. Preparation of the root canal system. In: Pitt-Ford TR, ed. *Harty's endodontics in clinical practice*. 5th ed. London: Butterworth-Heinemann; 2004. p.86.
52. Toledano M, Perdigao J, Osoria E, Osorio R. Influence of NaOCl deproteinisation on shear bond strength in function of dentine depth. *Am J Dent* 2002; 15:252–55.
53. Martin-De Las Heras S, Valenzuela A, Overall CM. The matrix metalloproteinase gelatinase A in human dentine. *Arch Oral Biol* 2000; 45:757–65.
54. al-Omari MA, Dummer PM. Canal blockage and debris extrusion with eight preparation techniques. *J Endod* 1995; 21:154–58.
55. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000; 14:2123–33.
56. Siqueira JF Jr, Lima KC, Magalhaes FA, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial cell number inside the root canal by three instrumentation techniques. *J Endod* 1999; 25:332–35.
57. Kishen A. Periapical biomechanics and the role of cyclic biting force in apical retrograde fluid movement. *Int Endod J* 2005; 38:597–603.
58. Laplace JM, Thuault M, Hartke A, Boutibonnes P, Auffray Y. Sodium hypochlorite stress in *Enterococcus faecalis*: influence of antecedent growth conditions and induced proteins. *Curr Microbiol* 1997; 34:284–89.
59. Yuichiro N, Atsushi E, Takashi K, Naoki T, Shigeyuki E. Participation of bacterial biofilms in refractory and chronic periapical periodontitis. *J Endod* 2002; 28:679–83.
60. Nair PNR, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; 99:231–52.
61. Stewart PS. Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrob Agents Chemother* 1996; 40:2517–22.
62. Mah TFC, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001; 9:34–9.

63. Michael RW, Anthony B, Smith W. Dormancy and persistence in chronic infection: role of the general stress response in resistance to chemotherapy. *J Antimicrob Chemother* 2001; 48:141–42.
64. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by *Enterococcus faecalis*. *J Endod* (in press).
65. Friedman S, Abitbol S, Lawrence HP. Treatment outcome in endodontics: The Toronto Study. Phase I: Initial Treatment. *J Endod* 2003; 29:787–93.
66. Portenier I, Waltimo TMT, Haapasalo M. *Enterococcus faecalis* – the root canal survivor and “star” in post-treatment disease. *Endod Topics* 2003; 6:135–59.
67. Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiol Immunol* 2004; 19:95–101.
68. Kayaoglu G, Erten H, Ørstavik D. Growth at high pH increases *Enterococcus faecalis* adhesion to collagen. *Int Endod J* 2005; 38:389–96.
69. Ferrari M, Mason PN, Goracci C, Pashley DH, Tay FR. Collagen degradation in endodontically treated teeth after clinical function. *J Dent Res* 2004; 83:414–19.
70. Tatsuta CT, Morgan LA, Baumgartner JC, Adey JD. Effect of calcium hydroxide and four irrigation regimens on instrumented and uninstrumented canal wall topography. *J Endod* 1999; 25:93–8.
71. Sukawat C, Srisuwan T. A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with *Enterococcus faecalis*. *J Endod* 2002; 28:102–04.
72. Siqueira JF Jr, Rocas IN, Lopes HP, Magalhaes FA, de Uzeda M. Elimination of *Candida albicans* infection of the radicular dentin by intracanal medications. *J Endod* 2003; 29:501–04.