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Effects of MTAD on Some Physical Properties of Enamel and Dentin

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Effects of MTAD on Some Physical Properties of Enamel and Dentin

by

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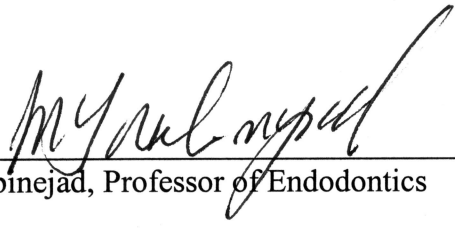

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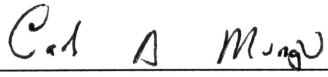

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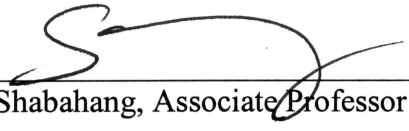

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ABSTRACT OF THE THESIS

Effects of MTAD on Some Physical Properties of Enamel and Dentin

by

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Dr. Mahmoud Torabinejad, Chairperson

Recently, a **M**ixture of **T**etracycline**,** an **A**cid, and a **D**etergent (MTAD) has been advocated to remove smear layer and disinfect canals. The purpose of this study was to evaluate the effect of MTAD the flexural strength and modulus of elasticity of dentin as well as its effect on the bond strength to enamel and dentin. In Part I, dentin bars were randomly assigned to eight groups treated either with various concentrations of NaOCl (5.25%, 2.65%, 1.31%, 0.66%), 17% EDTA, MTAD, saline, or according to a clinical protocol with 1.3% NaOCl and then 5 min of MTAD. Specimens were subjected flexural strength and modulus of elasticity tests. Data was analyzed using one-way ANOVA, Scheffés multiple comparisons procedure, and the two-sample *t*-test ($\alpha = 0.05$). In Part II, immediate enamel and dentin sheer bond strength was tested. Before bonding composite using Optibond Solo Plus (Kerr), one of the following surface treatments was used: 1 min NaOCl (5.25%)/ 1 min EDTA(17%); 1 min NaOCl (5.25%)/ 1 min MTAD; 15 s etch with H_3PO_4 (positive control); 2 min saline (negative control); or 20 min NaOCl(1.3%)/ 5 min MTAD (clinical protocol for MTAD). Data was analyzed using one-way ANOVA, Student-Newman-Keuls method, and the two-sample *t*-test ($\alpha = 0.05$). There was no significant difference in flexural strength and modulus of elasticity between the dentin bars exposed to saline, various concentrations of NaOCl or MTAD per clinical

protocol ($p>0.05$). Surface pre-treatment with acid etch resulted in the greatest enamel bond strengths ($p<0.05$). All surface pre-treatments were superior to saline for dentin bonding ($p<0.05$). Results indicated that MTAD could be used as prescribed for clinical use without affecting the tested physical properties of the tooth structure.

CHAPTER ONE

LITERATURE REVIEW

Effect of Irrigants on Dentin

Dentin is approximately 22% organic material by weight (Trowbridge and Kim, 1991). Most of this consists of type I collagen, which contributes considerably to the mechanical properties of dentin. It is reasonable to assume that the dissolution effect of sodium hypochlorite (NaOCl) would affect dentin (Grigoratos *et al.,* 2001). The possible mechanisms involved in dentin depletion and consequently the weakening effect of NaOCl have been investigated (Sakae *et al.,* 1988, Barbosa *et al.,* 1994).

Grigoratos and coworkers (2001) found that dentin bars treated with 3% and 5% NaOCl show a considerable difference in the mode of fracture as compared to controls. The fracture loads are much lower with considerable deformation of the dentin bars prior to fracture. There is a significant reduction in flexural strength and modulus of elasticity after treatment with 3% and 5% NaOCl for a total of 2 hours. The solution was changed every 15 minutes to prevent saturation by reaction products and to ensure all surfaces of the dentin bars were exposed.

Sim and coworkers (2001) studied the effects of NaOCl on mechanical properties of dentin and found that a 2 hour exposure of 5.25% NaOCl reduces the flexural strength and modulus of elasticity of dentin. The change in physical properties could be explained by the loss of the organic matrix within the dentin. Haikel and coworkers (1994) have shown that NaOCl efficiency increases with increasing concentration to reach a 70% protein desorption from the apatite surface. NaOCl remains the most commonly used

irrigant, despite the fact that several studies (Grigoratos *et al.*, 2001, Sim *et al.*, 2001) show that it has a negative effect on the properties of teeth.

EDTA has been advocated for smear layer removal (Yamada *et al.* 1983). It is reasonable to assume that the demineralizing effect of EDTA would affect dentin. EDTA is a chelating agent and its original use in endodontics was to negotiate calcified canals. Removing mineral salts by chelation leaves only a soften matrix (Patterson *et al.* 1963). Zerosi and Viotti (1958) confirmed that the extent of demineralization of root canal dentin is proportional to its exposure time. Therefore, extended exposure to EDTA could cause extensive demineralization. Çalt and Serper (2002) showed that even 10 minutes of exposure to EDTA causes extensive peritubular and intertubular dentinal erosion.

Effect of Surface Treatment on Bonding

It is believed that the efficacy of the current dentin adhesives depends upon the infiltration of those high-affinity hydrophilic monomers into the filigree of collagen fibers that make up the structure of acid-etched dentin. This entanglement of monomers with collagen fibers and a few residual hydroxyapatite crystals forms a hybrid tissue also known as resin-dentin interdiffusion zone (Van Meerbeek *et al.*, 1993). The depth of dentin demineralization has become an important issue in dentin bonding (Perdigão *et al.*, 2000). The standard method to produce the dentin bonding surface is to use the same acid conditioning, 30-37% phosphoric acid, as used in creating the ideal surface for bonding to enamel.

Several researchers (Barbosa *et al.*, 1994, Vargas *et al.*, 1997) have studied the role of NaOCl in dentin permeability and dentin adhesion. Depending on each testing

methodology and/or specific composition of each dentin adhesive, the application of NaOCl upon etching may increase (Inai *et al.*, 1998, Prukkanon *et al.*, 2000) or decrease (Perdigão *et al.,* 2000, Nikaido *et al.*, 1999) bond strength. Removal of the organic collagen layer following acid conditioning and subsequent bonding directly to the partially demineralized dentin layer may produce more durable adhesion to the hydroxylapatite component of the dentin substrate. It is theorized that dentin substrate conditioning with NaOCl would significantly enhance the long-term strength and durability of the resin to dentin bond for adhesive systems (Vargas *et al.*, 1997).

Prati and coworkers (1999) found that if acid-etching is followed by NaOCl treatment, high bond strengths can be achieved via "reverse hybrid layer" formation, a proposed new mechanism of micromechanical resin retention. In "reverse hybrid layer" formation, acid-etching removes the smear layer and exposes the collagen fibrils of the dentin matrix. This is followed by application of NaOCl, which not only removes the exposed collagen fibrils but also solubilizes the fibrils down into the underlying mineralized matrix to create submicron porosities within the mineral phase. Cylindrical channels previously occupied by collagen fibrils are now available for resin infiltration within the mineralized matrix (Prati *et al.*, 1999).

On the other hand, there appear to be changes in the crystallinity of dentin apatite upon NaOCl treatment: X-ray ion diffraction studies have suggest that recrystallization takes place after NaOCl application. The apatite crystals undergo substitution of certain ions in the crystal lattice (Inaba *et al.,* 1996). This re-crystallization might be responsible for changes in the surface tension of the substrate; therefore, it may compromise the bonding ability of the dentin surface. The effect of chemical irrigants on dentin bonding

is still unclear. However, NaOCl damages the organic components of dentin, mainly the collagen. This may influence the penetration of monomers into the demineralized dentin structure (Nikaido *et al.,* 1999). The effect of NaOCl on bonding still needs further clarification.

EDTA has also been used to prepare the surface for bonding. Cederlund and coworkers (2001) hypothesized that chelating agent such as EDTA in a saturated aqueous solution (24%) could function as a dentin conditioning agent with exposure times comparable to that of phosphoric acid without compromising shear bond strength. Their results indicate that EDTA gel conditioning of dentin surfaces need not exceed that of phosphoric acid in clinical practice to obtain an acceptable level of bond strength. Others have shown that the use of EDTA in combination with dentinal bonding agents results in significantly greater bond strength to dentin than does conventional acid etching (Blomlof *et al.*, 2001). A final flush with EDTA appears to remove the smear layer (McComb and Smith, 1975) and prepare the surface for bonding; however, EDTA is not antibacterial and erodes the dentin if the exposure time exceeds 1 minute (Çalt and Serper, 2002).

Smear Layer Removal and Chemical Conditioning

As a result of pathological changes in the pulp, the root canal system acquires the capability to harbor several species of bacteria, their toxins and their by-products. Kakehashi and coworkers (1965) and Möller and coworkers (1981) as well as other investigators have shown that pulpal and/or periradicular pathosis does not develop without the presence of bacterial contamination. Byström and Sundqvist (1981) have

shown that it is impossible to achieve complete removal or destruction of all bacteria utilizing solely mechanical root canal instrumentation with a saline irrigant. Microorganisms can be found in all areas of the root canal system and also in the dentinal tubules as shown by Siqueira *et al.* (1996), Perez *et al.* (1993), and Peters *et al.* (2001).

The search for an irrigating solution with antimicrobial properties, tissue-dissolving ability, and concomitant biocompatibility with the periapical tissues continues to be a subject of many studies (Taşman *et al.*, 2000). During endodontic preparation a smear layer is deposited on the canal walls that can reduce diffusion of an intracanal medicament by as much as 25 to 30% (Pashley *et al.*, 1978). The smear layer consists of a superficial layer of debris loosely attached to the dentinal wall and another layer of debris packed into the dentinal tubules (Cameron, 1983). There is no scientific consensus regarding the efficacy of smear layer removal in root canal treatment (Cergneux *et al.*, 1987, Gettleman *et al.*, 1991, Sen *et al.*, 1995); it has been suggested that retaining the smear layer may inhibit or delay bacterial colonization of the root canal by reducing dentin permeability.

However, currently the consensus is toward smear layer removal in order to reduce the microflora and associated endotoxins, enhance the sealing capability of obturation materials and decrease the potential of the bacteria to survive and reproduce (Di Lenarda *et al.*, 2000). Ørstvik and Haapasalo (1990) showed the importance of removal of the smear layer and the presence of patent dentinal tubules for the disinfecting effect of intracanal medications. When teeth are treated with sodium hypochlorite (NaOCl) alone, Marais (2000) found that the smear layer remains intact. One of the aims

of root canal preparation is to obtain access for the irrigation solution to allow intimate contact with the bacteria and debris (Marais, 2000).

Alteration of the intracanal smear layer was first reported by McComb and Smith (1975) who used EDTA. Yamada and coworkers (1983) later found that a final flush of 17% EDTA followed by rinsing with 5.25% NaOCl results in the cleanest canals. At present, chemical conditioning is the only way to obtain complete cleaning of dentinal walls. Smear layer removal requires a combination an organic solvent and substances that are active in removal of inorganic compounds (Di Lenarda *et al.*, 2000). Over 95% of practicing endodontists use NaOCl as an irrigant and about half use EDTA for smear layer removal (Inamota *et al.* 2002).

In addition to smear layer removal an ideal root canal irrigant should be able to disinfect the root canal system and the dentinal tubules, allow penetration of anti-microbial agents present in the solution into the dentinal tubules, and have sustained antibacterial effect after use. It is important that it be non-antigenic, non-toxic and non-carcinogenic to tissue cells surrounding the tooth and have no adverse effects on the physical properties of exposed dentin or the sealing ability of filling materials. Preferably, it should not discolor the tooth, be convenient to apply, and be relatively inexpensive.

A commonly used irrigant, sodium hypochlorite (NaOCl) has a good antimicrobial effect *in vitro*, good tissue dissolving capacity (Shih *et al.*,1970) and appears to be most effective undiluted at 5.25% concentration (Hand and Harrison, 1978) (Harrison *et al.*, 1981). Despite these attributes, NaOCl is not an "ideal" irrigant. It does not remove the smear layer, is not effective against *E. faecalis* (Siqueira *et al.*, 1997) and has been shown to be toxic especially at high concentrations (Spångberg *et al.*, 1973).

A primary function of an endodontic irrigant is to remove any loosened debris, as well as cleanse uninstrumented areas of the canal (Cameron, 1986). The irrigation solution must be brought in contact with the dentin wall and the debris (Abou-Ross *et al.*, 1982). Therefore, an ideal irrigant would also have a low surface tension that would increase its ability to penetrate into dentinal tubules (Taşman *et al.*, 2000). Previous studies indicate that decreasing the surface tension of irrigating solutions by adding a detergent may improve the efficacy of treatment (Abou-Ross *et al.*, 1982). The relatively lower surface tension values of NaOCl and EDTA may contribute to the high success rates achieved with the combined use of these irrigants (Taşman *et al.*, 2000).

A final flush with EDTA followed by NaOCl appears to remove the smear layer (Yamada *et al.*, 1983), but EDTA is not antibacterial (Heling and Chandler, 1998) and has the ability to erode the dentin if the exposure time exceeds one minute (Çalt and Serper 2002). Therefore, a new solution to this irrigation problem is necessary to remove the smear layer and disinfect the root canal system.

In a series of experiments, Torabinejad and associates have shown that a mixture of a tetracycline isomer, an acid and a detergent (MTAD) is an effective solution for the removal of the smear layer. Based on their findings it appears that it does not significantly change the structure of the dentinal tubules when used as a final irrigant in conjunction with low concentrations of NaOCl as a root canal irrigant (Torabinejad *et al.*, *In press*). MTAD is significantly more effective than 5.25% NaOCl in eradicating bacteria from infected root canals (Shabahang *et al.*, *In press*). They demonstrated that MTAD is still effective in killing *E. faecalis* at 200× dilution and that NaOCl ceases to exert its antibacterial activity beyond 32× dilution (Torabinejad *et al.*, *In press*).

These results are significant in that they demonstrate the efficacy of a single irrigant to remove most of the smear layer and kill a bacterial strain that has been shown to be resistant to many of the commonly used intracanal irrigants and dressings. In addition, they found that MTAD is a biocompatible material (Zhang *et al.*, *In press*) and has similar solubilizing effects on pulp and dentin to those of EDTA (Beltz *et al.*, *In press*). The major difference between EDTA and is a high binding affinity of doxycycline present in MTAD for the dentin (Beltz *et al.*, *In press*) that allows for a prolonged antibacterial effect (Wikesjö *et al.*, 1986).

In addition to antibacterial effect and biocompatibility, an "ideal" irrigant should: (1) Have minimal effect of the physical properties of the tooth and (2) Not adversely affect the restorative materials used subsequent to root canal therapy. Two separate experiments, Part I: Flexural Strength and Modulus of Elasticity and Part II: Bonding, were designed to test the following null hypotheses.

Part I:

The null hypothesis (H_0) is that there will be no difference in flexural strength or modulus of elasticity of dentin between the treatment groups. The alternative hypothesis (H_1) is that there will be a difference between the groups.

Part II:

The null hypothesis (H_0) is that there will be no difference in the bond strength to enamel and dentin between the treatment groups. The alternative hypothesis (H_1) is that there will be a difference between the groups.

CHAPTER TWO

Effect of MTAD on Flexural Strength and Modulus of Elasticity of Dentin

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The purpose of this study was to evaluate the effect of MTAD on the flexural strength and modulus of elasticity of dentin. Dentin bars were randomly assigned to eight groups treated either with various concentrations of NaOCl (5.25%, 2.65%, 1.31%, 0.66%), 17% EDTA, MTAD, saline, or according to a clinical protocol with 1.3% NaOCl and then MTAD. Specimens were subjected flexural strength and modulus of elasticity tests. Two-sample *t*-test (α =0.05) showed no statistically significant differences between various groups except for a reduction in transverse strength for the 2h MTAD group ($p = 0.002$) and the EDTA group ($p = 0.002$). A significant reduction of modulus of elasticity for the 2h MTAD group ($p < 0.001$),

EDTA group ($p < 0.001$), and 0.6% NaOCl ($p < 0.002$) was also noted. There was no significant difference in flexural strength and modulus of elasticity between the dentin bars exposed to saline or MTAD when applied according to the clinical protocol ($p>0.05$). These results indicate that MTAD can be used as prescribed for clinical use without affecting the physical properties of the dentin.

INTRODUCTION

As a result of pathological changes in the pulp, the root canal system acquires the capability to harbor several species of bacteria, their toxins and their by-products. Kakehashi *et al.* and Möller *et al.* as well as other investigators have shown that pulpal and/or periradicular pathosis does not develop without the presence of bacterial contamination.[1] Byström and Sundqvist have shown that it is impossible to achieve complete removal or destruction of all bacteria utilizing solely mechanical root canal instrumentation with a saline irrigant.[1] Microorganisms can be found in all areas of the root canal system and also in the dentinal tubules as shown by Siqueira *et al.*, Perez *et al.*, and Peters *et al.*[1]

Ørstvik and Haapasalo showed the importance of removal of the smear layer and the presence of patent dentinal tubules for the disinfecting effect of intracanal medications.[1] In addition to smear layer removal an ideal root canal irrigant should be able to disinfect the root canal system and the dentinal tubules, allow penetration of anti-microbial agents present in the solution into the dentinal tubules, and have sustained antibacterial effect after use. It is important that it be non-antigenic, non-toxic and non-carcinogenic to tissue cells surrounding the tooth and have no adverse effects on the

physical properties of exposed dentin or the sealing ability of filling materials. Preferably, it should not discolor the tooth, be convenient to apply, and be relatively inexpensive.[1]

A commonly used irrigant, sodium hypochlorite (NaOCl) has a good antimicrobial effect *in vitro*[2], good tissue dissolving capacity[3] and appears to be most effective undiluted at 5.25% concentration.[4] Despite these attributes, NaOCl is not an "ideal" irrigant. It does not remove the smear layer, is not effective against *E. faecalis*[5] and has been shown to be toxic especially at high concentrations.[6] A final flush with EDTA followed by NaOCl appears to remove the smear layer, but EDTA is not antibacterial and has the ability to erode the dentin if the exposure time exceeds one minute.[7-9] Therefore, a new solution to this irrigation problem is necessary to remove the smear layer and disinfect the root canal system.

In a series of experiments, Torabinejad and associates have shown that a mixture of a tetracycline isomer, an acid and a detergent (MTAD) is an effective solution for the removal of the smear layer. Based on their findings it appears that it does not significantly change the structure of the dentinal tubules when used as a final irrigant in conjunction with low concentrations of NaOCl as a root canal irrigant.[10] They investigated the ability of MTAD to disinfect contaminated root canals with whole saliva and compared its efficacy to that of NaOCl.[11] Their results show that MTAD is significantly more effective than 5.25% NaOCl in eradicating bacteria from infected root canals. They demonstrated that MTAD is still effective in killing *E. faecalis* at 200× dilution and that NaOCl ceases to exert its antibacterial activity beyond 32× dilution.[12] These results are significant in that they demonstrate the efficacy of a single irrigant to remove most of the smear layer and kill a bacterial strain that has been shown to be

resistant to many of the commonly used intracanal irrigants and dressings. In addition, they found that MTAD is a biocompatible material and has similar solubilizing effects on pulp and dentin to those of EDTA.[13] The major difference between EDTA and is a high binding affinity of doxycycline present in MTAD for the dentin that allows for a prolonged antibacterial effect. In addition the antibacterial effect and biocompatibility, an ideal irrigant should have minimal effect on the physical properties of the tooth. The purpose of this study was to evaluate the effect of MTAD on the flexural strength and modulus of elasticity of dentin.

MATERIALS AND METHODS

Teeth Selection and Embedding: One hundred-sixty dentin bars were made from 60 freshly extracted caries-free, unrestored human molars. Teeth were stored at 4 ^{O}C in 100% humidity containing 0.1 % chloramine T. Plano-parallel dentin bars were prepared using a multi-purpose diamond cutting disk and a special jig. This resulted in 1mm x 1mm dentin bars with at least 7mm of length. Each bar was evaluated for square-ness, adequate length, and defects. All the suitable dentin bars from the 60 teeth were mixed together to create the sample group. One hundred sixty (160) prepared dentin bars were randomly assigned to eight groups of 20 bars each.

Group 1: The dentin bars were placed in 30 ml of 5.25% NaOCl for a total of two hours.

Group 2: The dentin bars were placed in 30 ml of 2.63% NaOCl (1:1 dilution, 5.25% NaOCl: de-ionized water) for a total of two hours.

Group 3: The dentin bars were placed in 30 ml of 1.31% NaOCl (1:2 dilution) for a total of two hours.

Group 4: The dentin bars were placed in 30 ml of 0.66% NaOCl (1:4 dilution) for a total of two hours.

Group 5: The dentin bars were placed in 30ml of 17% EDTA for a total of two hours.

Group 6: The dentin bars were placed in 30 ml of MTAD for a total of two hours.

Group 7: The dentin bars were placed in 30 ml of saline solution for a total of two hours.

Group 8: The dentin bar were treated according to the MTAD clinical protocol: 20 min of 1.3% NaOCl, followed by 5 min of MTAD.

The solutions were agitated at 120 strokes per minute. For groups 1-7, the solution was changed every 15 minutes to prevent saturation by reaction products. At the completion of treatment, the bars were rinsed with copious amounts of de-ionized water and tested immediately.

Three-point flexural test: The specimens were tested on a three-point bend testing apparatus using a MTS Universal Testing machine (model 1125) at a cross-head speed of 1mm/min. The width and thickness of the dentin bars were measured using electronic calibers (Mitutoya), then the specimens were positioned on 2 points to create a 5mm test span with the cross-head centered in this span. The load required to fracture the specimens was recorded and expressed in MPas. The mode of fracture was recorded as either complete or incomplete fracture.

The Flexural Strength in MPa was calculated from the following equation[14]:

$$\text{Stress} = \frac{3 \times \text{Load} \times \text{Length}}{2 \times \text{Width} \times \text{Thickness}^2} \quad \text{or} \quad \sigma = \frac{3Pl}{2bh^2}$$

The Modulus of Elasticity in MPa was calculated from the following equation[14]:

$$\text{Deformation} = \frac{\text{Load} \times \text{Length}^3}{4 \times \text{Elastic Modulus} \times \text{Width} \times \text{Thickness}^3} \quad \text{or} \quad \delta = \frac{Pl^3}{4Ebd^3}$$

Data for the flexural strength and modulus of elasticity was analyzed using one-way analysis of variance (ANOVA). Scheffés multiple comparisons procedure was used to determine where the differences existed between the groups. The two-sample *t*-test ($\alpha = 0.05$) was used to compare each experimental group to the saline control.

RESULTS

The means and standard deviations for flexural strength and modulus of elasticity are listed in Table 2.1. A statistically significant difference was found between treatment groups ($p < 0.0001$). Schéffe's multiple comparisons procedure was used to determine differences between the groups for flexural strength and modulus of elasticity. No statistically significant differences were found between the divided groups, indicating by grouping that the values for flexural strength for EDTA and MTAD were different from the other groups (Table 2.2) and that the values for modulus of elasticity for MTAD, EDTA, and 0.6% NaOCl were different from the other groups (Table 2.3). When each experimental group was compared to the saline control using a two-sample *t*- test ($\alpha = 0.05$), no statistically significant differences were found between various groups except for a reduction in flexural strength for the 2h MTAD group ($p = 0.002$) and the EDTA group ($p = 0.002$). A significant reduction of modulus of elasticity for the 2h MTAD group ($p < 0.001$), EDTA group ($p < 0.001$), and 0.6% NaOCl ($p < 0.002$) was also noted. Table 2.4 shows the mode of fracture for the dentin bar specimens. All groups showed complete fracture of the dentin bars under load except for the EDTA and 2h

MTAD groups, where the majority of dentin bars showed incomplete fracture when loaded.

DISCUSSION

It is generally believed that endodontic treatment renders teeth weaker than normal vital teeth. A study by Reeh and coworkers revealed that endodontic procedures reduce the tooth stiffness by only 5%.[15] The reduction in tooth structure and the effect of dehydration on the dentinal tubules are widely considered to be the main reasons associated with increased weakness and brittle-ness of pulpless teeth.[16] Yet, the results of a study by Huang and coworkers did not support the theory that dehydration after endodontic treatment per se weakens dentin structure.[17] They found that dehydration increases stiffness and decreases flexibility in both normal vital tooth samples and treated pulpless tooth samples. In their study, wet dentin specimens from treated pulpless teeth generally showed a lower modulus of elasticity and proportional limit in compression than normal vital teeth. The question remains as to how these teeth were "treated". The treated pulpless tooth group consisted of teeth that had been endodontically treated at least one year before extraction, but no information was available regarding the materials used to complete the treatment.

The possible effects of materials, medications, and irrigants on the structure and physical properties of endodontically treated teeth have not been extensively investigated. In this study, the effect of different concentrations of NaOCl as well as the effect of EDTA and MTAD on flexural strength and modulus of elasticity of dentin were examined. Recently, it has been shown that NaOCl, one of the most widely used root canal irrigants, significantly lowers the flexural strength and modulus of elasticity of

dentin bars after a 2h exposure, thereby possibly contributing to the weakening of root-canal treated teeth.[18,19] In our investigation, the protocol was similar to these other studies and a 2h exposure time was chosen to account for a "maximum time" a tooth would be exposed to an irrigant for multi-visit endodontic treatment.[18,19] It was expected that the higher concentrations of NaOCl would have a greater effect on the properties of dentin than the lower concentrations of NaOCl. However, this did not occur.

There was a wide variation in the behavior of the dentin bars within groups. This can be explained by the variety of teeth and differences in their physical properties. However, in spite of these differences, the influences of the various treatments was dominant enough to show significant differences between groups ($p < 0.001$). When each of the experimental groups was compared against the saline control regarding flexural strength, no statistically significant difference was found between any of the NaOCl groups (5.25%, 2.6%, 1.3%, 0.6%) and the saline control. When each of the experimental groups was compared against the saline control regarding modulus of elasticity, only one statistically significant difference was found for the NaOCl groups. The reduction in value for the 0.6% NaOCl group was statistically significant. All of the specimens in all of the NaOCl groups exhibited complete fracture upon incremental loading. There was "brittle" snap and the two halves separated abruptly.

These findings appear to be in contrast to those reported by others who found that both 3% NaOCl and 5.25% NaOCl affect the dentin. [18,19] In the previous reports, exposure of the dentin bars to 0.5% NaOCl did not appear to alter the physical properties of the dentin and no statistically significant difference in flexural strength and modulus of elasticity was found between the 0.5% NaOCl group and the saline control. In addition,

Grigoratos and coworkers found that dentin bars treated with 3% and 5% NaOCl exhibit "green stick" fracture without displacement from the test span.[19] In some cases, their specimens did not facture at the maximum load, but continued deforming and fractured at a lower load. It is possible that the results of this study are different due to differences in the dentin substrate. The specimens used in the current investigation were obtained from freshly extracted teeth and then stored in Chloramine-T. The specimens for the other studies were stored in 4% formal-saline immediately after extraction. [18,19] The storage conditions of the teeth before testing may influence the results. In addition, there could have been variations within the dentin structure itself that affected the outcome.

The effect of 17% EDTA and MTAD on flexural strength and modulus of elasticity were also examined in this study. A 2h exposure time was used for these agents as well, again to simulate a maximum exposure time. The most common use of 17% EDTA is as a final rinse[7], but some clinicians advocate the use of 17% EDTA alternately with 5.25% NaOCl during instrumentation. If EDTA is used in this combination regimen, the maximum exposure time for multi-visit treatment would approach that for NaOCl used alone.

It was initially thought that MTAD could be used as an irrigant to replace NaOCl, but it was found that the cleanest canals were obtained if NaOCl was used prior to an MTAD final rinse.[10] Therefore, the effect of MTAD as recommended for clinical use (20 min 1.3% NaOCl / 5 min MTAD) was tested as well as a 2h exposure time to study the maximum effect of MTAD on dentin.

For the 2h 17% EDTA and 2h MTAD groups, the reduction in flexural strength and modulus of elasticity were statistically significant from the saline control group and

the majority of the specimens exhibited incomplete fracture upon incremental loading (Table 2.4). These specimens exhibited "green stick" fracture as described by Grigoratos and coworker in their 5.25% NaOCl group.[19] The specimens appeared to be "rubbery", bowed upon loading, and fractured without displacement from the test span.

The change in stiffness after root canal therapy is clinically relevant as it may predispose the tooth to fracture.[15] The decrease in flexural strength is also clinically relevant as it indicates that far less force is required for the cohesive bonds within dentin to fail.[18] Neither 17% EDTA or MTAD is recommended for use as an intracanal irrigant during instrumentation. The demineralizing effect of these solutions appears to affect the physical properties of dentin if the tooth is exposed to these agents for a long period of time. In addition to adversely affecting the flexural strength and modulus of elasticity of dentin, prolonged use of 17% EDTA and particularly when used in combination with NaOCl causes severe erosion at the expense of peritubular and intertubular dentin.[20]

When MTAD is utilized as recommended for clinical use (20 min 1.3% NaOCl / 5 min MTAD)[10], it does not have any adverse effects on flexural strength and modulus of elasticity of dentin. The mode of fracture of all the dentin bars treated with the MTAD clinical protocol showed complete fracture of the specimens as was seen in the saline control group (Table 2.4). No statistically significant differences were found between the MTAD clinical protocol group and the saline control group for flexural strength ($p = 0.865$), modulus of elasticity ($p = 0.858$), or mode of fracture. This suggests that MTAD as recommended for clinical use does not adversely affect dentin structure.

The use of 1.3% NaOCl is advocated during canal instrumentation prior to a final rinse of MTAD to achieve complete removal of the smear layer. Torabinejad and

associates found that 1.3% NaOCl is only slightly less effective than 2.6% NaOCl or 5.25% NaOCl for dissolving pulp[10]; therefore, they suggested using 1.3% NaOCl during instrumentation, instead of more toxic higher concentrations of NaOCl. This regimen removes some organic portion of the smear layer prior to using MTAD as a final rinse to remove mainly the inorganic component of the smear layer.

A potential concern with diluted NaOCl is reduction of its antimicrobial properties. Studies have shown that dilution of NaOCl to less than 2.5% significantly reduces the antimicrobial effect and tissue dissolving property when compared to 5.25% NaOCl.[3,4] Torabinejad and coworker found that a combination of 1.3% NaOCl with MTAD was effective in removing the smear layer.[10] The presence of doxycycline in the MTAD solution provides its antibacterial effect and eliminates a need for a higher concentration of NaOCl. This should reduce the chances for adverse effects as a result of accidental extrusion of NaOCl into the periradicular tissues during cleaning and shaping procedures.

Based on our results it appears that using the clinical protocol for MTAD causes no adverse effects on the physical properties of exposed dentin. The results of this *in vitro* study suggest that MTAD possesses most of the positive qualities of an "ideal" root canal irrigant. *In vivo* studies are in progress to assess its clinical effectiveness before its widespread clinical use in endodontics.

REFERENCES

1. Torabinejad M, Handysides R, Khademi A, Bakland LK. Clinical Implications of the Smear Layer in Endodontics: A Review. Oral Surgery, Oral Medicine, Oral Pathology. Oral Radiol Endod 2002;94:658-66.

2. Shih M, Marshall FJ, Rosen S. The bacterial efficiency of sodium hypochlorite as an endodontic irrigant. Oral Surg 1970;29:613-9.

3. Hand RE, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endodon 1978;4:60-4.

4. Harrison JW, Hand RE. The effect of dilution and organic matter on the antibacterial property of 5.25% sodium hypochlorite. J Endodon 1981;7:128-32.

5. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from root canal, *in vitro*. Int Endod J 1997;30:279-82.

6. Spångberg L, Engstrom B, Langeland K, Biologic effects of dental materials. III. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg 1973;36:856-71.

7. Yamada R, Aramas A Goldman M, Lin PS. A scanning electron microscope comparison of a high volume final flush with several irrigating solutions. Part 3. J Endodon 1983;9:137-42.

8. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8-14.

9. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endodon 2002;28:17-9.

10. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endodon. *in press*

11. Shabahang S, Pouresmail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and NaOCl. J Endodon. *Accepted for publication.*

12. Torabinejad M, Shabahang S, Aprecio R, Kettering JD. The antimicrobial effect of MTAD: An *in vitro* investigation. J Endodon *in press*

13. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using MTT tetrazolium method. J Endodon. *in press.*

14. Restorative Dental Materials, Eds. Craig RG, Ward ML. 10th Ed. Chapter 4: Mechanical Properties, pp 87-8. 1997: Mosby, St. Louis.

15. Reeh ES, Messer HH, Douglas WH. Reduction in tooth stiffness as a result of endodontic and restorative procedures. J Endodon 1989;15:512-6.

16. Tidmarsh BG. Restoration of endodontically treated posterior teeth. J Endodon 1976:2:374-5.

17. Huang TG, Schilder H, Nathanson D. Effects of moisture content and endodontic treatment on some mechanical properties of human dentin. J Endodon 1992;18:209-15.

18. 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.

19. 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;34:113-9.

20. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endodon 1987;13:147-57.

Table 2.1. Mean flexural strength (MPa) ± SD and mean modulus of elasticity (MPa) SD of dentin specimens (dentin bars).

	Flexural Strength	Modulus of Elasticity
5.25% NaOCl	138.942 ± 29.49	9.605 ± 1.59
2.6% NaOCl	184.513 ± 47.54	8.787 ± 2.21
1.3% NaOCl	170.013 ± 70.07	9.342 ± 2.01
0.6% NaOCl	164.944 ± 29.11	7.276 ± 1.43
EDTA	110.824 ± 28.04	4.437 ± 1.21
MTAD	111.698 ± 21.36	3.350 ± 0.78
Saline (control)	154.213 ± 46.34	9.246 ± 1.14
MTAD (clinical protocol)	156.515 ± 33.48	9.145 ± 1.37

Table 2.2. Using Schéffe's multiple comparisons procedure for flexural strength of dentin specimens.

EDTA	110.82						
MTAD	111.70			111.70			
5.25% NaOCl	138.94	(p = 0.163)		138.94		138.94	
Saline	154.21			154.21	(p = 0.051)	154.21	
MTAD protocol	156.52			156.52		156.52	(p = 0.166)
0.6% NaOCl				164.95		164.95	
1.3% NaOCl						170.01	
2.6% NaOCl						184.51	

Table 2.3. Using Schéffe's multiple comparisons procedure for modulus of elasticity of dentin specimens.

MTAD	3.35						
EDTA	4.44	(p = 0.9855)					
0.6% NaOCl				7.28			
2.6% NaOCl				8.79		8.79	
MTAD protocol				9.15	(p = 0.120)	9.15	
Saline				9.25		9.25	(p = 0.966)
1.3% NaOCl				9.34		9.34	
5.25% NaOCl						9.61	

Table 2.4. Mode of fracture for dentin bar specimens; recorded as complete or incomplete fracture.

(n=20)	Complete	Incomplete
5.25% NaOCl	17	0
2.6 % NaOCl	19	0
1.3 % NaOCl	18	0
0.6 % NaOCl	19	0
EDTA	7	**11**
MTAD	0	**17**
Saline (control)	18	0
MTAD (clinical protocol)	18	0

CHAPTER THREE

Effect of MTAD on Bond Strength to Enamel and Dentin

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The purpose of this study was to compare the effect of MTAD and phosphoric acid on the bond strength to enamel and dentin using a conventional OptiBond Solo Plus dentin adhesive system. One hundred flat surfaces (50 enamel / 50 dentin) for bonding were prepared. Specimens were embedded in acrylic with the flat surface exposed. One of the following surface treatments was applied before bonding composite with Optibond Solo Plus (Kerr Corp): 1 min NaOCl / 1 min EDTA; 1 min NaOCl / 1 min MTAD; 30 s etch with H_3PO_4 (positive control), 2 min saline (negative control), or 20 min NaOCl / 5 min MTAD (clinical protocol for MTAD). Sheer bond strength was tested 30 min after bonding. Data was analyzed using a one-way ANOVA followed by the Student-Newman-Keuls multiple

comparison method. Surface pre-treatment with acid etch resulted in the greatest enamel bond strengths ($p<0.05$). All surface pre-treatments were superior to saline for dentinal bonding ($p<0.05$). Based on our findings, it appears that teeth endodontically treated with the MTAD protocol for clinical use (20 min 1.3% NaOCl / 5 min MTAD) may not need any additional dentin conditioning before the application of the dental adhesive.

INTRODUCTION

Achieving predictable bonding to dentin has long been a goal and challenge in restorative dentistry. The high organic content of dentin, its tubular structure and the outward flow of fluid make the bonding of resin to dentin difficult to attain.[1] Recent advances in resin thru the use of a bifunctional molecule and improvements in the infiltration of the resin to a chemically altered dentin surface have made dentin adhesion easier. The standard method to create this micromechanical bond is to acid etch the dentin to remove the smear layer and decalcify the outer 5 – 7 micrometers of dentin. Then an amphiphilic primer is used which facilitates resin penetration into the demineralized dentin substrate.[2] Subsequent polymerization creates a transitional zone of resin-reinforced dentin called the hybrid layer, between the polymerized resin and the unaltered dentin.[3]

The standard method to produce the dentin bonding surface is to use the same acid conditioning, 30-37% phosphoric acid, as used in creating the ideal surface for bonding to enamel.[4] EDTA has also been utilized to prepare the dentin surface for

bonding. Some investigators have found that EDTA conditioning in combination with dentin bonding agents results in a significantly greater bond strength to dentin than conventional acid etching.[5,6] The use of sodium hypochlorite (NaOCl) for dentin surface treatment in combination with acid conditioning has also been suggested.[6] Removal of the organic collagen layer following acid conditioning and subsequent bonding directly to the partially demineralized dentin layer may produce more durable adhesion to the hydroxyapatite component of the dentin substrate.[7] It is theorized that dentin substrate conditioning with NaOCl would significantly enhance the long-term bond strength and durability of the resin adhesive system to dentin.

Immediate restoration of endodontically treated teeth is desirable to prevent coronal leakage. If the use of the root canal irrigant could aid in preparing the dentin surface for bonding, this would be an additional positive attribute. The majority of clinicians use varying concentrations of NaOCl to irrigate canals during root canal therapy. However, NaOCl does not remove the smear layer, does not have a complete antibacterial effect [8] and it is toxic especially at high concentrations.[9] A final flush with EDTA followed by NaOCl appears to remove the smear layer, but EDTA is not antibacterial and erodes the dentin if the exposure time exceeds one minute.[10] Therefore, a new solution with improved capabilities to disinfect the root canal system, remove the smear layer, and prepare the dentin surface for bonding is needed.

In a series of experiments, Torabinejad and associates have shown that a mixture of a tetracycline isomer, an acid and a detergent (MTAD) is an effective solution for the removal of the smear layer. Based on their findings it appears that MTAD does not significantly change the structure of the dentinal tubules when used as a final irrigant in

conjunction with low concentrations of NaOCl as a root canal irrigant.[11] They investigated the ability of MTAD to disinfect contaminated root canals with whole saliva and compared its efficacy to that of NaOCl.[12] Their results show that MTAD is significantly more effective than 5.25% NaOCl in eradicating bacteria from infected root canals. Furthermore, MTAD maintains its efficacy in killing *E. faecalis* at 200× dilution while NaOCl ceases to exert its antibacterial activity beyond 32× dilution.[13] These results are significant in that they demonstrate the efficacy of a single irrigant to remove most of the smear layer and kill a bacterial strain that has been shown to be resistant to many of the commonly used intracanal irrigants and dressings. In addition to these desirable properties, MTAD is a biocompatible material [14] and has similar solubilizing effects on pulp and dentin to those of EDTA.[15] A major difference between MTAD and EDTA is a high binding affinity of doxycycline present in MTAD for the dentin that allows for a prolonged antibacterial effect.

Due to the presence of citric acid in MTAD and its capability to remove the smear layer, it was hypothesized that this solution could also produce a bonding surface that would be equitable to the standard acid-etching. The purpose of this study was to compare the effect of MTAD and phosphoric acid on the bond strength to enamel and dentin using a conventional OptiBond Solo Plus dentin adhesive system.

MATERIALS AND METHODS

Tooth Selection and Embedding: The dentin and enamel substrate used for the bonding experiments were obtained from 100 freshly extracted caries-free, unrestored

human molars that were stored at 4 ^{O}C in 100% humidity containing 0.1 % chloramine T. The teeth were sectioned approximately 1mm below the cemento-enamel junction (CEJ) and then sectioned in half longitudinally in a buccolingual direction. On each tooth half, a small flat surface was created on the external uncut part of the crown. Fifty teeth had the flat area in enamel and the other 50 teeth were ground to expose dentin. All specimens were embedded in poly (methyl-methacrylate) using a Teflon mold leaving the flat surface exposed. The side with the flat bonding surface was polished to a 320 grit and then stored in deionized water.

Bonding: Prior to bonding, the surfaces were slightly refinished to expose fresh dentin or enamel. The teeth were randomly divided into five groups (1-5) of 10 specimens for dentin and five groups (6-10) of 10 specimens for enamel. Prior to bonding, the dentin and enamel surfaces were prepared in the following manner:

Groups 1,6: The bonding surface was irrigated with a solution of 5.25% NaOCl (Clorox, Oakland, CA) for 1min, followed by a 1 min surface treatment of 17% EDTA (REDTA, Roth Int. LTD., Chicago, IL)

Groups 2,7: The bonding surface was irrigated with a solution of 5.25% NaOCl for 1 min, followed by a 1 min surface treatment with MTAD.

Groups 3,8: The bonding surface was etched for 15 s with 35% H_3PO_4 (phosphoric acid, Ultra-Etch, Ultradent Products Inc., South Jordan, UT) as a standard surface treatment prior to bonding. (positive control)

Groups 4,9: The bonding surface was irrigated with a 0.9% saline solution (B. Braun Medical Inc., Irvine, CA) for 2 min (negative control).

Groups 5,10: The bonding surface was treated with the clinical protocol for root canal

disinfection with MTAD: 20 min of 1.3% NaOCl, followed by a 5 min application of MTAD

After treatment, the prepared bonding surfaces were rinsed with copious amounts of de-ionized water and treated with the bonding agent Optibond Solo Plus (Kerr USA, Orange, CA) according to the manufacturer's instructions (moist dentin, 20s scrub of bonding agent, 20s light cure). After the dentin bonding was polymerized the tooth was placed in a bonding jig (Ultradent Products Inc.). The jig had a teflon mold with a 2.378 mm opening that was positioned at the center of the specimen and secured to the tooth base assembly with the use of a grip that held the specimen and the teflon mold together. This allowed for a standardized amount of composite to be packed into the mold to a standardized surface area. Z-100 (3M/ESPE, St. Paul, MN) composite resin was packed to the treated surface and light polymerized with an Optilux 401 (Kerr Demetron, Danbury, CT) from the top of the mold for 40 seconds. The specimen was then removed from the jig and tested 30 minutes after bonding.

Testing Method: Shear bond strength was measured using an MTS Universal Testing machine (model 1125) at a crosshead speed of 1mm/min. A notched crosshead designed to match the diameter of the bonded specimen was used to apply the testing load. The specimens were placed in test base clamp, which was free to move to facilitate positioning under the load. The test base was then positioned so that the notched crosshead was placed against the dentin or enamel surface and the notch was fitted on the diameter of the bonded composite specimen. A load was applied to the point of failure. The mode of failure was classified by visual inspection as follows:

A: Adhesive failure, where the bond failed between the composite resin and the tooth

surface.

B: Adhesive/Cohesive failure, where the bond failed partially between the composite resin and the tooth surface (adhesive) and partially in the composite resin and/or tooth surface (cohesive).

C: Cohesive failure, where the failure occurred either completely within the tooth surface (dentin/enamel) or completely within the composite resin.

The load required to de-bond the specimens was recorded and the mean bond strength of each group (n=10) was calculated (load divided by the surface area of the bonded specimen) and expressed in MPa. Data for the bond strength for the dentin and enamel groups was analyzed using a one-way analysis of variance (ANOVA). The Student-Newman-Keuls method was used to identify the differences between the treatment groups. Two-sample *t*- test ($\alpha = 0.05$) was used to compare each experimental group to the acid etch standard treatment.

RESULTS

There was a statistically significant difference between the dentin groups ($p < 0.0001$) and between the enamel groups ($p = 0.001$). The means and standard deviations of the shear bond strength of Optibond Solo Plus to dentin and enamel are presented in Table 3.1. In the dentin series, the Student-Newman-Keuls multiple comparisons procedure showed that there were no statistically significant differences between the experimental surface treatment groups (NaOCl/EDTA, NaOCl/MTAD, MTAD clinical protocol) and the standard bonding surface treatment (acid etched) ($p = 0.331$). Each of the dentin surface treatments was compared to the standard bonding surface treatment

(acid etched). All of the experimental groups and the standard treatment (acid etched) had statistically significantly greater bond strengths than the negative saline control group ($p < 0.001$). In the enamel groups, the Student-Newman-Keuls multiple comparisons procedure showed that there was no statistically significant difference between the negative control (saline) and any of the experimental groups ($p = 0.104$). Each of the enamel surface treatments was compared to the standard bonding surface treatment (acid etched). The standard bonding surface treatment produced a significantly stronger shear bond strength compared to all of the groups except the NaOCl/ MTAD group ($p = 0.072$). Table 3.2 shows the mode of failure for the dentin and enamel bonding specimens. The majority of failures were adhesive failures, with very few adhesive / cohesive failures, and no cohesive failures.

DISCUSSION

Over the years, the total acid-etch-technique using 30-37% phosphoric acid has proven to provide a suitable surface for bonding to enamel. The resulting etch pattern is characterized by the formation of microporosities which allow the penetration of monomers to form resin tags that provide micromechanical retention.[4] In this study, the shear bond strengths for the majority of the experimental treatment groups for enamel were more similar to the negative control (Table 3.1) and these values were found to be statistically different from the standard acid-etch bonding surface treatment ($p > 0.05$). Since almost all of the failures were adhesive failures (Table 3.2), this indicates that the bond was weaker than either the enamel substrate or the composite resin material.

When each of the enamel experimental groups was compared to the 15s acid-etch control group, there was a statistically significant difference except for the 1 min 5.25% NaOCl / 1 min MTAD group ($p = 0.072$). It is possible that this treatment provided a surface that was more similar to that created by acid etching by removing organic debris with the sodium hypochlorite and then removing the inorganic mineral with the citric acid in the MTAD and creating a surface suitable for bonding. The MTAD protocol group, where the surface was treated with 20 min 1.3% NaOCl / 5 min of MTAD did not show as high of a bond strength. It is possible that using a diluted concentration of NaOCl (1.3%) resulted in incomplete removal of organic material and therefore the MTAD (pH 2) could not produce an enamel surface that was not as optimal for bonding.

Bonding to dentin has not yet achieved the ideal state. Researchers now believe that dentin adhesion relies primarily on the penetration of adhesive monomers into the filigree of collagen fibers left exposed by acid etching.[16] This is the ultimate goal; however, adhesion to dentin remains difficult and there appears to be some question as to what is the optimal treatment to provide an ideal bonding surface.

There is a controversy over whether to remove the smear layer before bonding. Some studies [17,18] promote smear layer retention for optimal dentin bond strength and others advocate its removal.[5,6] Not all dentin bonding agents have the same mechanism of action; therefore, the results of these studies could have varied depending on which materials were used. Some dentin adhesive agents may not show reduced bond strength when the smear layer is removed because they probably bond via dentinal collagen, but other agents interact primarily with dentinal calcium. Thus, removal of the smear layer for adhesives reliant on the presence of calcium is undesirable.[1]

Smear layer removal exposes the dentinal tubules and provides a network for adhesive resin tag formation within the dentinal tubules and the anastomosing of lateral canals which may be fundamental to the development of a stronger dentin / resin bond.[6] In the present study, it appears that the removal of the smear layer, whether by 35% phosphoric acid-etch, 17% EDTA treatment or MTAD treatment resulted in equitable dentin bond strengths (Table 3.1). The shear bond strengths for the experimental surface treatment groups and the standard bonding surface treatment, 35% phosphoric acid etch, were very similar and no statistical significant differences were found ($p = 0.331$). The majority of failures were adhesive failures (Tables 3.2). The dentin group did show adhesive / cohesive failure where part of the dentin surface was removed in the shear bond test. It is possible that the specimen was not accurately butted against the shearing blade resulting in not pure shear test, but a tensile/shear test. This action generates a large amount of force at the initial point of contact creating an S-type fracture taking a chunk of dentin with the fracture.

Dentin treatment before adhesion of composites is performed both to enhance adhesion and to remove the microbial contents of the smear layer.[19] It is important to eliminate any remaining bacteria that may be present on the cavity walls, in the smear layer, at the enamel-dentin junction, or in the dentinal tubules [20] to halt the deleterious effect of bacteria and their by-products. In addition, it seems that even the latest adhesive systems are not capable of producing a complete seal in clinical situations, so gaps form and bacteria and their toxic products readily penetrate. [21] Therefore, bonding systems that have intrinsic antimicrobial properties would be beneficial. In an *in vitro* study Settembrini and coworkers [22] found that the Single Bond etchant (3M Products, St. Paul,

MN) demonstrated antibacterial activity against several bacterial strains. However, the duration of the effect or whether this antibacterial effect is enough to inhibit bacteria when applied in a clinical situation is unclear. An advantage of using an agent such as MTAD to create the bonding surface is its antimicrobial properties. Torabinejad and coworkers [13] confirmed that EDTA possesses little or no bactericidal activity when tested using the minimum inhibitory concentration (MIC) method.

In the present study, sodium hypochlorite was used before EDTA and MTAD for several reasons. If sodium hypochlorite is used after the dentin surface is demineralized, it appears that the collagen fibril layer is completely removed. Perdaigão and coworkers [4] found that when a 10% NaOCl gel is applied for 60s to etched dentin, the morphological appearance of the hybrid layer loses its fibillar arrangement and results in reduction in shear bond strengths that are equivalent to 31% - 38% of that obtained by total acid-etching depending on which dentin bonding agents are used. They proposed that the integrity of the collagen fibrils that are left exposed upon acid-etching plays a major role in the mechanism of adhesion for certain adhesive systems and that the intermingling of the adhesive monomers with the filigree of collagen fibers or hybrid layer should be considered the main dentin bonding mechanism. On the other hand, Prati and coworkers [23] as well as Vargas and coworkers [7] found that some dentin bonding agents produce high bond strengths without the presence of exposed collagen fibrils. However, they also showed that the morphology of the dentin surface is completely different when NaOCl treatment follows acid-etching. With this protocol, the dentinal tubules are open and the surface is porous. Therefore, it appears that dentin is partially removed and that this sequence of treatment causes destruction of dentin.[23]

In an abstract, Cederlund and coworkers [24] suggested that shear bond strength is significantly decreased when surfaces are treated with NaOCl after acid etching or EDTA conditioning. This phenomenon seems reasonable because calcium (Ca) and phosphorous(P) are major inorganic components of dental hard tissue and alterations in the Ca/P ratio may change the original ratio between organic and inorganic components. This in turn could change the permeability and solubility characteristics of dentin [25] and may affect adhesion. Doğan and Çalt [26] found that the use of 10ml of 2.5% NaOCl alters the mineral content of root dentin significantly. NaOCl treatment causes mineral accumulation in human root dentin.[27] Furthermore, NaOCl may dissolve the organic components and leave a smear layer of mineralized tissue.[28] The smear layer could then be removed to increase bond strength.

In the present study, specimens were exposed for 1 min to 5.25% NaOCl before a 1 min exposure to 17% EDTA or MTAD. Some specimens were exposed for 20 min to 1.3% NaOCl before a 5 min exposure to MTAD as recommended for clinical use in performing root canal therapy.[11] It appears that using any of these methods produces a dentin surface that is equivalent to the standard treatment which is a 15s etch with 35% phosphoric acid. Therefore, it would be reasonable to suggest that any of these dentin treatments could be used before bonding. However, MTAD does have antibacterial properties and EDTA does not [13] and 17% EDTA has the potential for causing excessive peritubular and intertubular dentinal erosion if the application time exceeds 1 min.[10] In addition, the NaOCl / MTAD treatment used on enamel produced shear bond strengths similar to that obtained by the acid-etch control group (Table 3.1).

Based on our findings, it appears that teeth endodontically treated with the MTAD protocol for clinical use (20 min 1.3% NaOCl / 5 min MTAD) may not need any additional dentin conditioning before the application of the dental adhesive. Future research will focus on the possibility of using MTAD as a pre-bonding treatment for cavity and crown preparations.

REFERENCES

1. Pashley DH. Interactions of dental materials with dentin in Proceedings of Conference on Enamel-Dentin-Pulp-Bone-Periodontal Tissue interactions with dental materials. Transaction of the Academy of Dental Materials 1990;3:55-73.

2. Nakabayashi N. Resin reinforced dentin due to infiltration of monomers into dentin at the adhesive interface. Jour of Japanese Soc for Dent Mater 1982;1:78-81.

3. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by infiltration of monomers into tooth substrates. Jour of Biomed Mater Res 1982;16:265-273.

4. Perdigão J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater. 2000 Sep;16(5):311-23.

5. Blomlof J, Cederlund A, Jonsson B, Ohlson NG. Acid conditioning combined with single-component and two-component dentin bonding agents. Quintessence Int 2001 Oct;32(9):711-5.

6. Chappell RP, Cobb CM, Spencer P, Eick JD. Dentinal tubule anastomosis: a potential factor in adhesive bonding. J Prosthet Dent 1994 Aug;7(2):183-8.

7. Vargas MA, Cobb DS, Armstrong SR. Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. Oper Dent. 1997 Jul-Aug;22(4):159-66.

8. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endodon 1994;20:276-8.

9. Spångberg L, Engstrom B, Langeland K, Biologic effects of dental materials. III. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg 1973;36:856-71.

10. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endodon 2002 Jan;28(1):17-9.

11. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endodon 2003;29(4):233-9.

12. Shabahang S, Pouresmail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and NaOCl. J Endodon. Accepted for publication.

13. Torabinejad M, Shabahang S, Aprecio R, Ketting JD. The antimicrobial effect of MTAD: An *in vitro* investigation. J Endodon. *in press*

14. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using MTT tetrazolium method. J Endodon. *in press*.

15. Beltz RE, Torabinejad M, Pouresmail M. Quantitative analysis of the effect of EDTA, sodium hypochlorite, and MTAD on bovine pulp and dentin. J Endodon. Submitted for publication.

16. Perdigão J. Dentin bonding as a function of dentin structure. Dent Clin North Am. 2002;46(2):277-301, vi.

17. White GJ, Beech DR, Tyas MJ. Dentin smear layer: an asset or a liability for bonding? Dent Mater 1989 Nov:5(6):379-83.

18. Ishioka S, Caputo AA. Interaction between the dentinal smear layer and composite bond strength. Prosthet Dent 1989 Feb;61(2):180-5.

19. Molgatini S, Abate PF, Negroni MB, Machhi RL, Gonzalez MI. Bacterial inhibition produced by substances for dentin pretreatment. Acta ocontol latinoam 1993;7(2):3-11.

20. Brännstrom M, Nordenvall KJ. Bacterial penetration, pulpal reaction and inner surface of concise enamel bond. Composite filling in etched and unetched cavities. J Dent Res 1978;57:3-10.

21. Atac AS, Cehreli ZC, Sener B. Antibacterial Activity of Fifth-Generation Dentin Bonding Systems. J Endodon 2001 Dec;27(12):730-3.

22. Settembrini L, Boylan R, Strassler H, Scherer W. A comparison of antimicrobial activity of etchants used for a total etch technique. Oper Dent 1997;22:84-8.

23. Prati C, Chersoni S, Pashley DH. Effect of removal of surface collagen fibrils on resin-dentin bonding. Dent Mater. 1999 Sep;15(5):323-31.

24. Cederlund A, Blomlof J, Jonsson B. Presence of intact collagen fibers and its effect on dentin bond strength. Abstract 0178. IADR 2002.

25. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992;71(8):1530-40.

26. Doğan H, Çalt S. Effects of Chelating Agents and Sodium Hypochlorite on Mineral Content of Root Dentin. J Endodon 2001 Sep;27(9):578-80.

27. Inaba D, Ruben J, Takagi O, Arends J. Effect of sodium hypochlorite treatment on remineralization of human root dentine in vitro. Caries Res. 1996;30(3):218-24.

28. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endodon 1987;13:147-57.

Table 3.1. Mean shear bond strengths (MPa) and standard deviations for the dentin and enamel groups. **Bold** indicates no significant difference between the **bolded** values in the dentin and enamel groups.

	Dentin	Enamel
Saline (negative control)	20.895 ± 7.47	**18.742 ± 5.26**
NaOCl/EDTA	**36.124** ± 4.55	**21.997 ± 3.60**
NaOCl/MTAD	**36.990** ± 6.22	**23.357 ± 5.23**
MTAD (clinical protocol)	**35.427** ± 5.54	**21.275 ± 3.42**
Acid Etch (standard tx)	**39.900** ± 5.04	27.405 ± 4.18

Table 3.2. Mode of failure for dentin bonding specimens. A= Adhesive failure; B= Adhesive / Cohesive failure; C= Cohesive failure.

	Dentin			**Enamel**		
(n=10)	**A**	**B**	**C**	**A**	**B**	**C**
Saline	10	0	0	10	0	0
NaOCl / EDTA	9	1	0	9	1	0
NaOCl / MTAD	9	1	0	10	0	0
MTAD (clinical protocol)	10	0	0	10	0	0
Acid Etch (standard treatment)	8	2	0	10	0	0

CHAPTER FOUR

SEM ANALYSIS

Sample Preparation Protocol for SEM

Two dentin bars from seven of the flexural strength groups as well as two dentin specimens and two enamel specimens from four of the different bonding surface treatments were examined.

Flexural Strength Groups:

Group 1: 5.25% of NaOCl for a total of two hours.

Group 2: 2.63% of NaOCl for a total of two hours.

Group 3: 1.31% of NaOCl for a total of two hours.

Group 4: 0.66% of NaOCl for a total of two hours.

Group 5: 17% EDTA for a total of two hours.

Group 6: MTAD for a total of two hours.

Group 7: 0.9% saline solution for a total of two hours.

Bonding Groups: (groups 1 –4 are dentin, groups 6-9 are enamel)

Groups 1,6: 1 min 5.25% NaOCl followed by 1 min 17% EDTA

Groups 2,7: 1 min 5.25% NaOCl followed by 1 min MTAD

Groups 3,8: 15 s with 35% H_3PO_4 (positive control)

Groups 4,9: 2 min 0.9% saline solution (negative control).

Samples were prepared for Scanning Electron Microscopy (SEM) immediately following treatment. First the specimens were fixed by incubating them in a buffered fixative solution, 2% glutaraldehyde in Na cacohylate buffer (0.1 M, pH 7.2), for at least 24 h at 4 °C in small vials. The specimens were then rinsed 3 times in Na cacodylate

buffer (0.1 M, pH 7.2) using small cups. Then they were incubated in 1% Osmium Tetraoxide for 30 minutes in the refrigerator and for an additional 30 minutes at room temperature with the vial lids removed. Specimens were rinsed 2 times with Na cacodylate buffer prior to starting the dehydration procedure. Specimens were dehydrated in increasing percentages of ethanol:

Dehydration:

- 1 rinse in 30% ethanol for 10 min
- 1 rinse in 50% ethanol for 10 min
- 1 rinse in 70% ethanol for 10 min
- 1 rinse in 90% ethanol for 10 min
- 1 rinse in 95% ethanol for 10 min
- 2 rinse in 100% ethanol for 10 min

Following dehydration, specimens were placed on aluminum stubs with two-sided tape and colloidal graphite was applied to create a connection between the specimen and the aluminum stub. Specimens were then left in the dessicator for a minimum of 24 hours. Before SEM analysis specimens underwent a 4 min sputter coating with gold-palladium (final thickness = ~25 nm). A setting of 10 Kv was used for SEM analysis and pictures were taken at 2500x, 5000x, and 10,000x magnifications.

SEM Findings

The following figures show the SEM Findings for the Flexural Strength and Bonding specimens.

Flexural Strength

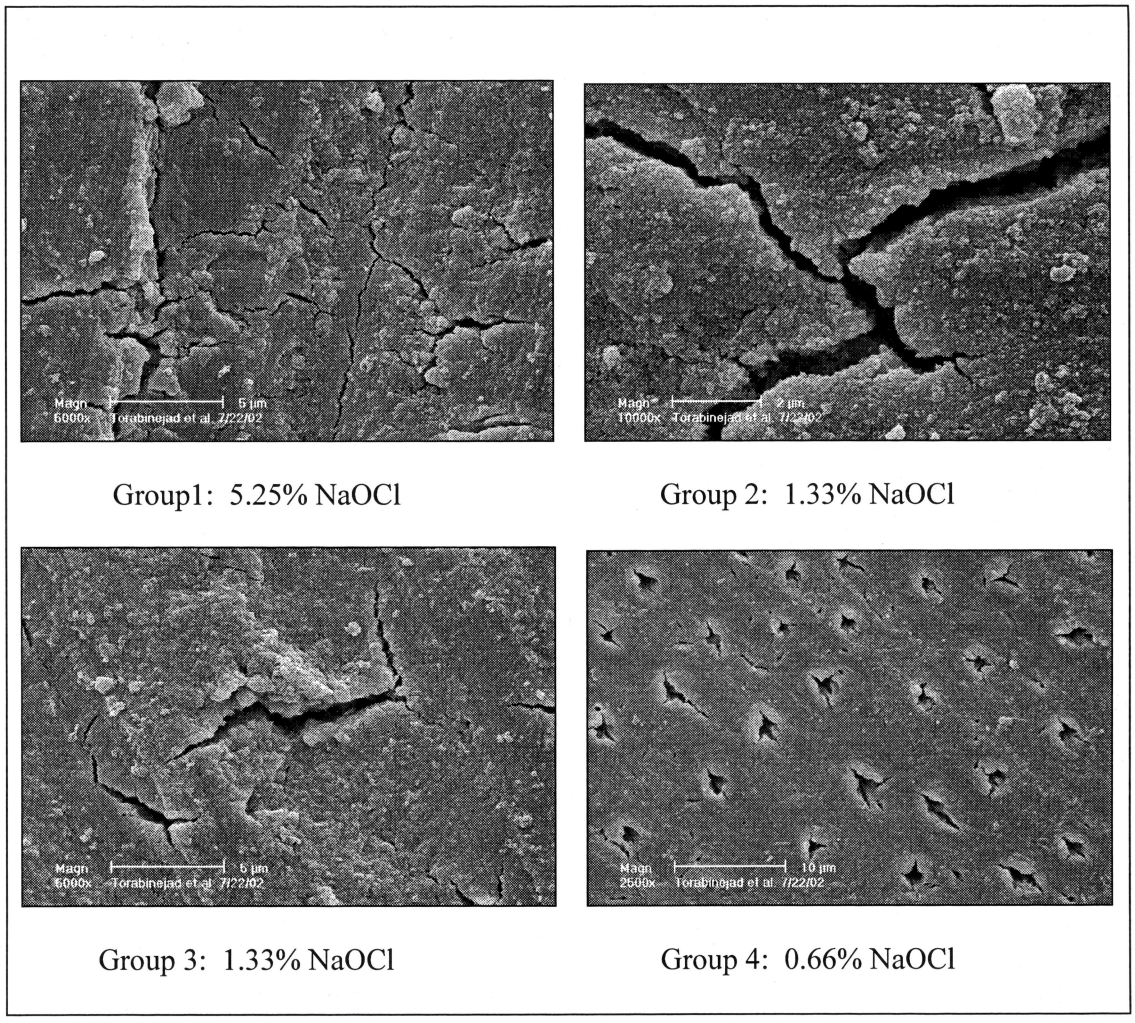

Figure 1. SEM Photographs of various concentrations of NaOCl at 2500x, 5000x and 10,000x magnifications.

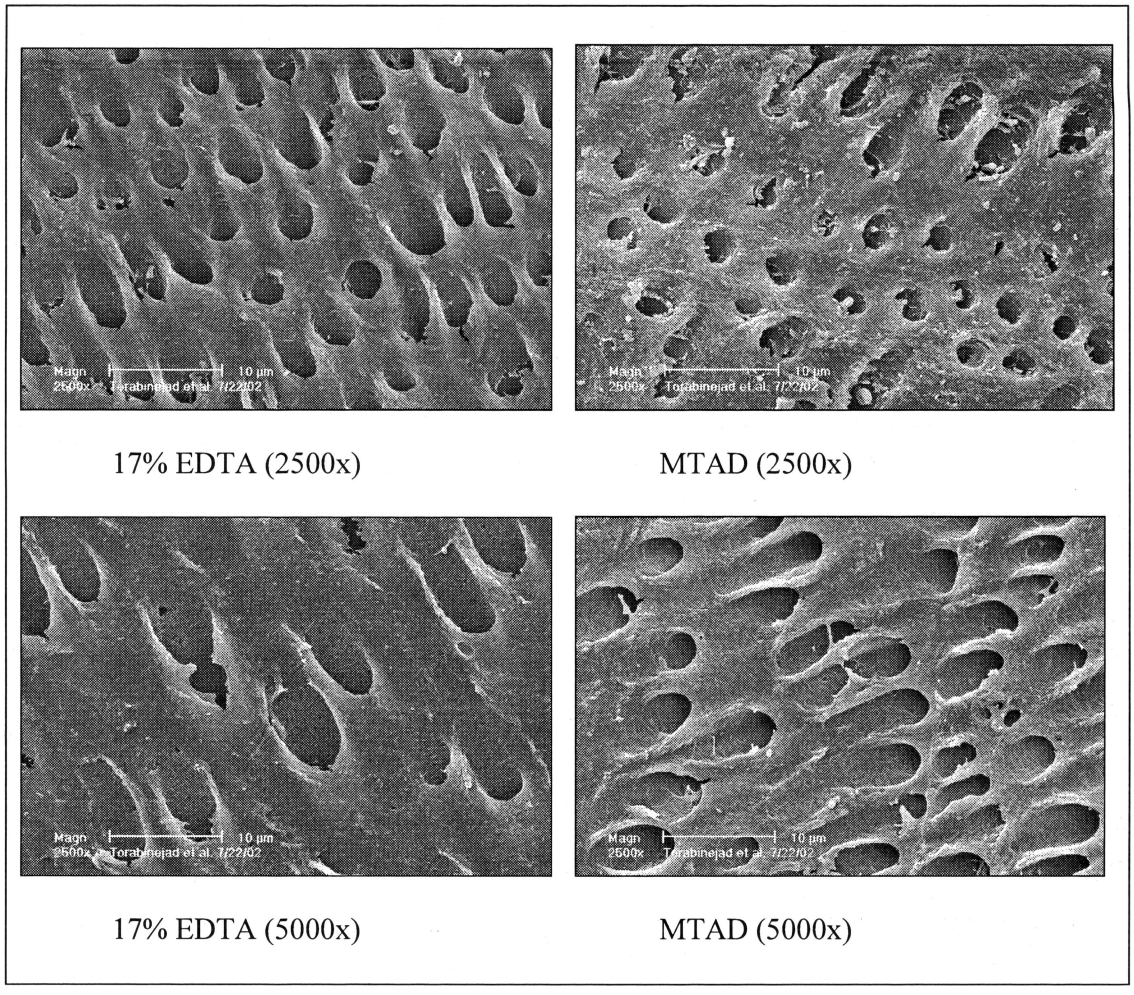

Figure 2. SEM Photographs of 17% EDTA (Group 5) and MTAD (Group 6) at 2500x and 5000x magnifications.

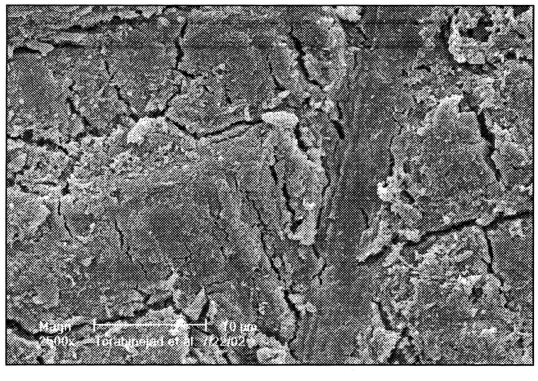

Figure 3. SEM Photograph of 0.9% Saline (Group 7) at 2500x magnification.

Bonding – Dentin

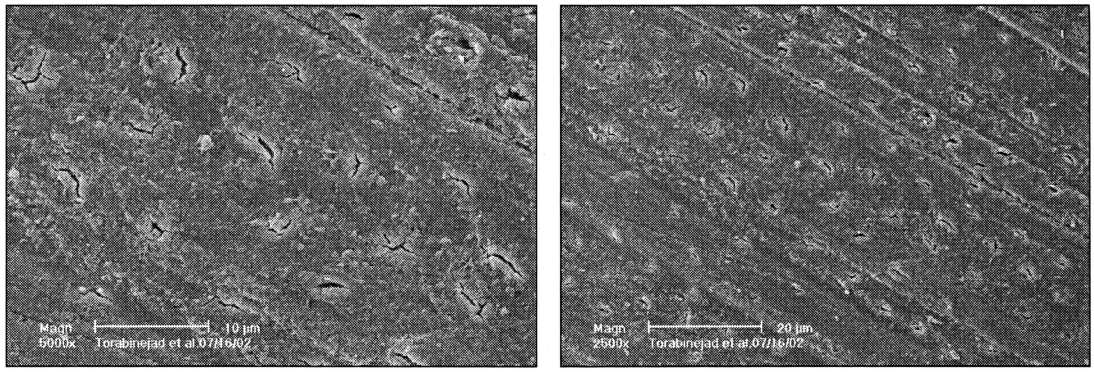

Figure 4. SEM Photographs of 0.9% Saline (Group 4) at 2500x and 5000x magnifications.

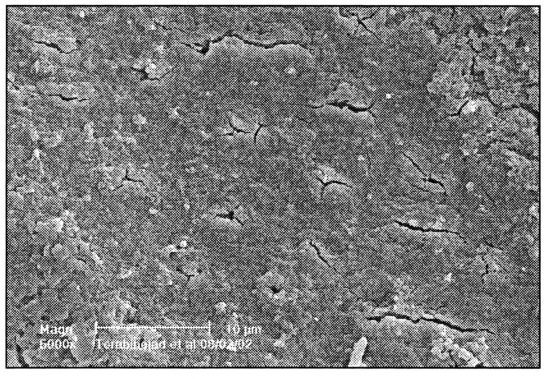

Figure 5. SEM Photograph of 1min 5.25% NaOCl / 1min 17% EDTA (Group 1) at 5000x magnification.

Bonding – Dentin

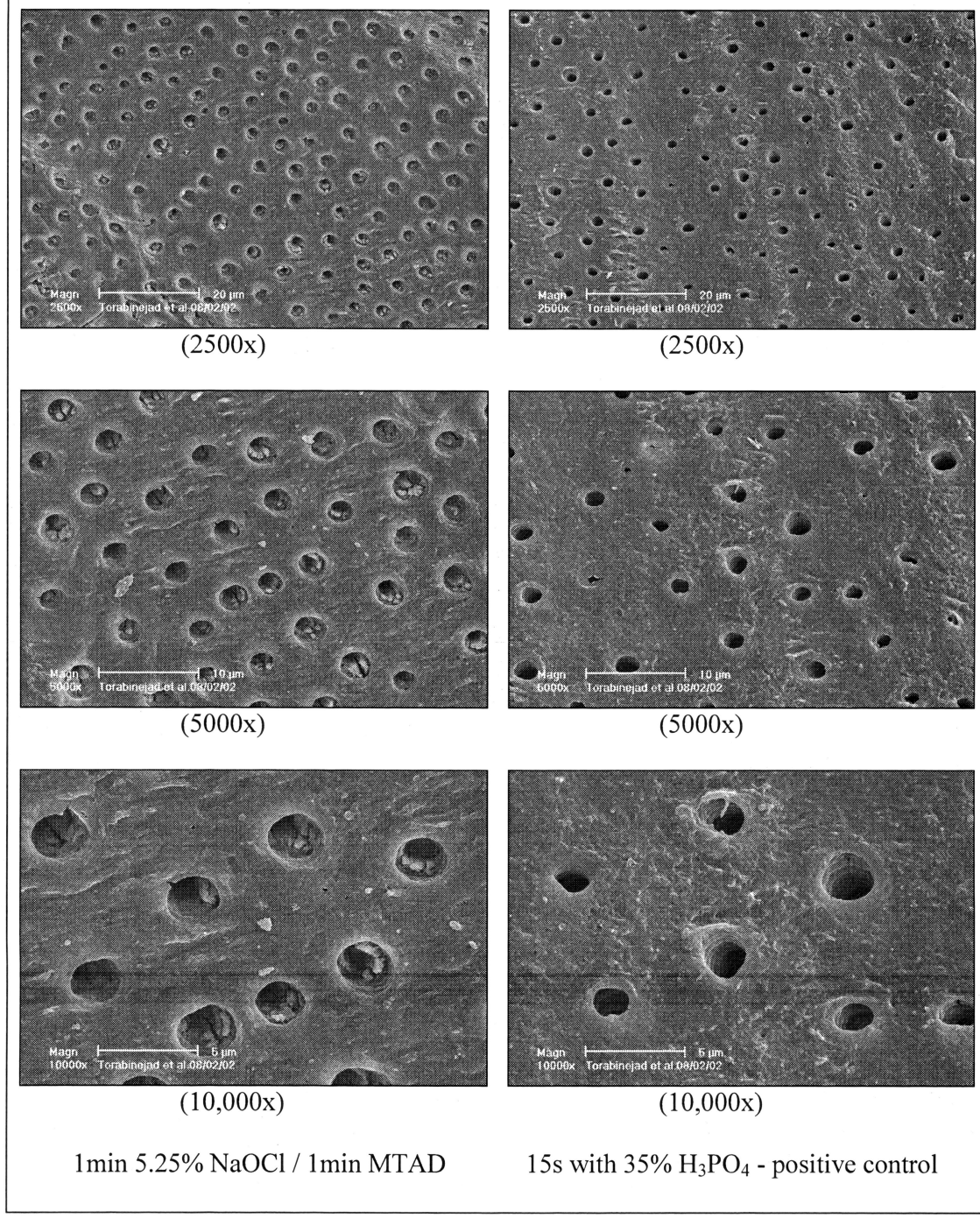

Figure 6. SEM Photographs of 1min 5.25% NaOCl / 1 min MTAD (Group 2) and 15s with 35% H_3PO_4 - positive control (Group 3).

Bonding – Enamel

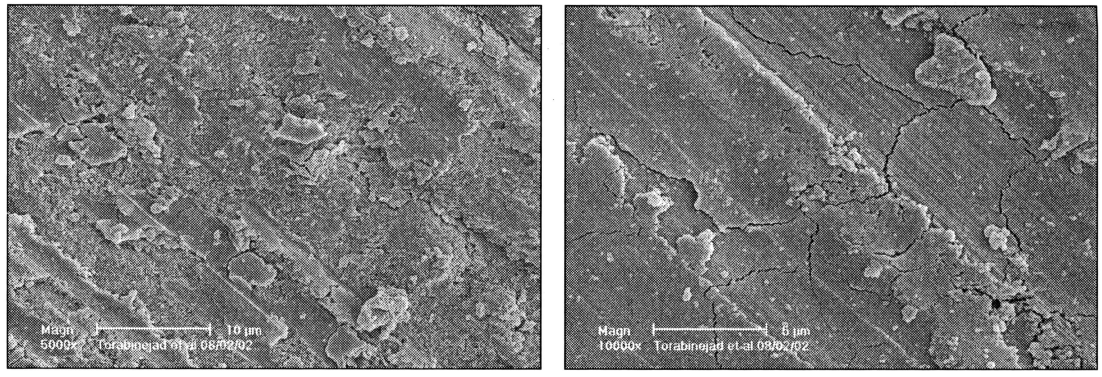

Figure 7. SEM Photographs of 0.9% Saline (Group 9) at 5000x and 10,000x magnifications.

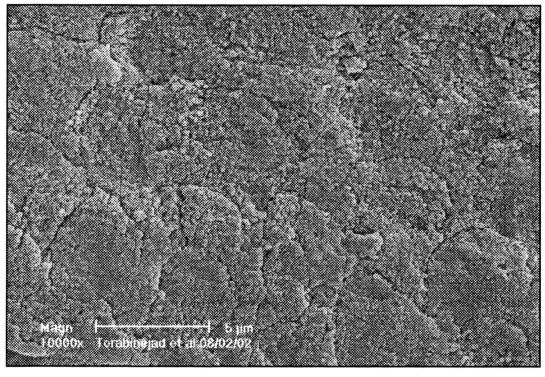

Figure 8. SEM Photograph of 1min 5.25% NaOCl / 1min 17% EDTA (Group 6) at 10,000x magnification.

Bonding – Enamel

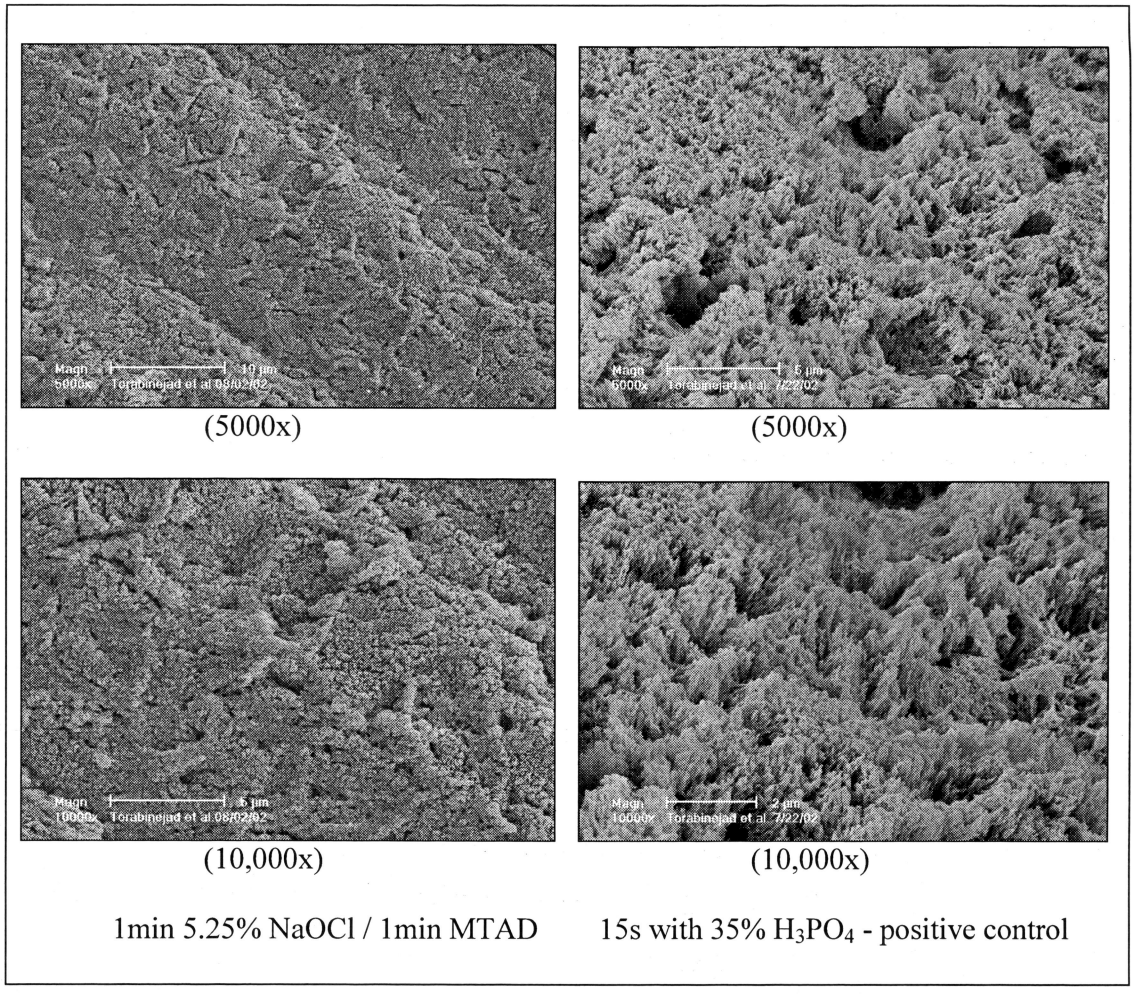

Figure 9. SEM Photographs of 1min 5.25% NaOCl / 1 min MTAD (Group 7) and 15s with 35% H_3PO_4 - positive control (Group 8).

CHAPTER FIVE

CONCLUSIONS

The one-way ANOVA rejects the null hypotheses for both Part I: Flexural strength and Part: Bonding. The differences in mean values among the treatment groups were greater than would be expected by chance. There was a statistically significant difference between treatment groups for flexural strength and modulus of elasticity ($p < 0.0001$). The values for the 2h EDTA and 2h MTAD groups were different from the other groups. Whereas, the MTAD clinical protocol group was not statistically different from the saline control. No statistically significant differences were found between the MTAD clinical protocol group and the saline control group for flexural strength ($p = 0.865$), modulus of elasticity ($p = 0.858$), or mode of fracture. This suggests that MTAD as recommended for clinical use does not adversely affect dentin structure.

There was a statistically significant difference between dentin groups ($p < 0.0001$) and between enamel groups ($p = 0.001$) for shear bond strength. Specimens were exposed for 1 min to 5.25% NaOCl before a 1 min exposure to 17% EDTA or MTAD. Some specimens were exposed for 20 min to 1.3% NaOCl before a 5 min exposure to MTAD as recommended for clinical use in performing root canal therapy (Torabinejad *et al.*, *in press*). It appears that using any of these methods produces a dentin surface that is equivalent to the standard treatment which is a 15s etch with 35% phosphoric acid. Therefore, it would be reasonable to suggest that any of these dentin treatments could be used before bonding. However, MTAD does have antibacterial properties and EDTA does not (Torabinejad *et al.*, *in press*) and 17% EDTA has the potential for causing

excessive peritubular and intertubular dentinal erosion if the application time exceeds 1 min (Çalt and Serper, 2002).

For enamel bonding, 1 minute 5.25% NaOCl followed by 1 minute MTAD produced shear bond strengths similar to that obtained by the acid-etch control group. It is possible that this treatment provided a surface that was more similar to that created by acid etching by removing organic debris with the sodium hypochlorite and then removing the inorganic mineral with the citric acid in the MTAD and creating a surface suitable for bonding.

Based on our findings, it appears that teeth endodontically treated with the MTAD protocol for clinical use (20 min 1.3% NaOCl / 5 min MTAD) may not need any additional dentin conditioning before the application of the dental adhesive. Future research will focus on the possibility of using MTAD as a pre-bonding treatment for cavity and crown preparations.

It appears that using the clinical protocol for MTAD causes no adverse effects on the physical properties of dentin. The results of these *in vitro* studies suggest that MTAD possess most of the positive qualities of an "ideal" irrigant. *In vivo* studies are in progress to assess its clinical effectiveness before its widespread use in endodontics.

REFERENCES

Abou-Ross M, Patonai FJ. The effects of decreasing surface tension on the flow of irrigating solutions in narrow root canals. Oral Surg 1982;53:524-6.

Atac AS, Cehreli ZC, Sener B. Antibacterial Activity of Fifth-Generation Dentin Bonding Systems. J Endod 2001 Dec;27(12):730-3.

Barbosa SV, Safavi KE, Spångberg SW. Influence of Sodium Hypochlorite on the permeability and structure of cervical human dentine. Int. Endodontic Journal, 1994, 27:309-12.

Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. Journal of Endodontics, 1992, 18: 605-12.

Baumgartner JC, Mader CL. Scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod 1987 Apr;13(4):147-57.

Beltz RE, Torabinejad M, Pouresmail M. Quantitative analysis of the effect of EDTA, sodium hypochlorite, and MTAD on bovine pulp and dentin. J Endodon. Submitted for publication.

Blomlof J, Cederlund A, Jonsson B, Ohlson NG. Acid conditioning combined with single-component and two-component dentin bonding agents. Quintessence Int 2001 Oct;32(9):711-5.

Brännstrom M, Nordenvall KJ. Bacterial penetration, pulpal reaction and inner surface of concise enamel bond. Compostie filling in etched and unetched cavities. J Dent Res 1978;57:3-10.

Buck R, Eleazer PD, Staat RH. In vitro disinfection of dentinal tubules by various endodontics irrigants. J Endod. 1999 Dec;25(12):786-8.

Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.

Byström A, Sundqvist G. The bacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endodontic J, 1985,18:35-40.

Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endodon 2002 Jan;28(1):17-9.

Cameron JA. The use of ultrasonics in the removal of the smear layer: a scanning electron microscope study. J Endodon 1983;9:289-92.

Cameron JA. The effect of a fluorocarbon surfactant on the surface tension of the endodontic irrigant, sodium hypochlorite. A preliminary report. Aust Dent J 1986;31:364-8.

Cederlund A, Blomlof J, Jonsson B. Presence of intact collagen fibers and its effect on dentin bond strength. Abstract 0178. IADR 2002.

Cederlund A, Jonsson B, Blomlof J. Shear strength after ethylenediaminetetraacetic acid conditioning of dentin. Acta Odontol Scand 2001;59(6):418-22.

Cergneux M, Chiucchi J, Dietschi JM, Holtz J. The influence of the smear layer on the sealing ability of canal obturation. Int Endodon Jour 1987;20:228-32.

Chappell RP, Cobb CM, Spencer P, Eick JD. Dentinal tubule anastomosis: a potential factor in adhesive bonding. J Prosthet Dent 1994 Aug;7(2):183-8.

Craig RG, Ward ML Eds. Restorative Dental Materials, 10th Ed. Chapter 4: Mechanical Properties, pp 87-8. 1997: Mosby, St. Louis.

Deardorf KA, Swartz ML, Newton CW, Brown CE Jr. Effect of root canal treatments on dentin permeability. J Endod. 1994 Jan;20(1):1-5.

Di Lenarda R, Cadenaro M, Sbaizero O. Effectiveness of 1 mol L-1 citric acid and 15% EDTA irrigation on smear layer removal. Int Endod J. 2000 Jan;33(1):46-52.

Doğan H, Çalt S. Effects of Chelating Agents and Sodium Hypochlorite on Mineral Content of Root Dentin. J Endod 2001 Sep;27(9):578-80.

Frankenberger R, Kramer N, Oberschachtsiek H, Petschelt A. Dentin bond strength and marginal adaption after NaOCl pre-treatment. Oper Dent. 2000 Jan-Feb;25(1):40-5.

Gettleman BH, Messer HH, El-Deeb ME. Adhesion of sealer cements to dentin walls with and without the smear layer. J Endodo 1991;17:15-20

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 Mar;34(2):113-9.

Goldsmith M, Gulabivala K, Knowles JC. The effect of sodium hypochlorite irrigant concentration on tooth surface strain. J Endodon 2002 Aug;28(8):575-9.

Haapasalo HK, Siren EK, Waltimo TM, Ørstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine: an in vitro study. Int Endod J. 2000 Mar;33(2):126-31.

Haikel Y, Gorce F, Alermann C, Voegel JC, In-vitro efficiency of endodontic irrigation solution on protein desorption. International endodontic J, 1994;27:16-20.

Hand RE, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endodon 1978;4:60-4.

Harrison JW, Hand RE. The effect of dilution and organic matter on the antibacterial property of 5.25% sodium hypochlorite. Journal of Endodontics, 1981;7:128-32.

Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8-14.

Huang TJ, Shilder H, Nathanson D. Effects of Moisture Content and Endodontic Treatment on Some Mechanical Properties of Human Dentin. J Endod 1992 May;18(5):209-15.

Inai N, Kanemura N, Tagami J, Watanabe LG, Marshall SJ, Marshall GW. Adhesion between collagen depleted dentin and dentin adhesives. Am J Dent. 1998 Jun;11(3):123-7.

Inaba D, Ruben J, Takagi O, Arends J. Effect of sodium hypochlorite treatment on remineralization of human root dentine in vitro. Caries Res. 1996;30(3):218-24.

Inaba D, Duschner H, Jongebloed W, Odelius H, Takagi O, Arends J. The effects of a sodium hypochlorite treatment on demineralized root dentin. Eur J Oral Sci. 1995 Dec;103(6):368-74.

Inamoto K, Kojima K, Nagamatsu K, Hamaguchi A, Nakata K, Nakamura H. J Endodon 2002;28(5):371-4.

Ishioka S, Caputo AA. Interaction between the dentinal smear layer and composite bond strength. Prosthet Dent 1989 Feb;61(2):180-5.

Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endodon 1994;20:276-8.

Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional lab rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.

Marais JT. Cleaning efficacy of a new root canal irrigation solution: a preliminary evaluation. Int Endod J. 2000 Jul;33(4):320-5.

McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endodon 1975;1:238-42.

Möller ÅJR, Fabricius L, Dahlen G, Öhman AE, Heyden G. The influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475-84.

Molgatini S, Abate PF, Negroni MB, Machhi RL, Gonzalez MI. Bacterial inhibition produced by substances for dentin pretreatment. Acta ocontol latinoam 1993;7(2):3-11.

Nakabayashi N. Resin reinforced dentin due to infiltration of monomers into dentin at the adhesive interface. Jour of Japanese Soc for Dent Mater 1982;1:78-81.

Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by infiltration of monomers into tooth substrates. Jour of Biomed Mater Res 1982;16:265-273.

Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically-treated teeth. Am J Dent. 1999 Aug;12(4):177-80.

Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-9.

Pashley DH. Interactions of dental materials with dentin in Proceedings of Conference on Enamel-Dentin-Pulp-Bone-Periodontal Tissue interactions with dental materials. Transaction of the Academy of Dental Materials 1990;3:55-73.

Pashley DH, Livingston MJ, Reeder OW, Horner J. Effects of the degree of tubule occlusion on the permeability of human dentin in vitro. Arch Oral Biol 1978;23:1127-33.

Patterson S. *In vivo* and *in vitro* studies of the effect of disodium salt of ethylenediamine tetra-acetate on human dentin and its endodontic implications. Oral Surg 1963;16:83-103.

Perdigão J. Dentin bonding as a function of dentin structure. Dent Clin North Am 2002;46(2):277-301, vi

Perdigão J, Frankenberger R, Rosa BT, Breschi L. New trends in dentin / enamel adhesion. Am J Dent 2000;13(Spec No):25D-30D.

Perdigão J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater. 2000 Sep;16(5):311-23.

Perez F, Rochd T, Lodter J-P, Calas P, Michel G. *In vitro* study of the penetration of three bacterial strains into root dentine. Oral Surg Oral Med Oral Pathol Oral Max Fax Radio Endod 1993;76:97-103.

Peters LB, Wesselink PR, Buiji JF, van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endodon 2001;27:76-81.

Phrukkanon S, Burrow MF, Hartley PG, Tyas MJ. The influence of the modification of etched bovine dentin on bond strengths. Dent Mater. 2000 Jul;16(4):255-65.

Pioch T, Kobaslija S, Schagen B, Gotz H. Interfacial micromorphology and tensile bond strength of dentin bonding systems after NaOCl treatment. J Adhes Dent. 1999;1(2):135-42.

Prati C, Chersoni S, Pashley DH. Effect of removal of surface collagen fibrils on resin-dentin bonding. Dent Mater. 1999 Sep;15(5):323-31.

Reeh ES, Messer HH, Douglas WH. Reduction in tooth stiffness as a result of endodontic and restorative procedures. J Endodon 1989;15:512-6.

Rostein I, Cohenca N, Teperovich E, Moshonov J, Mor C, Roman I et al. Effect of chloroform, xylene, halotene on enamel and dentine microhardness of human teeth. Oral Surgery, Oral Medicine and Oral Pathology. 1999;87:366-8.

Sakae T, Mishima H, Kozawa Y. Changes in bovine dentine mineral with sodium hypochlorite treatment. Journal of Dental Research 1988;67:1229-34.

Seidberg BH and Schilder H. An evaluation of EDTA in endodontics. Oral Surg. 1974;37(4):609-20.

Sen BH, Wesselink PR, Turkun M. The smear layer: a phenomenon in root canal therapy. Int Endodon Jour 1995;28:141-8.

Settembrini L, Boylan R, Strassler H, Scherer W. A comparison of antimicrobial activity of etchants used for a total etch technique. Oper Dent 1997;22:84-8.

Shabahang S, Pouresmail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and NaOCl. J Endodon. Accepted for publication.

Shih M, Marshall FJ, Rosen S. The bacterial efficiency of sodium hypochlorite as an endodontic irrigant. Oral Surgery 1970;29:613-9.

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 Mar;34(2):120-32.

Siqueira JF Jr., de Uzeda M, Fonseca MEF. A scanning electron microscopic evaluation of *in vitro* dentinal tubules penetration by selected anaerobic bacteria. J Endodon 1996;22:308-10.

Siqueria JF Jr, Machado AG, Silveira RM, Lopes HP, Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from root canal, *in vitro*. Int Endod J 1997;30:279-82.

Spångberg L, Engstrom B, Langeland K, Biologic effects of dental materials. III. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg 1973;36:856-71.

Taşman F, Cehreli ZC, Ogan C, Etikan I. Surface tension of root canal irrigants. J Endod. 2000 Oct;26(10):586-7.

Tidmarsh BG. Restoration of endodontically treated posterior teeth. J Endodon 1976:2:374-5.

Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove smear layer. J Endodon. *in press*

Torabinejad M, Handysides R, Khademi A, Bakland LK. Clinical implimations of the smear layer in endodontics: a review. Oral Surgery, Oral Medicine and Oral Pathology, Oral Radiol Endod 2002;94:658-66.

Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Shabahang S. A new solution to remove the smear layer. J Endodon. J Endodon 2003;29:170-5.

Torabinejad M, Shabahang S, Aprecio R, Ketting JD. The antimicrobial effect of MTAD: An *in vitro* investigation. J Endodon *in press*

Trowbridge HO and Kim S. Pulp development structure and function. In: Cohen S, Burns RC, eds. Pathways of the Pulp. 1991. St. Louis, MO, USA: Mosby Year Book

Van Meerbeek B, Dhem A, Goret-Nicaise M, Braem M, Lambrechts P, Vanherle G. Comparitive SEM and TEM examination of the ultrasonic structure of the resin-dentin interdiffusion zone. J Dent Res 1993;72:495-501.

Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992;71(8):1530-40.

Van Meerbeek B, Lambrechts P, Inokoshi S, Braem M, Vanherle G. Factors affecting adhesion to mineralized tissues. Oper Dent 1992;5:111-24.

Vargas MA, Cobb DS, Armstrong SR. Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. Oper Dent. 1997 Jul-Aug;22(4):159-66.

White GJ, Beech DR, Tyas MJ. Dentin smear layer: an asset or a liability for bonding? Dent Mater 1989 Nov:5(6):379-83.

Wikesjö UME, Baker PJ, Christersson LA, Genco RJ, Lyall RM, Hic S, DiFlorio RM, Terranova VP. A biochemical approach to periodontal regeneration: Tetracycline treatment conditions dentin surfaces. J Periodont Res 1986;21:322-9.

Yamada R, Aramas A Goldman M, Lin PS. A scanning electron microscope comparison of a high volume final flush with several irrigating solutions. Part 3. J Endodon 1983;9:137-42.

Zerosi C, Viotti L. L' EDTA in terapia radicolare. Pass Trimes Odontol 1958;39:683-6.

Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using MTT tetrazolium method. J Endodon. *in press*