In vitro Effects of Berberine versus Sodium Hypochlorite on Dentin Flexural Strength

BY

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THESIS

Submitted as partial fulfillment of the requirements for the degree of Master of Science in Oral Sciences in the Graduate College of the University of Illinois at Chicago, 2012

Chicago, Illinois

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This thesis is dedicated to my family, whose sacrifices have made my goals and dreams possible. It certainly would not have been accomplished without their continued support.
ACKNOWLEDGEMENTS

I would like to thank the members of my thesis committee, Dr. Ana Bedran-Russo, Dr. Mohamed Fayad, Dr. Bradford Johnson, and Dr. Christopher Wenckus for their support and assistance. Dr. Bedran-Russo and Dr. Fayad provided me with unparalleled wisdom throughout the length of this project. A special thank you to my program director, Dr. Johnson, whose door is always open, both literally and implicitly. Dr. Bedran-Russo permitted me the use of her spacious and well-equipped lab as well as the knowledge related to her specialized field of restorative dentistry. I would also like to thank Dr. Berdan Aydin and Dr. Lina Hassan who are research associates in Dr. Bedran-Russo’s lab. Drs. Aydin and Hassan sacrificed their time to train me in the DSC machine and collagen degradation test. To all that took part in this project, I am in your gratitude, thank you.

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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BBr</td>
<td>Berberine</td>
</tr>
<tr>
<td>CHX</td>
<td>Chlorhexidine</td>
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<tr>
<td>DSC</td>
<td>Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>MPa</td>
<td>Megapascals</td>
</tr>
<tr>
<td>n</td>
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<tr>
<td>NaOCl</td>
<td>Sodium hypochlorite</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Td</td>
<td>Temperature denaturation</td>
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<td>wt</td>
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SUMMARY

Chemical debridement through effective irrigating medicaments facilitates root canal disinfection and prevents reinfection. Today, the most commonly used irrigant is sodium hypochlorite, commonly known as bleach. Hypochlorite solutions may affect mechanical protein properties via the degradation of organic dentin components. Sim et al. (2001) demonstrated that irrigation with 5.25% NaOCl reduces the flexural strength of dentin. In addition, as a nonspecific oxidizing and proteolytic agent, NaOCl oxidizes the organic matrix and denatures the collagen components of the smear layer (Zhang et al., 2010). Berberine, a natural antimicrobial plant alkaloid, has demonstrated antimicrobial activity in an in vitro tooth model (Moussa et al., 2010; Xie et al., 2011). Demonstrating radicular dentin strength with berberine may ultimately indicate more stable dentin collagen, which is critical for the long-term stability of the endodontically treated tooth.

We hypothesize that berberine, used as an adjunct irrigating solution, would not affect the flexural strength when compared to a control. In addition, berberine, when used as an adjunct irrigating solution, would not affect the collagen structure when compared to a control. Root dentin strength was measured by evaluating flexural strength, collagen degradation, and collagen temperature denaturation after exposure to irrigating solutions of berberine chloride, sodium hypochlorite (NaOCl), or a berberine/NaOCl combination. Twenty-four caries, restoration and crack-free single-rooted human permanent teeth were decoronated and 7.0mm samples from
the coronal and middle 1/3 of the root were retained. Dentin beams (sections) were prepared by making 7mm x 3mm x 0.3mm slices through each root. Control beams for each group were prepared from the same tooth as the experimental group. Irrigation was performed on one surface of the tooth samples. Teeth were divided into four groups as follows: Group 1- 60 seconds irrigation with saline; Group 2- 60 seconds irrigation with 0.2% Berberine; Group 3- 120 seconds irrigation with 5.25% NaOCl; Group 4- 120 seconds irrigation with 5.25% NaOCl followed by 60 seconds irrigation with 0.2% Berberine. Specimens were then tested for flexural strength using a 3-point flexural device. From the same teeth, dentin disks were cut into 2mm x 2mm x 0.25mm to measure collagen degradation using a collagen digestibility method, and collagen temperature denaturation was examined by a differential scanning calorimeter (DSC). Statistical analysis was performed using one-way ANOVA (p<0.05). No statistically significant difference in flexural strength, collagen degradation, and collagen temperature denaturation among groups (p>0.05) was detected.

We concluded that the 2-minute exposure of 5.25% NaOCl and 1-minute exposure of 0.2% berberine did not affect the flexural strength of dentin when compared with saline and the biodegradation rates were not affected by the irrigants evaluated. Also the NaOCl and/or berberine irrigants had no effect on the thermal stability of the mineralized dentin. These results contradict other studies.
due to different methodologies, as other samples were immersed in irrigating solutions for an extended period of time compared to our study, which more closely resembles clinical use.
I. INTRODUCTION

A. Background & Hypotheses

Microorganisms are the primary etiologic factor in the development of periapical bone lesions and failure of endodontic treatment (Kakehashi, 1965; Sundqvist, 1998; Pinheiro, 2003). Successful root canal therapy depends on adequate mechanical preparation and chemical debridement (Schilder, 1967). Effective irrigating solutions enable root canal disinfection and aid in preventing reinfection. Ideal characteristics of an endodontic irrigant are to flush out debris during cleansing and shaping, possess a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic pulp tissue remnants, and inactivate endotoxin. Furthermore, it should prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed (Zehnder, 2006), and be systemically nontoxic, noncaustic to periodontal tissues with little to no potential of causing an allergic reaction.

Current regimens utilize sodium hypochlorite because of its effective antimicrobial activity and tissue dissolution property. However, at high concentrations, NaOCl is highly toxic and irritating to tissue (Spangberg et al., 1973). Moreover, NaOCl negatively affects the mechanical properties of dentin including the flexural strength and elastic modulus when compared to saline (Sim et al.,
Irrigation with 5.25% NaOCl for longer than 1 hour has been found to degrade the collagen matrix (Zhang et al., 2010).

The development of a new medicament in endodontic irrigation that is antibacterial, anti-inflammatory and exhibits minimal toxicity to human tissues, is of considerable significance. Berberine is a plant alkaloid isolated from a phytomedicine (goldenseal, *Hydrastis Canadensis*) in the U.S. As a natural agent, it has been proven to possess antimicrobial and anti-inflammatory activities along with non-toxicity to the host. More recently, it has been proven to effectively reduce the bacterial load of *Enterococcus Faecalis* in a single canal *in vitro* tooth model (Moussa et al., 2010). It is important to assess potential effects of berberine on tooth mechanical properties.

Publications are sparse on the use of natural products with strengthening properties in endodontics; therefore, it is imperative to analyze the use of a particular plant extract, *in vitro*, which has been documented to have attractive characteristics that may be advantageous in the practice of endodontics.

This research proposal tested the null hypotheses:

1. Berberine, used as an adjunct irrigating solution, would not affect the flexural strength when compared to a control.
2. Berberine, used as an adjunct irrigating solution, would not affect the collagen structure when compared to a control.
B. **Objectives of the Study**

The objectives of the present study are:

1- To investigate the flexural strength of radicular dentin after exposure to berberine with and without sodium hypochlorite.

2- To investigate the collagen biodegradation after exposure to berberine with and without sodium hypochlorite.
II. REVIEW OF LITERATURE

A. **Endodontic Success and Microbiology**

Root canal therapy is a common dental procedure; its success depends on adequate mechanical preparation of the canals and chemical debridement of the root canal system through the use of effective irrigating solutions followed by the total three-dimensional filling of the root canals and all accessory canals (Schilder, 1967). The ultimate goal of endodontic treatment is to control the microbial factor in complex root canal anatomy (Nair et al., 2005). Microorganisms have long been recognized as the primary causative component in the development of periapical bone lesions (Kakehashi et al., 1965) and failure of endodontic treatment (Sundqvist et al., 1998; Pinheiro et al., 2003). The classic study of Kakehashi et al. demonstrated that exposed pulpal tissues became necrotic in the presence of bacteria, resulting in the development of chronic pulpal inflammation; eventually, periapical granulomas were formed (Seltzer and Farber, 1994). Reinfection and continued periapical inflammation may occur from viable bacteria residing in the complex root canal system and dentinal tubules (Sjogren et al., 1997). The microorganisms available in the necrotic root canal emanate from the oral cavity. Anaerobic bacteria dominate primary apical periodontitis with only few or no facultative or aerobic species in the canals (Haapasalo et al., 2007). With previous endodontic treatment,
*Enterococcus faecalis* is the most common and occasionally, the only single isolated bacteria from root canals of teeth with persistent periapical periodontitis (Rocas et al., 2004).

Microbes in the root canal system of a necrotic tooth depend on the ecologic factors such as redox potential, the availability of nutrients found throughout the root canal system, and composition of the infective microflora, including bacterial interactions. Microorganisms located within the root canal system travel via a communication pathway, for example dentinal tubules in areas of resorbed root cementum, lateral canals, or apical foramina, to the surrounding periradicular tissue to cause periapical inflammation. The predominance of microbes in primary apical periodontitis are located in the main root canal while some have invaded deeper into the dentinal tubules (Peters et al., 2001; Matsuo et al., 2003) and accessory canals. With regard to secondary apical periodontitis (post treatment disease), the location of the microbes is influenced by additional factors such as the availability of nutrients due to coronal leakage and quality of the root filling (Haapasalo et al., 2007). In post treatment endodontic disease, the bacteria interact with the host defense to cause an inflammatory reaction resulting in destruction of the periodontium. Due to our knowledge of the etiology of apical periodontitis, there is a general agreement that elimination of the microbes through chemomechanical preparation is the main immediate goal of treatment. Irrigants play an instrumental role in accomplishing this goal.
B. **Significance of Chemical Debridement**

Chemomechanical preparation utilizes chemically active irrigating solutions in combination with mechanical cleansing. Chemical debridement through effective irrigating medicaments facilitates root canal disinfection and helps prevent reinfection. Different irrigants are currently used to flush out loose debris, destroy microbes and remove tissue remnants (Perez-Hedia et al., 2006; Zehnder, 2006). The early work of Hess and Zurcher, and Gutierrez and Garcia, to more recent studies (Kartel and Yanikoglu, 1992; Li et al., 2012) assist the dental community in appreciating the anatomic complexities of the root canal system. When performing endodontic therapy, the practitioner should keep in mind the anatomic irregularities, such as fins and isthmuses, and select an irrigant with the ideal
characteristics of a broad antimicrobial spectrum with no adverse effects on the physical properties of exposed dentin.

C. Ideal Characteristics of Endodontic Disinfecting Agents & Most Commonly Used Irrigant

In the past, countless compounds in aqueous solution have been suggested as root canal irrigants, including inert substances such as sodium chloride (saline) or highly toxic and allergenic biocides such as formaldehyde (Harrison, 1984). The main purpose of an irrigant is to flush debris from the root canal system during the cleansing and shaping process. To this end, root canal irrigants ideally should have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic pulp tissue remnants, inactivate endotoxin, and prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed (Zehnder, 2006). Additionally, they should be systemically nontoxic, noncaustic to periodontal tissues with little to no potential of causing an allergic reaction.

Today, the most commonly used irrigant is sodium hypochlorite (NaOCl), commonly known as bleach. Sodium hypochlorite was first recommended as an antiseptic solution by Henry Dakin (Dakin, 1915). During World War I, a 0.5% buffered solution was used extensively to irrigate wounds. Taylor and Austin, 1918, demonstrated the solvent action of NaOCl (Dakin’s solution) on nonvital tissue while noting that the 0.5% solution was only mildly inflammatory to normal tissue (Senia et al., 1971).
Sodium hypochlorite is considered the most ideal irrigant because of fulfilling key requirements in endodontic irrigation. Sodium hypochlorite has the unique property of dissolving necrotic tissue (Grossman and Meiman, 1941; Naenni et al., 2004), more effectively at greater concentrations and the dissolution of organic components of the smear layer (Baumgartner and Mader, 1987; Gutierrez et al., 1990), pulp tissue, and collagen. NaOCl solutions are very strong oxidizing agents. While in solution they comprise of hypochlorite ion (OCl⁻) and hypochlorous acid (HOCl) in differing proportions and in combination constitute the active chlorine content (Clarkson et al., 2011). Chlorine provides the protein dissolving ability and broad-spectrum anti-microbial activity (Hoffman et al., 1991). It was found that 5.25% NaOCl was the only irrigant capable of removing biofilm after only 5 minutes (Giardino et al., 2007). Sodium hypochlorite was found to inactivate bacterial endotoxin (Silva et al., 2004). Moreover, sodium hypochlorite solutions are inexpensive, easily available, and demonstrate good shelf life (Frais et al., 2001).

During endodontic therapy, NaOCl solutions are used in concentrations ranging from 0.5% to 6% (“full strength”). There is no consensus regarding the appropriate concentration of sodium hypochlorite solution for endodontic use, but the full strength concentration is common practice. At a higher concentration it is highly toxic and irritating to tissue (Spangberg et al., 1973). Severe reactions have occurred with the passage of NaOCl solution through the apical foramen, which could occur when the needle is momentarily wedged tightly into the canal during irrigation (Pashley et al., 1985). Sodium hypochlorite accidents result in immediate and severe pain for the patient, gradually increasing edema, and a profuse
hemorrhage both interstitially and through the tooth (Kleier et al., 2008). In 2008, Kleier et al. conducted a survey on the incidence of NaOCl accidents and found that 42% of board-certified endodontists reported experiencing a sodium hypochlorite accident. Sodium hypochlorite also possesses allergic potential (Zehnder, 2006). Moreover, a 5.25% solution significantly decreased the flexural strength and elastic modulus of human dentin compared to physiologic saline (Sim et al., 2001) and decreased its microhardness (Slutzky-Goldberg et al., 2004).

Table I

OVERVIEW OF THE FEATURES OF AQUEOUS IRRIGANTS FREQUENTLY RECOMMENDED FOR ENDODONTIC USE (ZEHNDER, 2006)¹

<table>
<thead>
<tr>
<th>Compound (recommended concentration)</th>
<th>Type</th>
<th>Action on Endodontic Taxa Biofilm</th>
<th>Tissue Dissolution Capacity</th>
<th>Endotoxin Inactivation</th>
<th>Action on Smear Layer</th>
<th>Caustic Potential</th>
<th>Allergic Potential</th>
</tr>
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<tr>
<td>Hydrogen peroxide (3-30%)</td>
<td>Peroxygen</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>D.O.C.</td>
<td>-</td>
</tr>
<tr>
<td>Sodium hypochlorite (1-5.25%)</td>
<td>Halogen-releasing agent</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++ on organic compound</td>
<td>D.O.C.</td>
<td>+</td>
</tr>
<tr>
<td>Iodine potassium iodide (2-5%)</td>
<td>Halogen-releasing agent</td>
<td>++</td>
<td>-</td>
<td>No information</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Chlorhexidine (0.2-2%)</td>
<td>Bisguanide</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>D.O.C.</td>
<td>+</td>
</tr>
<tr>
<td>Dequalinium acetate (0.5%)</td>
<td>Quaternary ammonium compound</td>
<td>No information</td>
<td>-</td>
<td>No information</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Ethylenediamine tetraacetic acid (10-17%)</td>
<td>Polyprotic acid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++ on inorganic compound</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citric acid (10-50%)</td>
<td>Organic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++ on inorganic acid</td>
<td>-</td>
<td>-</td>
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(-) absent or minor, (+) reported, (++) definitely present, (++++) strong, (D.O.C.) depending on concentration

¹Appendix B: Editor's letter of permission
D. **Other Commonly Used Endodontic Irrigants**

1. **Chlorhexidine**

Chlorhexidine (CHX) was developed more than 50 years ago. CHX is an antiseptic that is commonly used in controlling plaque in the oral cavity (Addy and Moran, 1997). A solution of 0.12% is recommended for this purpose, whereas a concentration of 2% is used in root canal irrigation (Zamany et al., 2003). Chlorhexidine has a broad-spectrum antimicrobial activity (Parson et al., 1980) as well as demonstrating substantivity (Greenstein et al., 1986), the ability to be retained in the matrices of dentin. Carilloho et al., 2010, concluded that the outstanding substantivity of CHX to dentin and its reported effect on the inhibition of dentinal proteases should explain why CHX could prolong the durability of resin-dentin bonds.

Chlorhexidine is commonly considered to be less toxic than NaOCl, however this may not be the case (Spangberg, et al., 1973). Even with its usefulness as a final irrigant, CHX cannot be advised as the main irrigant in standard endodontic cases because it is unable to dissolve necrotic tissue components (Naenni, et al., 2004) and is less effective on gram-negative than on gram-positive bacteria (Davies et al., 1954; Hennessey, 1973; Emilson, 1977). Further unfavorable effects include causing teeth and tongue discoloration, loss of taste, burning sensation of the oral mucosa, and subjective dryness of the oral cavity. There are no published reports on allergic reactions following root canal irrigation with chlorhexidine (Hulsmann et al., 2007).
2. Ethylenediamine tetraacetic acid

Ethylenediamine tetraacetic acid (EDTA) is a demineralizing agent recommended as an adjuvant to root canal therapy. The use of EDTA to remove calcium salts from dentin walls and facilitate instrumentation of occluded canals began as early as 1957 (Nygaard-Ostby, 1957). Since sodium hypochlorite is commonly used for dissolution of the organic component of the smear layer due to its proteolytic capabilities, EDTA is used to remove the inorganic portion through a chelation process (Hulsmann et al., 2003). Prolonged use of EDTA can lead to demineralization of dentin immediately below the smear layer, reducing its hardness (Hasegawa et al., 1989).

E. Berberine as a Natural Product

One natural product of keen interest is berberine, a quaternary ammonium salt from the group of isoquinoline. It is found in such plants as berberis, goldenseal (Hydrastasis canadensis) and Coptis chinensis, usually in roots, rhizomes, and stem bark. Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and chlamydia (Peter et al., 2000). The medicinal uses of berberine are anti-diarrheal, anti-inflammatory and antimicrobial for the treatment of diarrhea, skin inflammation and liver disease (Huang et al., 1999).
Berberine has also been found to possess anti-carcinogenic properties by inhibiting tumorigenesis through inhibition of inflammation (Kuo et al., 2004).

Its use in dentistry is limited to the inhibitory effect on periodontopathogenic bacteria; the results suggest the possibility of a potential clinical application for the treatment of periodontal diseases (Hu et al., 2000). Berberine was found to have no significant side effects or toxicity on humans and was not found to induce adverse effects in the gastrointestinal tract (Rabanni et al., 1987). Toxicological data from the National Toxicology Program (NTP) regarding Hydratis and two of its major alkaloids, berberine and hydrastine demonstrated no short-term toxicity on mice, nor developmental toxicity in fetus. The National Toxicology Program reports on the toxicology of chemicals used in the United States.

F. Evaluation of Efficacy of Berberine Against E. faecalis and Multiple Species

In the study by Moussa et al. (2010), the in vitro bactericidal activity of berberine and the berberine/chlorhexidine (CHX) combination against Enterococcus faecalis was evaluated and compared to conventional irrigants in a single tooth model. With regards to viable E. faecalis counts in dentin, irrigation with NaOCl, CHX, berberine, or berberine/CHX significantly reduced the counts compared to saline (p<0.05). Berberine demonstrated bactericidal activity better than saline but not as effective as the other irrigants. The berberine/CHX combination demonstrated superior bactericidal efficacy against E. faecalis in the dentin. Berberine did not demonstrate antagonistic interaction when used in combination
with CHX. In conclusion, the data indicated that the use of berberine could represent an alternative or natural adjunct endodontic irrigant.

A follow-up study (Xie et al., 2011) involved evaluating the antimicrobial efficacy of berberine solution as an irrigant against selective endodontic pathogens using a multi-species biofilm tooth model. The bacterial species used were *Fusobacterium nucleatum, Enterococcus faecalis*, and *Prevotella intermedia*. The authors found that the berberine and CHX combination presented with similar bactericidal efficacy as NaOCl and CHX in regards to the bacterial reduction in dentin. Berberine is more effective than saline as an endodontic irrigant against selective endodontic pathogens *in vitro*, and when combined with CHX was comparable to NaOCl in its bactericidal efficacy.

G. **Dentin Composition**

Dentin is composed of 22 wt % hydrated organic matrix and 10 wt % water, with the remaining being an inorganic reinforcing phase of carbonated hydroxyapatite (Pashley, 1989). The organic matrix consists of proteins, of which type I collagen accounts for 90% of dentin protein (Qin et al., 2006). Type I collagen contributes considerably to the mechanical properties of dentin. Dentinal tissue consists of closely packed tubules, 38-40,000 per square mm present in the coronal portion of the tooth, decreasing to 18-20,000 per square mm in the root portion.

The dentinal tubules are surrounded by highly mineralized peritubular dentin and intertubular dentin (Haapasalo et al., 2007). Located between the dentinal tubules is the intertubular dentin and accounts for the majority of the dentin. Its
organic matrix is composed largely of collagen fibrils that are well mineralized and provide tensile strength to dentin (Kinney et al., 2003). Collagen fibrils are composed of self assembled aggregations of collagen molecules (Pashley et al., 2003). Their mechanical properties are due to cross-links (Knott and Bailey, 1988) and molecular intertwining (Silver et al., 2000).

Intratubular, or peritubular, dentin lines the inner walls of the dentinal tubules. Peritubular dentin is highly mineralized and has fewer collagen fibrils and a high amount of sulfated proteoglycans and mineral. As a result of its lower quantity of collagen, peritubular dentin is harder than intertubular dentin and thus more quickly dissolved with acid-etching agents such as ethylenediamine tetraacetic acid (Kinney et al., 1996). EDTA makes dentin more permeable by enlarging the openings of dentinal tubules. The dentinal tubules usually undergo closure or blockage with aging or constant provocation from bacteria, restorative procedures, or restorative materials.

H. Mechanical Properties of Dentin

1. Flexural Strength

There is a widely held belief that root-treated teeth are weakened and more susceptible to fracture than vital teeth (Rosen, 1961; Johnson et al., 1976; Gher et al., 1987). The main causes may be divided broadly into three areas: loss of tooth tissue, altered physical properties of dentin, and altered proprioception/nociception (Gutmann, 1992; Gulabivala, 1995). One of the most important physical properties
of dentin is flexural strength. Flexural strength is defined as the ability of the material to resist deformation under load. The decrease in flexural strength is clinically relevant; it indicates that fewer forces are required for the cohesive bonds within dentin to fail (Sim et al., 2001).

Sodium hypochlorite is utilized in root canal treatment largely because of its antimicrobial properties and its dissolution of the organic components of the smear layer. The elevated concentration makes tissue disintegration more effective but also has a deleterious effect on the properties of dentin. Sim et al. (2001) exhibited that irrigation with 5.25% NaOCl as compared to saline solution reduces the flexural strength and the elastic modulus of dentin. Pascon et al. (2009) conducted a review on the effect of sodium hypochlorite on the mechanical properties of root dentin; the studies showed reductions in the flexural strength of dentin after irrigation of the canals with 2.5%, 3%, 5%, 5.25% and 9% NaOCl from 24 minutes to 2 hours. The potential mechanisms in dentin depletion and hence the weakening effect of NaOCl have been analyzed by Barbosa et al., 1994.

Zhang et al. (2010) evaluated different irrigation regimens on collagen denaturation, degradation and flexural strength of mineralized dentin. To determine the flexural strength, human third molars sectioned into dentin disks were immersed in 1.3% or 5.25% NaOCl for 10-240 minutes. All sections, except for the control, were immersed for the respective experimental time followed by a final rinse with 17% EDTA for 2 minutes. Hydrated dentin beams were exposed to a 3-point flexure test. A significant reduction was noticed when 5.25% was used as the initial irrigant for longer than 60 minutes. Changes were observed after 4 hours
with 1.3% NaOCl. The effects of sodium hypochlorite on dentin appear to be concentration and time dependent and not associated with demineralization.

Another study (Marending et al., 2007) demonstrated the impact of different irrigation sequences of NaOCl (24 minutes) and EDTA (3 minutes) on the flexural strength with standardized human root dentin. Exposures to exclusively EDTA, NaOCl, and water were used as control treatments. The 24-minute immersion of the entire dentin disks to the hypochlorite solution caused a drop in flexural strength compared with water or EDTA treated controls. In addition, the minimal exposure to EDTA did not impact the mechanical dentin parameters regardless of the irrigant sequence.

In 2001, Grigoratos et al. evaluated the effects of 3% and 5% solutions of sodium hypochlorite on the flexural strength and modulus of elasticity of standardized dentin bars. The dentin bars were treated by exposure to the different solutions of NaOCl by immersing them for 2 hours and subsequent loading to failure in a 3-point bending test. Subjections to both solutions reduced the flexural strength and modulus of elasticity.

Sim et al. conducted further testing on the effects of sodium hypochlorite on root dentin. They tested dentin bars by immersing them in either 0.5% NaOCl or 5.25% NaOCl for 2 hours and then subjecting them to a three-point bending test. The authors noticed a decrease in flexural strength of the dentin in the 5.25% NaOCl group compared to the 0.5% NaOCl group. Consequently, it is important to identify the possible effects of irrigants, medicaments and materials on the physical properties of teeth with endodontic therapy.
2. **Collagen Degradation**

Treatment of root canals with differing irrigants produces modifications in the chemical and structural composition of human root dentin. The mineral constituent in hard connective tissue is a factor in its strength and elastic modulus; whereas, the collagen unit contributes to the toughness of the tissues (Wang et al., 2001). The ideal balance between stiffness and toughness contributes to the structural integrity of hard tissues such as dentin. Type I collagen fibrils are stabilized by covalent intramolecular and intermolecular crosslinks. Increased focus has been placed on collagen crosslinking to stabilize dentin collagen. Increasing the intra and intermolecular bonds by cross-linking of collagen has been demonstrated to increase the ultimate tensile strength (Bedran-Russo et al., 2007, 2011). Zhang et al. analyzed the degradation, or withdrawal, of intact collagen from mineralized dentin powder by sodium hypochlorite. Radicular dentin powder was immersed in 1.3% or 5.25% NaOCl for 10-240 minutes and later washed with 17% EDTA as the final irrigant for 2 minutes. The dentin powder specimens were studied by using a Fourier transform infrared spectroscopy to evaluate their relative subsurface intact collagen content with the apatite/collagen ratio. The results revealed that collagen degradation was significantly increased after the use of 5.25% NaOCl as the initial irrigant for more than 1 hour. On the other hand, changes were insignificant when the 1.3% NaOCl solution was used as the initial irrigant for up to 4 hours.
The degradation of collagen fibrils may also be attributed to matrix metalloproteinases (MMPs). MMPs form a group of enzymes involved in the extracellular matrix degradation. These proteinases play a role in biological processes such as normal tissue remodeling, wound healing, and angiogenesis (Visse & Nagase, 2003). MMP -2, -8, and -9 have been discovered in human crown dentin (Mazzoni et al., 2007) as well as human radicular dentin (Santos et al., 2009). The release and activation of MMPs may play a part in the organic matrix degradation during caries (Chaussain-Miller et al., 2006) and along side resin-dentin-bonded interfaces (Carillho et al., 2007).

An increase in collagen crosslinks will improve the stability of collagen and in turn enhance the bond strength. Macedo et al. (2009) examined the addition of chemical cross-linking agents, glutaraldehyde and grape-seed extract, to dentin collagen. Their results demonstrated that the application of glutaraldehyde or grape-seed extract to demineralized dentin powder previous to the bonding procedure increased the dentin bond stability. Along with biochemical cross-linking, strengthening of the collagen matrix can be accomplished by incorporating biopolymers, carboxymethylchitosin (CMCS), that can be cross-linked with collagen fibrils (Shrestha et al., 2011).

3. **Temperature Denaturation of Collagen**

There is increasing appeal in the thermal stability of mineralized and demineralized dentin. During cleaning and shaping, root canals become vulnerable
to chelating agents like EDTA that can demineralize the dentin surface exposing the collagen matrix. Armstrong et al. (2008) were the first to use human teeth to evaluate temperature denaturation on collagen matrices; preceding reports were performed on rat-tail tendon as it consists mainly of unmineralized type I collagen. The denaturation temperature of dentin collagen in mineralized and demineralized dentin matrices, as a function of age and extent of dehydration, was examined. It was concluded that the presence of minerals, apatite crystallites, increased the denaturation temperature of demineralized dentin collagen. The temperature denaturation, of dentin samples treated with chemical cross-linkers, were also examined by Bedran-Russo et al. (2011). Modifications of collagen dentin matrices using chemical agents affect the collagen biochemistry and also the proteoglycans, a major group of non-collagenous proteins in dentin.
III. MATERIALS AND METHODS

A. Material Selection and Experimental Design

Extracted human anterior and single-rooted premolar teeth were kept frozen for no longer than 6 months and were used under a protocol approved by the University of Illinois at Chicago. The experimental protocol was deemed exempt by the University of Illinois at Chicago (UIC) Institutional Review Board (Protocol # 2011-0426).

The following agents were used in this study: 5.25% sodium hypochlorite (NaOCl, James Austin Co., Mars, PA, pH 12.0), 2mg/ml berberine chloride- (Sigma-Aldrich Corp. Saint Louis, MO, purity 98%, TLC, pH 4.76) and saline- (Baxter Health Care, Deerfield, IL, pH 7.0). The pH reading of berberine was measured using the Mettler-Toledo digital bench top pH meter (Mettler-Toledo Inc., Columbus, OH).

The following groups and exposure times were assigned: Group 1: saline (60 seconds); Group 2: 2mg/ml (0.2%) berberine (60 seconds); Group 3: 5.25% NaOCl (120 seconds); Group 4: 5.25% NaOCl (120 seconds) followed by 0.2% berberine (60 seconds). In group 4, the berberine was applied immediately following the NaOCl exposure with no water rinse in between. Following irrigation in all 4 groups, the beams were rinsed with distilled water to prevent continuous contact with the irrigant. The concentration of berberine was decided to be 0.2% to be consistent with past studies using berberine as a potential endodontic irrigant (Moussa et al., 2010; Xie et al. 2011).
Power analysis was performed to determine the sample size in each group, power=0.8. It was determined a minimum of twenty-four teeth were needed for the flexural strength test and a minimum of ten teeth for the collagen degradation test. Five teeth were used for the collagen digestibility test in accordance with past studies. All teeth served as their own control. In addition, irrigation was performed on one surface of the tooth samples. Dentin disks were placed in paraffin wax to keep the irrigants from spreading to other areas.

B. **Methodology**

1. **Tooth Selection and Preparation**

Twenty-four permanent single-rooted human teeth were collected and stored in 0.1% thymol solution until use. The exclusion criteria included no root curvatures and no root fractures. External root surfaces were debrided of bone, calculus and soft tissue using a Gracey curette. The teeth were then decoronated using a water-cooled Isomet saw (Buecher Ltd, Lake Bluff, IL) and 7mm of the coronal and middle 1/3 of each root were retained. The root was placed in a mesial-distal direction and slices, 0.3 mm thickness, were made through the entire root. Dentin beams were prepared by making 7mm x 3.0mm x 0.3mm slices through each root. Control sections for each group were prepared from the same tooth as the experimental group. The beams were maintained in a hydrated state in distilled water according to teeth number.
Within one week of dentin beam preparation, the dentin disks were removed from the distilled water and divided into four groups according to the irrigant to be used.
24 extracted single-rooted human permanent teeth → Teeth decoronated and 7.0mm of coronal and middle 1/3 of root retained → Dentin beams prepared by making 7.0mm x 3.0mm x 0.3mm slices through each root

Negative control: all teeth served as their own control

Group 1: 60 second irrigation with sterile saline (NaCl)

Group 2: 60 second irrigation with 0.2% BBr

Group 3: 120 second irrigation with 5.25% NaOCl

Group 4: 120 second irrigation with 5.25% NaOCl followed by a 60 second irrigation with 0.2% BBr

Specimens (n=24) tested for flexural strength using a 3-point flexural device with a 5mm support span

Specimens (n=11) measured for collagen degradation using a collagen digestibility method

Specimens (n=5) measured for collagen denaturation using a differential scanning calorimeter

Figure 2. Flowchart of the methodology used in this study
2. Flexural Strength

An in vitro tooth model for evaluating flexural strength was performed by using dentin from the coronal and middle thirds of 24 teeth to ensure collection of an adequate amount of testable substrates and consistency in dentin tubular orientation. The dentin disks of 3.0mm thickness were prepared perpendicular to the longitudinal axis of each tooth. The 7mm x 3.0mm x 0.3mm beam was prepared from the center of each disk to ensure that dentinal tubules were oriented parallel to the plane of maximum stress during 3-point flexure. The dimensions of each beam were measured to the nearest 0.01mm. Four beams from each tooth were divided into the groups. Subsequently, one surface of the dentin samples were irrigated for 60 seconds with saline, 60 seconds of 0.2% berberine chloride, 120 seconds of 5.25% NaOCl, or a combination of 5.25% NaOCl for 120 seconds followed by 0.2% berberine chloride for 60 seconds.

Flexural strength was performed using a miniature 3-point flexure device with a 5mm support span. Each 7mm long beam was placed on top of the support span and loaded to fracture under water by using a universal testing machine at a crosshead speed of 1mm/min. Flexural strength (MPa) is calculated with the formula 3PL/2bd², where P=load of fracture (N), L=length of support span (mm), b=beam width (mm), and d=beam thickness (mm).
3. **Collagen Digestibility**

Using the same teeth used in the flexural strength part of this study, dentin beams from ten teeth were used for the collagen digestibility test. A protocol by Macedo et al., 2009, was followed with some modifications. The 7mm dentin discs were prepared to be consistent in size of 2.5mm x 2.5mm x 0.3mm. Dentin discs were treated with irrigants from the four groups. Afterwards, each root dentin disc was decalcified with 1.5ml 10% phosphoric acid for 5 hours at room temperature. Thereafter, samples were placed in a vortex shaker positioned horizontally. The insoluble residue was washed with distilled water by repeated cycles of distilled water. The samples were then placed in a vacuum overnight to dry. The following day, samples were weighed and subsequently rehydrated with distilled water for 30 minutes. Each sample was later suspended in 1.5ml of collagenase for 24 hours at 37°C, horizontally and in a shaker. Then, samples were placed in the desiccator overnight and weighed. The difference in weight was evaluated and expressed as a percent mass loss of digested matrix.

4. **Collagen denaturation**

To measure the denaturation temperature of the dentin matrix, differential scanning calorimetry (DSC) was used in a similar technique to Armstrong et al.
2006. Dentin discs measuring 0.25mm thick were cut from the coronal and middle third of the root, using an Isomet saw (Buehler Ltd., Lake Bluff, IL), from 5 teeth of the 24 used in the flexural strength part of the study. These disks were then cut into 2mm x 2mm dentin blocks using a medium diamond bur in a high-speed dental handpiece with copious air-water spray. After the blocks were cut, they were stored in distilled water.

Specimens were treated with irrigants from the four groups. The mineralized dentin blocks were weighed wet and quickly sealed in high-pressure DSC pans (PerkinElmer, Shelton, CT). All the blocks were subsequently weighed and a volume of the respective irrigant content of the matrix was acquired. Specimens sealed in high-pressure pans were placed in a DSC (Model DSC-7, Perkin-Elmer Life and Analytical Sciences, Inc., Wessleyan, MA) and scanned from 25-200°C at a rate of 10°C/min. Subsequent cooling and reheating confirmed that the collagen denaturation was irreversible. Calibration of the DSC was performed prior to use.

C. Statistical methods

Data were analyzed using Statistical Package for Social Sciences (SPSS, Chicago, IL, 2011) version 19.0. The value p<.05 was considered as a stop level for statistical significance. The flexural strength, collagen digestion, and collagen temperature denaturation were investigated. Since the data sets were found to be normally-distributed, the data were analyzed using parametric statistical methods. One-Way
Analysis of Variance (ANOVA) was used.

D. **Pilot Study**

Preceding the proposed thesis research, pilot studies were completed. From three teeth, data analysis (Tables II & III) was performed using one-way ANOVA and revealed no statistically significant differences in flexural strength and collagen degradation among groups (p>0.05). Based on this pilot study, irrigation with berberine did not alter radicular dentin strength when compared to sodium hypochlorite and saline. Also, the percent mass loss of saline was more than with berberine or sodium hypochlorite. It was determined that the small sample size was a potential contributor to the statistically insignificant findings.

**TABLE II.**

MEANS TABLE FOR FLEXURAL STRENGTH
PILOT TEST

<table>
<thead>
<tr>
<th>IRRIGANT</th>
<th>n</th>
<th>MEAN (MPa)</th>
<th>SD (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3</td>
<td>181.9</td>
<td>24.5</td>
</tr>
<tr>
<td>0.2% Berberine</td>
<td>3</td>
<td>205.7</td>
<td>27.9</td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>3</td>
<td>224.1</td>
<td>91.1</td>
</tr>
<tr>
<td>5.25% NaOCl + 0.2% Berberine</td>
<td>3</td>
<td>170.2</td>
<td>19.7</td>
</tr>
</tbody>
</table>

n= sample size; NaOCl= sodium hypochlorite; SD= standard deviation; MPa= megapascals
TABLE III.
MEANS TABLE FOR COLLAGEN BIODEGRADATION
PILOT TEST

<table>
<thead>
<tr>
<th>IRRIGANT</th>
<th>n</th>
<th>MEAN (% Mass Loss)</th>
<th>SD (% Mass Loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3</td>
<td>33.2</td>
<td>13.5</td>
</tr>
<tr>
<td>0.2% Berberine</td>
<td>3</td>
<td>28.9</td>
<td>3.4</td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>3</td>
<td>31.4</td>
<td>12.7</td>
</tr>
<tr>
<td>5.25% NaOCl + 0.2% Berberine</td>
<td>3</td>
<td>51.6</td>
<td>8.7</td>
</tr>
</tbody>
</table>

n= sample size; SD= standard deviation; NaOCl= sodium hypochlorite
IV. RESULTS

A. Experimental Study

1. Flexural Strength

The means and standard deviations of the flexural strength (Table IV) are presented. The flexural strength measurements in the four groups were compared using the One-way Analysis of Variance (ANOVA) test. There were no significant differences (p=0.142) between the four groups. The different irrigant solutions had no effect on flexural strength when compared to the control, saline.

TABLE IV.
MEANS AND STANDARD DEVIATIONS OF FLEXURAL STRENGTH FOR EACH GROUP, INCLUDING THE STATISTICAL ANALYSIS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>n</th>
<th>MEAN ± SD (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>27</td>
<td>191.3 ± 54.3</td>
</tr>
<tr>
<td>0.2% Berberine</td>
<td>27</td>
<td>200.5 ± 36.8</td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>27</td>
<td>217.7 ± 58.2</td>
</tr>
<tr>
<td>5.25% NaOCl + 0.2% Berberine</td>
<td>27</td>
<td>184.0 ± 68.1</td>
</tr>
</tbody>
</table>

n= sample size; SD= standard deviation; MPa= megapascals; NaOCl= sodium hypochlorite
Figure 3. Flexural strength values expressed in MPa (mean). Test reagents include Saline, Berberine (BBr) and Sodium hypochlorite (NaOCl).
2. **Collagen Degradation**

The means and standard deviations of the collagen degradation (digestion) test are exhibited in Table V. The analysis of variance test revealed results with no statistical significant differences among the four groups (p=0.515). The mean percentage of mass loss of berberine (44.2 ± 18.3) was similar to saline (43.3 ± 21.7) and the berberine/NaOCl combination (45.6 ± 14.6) and higher than NaOCl (34.4 ± 18.2).

**TABLE V.**

MEANS AND STANDARD DEVIATIONS OF COLLAGEN DIGESTION FOR EACH GROUP, INCLUDING THE STATISTICAL ANALYSIS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>n</th>
<th>MEAN ± SD (% MASS LOSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11</td>
<td>43.3 ± 21.7</td>
</tr>
<tr>
<td>0.2% Berberine</td>
<td>11</td>
<td>44.2 ± 18.3</td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>11</td>
<td>34.4 ± 18.2</td>
</tr>
<tr>
<td>5.25% NaOCl + 0.2% Berberine</td>
<td>11</td>
<td>45.6 ± 14.6</td>
</tr>
</tbody>
</table>

n= sample size; SD= standard deviation; NaOCl= sodium hypochlorite
Figure 4. Collagenase biodegradation values expressed in percent mass loss (mean). Test reagents include Saline, Berberine (BBr) and Sodium hypochlorite (NaOCl).
3. **Collagen Temperature Denaturation**

The results of the third and final component of the research are summarized in Tables VI. For mineralized and fully hydrated root dentin among the four groups the p value was not statistically significant (p=0.056). Using differential scanning calorimetry, the temperature denaturation (Td), of mineralized dentin exposed to saline (123.6 ± 19.1°C) was shown to be similar to berberine (122.1 ± 13.8°C) and lower than sodium hypochlorite alone (154.2 ± 27.3°C) or the berberine/NaOCl combination (145.3 ±18.4°C).

**TABLE VI.**

**MEANS AND STANDARD DEVIATIONS OF COLLAGEN DENATURATION FOR EACH GROUP, INCLUDING THE STATISTICAL ANALYSIS**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>n</th>
<th>MEAN ± SD (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5</td>
<td>123.6 ± 19.1</td>
</tr>
<tr>
<td>0.2% Berberine</td>
<td>5</td>
<td>122.1 ± 13.8</td>
</tr>
<tr>
<td>5.25% NaOCl</td>
<td>5</td>
<td>154.2 ± 27.3</td>
</tr>
<tr>
<td>5.25% NaOCl + 0.2% Berberine</td>
<td>5</td>
<td>145.3 ± 18.4</td>
</tr>
</tbody>
</table>

n= sample size; SD= standard deviation; NaOCl= sodium hypochlorite
Figure 5. Temperature denaturation values expressed in °C (Mean). Test reagents include Saline, Berberine (BBr) and Sodium hypochlorite (NaOCl).
V. DISCUSSION

A. **Use of Berberine as an Endodontic Irrigant**

The use of berberine as a natural product for endodontic irrigation is attractive due to its rich source of antimicrobial compounds. Berberine is known to possess antidiarrheal, antimicrobial, fever-reducing, anti-inflammatory, anti-proliferative, and anti-oxidative activities. This is an age of evidence-based dentistry (Zehnder, 2006) and any new concepts and techniques to be used in the clinic should be assessed in randomized clinical trials. Nonetheless, this is not always possible in endodontic research and particular attention must be given to *in vitro* studies.

Past studies demonstrated antimicrobial activity against selected oral pathogens. Berberine was shown to be more effective than saline as an endodontic irrigant against selective endodontic pathogens *in vitro*, and when combined with CHX was comparable to NaOCl in its bactericidal efficacy. The possibility for berberine to bring about radicular dentin structural changes was investigated in this study. In the present study, we did not include irrigants other than berberine and NaOCl. We felt it was important to demonstrate whether berberine, used following sodium hypochlorite, can serve to strengthen radicular dentin since NaOCl has been found *in vitro* to weaken radicular dentin.
B. *In vitro Treatment of Dentin Specimens*

The irrigation treatment we developed was unique. The dentin specimens used in this study were exposed to irrigants under investigation from one side of the dentin beams, which more closely corresponds with the situation in the root canal. In other studies (Marending, et al., 2007; Sim et al., 2001; Zhang et al., 2010) the dentin specimens were exposed to the irrigants from all four sides. In addition, the exposure time for sodium hypochlorite in those studies was longer, ranging from 24 minutes to 24 hours.

In the Marending et al. study, the dentin beams were immersed for 24 minutes in 2.5% NaOCl. The 24-minute subjection caused a significant decrease in flexural strength compared with water or EDTA-treated controls. Mineralized dentin beams in the Zhang et al. study were immersed anywhere from 10-240 minutes in 1.3% or 5.25% NaOCl and then rinsed with 17% EDTA as the final irrigant for 2 minutes. The results of the investigation revealed significant increases in the degradation of collagen with a notable decrease in flexural strength with 5.25% NaOCl as the initial irrigant for 1 hour. At the same time, no change was detected with 1.3% NaOCl as the initial irrigant for up to 4 hours. The findings indicated there was a slow, continuous degradation and/or extraction of intact collagen from the mineralized dentin by 5.25% NaOCl.

In 2001 Sim et al. conducted a study to determine the effects of sodium hypochlorite on the mechanical properties of dentin and how it contributes to the
weakening of the root-treated teeth. The effects of two concentrations (0.5% and 5.25%) of sodium hypochlorite on immersed dentin bars were evaluated. The solutions, subjected to constant agitation, were changed every ten minutes with a total immersion time of 2 hours. A significant decrease in flexural strength was found in the 5.25% NaOCl group compared to the 0.5% NaOCl group. The results from the afore-mentioned studies demonstrate that degradation of collagen is concentration-dependent and time-dependent.

In root canal irrigation, we lubricate and disinfect the canal with 5.25% sodium hypochlorite. Only the inner surface of the canal is subjected to this irrigant and for this reason irrigation to one surface of our dentin beams was chosen. If we had exposed all dentin surfaces, more collagen surface structure would be exposed and degraded and possibly show different results.

The mineral phase of dentin serves to protect the collagen matrix. Mineralized dentin that is encapsulated by hydroxyapatite is less susceptible to destructive effects of NaOCl; but when NaOCl is used for a lengthy time, the organic components will in time be deproteinized by NaOCl (Di Renzo et al., 2001). In addition, the short exposure time (1 minute) of EDTA is self-limiting (von der Fehr & Nygaard-Ostby, 1963; Machado-Silveiro et al., 2004) due to dentin’s excellent buffering capacity (Camps & Pashley, 2000). We chose not to use EDTA in the present research.

The exposure time in this research is minimal compared to what is actually applied during cleaning and shaping of endodontic therapy. In the present study we chose a short exposure time, of two minutes, for NaOCl to serve as a baseline from
which other studies can develop. Two minutes does not serve to be clinically relevant. The one-minute time selected for berberine was in conjunction with other adjunct endodontic irrigants, such as EDTA and CHX.

C. Study Findings

Within the limits of the present study, it may be concluded that the effects of the respective test agents did not possess significant effects on the mechanical properties of radicular dentin. As a result, the null hypothesis that berberine, used as an adjunct irrigating solution, would not affect the flexural strength and collagen structure when compared to a control was accepted. The current data were obtained in a controlled laboratory environment. Under the conditions of the current study, berberine and sodium hypochlorite independently and in combination did not mediate dentin collagen degradation at the exposed specimen surface.

Furthermore, no precipitate resulted from the application of berberine following NaOCl. Only a slight yellowish discoloration was seen when berberine was applied in Groups 2 and 4.

1. Flexural Strength

In this study, we analyzed the effects of berberine and sodium hypochlorite on the flexural strength of the coronal and middle one-third of radicular dentin. The
outcome of the 1-minute berberine and 2-minute NaOCl irrigation independently
and in combination, on one side of the dentin beams, did not affect the flexural
strength. It has been described that sodium hypochlorite can remove the organic
material in addition to the carbonate and magnesium ions from the dentin crystal
structure (Sakae et al., 1988). The exposure time of 5.25% NaOCl probably produced
changes in dentin deproteinization but not to the extent of affecting the flexural
strength. Prolonging the subjection time of NaOCl and/or berberine would likely
have a more recognizable effect in dentin penetration compared with the minimal
exposure time. Furthermore, the attenuated total reflection Fourier transform
infrared (ATR-FTIR) spectroscopy technique can be used to investigate the effects of
berberine on the surface chemistry of human dentin disks. It is a simple,
nondestructive, effective, and sensitive technique for measuring surface chemistry
changes.

The mechanical properties of mineralized dentin are unfavorably affected by
its prolonged proximity with 3%-5% NaOCl (Sim et al., 2001; Marending et al.,
2007). In vitro studies have demonstrated a significant decrease in flexural strength
when immersing the dentin beam into a high concentration (5.25%) of hypochlorite
solution for over 1 hour (Zhang et al., 2010; Mai et al., 2010). Clinically, milder
results would be expected when exposure time is minimal and to only one side of
the dentin beam. Longer contact time would allow the sodium hypochlorite to
penetrate deeper into intact dentin generating biochemical damage to the organic
components of dentin (Mai et al., 2010). Mai et al. tested whether the usage of
ethylenediamine tetraacetic acid as a final irrigant caused canal wall erosion only
after prolonged use of 5.25% sodium hypochlorite as the initial irrigant. Two irrigation protocols were employed. A 5.25% NaOCl solution as the initial irrigant for 10 minutes and a 17% EDTA solution as the final irrigant for 2 minutes; and a 5.25% NaOCl solution for 60 minutes followed by a 17% EDTA solution for 2 minutes. Flexural strengths of dentine beams prepared from mid-coronal dentin were evaluated using a miniature three-point bending device after they were irrigated with either protocol. The 60-minute NaOCl exposure resulted in a decrease in flexural strength of significance; whereas, the 10-minute NaOCl exposure did not result in a significant reduction in flexural strength.

In past studies, the overzealous use for testing an irrigant and the immersion of all sides of the dentin disk are not clinical relevant. One should interpret these studies that have employed this methodology with caution. For if we carry their findings to the in vivo scenario, our belief might be that root-canaled treated teeth are more prone to vertical fracture; this is not the case.

2. **Collagen Structure Degradation**

We hypothesized that berberine, used as an adjunct irrigating solution, would not affect the collagen structure when compared to a control. The collagen digestibility tests performed indicate that dentin treated with berberine was insignificantly susceptible to collagenase digestion along with the other test groups.

In our protocol, we exposed irrigants to 2mm x 2mm x 0.25mm dentin disks rather than pulverizing the dentin as in other studies (Zhang et al., Santos et al.,
2009). Pulverizing the dentin disks would increase the surface area of exposure to the irrigant. Also, our exposure time of the irrigants was shorter than others at 1 hour (Macedo et al., 2009) and 4 and 40 hours (Bedran-Russo et al., 2006). Zhang et al. (2010) pulverized dentin and exposed the powder to 1.3% or 5.25% NaOCl for 10-240 minutes followed by a rinse with 17% EDTA for 2 minutes. Dentin powders were examined by Fourier transform infrared spectroscopy to examine the collagen content with the apatite/collagen ratio. Collagen degradation was significantly increased after the use of 5.25% NaOCl for 1 hour. Bedran-Russo et al. (2006) used gluteraldehyde, a chemical crosslinker, on undemineralized and demineralized dentin beams (0.5mm x 0.5mm) treated for 4 and 40 hours. The results showed no difference in tensile strength of the gluteraldehyde-treated group compared to the control.

Matrix metalloproteinases are located in coronal and radicular dentin with an increased presence in demineralized root dentin and with age of the tooth (Santos et al., 2009). MMP-2 and -8 are mainly detected in mineralized dentin while MMP-9 protein more so in non-mineralized dentin (Santos et al., 2009). In mineralized dentin, MMP-9 is found in dentinal tubules and in the mineral-organic matrix interface requiring substantial demineralization (96 hours) for its withdrawal. MMPs are found naturally as disulfide bonds with non-collagenous proteins in dentin (Gutierrez-Fernandez et al., 2007). Dentin-bound MMPs are involved in caries progression through degradation of dentin matrix (Hannas et al., 2007). In addition, etchant adhesives during dentin bonding elicit the collagenolytic activities of MMPs (Mazzoni et al., 2006) which is concerning since adhesive
procedures could be used in endodontics for bonding root fillings. The application of 2% chlorhexidine inhibits the endogenous collagen-bound proteases (MMPs) and preserves the cohesion of the hybrid layer (resin and dentin matrix) (Carillho et al., 2007). In the present study, demineralizing agents were not used prior to irrigation and we believe this is why MMPs were not triggered. As a result, MMP-induced collagenolytic activity was suppressed and the dentin collagen extracellular matrix remained intact.

3. **Collagen Temperature Denaturation**

Interpeptide hydrogen bonding within collagen fibrils stabilizes them against thermal challenges (Armstrong et al., 2006). Prior to the Armstrong et al. study, most research analyzed temperature increases from endodontic heat sources along the external root surface (Weller et al., 1996; Lee et al., 1998; Lipski, 2005). The potential damage to the tooth-supporting structures is a concern and should be understood. Using high-speed burs with no water coolant burns mineralized dentin and turns its pale yellow color to dark brown. This may give rise to localized denaturation of the mineralized root dentin. These dehydrated, denatured regions may perhaps become more brittle (Kishen and Asundi, 2005), where cracks are instituted within endodontically-treated roots. Within the limits of this study, there was no statistical significance between the berberine (122.1±13.8°C) group compared to the NaOCl (154.2±27.3°C) and the berberine/NaOCl (145.3±18.4°C)
groups. The NaOCl and/or berberine irrigants had no effect on the thermal stability of the mineralized dentin.

D. **Relevance of Study to Clinical Practice**

Irrigation has a key role in successful endodontic treatment with no single irrigant accomplishing all the tasks required by irrigation. Since the chemical and structural composition of radicular root dentin is altered with different irrigants, an effective root canal irrigant should maintain superior radicular dentin strength following cleaning and shaping.

The methodology of the present study varied from past methodologies. The exposure time and application to one surface of the dentin beams, used in the current study, were more clinically relevant than what was used in previous studies. In this study, berberine was used at a concentration of 2mg/ml since this was used in past studies testing berberine’s antimicrobial efficacy. Sodium hypochlorite at a 5.25% concentration was utilized due to its routine use in clinical practice.

The decreased flexural strength of NaOCl-treated dentin beams is well documented in literature. However, in our study we found it to have no significant effect. Equally the effect of berberine on radicular dentin was noted. We deduced that 0.2% berberine alone and in combination with 5.25% NaOCl did not have a statistically significant influence on the mechanical properties of radicular dentin.
E. **Strengths and Weaknesses**

Weaknesses of the study could be a reduced sample size for the collagen denaturation test, the short exposure time of the sodium hypochlorite, and not applying a demineralizing agent prior to treatment of the NaOCl. Strengths of the study include all samples coming from the same group of teeth and each tooth serving as its own control. Furthermore, the present research is more clinically relevant than previous studies in exposing only one surface of the dentin beam, as in an *in vivo* scenario.

F. **Future Research Direction**

The supportive data acquired in this study have been promising and keep the door open for further research using berberine, a natural antimicrobial plant alkaloid. Future studies should aim to outline guidelines for the exposure time of endodontic irrigation to obtain a clean root canal system without creating unforeseen effects to the tooth. In addition, exposure to one surface should remain part of the protocol; for when we irrigate, the subjection is limited to one surface of the root canal system. Furthermore, validation of the advantageous characteristics of berberine, if any, needs to be performed through *in vivo* clinical trials. Finally, when this study is repeated an increased sample size will be used to further increase the power of this study. The potential for future research in irrigants is limitless.
VI. CONCLUSIONS

All test irrigants in the present study did not alter radicular dentin strength when compared to saline. The 2-minute exposure of 5.25% NaOCl and 1-minute exposure of 0.2% berberine did not cause a significant drop in flexural strength. It was evident that due to the different methodology used in this study that the results would be milder than past studies; thus the deleterious effects attributed to NaOCl are time dependent. The use of berberine and/or NaOCl did not affect the mechanical properties of demineralized dentin. The biodegradation rates of collagen were not affected by the irrigants evaluated. Also, the NaOCl and/or berberine irrigants had no effect on the thermal stability of the mineralized dentin. Due to these findings, berberine may represent an alternative and natural adjunctive endodontic irrigant. These results contradict other studies due to different methodologies, as other samples were immersed in irrigating solutions for an extended period of time, compared to our study, which more closely resembles clinical use.


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*20110426-60870-1*

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ABSTRACTS: Bahrani, Z., Fayad M., Johnson B., Wenckus C., Bedran-Russo A.: *In vitro* Effects of a Natural Plant Alkaloid versus Sodium Hypochlorite on Radicular Dentin, (AAE Annual Convention) #PR 03, April 2012