Quantitative Analysis of the Effect of Irrigation Sequences on Root Canal Wall Dentin Erosion

by

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Abstract

Objectives: The purpose of the present study was to examine the effect on root canal wall dentin and compare the level of erosion caused by different irrigation sequences.

Material and methods: Dentin specimens of the middle third of the root of extracted teeth with one root canal were instrumented and randomly divided into five groups. Each group was subjected to 17% ethylenediaminetetraacetic acid (EDTA), 17% ethylene-glycoltetraacetic acid (EGTA), or 10% citric acid (CA) and 5.25% sodium hypochlorite (NaOCl) varying the time of irrigation and the order of the irrigants. The blocks were prepared for and examined by scanning electron microscopy. Digital images at a magnification of 2000× were taken at randomized areas on the root canal dentin surface, and the area of tubule openings was measured by a semi-automatic method using image analysis software image-Pro Discovery 5.0.

Results: Erosion of peritubular and intertubular dentin was detected when EDTA, EGTA or CA were used as the initial rinse (even for 30 s), followed by 5.25% NaOCl. The area of dentin tubule opening increased markedly when compared to the sequences where NaOCl was used first, before the chelators or CA ($P<0.05$). An initial rinse with the chelators or CA for 5 min, followed by a final rinse with NaOCl, regardless of the

duration of the NaOCl rinse (1 - 5 min), resulted in over 100% increase in the area of dentin tubule openings ($P<0.01$).

Conclusions: Irrigation using 5.25% sodium hypochlorite after demineralization agent(s) on root canal wall dentin with smear layer causes marked erosion at the dentin surface.

Preface

This research project has been written into manuscript, and accepted for publishing in the Journal of Endodontics. Currently it is in corrected proof: Qian W, Shen Y, Haapasalo M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. J Endod (2011) . doi:10.1016/j.joen.2011.06.005. Research question of the project was identified and project was designed byWei Qian under the guidance of Dr. Markus Haapasalo and Dr. Ya Shen. All the sample preparation and image acquiring were done by Wei Qian. Data were analyzed by Wei Qian and Dr. Ya Shen. Manuscript was prepared by Wei Qian, edited by Dr. Ya Shen and Dr. Markus Haapasalo.

The study was approved by the UBC Clinical Research Ethics Board (UBC CREB number: H05-70207)

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CA: citric acid

EDTA: ethylenediaminetetraacetic acid

EGTA: ethyleneglycoltetraacetic acid

NaOCl: sodium hypochlorite

SEM: scanning electron microscopy

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To my wife Jiangtao and my mother Xinhua.

Chapter 1. Review of the literature

Pulpitis is caused by microbial antigens entering the pulp from a carious lesion or a leaking filling through dentinal tubules. As long as the pulp remains vital, the number of bacteria in the pulp is considered low and of no clinical significance in cases where root canal treatment is performed. Consequently, the treatment of vital cases should focus on the prevention of infection entering a primarily sterile environment, which is the apical portion of the root canal (Haapasalo & Qian 2008).

However, with proceeding infection, the entire root canal system becomes invaded by bacteria, and necrosis and apical periodontitis will develop. It has been shown without doubt that microorganisms are the etiological factor of apical periodontitis (Kakehashi et al. 1966, Bergenholtz 1974, Sundqvist 1976). The goal of endodontic treatment is therefore prevention and/or elimination of a microbial infection in the root canal system, more specifically, to remove and kill all microorganisms in the root canal and to neutralize any antigens that may be left in the canal after killing the microbes (Orstavik & Pitt Ford 1998).

In current endodontic treatment, the mostly practiced way of such disinfection is achieved by instrumentation, irrigation and sometimes also use of local inter-appointment disinfecting agents placed into the root canal(s). The goal of hand and rotary instrumentation and irrigation is to remove all necrotic and vital organic tissue, as well as some hard tissue including dentin chips created by instrumentation, from the root canal system and give the canal system a shape that facilitates optimal irrigation, debridement and placement of local medicaments. In addition, instrumentation shapes the root canal to accept permanent root filling, which is supposed to form a tight seal to prevent invasion and re-colonization by oral microbiota in case of a new caries lesion or leaking filling allows penetration and new threat by bacteria.(Schilder 1974) Because of the complex anatomy of root canal systems, with multiple fins and ramifications, antisepsis in necrotic teeth and teeth with failed root canal treatments is more challenging than in vital teeth, both from a technical and a microbiologic point of view (Zehnder 2006). Besides the risk of instrument separation and preparation errors, instrumentation has also two other drawbacks: one is the production of smear layer. A smear layer is produced on the instrumented walls of the main root canal, and it is comprised of inorganic and organic material such as dentin filings and pulp tissue remnants (McComb & Smith 1975). This deposit can also contain bacteria and microbial antigens and it may offer protection to biofilms adhering to root canal walls. Furthermore, the smear layer interferes with a tight adaptation of currently used root canal sealers to dentin walls, and may therefore promote microleakage (Kokas et al. 2004). The second drawback is that mechanical

instrumentation in combination with a chemically inert irrigating solution cannot adequately reduce the number of viable microorganisms in the infected root canal system, nor can the formation of a smear layer be prevented (Bystrom & Sundquist 1981)

Irrigation has a central role in endodontic treatment. The irrigants facilitate removal of microorganisms, tissue remnants, and dentin chips from the root canal space during and after instrumentation through a flushing mechanism. Irrigating solutions also help prevent packing of the hard and soft tissue in the apical root canal, which could otherwise cause a variety of complications such as transportation, zipping, and extrusion of infected material into the periapical tissues. Some irrigating solutions can dissolve organic or inorganic tissue. Several solutions also have antimicrobial activity and they kill bacteria and yeasts when allowed in direct contact with the microorganisms. On the other hand, many of these solutions have potentially harmful effects, too. Several irrigating solutions have cytotoxic potential(Spangberg et al. 1973), and they may cause severe pain if they gain access into the periapical tissues (Becker et al. 1974). Because of the central role of irrigation in endodontic treatment and the multitude of tasks the irrigants are supposed to do, there is a continuing search for an optimal irrigating solution and irrigation strategy. An optimal irrigant should have all or most of the characteristics listed below, but none of the negative or harmful properties. None of the presently available irrigating solutions

can be regarded as optimal. However, using a combination of products in a correct sequence contributes to successful treatment outcome.

Desired functions of irrigating solutions:

- Washing action (helps remove debris)
- Reduce instrument friction during preparation (lubricant)
- Facilitate dentin removal (lubricant)
- Dissolve inorganic tissue (dentin)
- Penetrate to canal periphery (fins, anasthomoses, lateral canals)
- Dissolve organic matter (dentin collagen, pulp tissue, biofilm)
- Kill bacteria and yeasts (both planktonic and biofilm microorganisms)
- Do not irritate or damage vital periapical tissue, no caustic or cytotoxic effects
- Are not allergenic
- Do not weaken tooth structure
- Do not stain dental hard tissues
- Cheap to manufacture
- Long shelf live
- Simple to use

An optimal irrigant should have all or most of the positive characteristics listed above, but none of the negative or harmful properties. While none of the available irrigating solutions fill all the criteria presented in Table 1, a selective use of the existing solutions in correct combinations and sequence can greatly contribute to the successful outcome of the treatment. NaOCl and EDTA are among the most popular and effective irrigants used in root canal treatment.

1.1 Sodium hypochlorite

1.1.1 History

Sodium hypochlorite (NaOCl) has an extensive history in medicine and dentistry. As early as in 1785, a solution of chlorine gas in water was used to bleach textiles. Potassium hypochlorite (Eau de Javel) was prepared by Berthollet in 1789 to replace chlorine. In 1820, French chemist Labarraque replaced potash liquor by the cheaper caustic soda liquor and lead to the invention of sodium hypochlorite. At the end of the 1820s, Collins and Holmes showed that childbed fever frequency decreased when midwives wash their hands in chlorinated water. In 1881, German bacteriologist Koch demonstrated under controlled laboratory conditions that pure cultures of bacteria may be destroyed by the use of hypochlorite. Few years after, in 1894, Traube established the purifying and disinfecting properties of hypochlorite in water treatment. In World War I, British chemist Dakin uses a buffered 0.5% sodium hypochlorite solution to irrigate

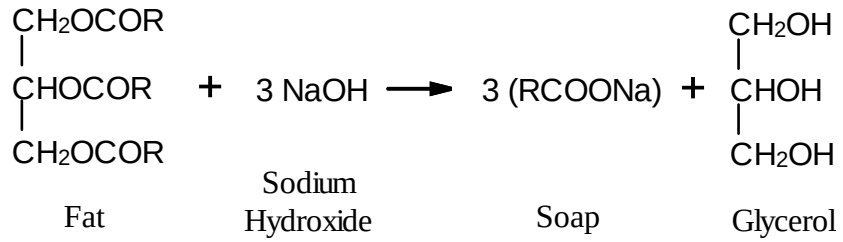
infected wounds. The use of sodium hypochlorite solution in endodontics as the main irrigant was recommended by Coolidge in 1919 (Coolidge 1919). Walker (1936) introduced the use of double-strength chlorinated soda (5% NaOCl) solution as a root canal irrigant.

1.1.2 Mechanism of action

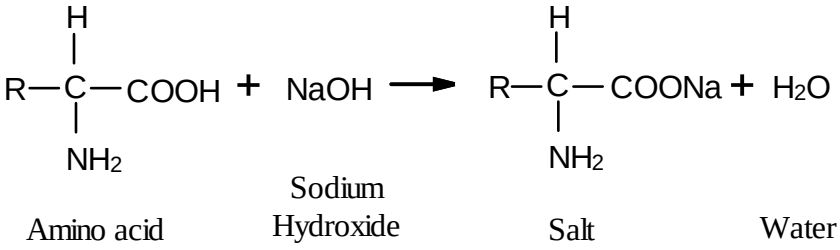
NaOCl ionizes in water into Na+ and the hypochlorite ion, OCl^-, establishing equilibrium with hypochlorous acid (HOCl). At acidic and neutral pH, chlorine exists predominantly as HOCl; at pH 9 and above, OCl^- predominates. Hypochlorous acid is responsible for the antibacterial activity; the OCl^- ion is less effective than the undissolved HOCl (Randtke 2010). Morris (1966) found that OCl^- ion possesses approximately 1/80th of the germicidal potency of HOCl in killing *Escherechia coli*.

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$$

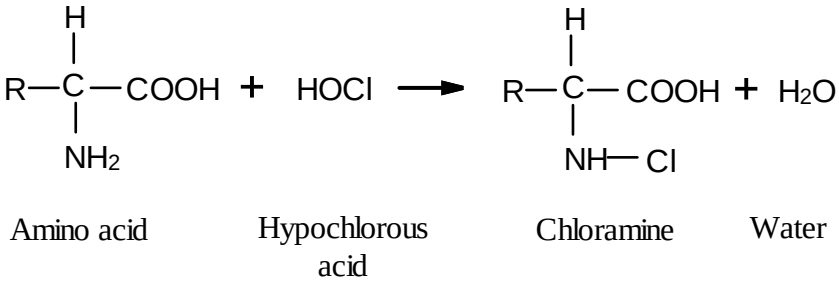
The antimicrobial mode of action of hypochlorite is not fully understood, but several mechanisms have been proposed. These include cell wall effects (Pulvertaft & Lumb, 1948), membrane effects (Chang 1944), inhibition of sulfhydrylenzymes (Green & Stumpf 1946) and activity against DNA (Haas & Engelbrecht 1980). The chemical reactions between organic tissue and NaOCl are rather complicated due to the huge varieties of organic molecules in tissue and biofilm. Esterla et al. (2002) proposed three reactions as below:



Scheme 1. NaOCl acts as a fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol (alcohol), which reduces the surface tension of the solution.



Scheme 2. NaOCl neutralizes amino acids forming water and salt. With the consumption of hydroxyl ions, the pH decreases.



Scheme 3. Hypochlorous acid reacts with the protein amino group to form chloramines. This leads to amino acid degradation and hydrolysis. The chloramination reaction between chlorine and the amino group (NH) forms chloramines that interfere in cell metabolism. Chlorine (a strong oxidant) has an antimicrobial action, inhibiting bacterial

enzymes and leading to an irreversible oxidation of sulfhydryl groups (SH) of essential bacterial enzymes.

NaOCl is a potent antimicrobial agent, killing most bacteria instantly on direct contact. It also effectively dissolves pulpal remnants and collagen, the main organic components of dentin. Hypochlorite is the only root-canal irrigant of those in general use that dissolves necrotic and vital organic tissue. It is difficult to imagine successful irrigation of the root canal without hypochlorite.

1.1.3 Antibacterial property

NaOCl is commonly used in concentrations between 0.5% and 6%. In some articles hypochlorite is reported to kill the target microorganisms in seconds, even at low concentrations, although other reports have published considerably longer times for the killing of the same species (Waltimo et al. 1999, Gomes et al. 2001, Radcliffe et al. 2004, Vianna et al. 2004). Such differences are a result of confounding factors in some of the studies. The presence of organic matter during the killing experiments has a great effect on the antibacterial activity of NaOCl. Haapasalo et al. (2000) showed that the presence of dentin caused marked delays in the killing of *Enterococcus faecalis* by 1% NaOCl. Dunavant et al. (2006) compared the efficacy of 1% or 6% NaOCl and 2% CHX, against *E. faecalis* biofilms in vitro model system. Their model consisted of biofilms grown in a

flow cell system. Biofilms were immersed in test irrigants for 1 or 5 minutes. Results showed that both concentrations of NaOCl provided statistically significantly better biofilm kill than any other of the tested agents. Biofilm cell was also removed by 6% NaOCl. Clegg et al. (2006) in an ex vivo biofilm study, demonstrated a difference in the effectiveness against biofilm bacteria by 6%, 3% and 1% NaOCl. They showed that 6% NaOCl and 3% NaOCl were capable of disrupting and removing the biofilm; 1% NaOCl and 1% NaOCl followed by MTAD were capable of disrupting the biofilm, but not eliminating bacteria; 2% CHX was not capable of disrupting the biofilm. Viable bacteria could not be cultured from specimens exposed to 6% NaOCl, 2 % CHX, or 1% NaOCl followed by BioPure MTAD. So it was concluded that 6% NaOCl was the only irrigant capable of both rendering bacteria nonviable and physically removing the biofilm.

Bacterial cells in the root canal encounter a harsh ecologic milieu. Recently, one in vitro study (Liu et al. 2010) evaluated the biofilm formation capability of starved *E. faecalis* cells on human dentin and the susceptibility of the biofilm to 5.25% NaOCl. The findings showed that *E. faecalis* cells in the starvation phase could develop biofilm on human dentin. Biofilms of starved cells were more resistant to 5.25% NaOCl than those of stationary cells. Many of the earlier studies were performed with planktonic bacteria in the presence of an unknown amount of organic matter (e.g. nutrient broth) or without controlling the pH of the culture, both of which affect the result. When the confounding

factors are eliminated, it has been shown that NaOCl kills the target microorganisms rapidly even at low concentrations of less than 0.1% (Vianna et al. 2004, Portenier et al. 2005). However, in vivo the presence of organic matter (inflammatory exudate, tissue remnants, microbial biomass) consumes NaOCl and weakens its effect. Therefore, continuous irrigation and sufficient time are important factors for the effectiveness of hypochlorite.

1.1.4 Antifungal activity

Candida albicans is the fungal species most commonly detected in the oral cavity of both healthy (30-45%) and medically compromised (95%) individuals. Fungi have occasionally been found in primary root canal infections, but they seem to be more common in the root canals of obturated teeth in which the treatment has failed (Siqueira et al. 2004). Overall, the occurrence of yeasts reported in infected root canals varies between 1% and 17% (Waltimo et al.1997). Since fungi may be involved in cases of persistent and secondary infections associated with recalcitrant periradicular lesions, the spectrum of antimicrobial activity of endodontic medicaments and irrigants should include these microorganisms. Research had shown that the antifungal activity of NaOCl is superior to or at least equal to other common irrigation solutions, such as 2% CHX, MTAD and 17%EDTA (Ayhan et al. 1999, Radcliffe et al. 2004, Ruff et al. 2006).

1.1.5 Increasing the efficacy of NaOCl solutions

In aqueous solution, hypochlorous acid partially dissociates into the hypochlorite anion (OCl^-):

$$HOCl \leftrightarrow H^+ + OCl^-$$

The available chlorine is the electrochemical equivalent amount of elemental chlorine, as a measurement of oxidizing capacity. It is the sum of the HOCl and OCl^- concentrations in the solution.

1.1.5.1 Altering the pH

HOCl dissociation depends on pH, with the clinical equilibrium between HOCl and OCl− being maintained as HOCl is consumed through its germicidal function. Baker (1959) illustrated the relationship between HOCl, OCl^-, and pH. At pH 10, basically all chlorine is in the OCl^- form; the reverse occurs at a pH of 4.5, when all chlorine is in the form of HOCl. The disinfecting properties decrease with higher pH, paralleling the concentration of dissociated HOCl. So it is obvious that to increase the antimicrobial efficacy of hypochlorite solutions could thus be to lower the pH. It has also been suggested that such solutions would be less toxic to vital tissues than nonbuffered preparations (Dakin 1915, Cotter et al. 1985). However, buffering hypochlorite with bicarbonate makes the solution unstable so that its shelf life shortened to less than 1 week (Cotter et al. 1985). Changing the amount of the bicarbonate in the mixture and therefore

the pH value, the antimicrobial efficacy of a fresh bicarbonate buffered solution is only slightly higher (Cotter et al. 1985) or not elevated at all compared to that of a nonbuffered preparation (Zehnder et al. 2002). Above all, the caustic potential of hypochlorite solutions appears to be influenced mainly by the available chlorine rather than by pH or osmolarity (Zehnder et al. 2002).

1.1.5.2 Temperature

As early as 1936, the effect of NaOCl temperature on Mycobacterium tuberculosis survival was demonstrated (Costigan 1936). Using 50 ppm available chlorine hypochlorite solution at pH 8.35, he obtained complete kill in 30 seconds at 60 ℃, in 60 seconds at 55 ℃, and in 2.5 minutes at 50 ℃. Under the same test conditions, 200 ppm available chlorine solutions at pH 9 destroyed the organism in 60 seconds at 50 ℃ and in 30 seconds at 55 ℃. A recent study using steady-state planktonic *E. faecalis* cells found a temperature raise of 25 ℃ increased NaOCl efficacy by a factor of 100 (Sirtes et al. 2005).

Cunningham et al. (1980) found that 2.6% NaOCl solution at a temperature of 37 °C was equally effective as a collagen-dissolving agent when compared to 5.2 % NaOCl at either 21 ℃, and the time interval for 2.6 % NaOCl to produce sterility of in vitro bacterial cultures was significantly reduced at 37 °C compared to 22 ℃. Thus, for low-

concentration hypochlorite irrigants, one way to improve their effectiveness in the root canal system is to increase the temperature. This improves their immediate tissue dissolution capacity (Abou-Rass et al. 1981). Stojicic et al. (2010) used an in vitro model to compare tissue dissolution capability of 1-5.8% NaOCl at room temperature, 37 ℃ and 45 ℃, and found 30-300% increase depending on the concentration, temperature, and type of hypochlorite. The capacity of a 1% NaOCl at 45 ℃ to dissolve human dental pulps was found to be equal to that of a 5.25% solution at 20 ℃. Heated hypochlorite solutions also remove organic debris from dentin shavings more efficiently than unheated solutions (Kamburis 2003).

With similar short-term efficacy in the immediate environment as the root canal system, the systemic toxicity of preheated NaOCl irrigants should be lower than the one of more concentrated non-heated solutions as a temperature equilibrium is reached relatively quickly (Cunningham et al. 1980). However, there is still a lack of clinical studies supporting the use of heated sodium hypochlorite.

1.1.5.3 Ultrasonic agitation

The use of ultrasonic energy for cleaning of the root canal and to facilitate disinfection has a long history in endodontics. The comparative effectiveness of ultrasonics and hand-instrumentation techniques has been evaluated in several earlier studies (Martin et al.

1976, Cunningham et al. 1982, Plotino et al. 2007). Most of these studies concluded that ultrasonics, together with an irrigant, contributed to a better cleaning of the root-canal system than irrigation and hand-instrumentation alone. Cavitation and acoustic streaming of the irrigant contribute to the biologic-chemical activity for maximum effectiveness (Martin et al. 1985). Analysis of the physical mechanisms of the hydrodynamic response of an oscillating ultrasonic file suggested that stable and transient cavitation of a file, steady streaming, and cavitation microstreaming all contribute to the cleaning of the root canal (Roy et al. 1994). Ultrasonic files must have free movement in the canal without making contact with the canal wall to work effectively (Lumley et al. 1992). Several studies have indicated the importance of ultrasonic preparation for optimal debridement of anastomoses between double canals, isthmuses, and fins (Goodman et al 1985, Sjogren et al. 1987, Archer et al. 1992). The effectiveness of ultrasonics in the elimination of bacteria and dentin debris from the canals has been shown by several studies (Spoleti et al. 2002, Sabins et al. 2003, van der Sluis et al. 2005& 2007). However, not all studies have supported these findings (Sjogren et al. 1987).

Van der Sluis and colleagues (van der Sluis et al. 2005) suggested that a smooth wire during ultrasonic irrigation is as effective as a size 15 K-file in the removal of artificially placed dentin debris in grooves in simulated root canals in resin blocks. It is possible that preparation complications are less likely to occur with an ultrasonic tip with a smooth,

inactive surface. In a recent study, Stojicic et al. (2010) showed that sonic agitation and even agitation with conventional syringe-needle irrigation were equally effective in improving tissue dissolution by sodium hypochlorite solutions at different concentrations. The results of the study indicated that refreshment of the NaOCl solution on the substrate surface is the key for improved effect, and that this effect can be obtained with various means (Stojicic et al. 2010).

1.1.6 Effect of NaOCl on the composition and structure of dentin

Dentin is composed of approximately 20% organic material by weight. Most of this consists of type I collagen, which forms the matrix for inorganic component to deposit. Sodium hypochlorite is known to fragment long peptide chains and to chlorinate protein terminal groups; the resulting N-chloramines are broken down into other species (Stoward, 1975). Consequently, hypochlorite solutions may affect the mechanical properties of dentin by the degradation of organic dentin components (Marending et al 2007). A study on bovine dentin suggested that, within the time frame of a root canal treatment, concentrated hypochlorite solutions cause untoward effects on dentin biomechanics. Slutzky-Goldberg et al. (2004) evaluated the effect on root dentin microhardness of 2.5% and 6% NaOCl solutions for various irrigation periods (5, 10, or 20 min). They found that there was a significant difference in groups irrigated for 10 or 20 min. Furthermore, the decrease in microhardness was more marked after irrigation

with 6% NaOCl than 2.5% NaOCl. A 2 h exposure of dentin to NaOCl solutions of more than 3% (w/v) significantly decreases the elastic modulus and flexural strength of human dentin compared to physiological saline.

Mountouris et al. (2004) evaluated the deproteination potential of 5% aqueous NaOCl solution applied with a rubbing action on the molecular composition and morphology of smear-layer covered and acid-etched human coronal dentin surfaces. They found that in both groups, NaOCl treatment reduced the organic matrix (amide I, II, III peaks), but did not affect carbonates and phosphates. Di Renzo et al. (2001) evaluated chemical alterations on the dentin surface after treatment with NaOCl using a photoacoustic FTIRS (PA-FTIRS) technique. Results showed that NaOCl-treated dentin samples demonstrated a slow and heterogeneous removal of its organic phase, leaving calcium hydroxyapatite and carbonate apatite unchanged. A combined sequential 2 min treatment of dentin with both maleic acid and NaOCl indicated that this treatment could produce a surface region which was neither significantly demineralised nor deproteinated. In another study, the effects of NaOCl on dentin collagen and glycosaminoglycans were evaluated immunohistochemically. The results showed that 5% NaOCl induced alterations in dentin collagen and glycosaminoglycans and hydroxyapatite demonstrated a protective role of on organic matrix stability. Recently, Marending et al. (2007) evaluated the effects of NaOCl on the structural, chemical and mechanical properties of human root

dentin. They found that NaOCl caused a concentration-dependent reduction of elastic modulus and flexural strength in human root dentin. Furthermore, both the carbon and nitrogen content of the specimens were significantly reduced. In addition, intertubular dentin permeability to basic fuchsin dye and the peripheral dentin matrix were altered, although no effect on inorganic dentin components could be detected in backscattered SEMs.

1.1.7 Effect of NaOCl on bonding to dentin

Dentin is degenerated by NaOCl treatment because of the dissolution of dentinal collagen (Ishizuka et al. 2001). Moreover, residual NaOCl may interfere with polymerisation of bonding resin due to oxygen generation. The bond strength of resin following NaOCl treatment before etching decreased when a MMA-TBB resin system was employed (Kataoka et al. 1999). The decreased bond strength is improved when an ascorbic acid or a sodium thiosulfate solution is applied after NaOCl treatment. These solutions remove NaOCl by the oxidation-reduction reaction. Nikaido et al. (1999) evaluated the bonding strength at the buccal dentin surface after NaOCl treatment on the root canal wall dentin; the bonding strength of single bond (SB) was significantly decreased after NaOCl treatment, while that of self-etching primer system (Clearfil MegaBond) did not change. Contrary to this, Ishizuka et al. (2001) found that the bonding strength of self-etching primer system decreased following NaOCl treatment whereas that of SB did not change.

Perdigao et al. (2000) assessed the effect of 10% commercial NaOCl gel on the dentin shear bond strengths and hybrid layer ultra-morphology of two total-etch adhesive systems (Prime & Bond NT and Single Bond). Results demonstrated that the increase in the NaOCl application time resulted in a progressive decrease in shear bond strengths for both dentin adhesives.

Frankenberger et al. (2000) compared the dentin bond strength and marginal adaptation of direct composite resins with and without additional NaOCl treatment after the etching process. They found that after hypochlorite treatment, dentin bond strength and marginal adaptation decreased significantly. Saboia et al. (2006) investigated the effect of 10% NaOCl for one minute after acid conditioning on the shear bond strength of two acetone-based single-bottle adhesive systems and found that collagen removal improves the bond strength for these systems. Pioch et al. (1999) evaluated the effect of NaOCl treatment of acid-etched dentin on the tensile bond strength of adhesive resins. They found that the removal of the collagen layer with NaOCl could enhance or decrease bond strengths, depending on the bonding agent used. Osorio et al. (2002) evaluated the effect of NaOCl treatment on the shear bond strength and microleakage of a polyalkenoic acid-containing adhesive system. Results showed that adverse chemical interactions could have occurred between the remnant collagen matrix and/or mineralised dentin after NaOCl treatment. There was no additional advantage in using NaOCl treatment with this adhesive. Ari et al.

(2003) evaluated the effect of NaOCl on the regional bond strengths of four adhesive systems to root canal dentin. They found that, depending on the adhesive system, NaOCl enhanced the bond strength. Erdemir et al. (2004) indicated that NaOCl, decreased bond strength of C&B Metabond to root canal dentin significantly.

Shinohara et al. (2004) found that depending on the adhesive system used, the application of NaOCl increased microleakage along dentin margins. Correr et al. (2004) demonstrated that dentin surface treatment with NaOCl did not affect the resin-dentin bonding strength in primary teeth. Vongphan et al. (2005) indicated that NaOCl significantly reduced the bond strengths of the adhesive when a total etching was applied. The application of sodium ascorbate on NaOCl treated dentin significantly improved the bond strengths. Wachlarowicz et al. (2007) examined the effects of commonly employed endodontic irrigants on Epiphany (root canal sealer)-dentin bond strengths. They found that only NaOCl improved the bond strengths.

1.1.8 Influence of NaOCl on endodontic instrument metals

Hypochlorite is that is potentially very corrosive against metals. The corrosion process involves selective removal of nickel (the more reactive element of the alloy) from the material surface, creating micro corrosion pits (Sarkar et al. 1983). It is assumed that these microstructural defects can lead to areas of stress concentration and crack

formation, weakening the structure of the instrument. O'Hoy et al. (2003) evaluated the effect of cleaning procedures using NaOCl, and detected significant corrosive phenomena of NiTi instruments exposed to 1% NaOCl for up to 10 cleaning cycles. However, no significant reduction of torque at fracture or number of revolutions to flexural fatigue was found. Busslinger et al. (1998) used 5% NaOCl for 30 or 60min and Lightspeed rotary instruments, and found corrosion patterns, even if the authors were not sure of the clinical implications. Berutti and Marini (1996) evaluated the influence of immersion in NaOCl on resistance to cyclic fatigue fracture and corrosion of ProTaper NiTi rotary instruments. They concluded that if NiTi rotary instruments operate immersed in a NaOCl solution contained in the pulp chambers of teeth restored with metals or alloys having different electrochemical nobility values, galvanic corrosion may occur. These coupling phenomena may cause pitting and cracks that alter the integrity of the instrument surface, decreasing its resistance to fracture because of cyclic fatigue. Haikel et al. (1998 A&B) reported that the mechanical properties of Ni-Ti instruments were not affected by NaOCl, nor was the cutting efficiency. This is probably the result of very slow corrosion of NiTi alloy alone, when the galvanic effect does not potentiate it, as occurs where other metals are present. Peters et al. (2007) found a negative effect on the fatigue resistance of NiTi rotary files that had been immersed for up to 2 hours in 5.25% hypochlorite solution before fatigue testing the instrument in air afterwards. In conclusion, although prolonged exposure to hypochlorite can have a dramatic effect on

the NiTi metal, it seems that in practice the limited time of exposure is not enough to impact the performance and durability of the files to the level that should cause alarm.

1.1.9 Interaction of NaOCl and chlorhexidine

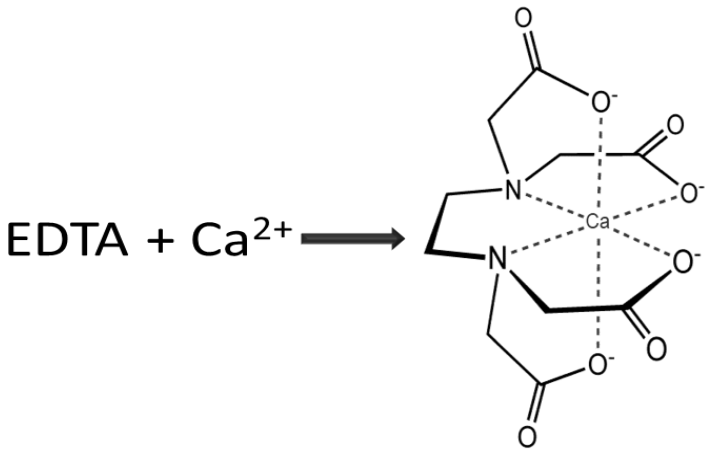
The mixing of NaOCl with CHX produces an orange-brown precipitate (Basrani et al. 2007, Bui et al. 2008). The precipitate is an insoluble neutral salt formed by the acid-base reaction between NaOCl and CHX. Parachloroanaline (PCA) is the main product of the interaction of NaOCl and CHX, with the molecular formula C_6H_6NCl as analyzed by mass spectrometry. When mixed together with NaOCl, CHX molecules become hydrolyzed into small fragments, each of which being able to react and form byproducts. At the beginning, the bonds between carbon and nitrogen are broken because the bond dissociation energy required between these two atoms is low.

PCA has shown to be cytotoxic in rats (Chhabra et al. 1991) and possibly carcinogenic in humans (International Agency for Research on Cancer group 2B). Leaching of parachloroanaline from the insoluble precipitate into periradicular tissues is of concern. This precipitate also coats the canal surface and can even occlude the openings of the dentin tubules (Bui et al. 2008), which may preventing the penetration of the intracanal medicaments and compromises the seal of the obturated root canal. Also, its presence imparts color to the canal wall and causes tooth discoloration affecting esthetics.

In summary, despite of some potential problems related to the use of NaOCl, it is the only solution in endodontics that can at least to some extend dissolve biofilm in addition to direct killing of microbes inside the biofilm, it should be regarded as the main disinfecting solution during chemomechanical instrumentation of the infected root canals.

1.2 EDTA

Chelators were first introduced to endodontics by Nygaard-Østby (1957), who recommended the use of a 15% EDTA solution (pH 7.3) with the following composition: Disodium salt of EDTA (17 g); Distilled water (100mL); 5M sodium hydroxide (9.25 mL) According to Nygaard-Østby, the main mineral components of dentin, phosphate and calcium, are soluble in water. When the disodium salt of EDTA is added to this equilibrium, calcium ions are removed from the solution. This leads to the dissolution of further ions from dentin so that the solubility product remains constant. Thus, chelators cause decalcification of dentin.



A few years later, a detergent was added in order to increase the cleaning and bactericidal potential of the EDTA solution, the new composition being known as EDTAC (Von der Fehr & Nygaard-Østby 1963). EDTAC is produced when EDTA is mixed with 0.84 g of a quarternary ammonium compound. This addition is aimed at reducing the surface tension of the irrigant, facilitating the wetting of the entire root canal wall and thereby increasing the ability of the chelators to penetrate the dentin. EDTA in its pure form already has a lower surface tension than 1 or 5% sodium hypochlorite (NaOCl), saline solution or distilled water (Tasman et al. 2000). Furthermore, EDTAC should have a greater antimicrobial effect than EDTA. In contrast to this, no difference in the chelating effectiveness of EDTAC and EDTA has been reported (Weinreb & Meier 1965).

Initially, chelators were used as liquids for irrigation during mechanical instrumentation of the root canal system. In 1969, Stewart et al. presented RC-Prep (premier Dental; Philadelphia, PA, USA), probably the best known paste-type chelating agent. Although the efficacy of liquid and paste-type EDTA preparations in softening root dentin has been a point of controversy, chelator preparations have been advocated frequently as adjuncts for root canal preparation, especially in narrow and calcified root canals (Lovdahl & Gutmann 1997), and for removal of the smear layer (McComb & Smith 1975, Goldman et al. 1985, Baumgartner & Mader 1987, Çalt & Serper 2000). Recently, paste-type chelators have regained popularity as many manufacturers of nickel–titanium instruments

recommend their use as a lubricant during rotary root canal preparation, presumably to reduce the risk of instrument separation.

1.2.1 Time duration for smear layer removal

It should be noted that EDTA alone cannot completely remove the smear layer. This is because smear layer contains also organic matter (e.g. predentin and dentin collagen fibers), and EDTA only affects the inorganic part of the layer. For complete removal of smear layer, use of hypochlorite is also necessary (Baumgarterner et al. 1987).

1.2.2 Effect on tooth surface strain

Rajasingham et al 2010 showed that irrigation with 5% NaOCl alone or alternated with 17% EDTA significantly increased tooth surface strain. The alternated regimen showed significantly greater changes in tooth surface strain than NaOCl alone. Irrigation with 3% NaOCl and 17% EDTA individually or in combination did not significantly alter the tooth surface strain.

1.2.3 EDTA with ultrasonics

A 1-min application of 17% EDTA combined with ultrasonics is efficient for smear layer and debris removal in the apical region of the root canal. EDTA performed significantly better than NaCl and NaOCl in smear layer removal and dentinal tubule opening.

1.3 EGTA

EGTA is able to bind Ca^{2+} more specifically and its chelating capability is weaker than EDTA (Calt et al. 2000). Studies verified that 17% EGTA had less calcium extraction ability compared to 17%EDTA (Sauin et al. 2007, Marques et al. 2006) and it can be effectively used as an alternative chelator for the removal of the smear layer (Calt & Serper 2000, Viswanath et al. 2003). Both EGTA and EDTA had similar biocompatibility (Sousa et al. 2005). EGTA also have less inhibitory effect on substrate adherence capacity of rat inflammatory macrophages than EDTA (Segura-Egea et al. 2003)

1.4 Citric acid

Citric acid is a chelating agent that reacts with metals to form a nonionic soluble chelate. Loel (1975) suggested its use as a cleaner in root canal treatment. Goldman et al. (1981) reported that the effects on the removal of the smear layer obtained with citric acid were similar to those by EDTA. Ando (1985) reported that citric acid is less cytotoxically irritable to tissue than EDTA. Yamauguchi et al. (1996) showed that 0.5 M, 1 M and 2 M citric acid had better decalcifying capability than 0.5 M EDTA and they all have some antibacterial activities. Its decalcifying action depends on both acidity and chelation. Wayman et al. (1979) showed that 10% used with 2.5% NaOCl is effective in producing a clean canal.

1.5 Present recommendations

The combination of EDTA and sodium hypochlorite (NaOCl) have been advocated as an effective irrigation regimen to remove the organic and inorganic matter (Baumgartner et al 1987, Perez-Heredia et al. 2006, Zehnder, 2006), but there is no clear consensus regarding the ideal irrigation sequence, volume and application time in the literature. While NaOCl is used during instrumentation, EDTA is preferably used at the end of instrumentation to complete the removal of the smear layer (Yamada et al. 1983, Zehnder et al. 2002). Sufficient time and volume of NaOCl ensures high disinfecting efficacy and enables NaOCl to penetrate into dentin. On the other hand, a final flush of NaOCl has also been advocated, as after smear layer removal NaOCl could better reach areas previously covered by the smear layer (Goldman et al. 1982).

1.6 Challenge

Root canal irrigation with the above solutions can lead to structural changes, as evidenced by reduction of dentin strength, microhardness and changes in surface roughness (Eldeniz et al. 2005, Marending et al. 2007 A & B). Baumgartner & Mader (1987) reported that when EDTA and NaOCl solutions were alternately applied to uninstrumented root canal wall, dentin showed an eroded appearance, and tubular orifice diameters were enlarged. Conflicting results have been obtained from some in vitro studies regarding the dentin surface after different irrigation protocols (Niu et al. 2002,

Torabinejad et al. 2003, Perez-Heredia et al. 2006). Many studies have suffered from specific limitations, such as qualitative evaluation based on non-randomized selection of the observation areas, and analysis done by scores. The lack of standardization of many of the studies makes it difficult to fully understand the effect of irrigation sequences on dentin surface.

1.7 Objectives

The purposes of the present study were

1. To examine the effect of different irrigation protocols on root canal wall dentin.

2. To quantify and compare the level of erosion caused by irrigation sequences, using a quantitative analysis with a semi-automatic method.

1.8 Hypothesis

Using 5.25% NaOCl after demineralizing solutions will cause significant dentin erosion measured by average dentinal tubule opening area.

Chapter 2. Materials and methods

2.1 Dentin block preparation

Eighty-seven extracted single-rooted human teeth with one root canal without previous endodontic treatment were selected for the study. The study was approved by the University research ethics board (UBC CREB number: H05-70207). The teeth were stored in 0.01% NaOCl at 4˚C after the root surface was cleaned with curettes. The teeth were thoroughly rinsed with distilled water before the experiments. The crowns were removed at the cemento-enamel junction using high-speed bur under water-cooling. At least 3 mm of coronal and apical root were removed, only the 5 mm long middle part was used in the experiment. Residual pulp tissue was removed by fine barbed broaches. The canal wall was then instrumented by circumferential filing using #40 Hedstrom hand files (Dentsply Maillefer, Ballaigues, Switzerland) for one minute. After instrumentation, external grooves were made on bucco-lingual surfaces of the roots with diamond disc, and the tooth blocks were split into two halves by a single edge razor blade and a hammer.

2.2 Irrigants treatment

The dentin blocks were randomly divided into the experimental groups and treated with 5.25% NaOCl (VWR, Mississauga, Canada), 17% EDTA pH 7.0, 17% EGTA pH 7.0,

and 10% citric acid (Fisher Scientific, Ottawa, Canada) with designated sequence and timing as shown in Table 1. Each tooth block was put into a 15 mL capped test tube containing 10 mL of one of the above solutions, and the tube was constantly rocked for mixing. The specimen was rinsed thoroughly with distilled water and dried with lint-free Kimwipes between and after the treatments.

2.3 Scanning elecltron microscope sample preparation , observation and image acquisition

After the treatments, the samples were dehydrated with 100% alcohol overnight, followed by 3 hours in a 58 ℃ oven. The dried blocks were mounted on aluminum stubs and coated with gold-palladium in a Hummer VI sputter (Technics West Inc., San Jose, CA). Three samples from each group were observed with a scanning electron microscope (Stereoscan 260, Cambridge, UK) operating at 8kV at a magnification of 2000x. A motorized specimen stage was used to acquire five random images of each sample from the canal wall dentin surface. The area to be analyzed was selected at low magnification of 200x at which dentinal tubule openings could not be seen. The magnification was then increased to 2000x without moving the microscope and the first area was thus chosen. The other four areas were selected by moving the SEM field according to a predetermined plan. Images obtained by the SEM were transferred to a computer and examined.

2.4 Image analysis and statistical calculation

Surface area of a minimum of 500 tubule openings in each group was measured. Every tubule contour was autosegmented by ImagePro Discovery 5.0 (Media Cybernetics, Silver Spring, MD, USA) software at high magnification. The area of tubule opening was auto-calculated. Finally, to assess the reproducibility of the semi-automatic method the SEM images were randomly coded and evaluated individually by two dentists experienced in SEM. The interclass correlation coefficient was submitted to statistical analysis. Data were analyzed using AVOVA test (SPSS for Windows 11.0, SPSS, Chicago, IL, USA).

Chapter 3. Results

The inter-class correlation coefficient was 0.96. Examination of the surface of root canal walls exposed to different irrigation sequences revealed differences in dentin surface structure. The use of both irrigation sequences (NaOCl before or after chelators or CA) resulted in complete removal of the smear layer (Figure. 1). Specimens irrigated first with 5.25% NaOCl followed by the three demineralizing agents had smooth, nonporous intertubular dentin surface, and dentinal tubule orifices were regular and well separated from each other. The average area of tubule openings in Groups I - IV (hypochlorite first) was smaller than that in Groups V-IX (hypochlorite last) ($P<0.05$) (Table 1). The tubular openings were slightly larger when citric acid was used after NaOCl than when EDTA or EGTA were used as the final rinse, but the differences were not statistically significant (Table 1, groups II - IV). Erosion of peritubular and intertubular dentin were detected when 17% EDTA, 17% EGTA or 10% citric acid were used as the initial rinse (even for 1 minute only), followed by 5.25% NaOCl (Figure 2-5). Dentinal tubule orifices became irregularly enlarged and rough in appearance. Extending the time of either the demineralizing agent (EDTA, EGTA or CA) or the hypochlorite both increased the severity of the dentin erosion (Table 1, groups VI - IX). This erosion was extensive in some regions with merging of adjacent tubules and exposure of subsurface tubular structures, especially in the CA - NaOCl group (Figure 4). There were no significant differences among 17% EDTA, 17% EGTA or 10% citric acid (5 min) when they were

used after NaOCl, however, significant differences among them were measured when NaOCl was used as the final rinse ($P<0.05$) (Figure 6-9). There was less erosion in the EGTA - NaOCl group than in the EDTA - NaOCl and CA - NaOCl groups.

Table 1 Average area of dentinal tubule openings after different irrigation sequences and times. N: 5.25%NaOCl, E: 17%EDTA, C: 10%Citric acid, G: 17%EGTA. Number after the letter indicates time of exposure, e.g. N5E1= 5%NaOCl for 5min followed by 17%EDTA for 1min.

Group		Area (μm^2)
I	N1E1	7.95 ±1.7
	N1E5	8.61 ±1.67
	N1E10	10.67 ±1.42
II	N5E1	7.70 ±1.64
	N5E5	8.67 ±1.62
	N5E10	10.31 ±2.23
	N5E15	12.44 ±3.10
III	N5C1	8.38 ±1.92
	N5C5	9.27 ±1.13
	N5C10	11.22 ±2.11
IV	N5G1	7.86 ±1.25
	N5G5	8.18 ±1.5
	N5G10	10.44 ±2.12
V	E0.5 N5	8.87±1.07
	E1N1	14.22 ±2.96
	E1N5	15.51 ±2.01
	E1N10	16.01 ±3.10
VI	E5N1	18.15 ±2.32
	E5N5	18.13 ±2.49
	E5N10	19.10 ±2.88
VII	C1N1	15.26 ±1.64
	C1N5	16.08 ±1.36
	C1N10	17.33 ±2.22
VIII	C5N1	24.01 ±3.77
	C5N5	>30*
	C5N10	>30*
IX	G5N1	12.25 ±1.73
	G5N5	13.28 ±2.44
	G5N10	13.50 ±2.82

*The tubule openings had joined so that measurement of separate canal orifices was no longer possible.

Figure 1. SEM images of root canal wall surface dentin after irrigation. Non-erosion appearance. The smear layer is completely removed and peritubular and intertubular surface dentin appear smooth and flat (specimen from Group II: N5E5).

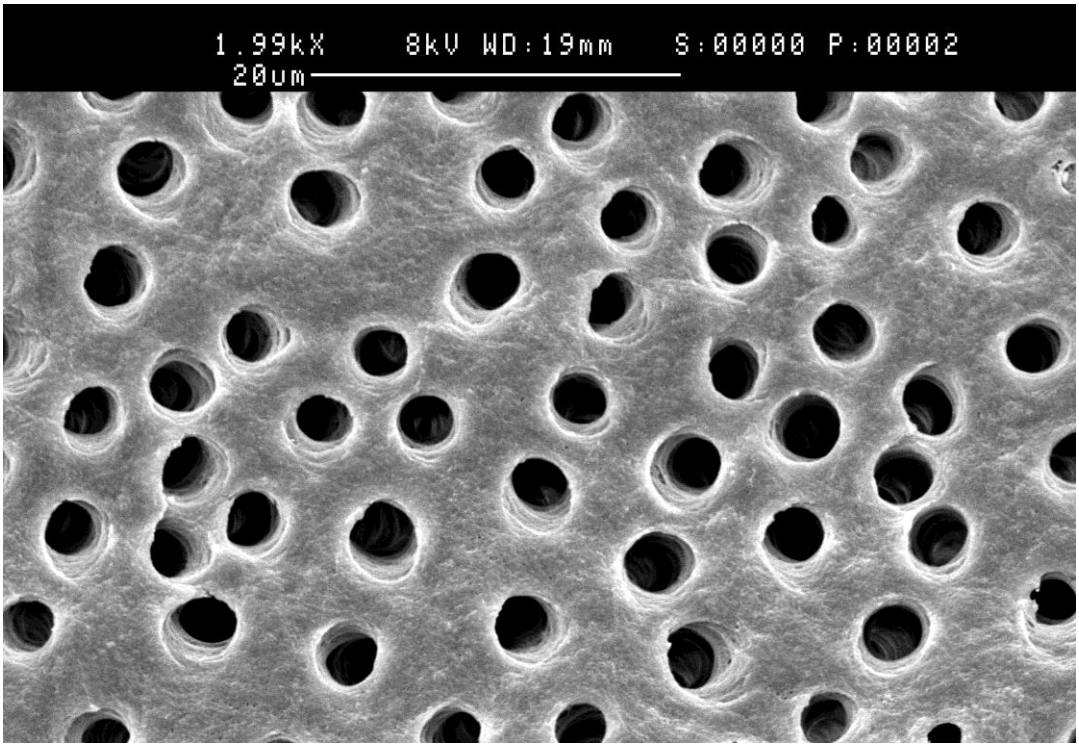


Figure 2. Weak erosion of peritubular and intertubular dentin. Dentin tubule orifices are irregularly enlarged and rough in appearance (specimen from Group II: E0.5N5).

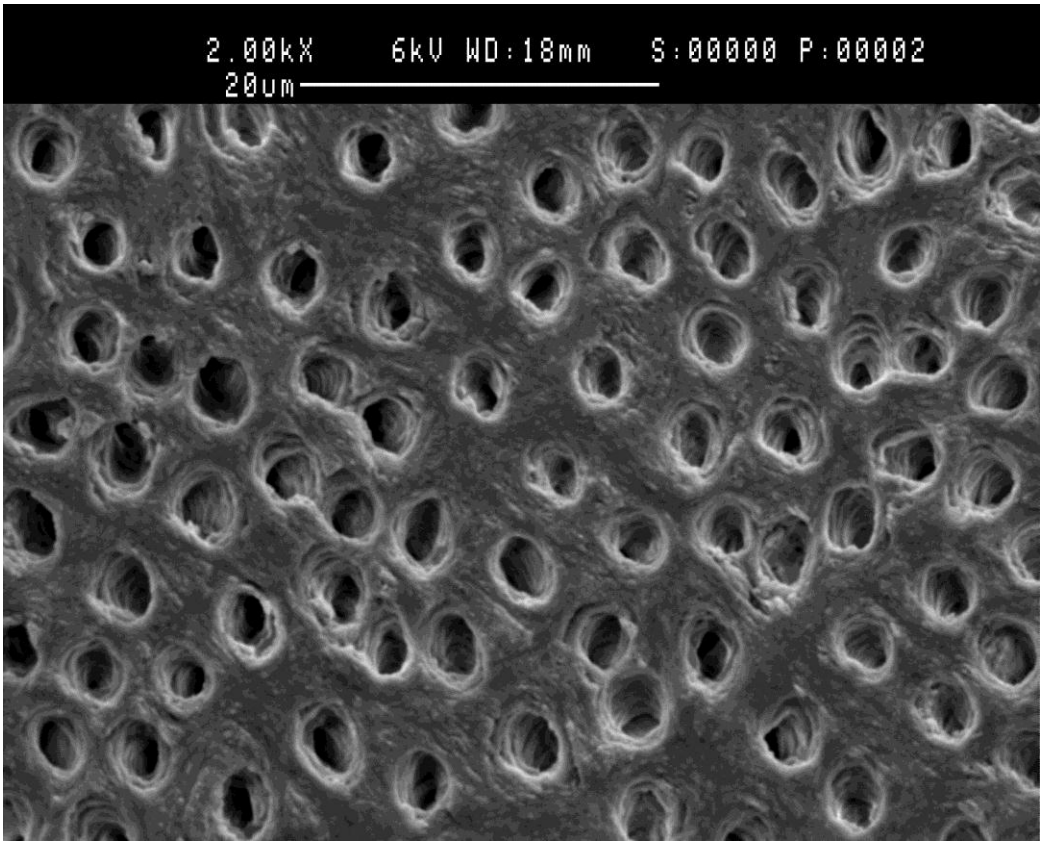


Figure 3. Strong erosion of root dentin surface. Marked loss of inter- and peri-tubular dentin (specimen from Group VI: E5N5).

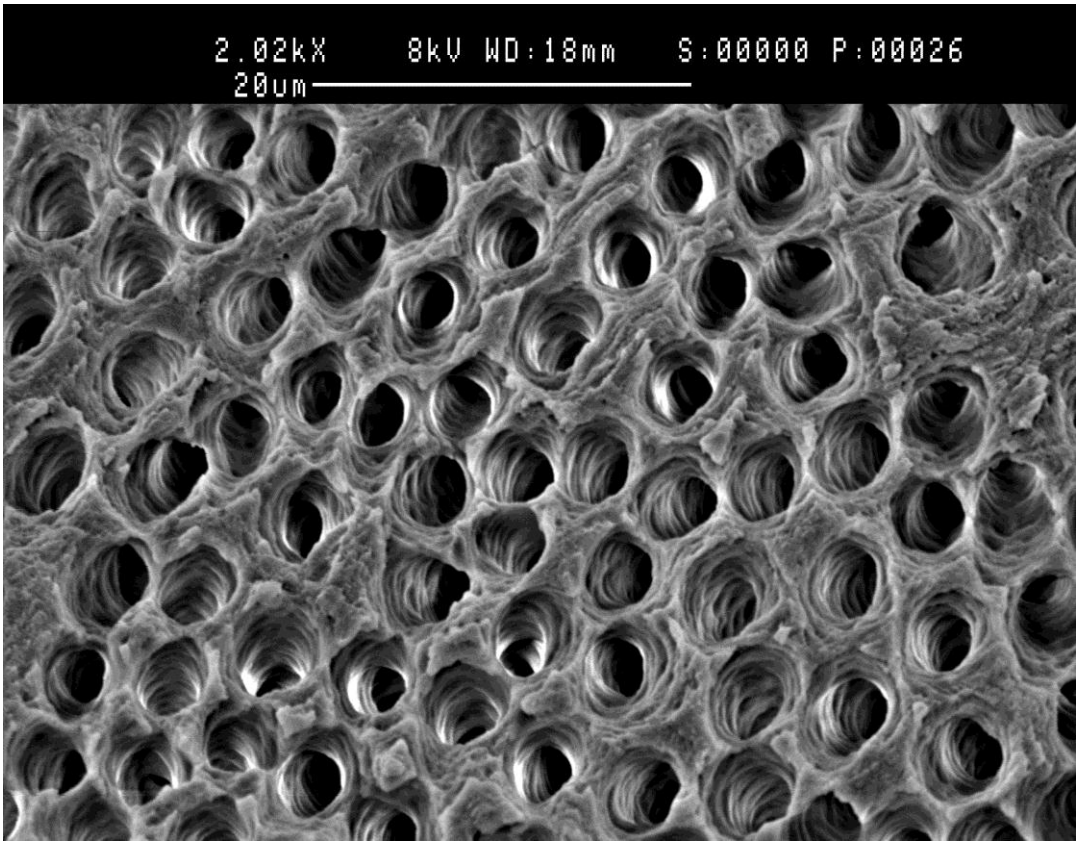


Figure 4. Image analysis by software. Irregular boundaries of the tubule openings were auto-segmented one by one using ImagePro software, and labeled with a sequenced combination of letters and numbers (such as PG1) for identification and calculation purposes.

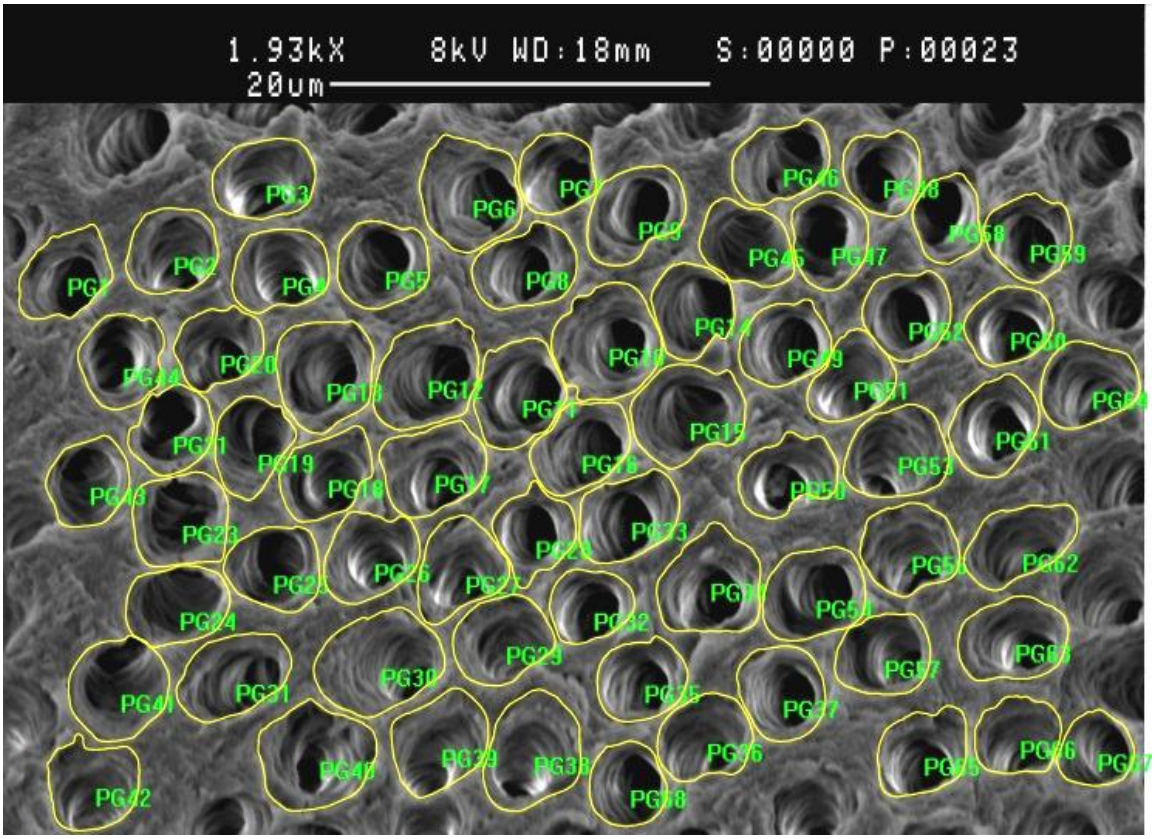


Figure 5. Tilted view of eroded dentin surface in Group VIII (C5N5).
Massive erosion caused by 5 minute exposure to hypochlorite after 5 min of 10% citric acid.

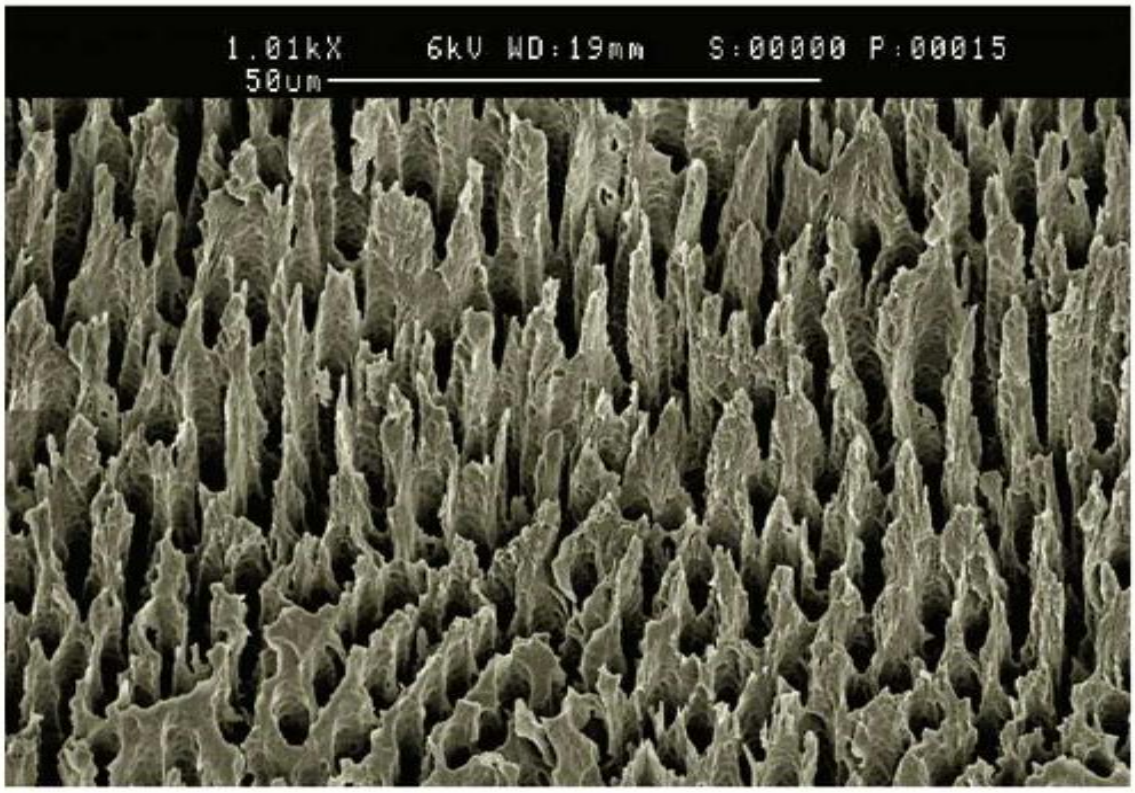


Figure 6. Column chart for average area of dentinal tubule opening (μm^2) after different irrigation protocols.

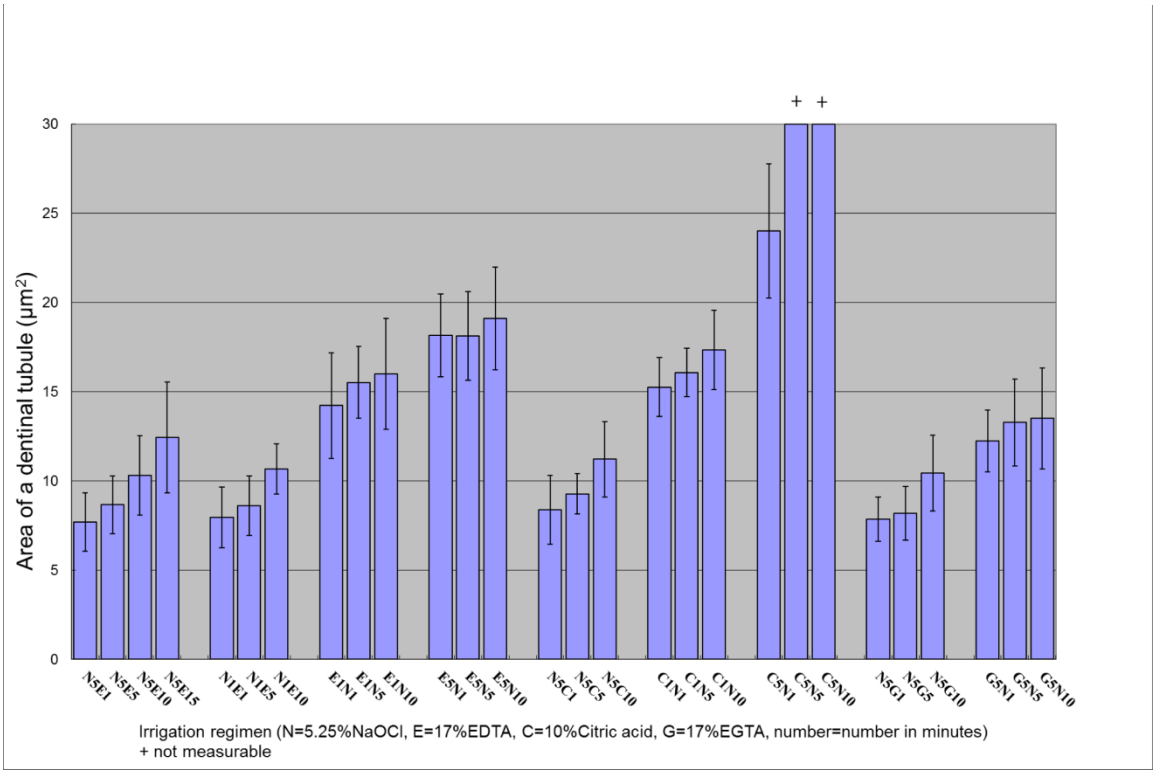
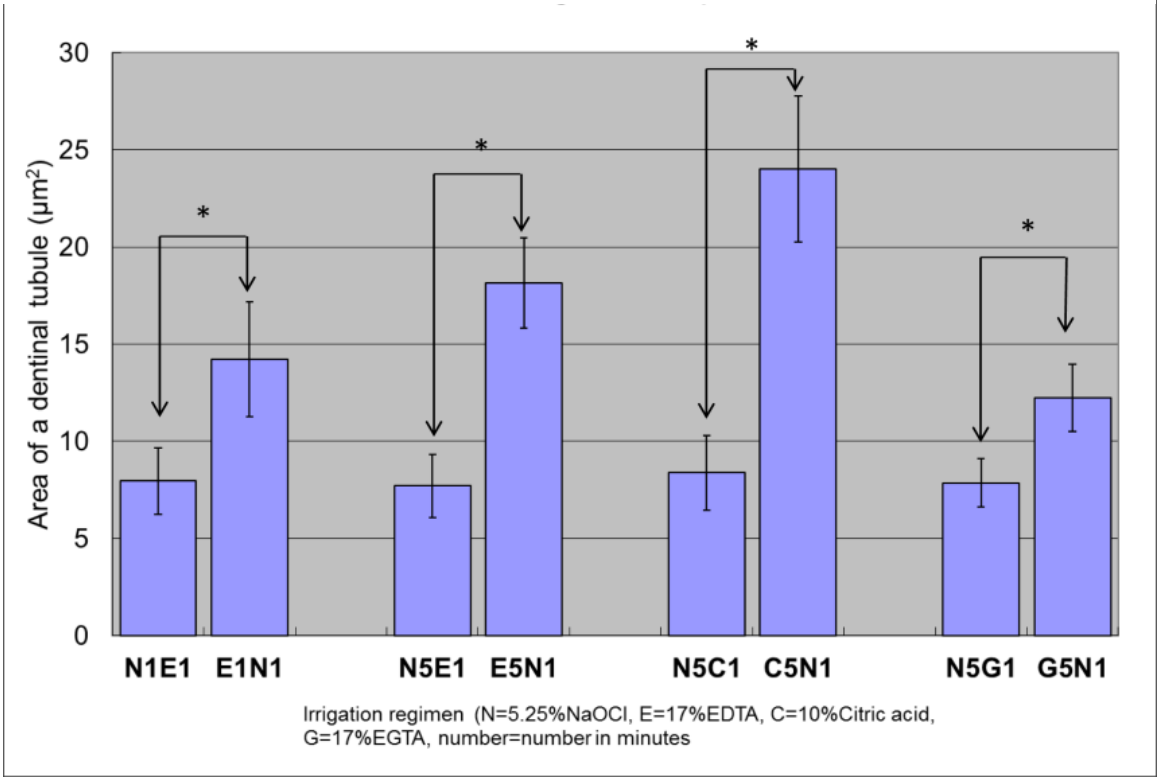
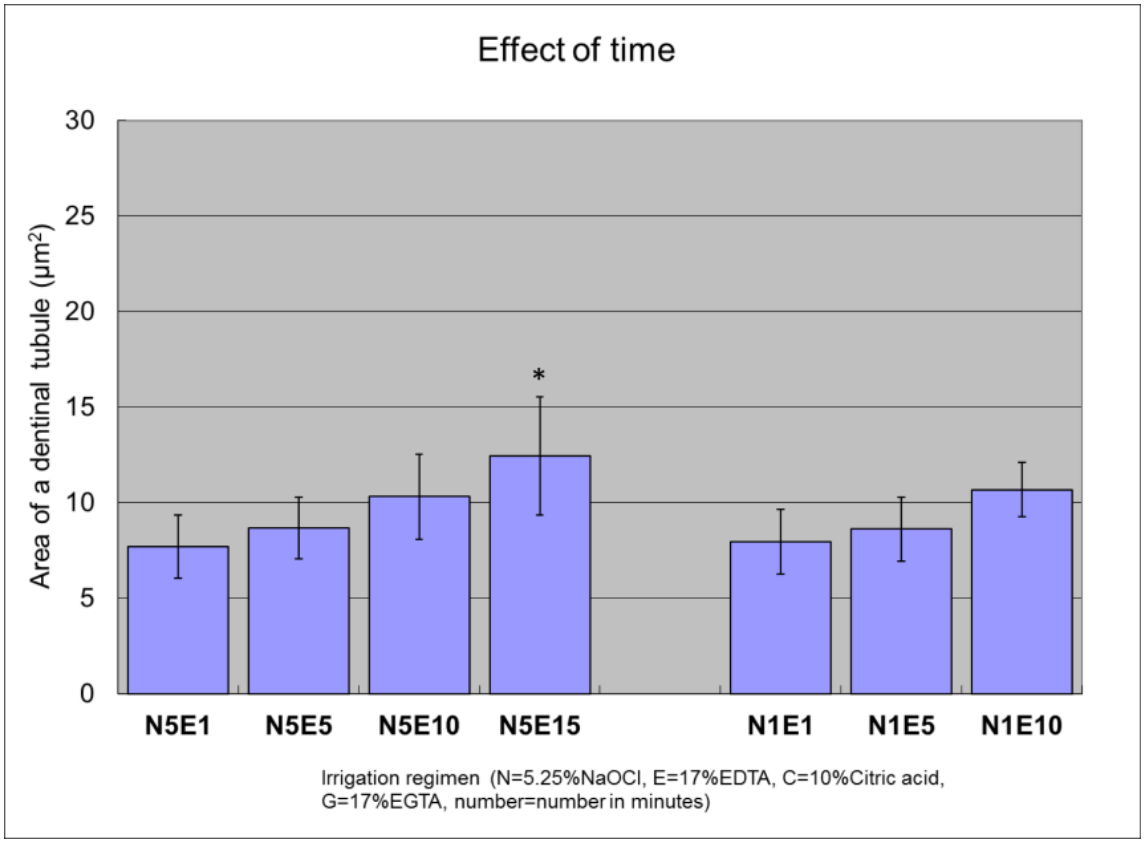


Figure 7. Effect of irrigation sequence on dentin erosion. Irrigant treatment with reversed sequence showed significant statistical differences for NaOCl with EDTA, EGTA or CA.



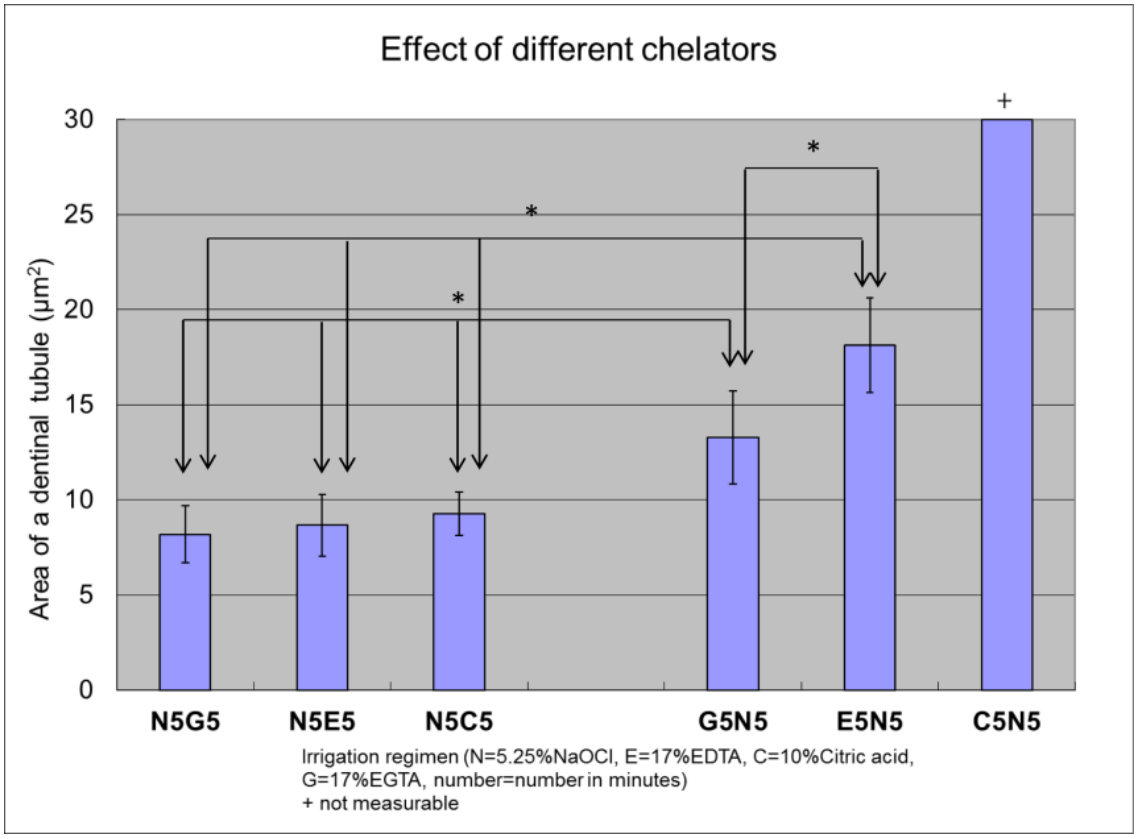
*denote statistical significance of $p<0.05$

Figure 8. Effect of time on dentin erosion. For treatment using NaOCl first, 1,5,or 10 mins of 17%EDTA afterwards, did not have significant impact on tubule opening area. Only at 15min, the enlargement showed statistical difference.



*denote statistical significance of $p<0.05$

Figure 9. Effect of different chelates on dentin erosion.When NaOCl was used first, there were no significant differences between dentinal tubule opening area after 5 minutes of irrigation with EDTA, EGTA or CA. However, if the above sequences were reversed, the dentinal tubule area increased significantly with the order of 17% EGTA < 17% EDTA < 10% CA, in accordance with their demineralising capability.



Chapter 4. Discussion, and conclusion

4.1 Image analysis methods

Several different methods have been used to score the amount of smear layer remaining after biomechanical preparation (Hulsmann et al. 1997, Prati et al. 2004). Modern measurement techniques making use of computerized systems for quantitative analysis of dental tissue are becoming increasingly popular (Coicca et al. 2007, De-Deus et al. 2007, George et al. 2008). In 2007, Ciocca et al. (2007) found that a completely automated analysis technique could be considered reliable, operator insensitive and save time in counting the dentinal tubules. They did the calculations by means of images with tubule outlines contrasting the intertubular dentin, and with specimens taken from cross sections where tubule openings were at right angles to the plane of observation. De-Deus et al. (2007) used co-site digital optical microscopy and image analysis to evaluate the process of dentin demineralization. They reported that the method was fast, robust and reproducible. A follow-up study by the same group showed erosion of human dentin specimens treated with high concentrations of citric acid for 60 s (Reis et al. 2008). According to the authors the images were slightly blurred because of dentin surface roughness and restricted depth of focus by the electron microscope. Therefore, they only performed automated analysis on specimens which were exposed to irrigants for no more than 60 s.

4.2 Experimental quality control measures

In order to reduce human operator variability, both manual and automatic approaches were applied in the present study. Two experienced endodontists highly skilled in SEM measured the tubular openings independently. Professional image software was used to draw every tubule outline automatically in high magnification, which eliminated the risk for bias by subjective assessment for the measurement. The operator can only control the software's performance by distinguishing between real tubules and any other defects in the digitalized images. This semi-automatic method provides operator-independent quantitative results and avoids the limitation of completely automated analysis technique, in which software considers every single pixel on the whole image and identifies the maximum local gradient of the images that go beyond a threshold value. Even though the area of dentinal tubule opening is a two-dimensional surface parameter, and cannot be simply related to the 3D structure of the tubules, it corresponds to an important microstructural feature for dentin analysis, as it reveals the opening of the tubules.

4.3 Dentin erosion

The results of the present study clearly showed that the sequence in which NaOCl and EDTA are used for canal irrigation has a major impact on the level of dentin erosion on the main root canal wall. Although the effect of dentin erosion on dentin and root strength or dentin bonding is not fully understood, there is a general consensus that

dentin erosion should be avoided. Recent studies have also shown that long term irrigation of dentin blocks prepared from root dentin with high concentration hypochlorite causes major changes in the physical properties of dentin. For example, dentin elasticity and flexural strength can be reduced by even 50% (Marending et al. 2007). However, the study was done using dentin pieces cut out of root dentin in such a way that dentinal tubules in the blocks may have been open from both ends. Such a situation may facilitate rapid penetration of hypochlorite into dentin, which may not be the case in vivo when the cement layer covers dentin in the other end. In the present study cement layer was present on the dentin surface preventing the solution from flowing through the dentin pieces. On the other hand, there are limitations in the present study as well. Root canal treatment with different irrigants and delivery system causes alterations in the chemical and structural composition of dentin (Baumgartner et al. 1987, Marending et al. 2007, Zhang et al. 2010). The in vivo root canal is a closed-end channel, which often causes gas entrapment at the apical end, producing a vapor lock effect during irrigant delivery. This and anatomical variations mean that different parts of the root canal wall are unevenly affected by irrigation. In the present study, instead of irrigation, as it happens in the root canal, dentin blocks were tested in an open system providing equal exposure and adequate irrigant replacement all over the specimens. This together with different hydrodynamic behaviour or the solutions in vivo and in the present study model could result in differences in the chemical action of the solutions on dentin; therefore caution is necessary when drawing conclusions from the results of the present

study. Apical root was removed from all teeth and not used for the experiments. The main reason for this was that sclerotic dentin is common in the apical third of the root canals (Paque et al. 2006) and comparison of dentin erosion by the solutions in this part to middle and coronal areas would be difficult or impossible to do properly.

4.4 Possible mechanisms of dentin erosion

The mechanism of dentin erosion and the effect of the irrigant sequence on the erosion are probably related to the structural architecture of dentin. Dentin, like bone, has a dense collagen network (Figure 7) covered by a hydroxyl apatite coating (Di Renzo et al. 2001). In other words, an organic core is covered by an inorganic outer sheath. NaOCl is a strong base and nonspecific oxidizer. It reacts with amino acids by neutralization and chloramination reactions, leading to degradation of the amino acids (Jones et al. 1968, McDonnell et al. 1999). An immunohistochemical study demonstrated that type I collagen and glycosaminoglycan lost their immunoreactivity after NaOCl treatment when a demineralized dentin model was used (Oyarzun et al. 2002). When hypochlorite is used before EDTA, the hydroxyapatite coating seems to protect the collagen fibers from the dissolving action of hypochlorite. However, in the opposite situation, when hypochlorite follows EDTA, EGTA or CA, hypochlorite can directly attack collagen which has been already exposed by the demineralizing agents. That also explains why there was no

corresponding erosion of dentin when the three demineralizing agents were used as the final rinse after the initial exposure to NaOCl.

It's important to understand the mechanism of dentin matrix destruction by chemical solutions. EDTA removes calcium ions (Ca^{2+}) from mineral tissue, including dentin. Treatment with 17% EDTA + 2.5% NaOCl for 1 min has been shown to remove significantly more Ca^{2+} from root canal dentin than treatment by 17% EDTA alone (Sayin et al. 2007). When EDTA alone is used for irrigation, the organic matrix of dentin is the limiting factor on the dissolution of dentin, because it accumulates on the canal surface, thereby preventing further dissolution (Oyarzun et al. 2002). Subsequent application of NaOCl may facilitate further exposure of the inorganic material through removal of the organic matrix (Perdigao et al. 2001, Dogan et al. 2001), and thus increase the demineralizing effect. Deproteinization transformed demineralized, collagen-rich dentin into a porous structure with multiple irregularities in peri- and intertubular dentin. Tubule surface area of the deproteinized substrate increased and had a funnel shaped three dimensional configuration. Increasing the times of exposure, both to the demineralizing agents (first) and to hypochlorite (last), increased the erosion significantly as suggested by previous studies (Niu et al. 2002, Calt et al. 2002). However, these studies did not study the importance of irrigation sequence, which is the main finding of the present study.

Calt and Serper (2002) recommended that root canals should be rinsed with 17% EDTA for no longer than 1 min to avoid dentin erosion. A previous study (Kinney et al. 1995) in which demineralization was monitored dynamically with synchrotron radiation computed tomography, showed that dentin demineralization appeared to occur in two stages, each governed by an unique rate. About 70- 75% of the mineral was removed rapidly, while the remaining mineral etched at a significantly slower rate. Hence, it is not surprising that dentin erosion occurred when NaOCl followed EDTA, even when EDTA was used for a very short time of 30 s (Table 1, group V: E0.5 N5).

4.5 Clinical implications

Optimally, after irrigation the root canal should be free of all organic debris, microorganisms and smear layer. In addition, bacteria that have penetrated into dentinal tubules should have been killed, while dentin characteristics (strength, composition, etc.) should not be affected in any negative manner. The present study shows that the sequence of use of the common endodontic irrigants, hypochlorite and the demineralizing agents (EDTA, EGTA or citric acid), is a key factor in determining the level of erosion in root canal wall dentin. Although erosion occurred already after one minute of hypochlorite exposure when done as the final rinse after the demineralizing agent and increased with increasing times of exposure to the irrigants, it is not known presently

whether such erosion is harmful for the root dentin and the tooth. It is well known that the mineral component in hard connective tissues contributes to strength and elastic modulus, whereas collagen is responsible for toughness (Wang et al. 2001, Calt et al. 2002, Marending et al.2007 A & B, Zhang et al. 2010). Theoretically, the observed erosion could be a contributing factor in vertical root fracture depending on the depth of erosion, thickness of the root and the amount of sclerotic dentin in the root, e.g. on the other hand, erosion may also help in achieving a maximally clean root canal wall surface, free of debris and bacteria.

So based on this study and other related investigations, the following irrigation sequence would be recommended during root canal treatment:

Full strength(5.25-6%) of NaOCl should be used during instrumentation. After the instrumentation is finished, 17% EDTA should be used. Chlorhexidine irrigation could be used for additional disinfection after EDTA. Agitation during irrigationshould also be helpful to achieve a cleaner canal.

4.6 Future directions

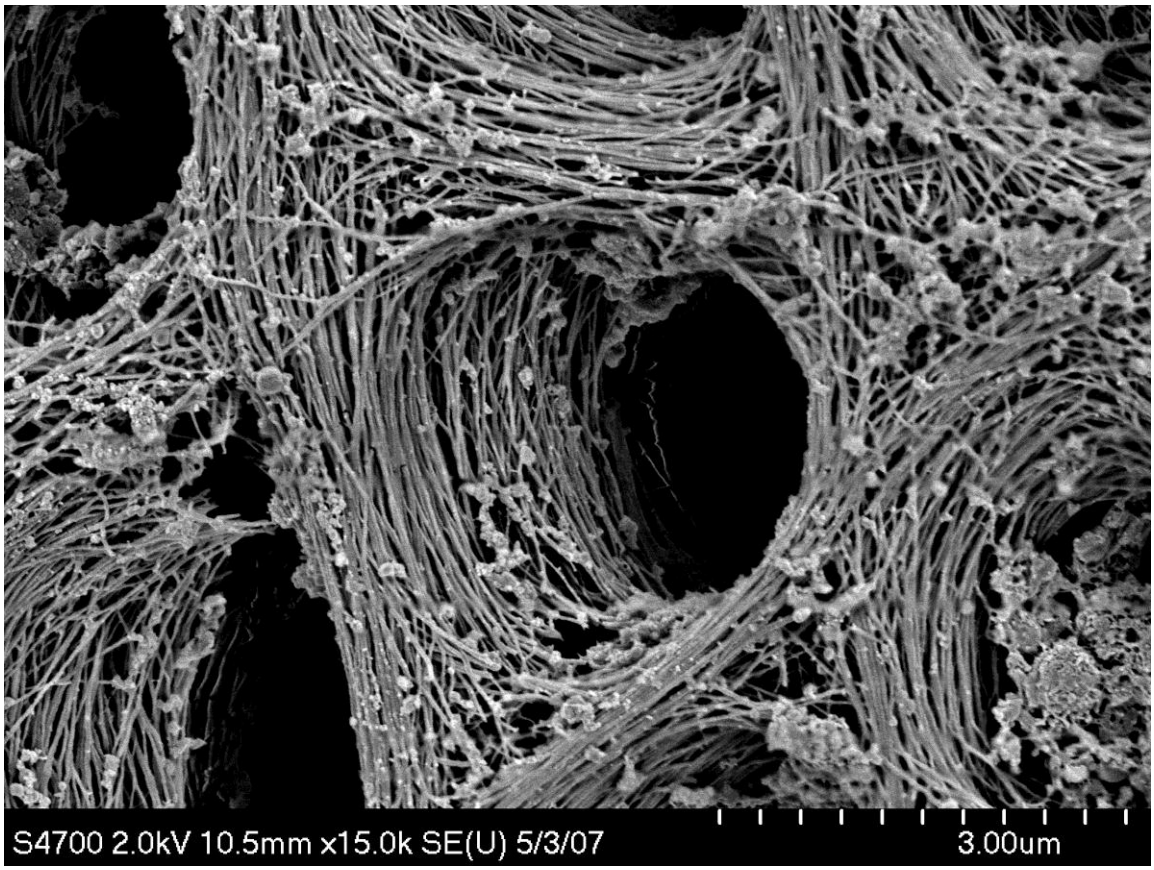
Future studies will be focused on:

1. Dentin hardness at different levels underneath the root canal surface after the use of different irrigation protocols
2. Effect of erosion on dentin bonding interface micro-morphology and strength.

4.7 Conclusion

Irrigation with 5.25% sodium hypochlorite after demineralization agent(s) on root canal wall dentin with smear layer causes marked erosion at the dentin surface.

Figure 10. SEM photo of collagen matrix at human root canal dentin surface.
In this specimen the hydroxyl apatite coating on the collagen fibers was dissolved by organic acids produced by bacteria grown on dentin surface.



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