EFFECT OF ANTIBIOTIC PASTES ON CHEMICAL STRUCTURE AND MICROHARDNESS OF RADICULAR DENTIN

by

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Submitted to the Graduate Faculty of the School of Dentistry in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University School of Dentistry, June 2014.

Thesis accepted by the faculty of the Department of Endodontics, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

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ACKNOWLEDGMENTS

I would like to acknowledge Ghaeth Yassen first and foremost for his contributions to this project. His previous work laid the foundation for the present study. He was vital to this study from concept to experiment design, to tooth collections and sample treatments, to data gathering and evaluation. You were a pleasure to work with and I owe much of the credit for this project to your training and guidance.

To my family, I owe the person I am today to your unconditional and undying love. I must first thank my parents, Tom and Pam, for a lifetime of emotional, academic, and financial support. I want to thank my wife, Erin, for her tremendous support during my endodontic residency. Your daily encouragement, love, and words of wisdom are invaluable to me.

To my endodontic and dental school colleagues, you are an extension of a family to me. I would like to thank Dr. Spolnik for affording me the opportunity to practice and to learn in the Graduate Endodontic department for two years. Thank you for your genuine devotion to provide academic, clinical, and professional guidance to the residents of this program. Your wisdom and advice is a large part of what makes Indiana University one of the premier endodontic programs in the country. I take comfort in knowing that every graduate of the program has been capable, knowledgeable, and successful.

I want to thank Dr. Ehrlich for his large contribution to this work. He took much of the responsibility for reviewing and editing proposals, presentations, abstracts, and the thesis. His encouragement also helped to keep the project on track and on time.

Thank you to Dr. Platt for his supervision and mentorship. His expertise was crucial to a successful experiment design. He took the time to critically evaluate every write-up along the way to make sure the project was accurate and articulate.

Thank you to Drs. Legan and Zunt for your support, advice, and time invested in evaluating and editing the writings for this thesis as members of my research committee. I am also grateful to Drs. Vail and Bringas for their efforts and enthusiasm in both the academic and clinical training during the residence. I would also like to thank all my classmates for your support and friendship over the past few years.

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INTRODUCTION

Pulp necrosis of an immature permanent tooth can be problematic because the development of the root is terminated, leaving the roots thin and weak. Fracture and the resultant extraction of such a tooth are problematic because of the inability to replace it with a fixed prosthesis for many years until completion of the child's growth and development. This creates not only an esthetic problem, but also may allow undesirable tooth movement and loss of arch length. One traditional approach to the necrotic immature tooth has been long-term calcium hydroxide treatment to induce apexification.[1, 2] A recent systematic review, however, has shown that exposure to calcium hydroxide for five weeks or longer reduces the mechanical properties of radicular dentin.[3] This effect is especially undesirable in a tooth with a weak, undeveloped root. More recently, MTA has been used to create an artificial barrier for apexification.[4] Though apexification has been shown to be successful,[5] it is challenging to maintain the obturation material within the root canal space. Another traditional treatment for the immature necrotic tooth has been apicoectomy, but this further decreases root length.[6, 7] While both apexification and surgery may resolve a periapical infection and provide a barrier against which to obturate, further development of length and width of the root is discontinued, leaving it prone to fracture.

Several recent case studies and case reports have detailed clinical protocols that have not only healed periapical infections in immature, necrotic permanent teeth, but have also successfully induced continued root development through the regeneration of vital tissue within the canal.[8-12] After a review of available case studies, the American

Association of Endodontics released a suggested protocol for a multiple appointment regenerative endodontic procedure. On the first appointment, the canal is irrigated, followed by application of either calcium hydroxide or a low concentration of an antibiotic paste as an antibacterial medicament. On a subsequent visit in three to four weeks, anesthesia without epinephrine is obtained, the medicament is rinsed from the canal with 17-percent EDTA, and bleeding is evoked by instrumentation beyond the apex. MTA is then placed over the blood clot and the canal is sealed with a permanent coronal restoration.[13]

In traditional endodontic techniques, the mere lowering of bacterial load and the prevention of reinfection of periapical tissues is conducive to healing. However, Fouad[14] states that the canal has to be disinfected to a "higher level of efficacy than is needed in clinical endodontics." Thus, it is necessary to maintain an aseptic environment in the pulp space following disinfection procedures to allow the new tissue sufficient time to establish itself in the root canal environment. Sodium hypochlorite significantly decreases bacterial loads, but it cannot penetrate dentinal tubules and render the canal completely bacteria free.[15] A commonly used medicament to accomplish a greater level of disinfection for regeneration is a triple antibiotic paste (TAP) composed of metronidazole, ciprofloxacin, and minocycline. In a 1996 in-vitro study, Hoshino, et. al.[16] found each of these drugs alone to substantially decrease bacteria from infected root dentin, but the combination eradicated all bacteria. The same study also found that the triple antibiotic paste deeply penetrated into dentinal tubules, which provides an advantage over sodium hypochlorite irrigation alone. TAP has since been found to be effective against endodontic pathogens in vivo.[17]

While triple antibiotic paste has favorable antibacterial properties, it also has several disadvantages. Crown discoloration has been associated with the use of TAP due to the minocycline content.[18,19] To address this issue, a modified triple antibiotic paste (MTAP) that replaces minocycline with clindamycin has recently been formulated and was successfully used as intracanal medicament in endodontic regeneration.[20]

Recent in-vitro studies have shown the potentially cytotoxic effects of TAP as well as minocycline and ciprofloxacin alone.[21] TAP has a concentration dependent cytotoxicity on stem cells of the apical papilla in vitro. Concentrations that are lower than that used in the clinical setting were found to have a detrimental effect on stem cells of the apical papilla[22] and dental pulp cells.[23] The concentration of TAP that was initially used clinically was about 1g/mL.[16] Research has focused on antibiotic mixtures containing lower concentrations of antibiotics that are antimicrobial and have a minimal cytotoxic effect on dental stem cells. However, at low concentrations of antibiotics, the mixture is very dilute. A thickening agent, like methylcellulose has to be added to improve the handling properties of the mixture. This creates a paste that can be more clinically applicable in endodontics.

Another concern is the impact of regenerative endodontic procedures on a tooth's fracture resistance. In several reported case studies, the increase in root thickness after regeneration has been confined to the apical and coronal thirds, which does not increase the tooth's resistance to fracture near the CEJ.[24] Additionally, long-term exposure to antibiotic pastes may also negatively affect the root's mechanical properties through demineralization and chelation. Minocycline has been shown to chelate calcium, leading to demineralization. [25] In regenerative endodontics the antibiotic pastes are placed in the

canal for several weeks. These longer exposure times to antibiotic pastes result in significantly greater demineralization compared with shorter time periods.[26]

This study will examine the effect of two concentrations of TAP and MTAP medicaments on the microhardness and chemical structure of radicular dentin using Vickers microhardness and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). Microhardness testing measures the resistance of the dentin to deformation caused by penetration of an indenting stylus. This can give insight into the effect antibiotic medicaments have on the mechanical properties of dentin. A recent study measured microhardness of radicular dentin cylinders after treatment with triple antibiotic paste, double antibiotic paste (metronidazole and ciprofloxacin), and calcium hydroxide. Triple antibiotic paste and double antibiotic paste caused a significant and continuous decrease in the microhardness of root dentin. This decrease was seen after the paste was applied for both a one-month and three-month period.[27] Another recent in-vitro study used ATR-FTIR to measure the effect of triple antibiotic paste, double antibiotic paste, and calcium hydroxide on the chemical structure of radicular dentin and found superficial collagen degradation caused by calcium hydroxide and a demineralization caused by antibiotic pastes. Four-week antibiotic paste samples had a significantly greater demineralization compared with one- and two-week samples.[26] This study showed that ATR-FTIR can measure the amount of demineralization relative to untreated control samples. Both of these in-vitro studies used concentrations of antibiotic paste that are approximately 1 g/mL. The effects of MTAP in this concentration, and the effect of lower concentrations of TAP and MTAP on the microhardness and chemical structure of radicular dentin have yet to be determined.

The aim of this study was to investigate the effects of two intracanal medicaments used during pulp regeneration on the microhardness and chemical structure of radicular dentin. These medicaments are triple antibiotic paste (TAP), and modified triple antibiotic paste (MTAP), at concentrations of 1 g/mL and 1 mg/mL.

CLINICAL SIGNIFICANCE

Antibiotic pastes are used for their antibacterial properties. However, they can also affect the biomechanical properties of dentin. It is of clinical interest to find the optimal concentration and optimal treatment time that maximizes the disinfection of the canal without compromising the properties of the remaining dentin. The demineralization properties of the antibiotic pastes expose collagen fibrils, and this may enhance the attachment of dental pulp stem cells during endodontic regenerative procedures.

HYPOTHESES

1) Null: TAP and MTAP have no significant effect at either 1 g/mL or 1 mg/mL concentrations on the microhardness of radicular dentin. Alternative: TAP and MTAP at concentrations of 1 g/mL or 1 mg/mL significantly decrease the microhardness of radicular dentin.
2) Null: TAP and MTAP have no significant effect at either 1 g/mL or 1 mg/mL concentrations on the chemical structure of radicular dentin measured by ATR-FTIR. Alternative: TAP and MTAP at concentrations of 1 g/mL or 1 mg/mL have a significant demineralization effect on the chemical structure of radicular dentin measured by ATR FTIR.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

In just over three centuries, the practice of dentistry has made remarkable advances, and currently has new developments on almost a daily basis. Historically, dental pain has been treated by extraction of the offending tooth. Dating back to 1687, Charles Allen penned the first book in the English language that was entirely devoted to dentistry. In its pages, he writes of a different option for treating problematic teeth by a type of transplantation that involves "taking out the rotten teeth or stumps and putting in their places some sound ones drawn immediately out of some poor body's head." [28]

Later, other methods were explored to find relief from pulpal and periapical disease while maintaining the patient's natural tooth. Many of these ideas were introduced by Pierre Fauchard, who is regarded as "the father of modern dentistry."[28] In his 1728 book The Surgeon Dentist, Fauchard described dental procedures that cultivated several concepts of modern endodontics. One such procedure was the relief of dental abscesses by creating an access hole into the pulp chamber and leaving it open for several months to drain. The chamber and canals were then sealed with lead foil, making Fauchard's endodontic procedure the first to include obturation of the canals.[28,29] Fauchard details a pulpectomy procedure in which a small pin was used to extirpate the pulp. He also describes treatment of deep carious lesions with the application of cloves or cinnamon, the beginnings of pulp capping.[28,30]

Twenty-eight years later in Germany, Phillip Pfaff expanded on this idea of pulp capping. In an attempt to protect the vitality of an exposed pulp, he cut a concave piece

of gold or lead to fit the opening over the pulp.[28] Other attempts at maintaining pulp vitality through pulp capping were also made during the 18th century. Codman was the first to define the objective of pulp capping. He identified secondary dentin obtained at the site of pulp exposure.[31]

The first endodontic procedures in the US are credited to Robert Woofendale in the middle of the 18th century. To alleviate pulpal pain, he cauterized the pulp with a hot instrument and placed cotton pellets into the canals.[32] Toward the end of the century, Frederick Hirsch began to elucidate the effects of periapical disease by percussion testing. He noted that diseased teeth responsible for dental pain were often painful to tapping. His treatment recommendation involved inserting a hot probe into the pulp, similar to the treatment protocol of Woofendale.[30]

The 19th century marked the emergence of "The Vitalistic Era," in which the dental community began to realize the importance of, and the subsequent effects of various treatments on pulp vitality.[28] Charles Bew described pulpal circulation, with blood flow into the pulp through the apical foramen and leaving through the periodontal membrane.[33] Leonard Koecker wrote Principles of Dental Surgery in 1826. In this book, he claimed that removal of the pulp would cause the entire tooth to die, rendering it a foreign body.[33] In order to avoid the subsequent necessity of removing this foreign body, Koecker promoted a pulp capping procedure similar to that of Pfaff.[31,33]

A few years later in 1829, SS Fitch described the "vitalistic" theory in his book System of Dental Surgery.[28] He believed that the entire tooth was vital, similar to a hollow bone. The pulpal circulation supplied the crown of the tooth, while the root was supplied by circulation from both the pulp and periodontal ligament. This led to the

practice of decoronation after pulp extirpation because its blood supply had been removed. The root was left in its socket to be restored by a crown. In contrast, those advocating a "nonvitalistic" concept believed the enamel and dentin to be, as the term nonvital implies, devoid of circulation, sensibility, and self-repair capabilities. Removal of the pulp would not, therefore, affect the remainder of the tooth. [33]

The decades to follow would bring about the use of various new medicaments during endodontic treatment. In 1836 New York, Shearjashub Spooner used arsenic trioxide to devitalize the pulp prior to pulpectomy.[34] This approach, which had been used in ancient Chinese medicine, soon became very popular because of its high success rate at minimizing or eliminating the pain associated with a vital pulpectomy. This treatment remained in practice until the 1920s.[35] John P. Buckley introduced formocresol as a means of pulp tissue fixation,[36] a concept that was used routinely for over 50 years, and to some extent remains in practice today. Jacob and Joseph Linderer advocated the use of essential or narcotic oil over pulp exposures.[37]

During the 19th century, various methods for filling and sealing the root canals were developed. In 1809, Edward Hudson, an Irish clinician practicing in Philadelphia, designed instruments to pack gold foil into root canals.[34] Baker expanded on this idea by describing removal of the nerve, cleaning the canal, and filling the canal with gold foil in the 1839 American Journal of Dental Science. He is credited with the first description of pulpal extirpation, canal cleaning, and root canal filling.[28] In the 1850s, a common method of obturation was to fill the canals with plugs of wood that had been impregnated with beechwood creosote.[29]

Finally in 1867, Dr. G. A. Bowman introduced the modern root-filling material of choice, gutta-percha.[29] Clarke Dubuque would also introduce an obturation method using hot gutta-percha.[28] In the closing years of the 19th century, Dr. Bowman developed a new method of obturation with gutta percha that would gain popularity for several following decades. He introduced chloropercha, a combination of gutta percha and chloroform. This solution was used with gutta percha cones for better adaptation and more complete three-dimensional obturation of the canals.[34]

Basic endodontic instruments still used today were invented in the 1800s, including endodontic files and the rubber dam. The first instrument to remove pulp from the canal was made by Edwin Maynard. He filed a watch spring to form a fine, four-sided broach for pulp extirpation. Sanford Barnum of Monticello, NY, introduced the rubber dam for the purpose of isolation during placement of gold foil restorations.[29] This invention would later find its place as a necessary component of modern endodontics because of its ability to create a saliva-free, aseptic environment.

This aseptic environment would become a necessary aspect of endodontic treatment as our understanding of microorganisms' role in pulp and periapical pathoses developed. Dr. G. O. Rodgers suggested, in his 1878 Dental Cosmos article, that pathogenic organisms might be the most common cause of pulpal pathoses. Successful treatment, therefore, required complete destruction and elimination of these microorganisms.[38] Arthur Underwood expanded on this theory in 1882, suggesting the use of antiseptic agents in the pulp space to eliminate pathogens, and to treat suppuration of the pulp.[28]

Groundbreaking advances in dentistry, and specifically in endodontics, came at the turn of the 20th century. With the development of procaine (Novocaine) in 1905, dentists had a much-needed alternative to the previously used anesthetic agent, cocaine. Block anesthesia techniques would be developed over the following decades for more effective local anesthesia.[35,39] One of the most important medical advancements of the time would have a significant role in endodontic therapy, the dental x-ray unit. Introduced in 1913, the dental x-ray unit became commercially available in 1919 after the advent of the Coolidge tube, which created a more focused x-ray beam.[40] Our understanding of the endodontic disease process developed as periapical radiolucencies could now be visualized as a manifestation of pulpal and periapical disease.[41] Radiographs also allowed for more accurate root length determination for cleaning and obturation purposes.[36] This would enhance our understanding of root canal anatomy, allowing for more precise cleaning and shaping, and more accurate sealing of the root canal space.

Also during the turn of the 20th century, however, the practice of endodontics fell under scrutiny due to the percolation of ideas that would become known as the "Focal Theory of Infection." This theory postulates that various diseases can be caused or exacerbated by the dissemination of microorganisms or toxic products that arise endogenously from a focus of infection.[42] This theory became particularly popular after a lecture by British physician and pathologist William Hunter at McGill University in Montreal entitled, "The Role of Sepsis and Antisepsis in Medicine," was published in the Lancet in 1911.[40,42] In this lecture, Hunter stated that "gold fillings, gold caps, gold bridges, gold crowns, fixed dentures, built in, on, and around diseased teeth, form a

veritable mausoleum of gold over a mass of sepsis to which there is no parallel in the whole realm of medicine or surgery."[43] In the wake of this movement, many physicians began to recommend extraction of all endodontically treated or non-vital teeth, while others even prescribed removal of all teeth for prevention or treatment of systemic diseases.[42] In the 1930s and 1940s, it was shown that there was not a clear cause-and-effect relation between dental infection and systemic disease. The theory of focal infection began to lose ground in the medical and dental community during what is referred to by some as "the scientific era."[40]

In the 1920s and 1930s, Hermann began the use of calcium hydroxide as medicament for pulp capping, pulpotomies, and as an intracanal medicament. He deduced that toxic materials from medicaments would find their way through the tooth and into surrounding oral tissues. Calcium hydroxide thus provided a more biocompatible alternative to the more caustic medicaments that had been used previously. He also demonstrated dentinal bridge formation in response to calcium hydroxide.[40]

U.G. Rickert developed the first obturation cement. The gutta percha cone was coated with sealer and inserted into the canal. This technique was soon improved by the invention of instruments to laterally condense the gutta percha points. In the same year, Lentulo introduced a rotary paste inserter that could be used for insertion of sealer, as well as calcium hydroxide.[40]

In the 1940s Adams and Grossman introduced antibiotic use as an adjunct to root canal therapy and were the first to use penicillin in pulp canal therapy.[40] Grossman recommended that a non-aqueous preparation of penicillin for intracanal use would be more stable. He later used impregnated paper points in the canal to sterilize the canal

space.[34] This generated interest in the chemotherapeutic treatment of root canals, not just the mechanical preparation. Antibiotic therapy, however, cannot completely sterilize the canal space. This idea of chemotherapeutic treatment was later combined with the concepts of mechanical preparation, to create the idea of chemo-mechanical preparation.[44] These ideas form the foundation of the shaping and cleaning goals of modern endodontic therapy.

In 1943, organized endodontics came into existence with the foundation of the American Association of Endodontists (AAE) in Chicago, Illinois. The AAE began exploring the idea of establishing a specialty board in 1949, and as a result the American Board of Endodontics (ABE) was formed in 1956.[45] Through the hard work of its members and leadership and growing numbers of board certified specialists, the American Dental Association recognized endodontics as a specialty in 1963. Two years later, 1965 marked the first examination and certification of Diplomates.[40]

THEORY OF ENDODONTICS

In 1965, Kakehashi, Stanley, and Fitzgerald conducted a landmark study that forms the basis for all current endodontic theories and practices. The experiment showed that pulps of germ-free rats, when exposed and left open, remained vital. Despite trauma from food impaction and exposure to the oral cavity, the pulp did not necrose and periapical tissues remained healthy. Rats under identical conditions that were not germ-free showed pulp necrosis and periapical pathosis.[46] These results illuminated the role that microorganisms play in endodontic infections.

The goal of treatment for both primary and secondary endodontic infections is to achieve maximal reduction in microorganisms and their toxins, eliminating microbial

insult to the pulpal and periapical tissues.[47,48] This is achieved through cleaning and shaping of the root canal system.[47,48] Bacteria and their byproducts within the canal space can exit to the periodontium through the apex and lateral, or accessory canals, causing a periradicular inflammatory response.[49] Failure to achieve sufficient reduction in microorganisms and their toxins could result in periradicular periodontitis, which is defined as inflammation and destruction of the periodontium that may or may not produce symptoms.[50] Endodontic success is therefore directly correlated to the reduction of pathogenic microorganisms.[46,51]

In 1955, Stewart emphasized three major phases of endodontic treatment: chemomechanical preparation, microbial control, and obturation of the root canal.[52] Of the three phases, chemomechanical preparation was identified as the most important. Grossman later confirmed that chemomechanical preparation effectively eliminates bacteria and debris from the root canal system. Grossman also identified the following 13 principles of effective root canal therapy:

1. Aseptic technique.
2. Instruments should remain within the root canal.
3. Instruments should never be forced apically.
4. Canal space must be enlarged from its original size.
5. Root canal system should be continuously irrigated with an antiseptic.
6. Solutions should remain within the canal space.
7. Fistulas do not require special treatment.
8. A negative culture should be obtained before obturation of the root canal.
9. A hermetic seal of the root canal system should be obtained.

10. Obturation material should not be irritating to the periapical tissues.

11. If an acute alveolar abscess is present, proper drainage must be established.

12. Injections into infectious areas should be avoided.

13. Apical surgery may be required to promote healing of the pulpless tooth.

Schilder discussed in 1967 the ultimate objective of endodontic therapy, which is the removal of diseased tissue and contents of the infection and inflammation of periapical tissues. He stressed the importance of chemomechanical preparation with instruments and antiseptics, along with three-dimensional obturation to the cementodentinal junction, which exists approximately 0.5 mm to 1 mm from the radiographic apex.[53] Ford expanded on Schilder's concepts, citing three reasons for a three-dimensional obturation. First, it leaves less space for bacterial colonization. Second, a three-dimensional obturation prevents apical contamination. Lastly, it prevents movement of bacteria along the walls of the canal. Along with these obturation concepts, Ford stressed the necessity for aseptic technique. This includes the use of rubber dams, adequate coronal restoration, and appropriate recall of endodontically treated teeth.[54] Proper debridement of the canal to reduce bacterial load; three-dimensional obturation to seal the root canal system; adequate coronal restoration to protect the tooth and prevent recontamination through coronal leakage; and appropriate maintenance are all important factors for successful endodontic therapy.

APEXOGENESIS

The goal of apexogenesis is to maintain vital pulp tissue in the root canal system to allow for continued root development.[55] This treatment is reserved for vital teeth with open apices. Indications for apexogenesis include pulpitis from carious or traumatic pulp

exposures in immature permanent teeth. The clinical procedure consists of a shallow or full pulpotomy to remove inflamed pulp tissue, followed by a pulp dressing and restoration.[56] The size of exposure and length of time before treatment determine the depth of pulpotomy. The traditional pulp dressing has been calcium hydroxide. Disadvantages of calcium hydroxide, however, are an incomplete, irregular reparative dentin layer and pulpal inflammation. MTA has shown superior pulp-capping abilities to calcium hydroxide because it forms a complete reparative dentin layer and does not induce pulpal inflammation.[57] The disadvantages of MTA are an increased cost, long setting time, and tooth discoloration.[58] After placement of a pulp dressing, the access preparation is restored with a permanent restoration, usually resin composite, to maintain a coronal seal. The patient is recalled and monitored for symptoms and for radiographic root development. Apexogenesis is currently the preferred treatment for teeth with a vital pulp and open apices that need pulp therapy due to a traumatic or carious pulp exposure. If the pulp becomes necrotic and the root does not continue to develop, further treatment such as apexification or regenerative endodontic procedures must be rendered.

APEXIFICATION

The traditional approach to apexification, introduced in the 1960s, is a procedure that aims to create a calcified barrier at the apex of a non-vital permanent tooth with an open apex through long-term treatment with calcium hydroxide.[59] The purpose of this barrier is to form a matrix against which to condense obturation materials without extruding them into the periapical tissues. The clinical procedure consists of accessing the tooth under rubber dam isolation and radiographic working length determination. Disinfection is achieved through mostly irrigation with minimal mechanical

instrumentation so as to not further weaken the already thin dentin root walls. Calcium hydroxide is then placed in the canal for several months. The patient is usually recalled at three-month intervals to determine when an apical barrier has formed. Treatment times usually span 9 months to 24 months. When an adequate apical barrier has developed, the canal is obturated with MTA and/or gutta percha and a permanent coronal restoration is placed to prevent coronal leakage.[55]

The traditional apexification procedure results in closure of the root apex at best, and does not induce development of root wall length or thickness. The calcified material at the apical foramen has been identified as osteoid or cementoid material, and has minute communications with the periapical tissues.[59,60] Another disadvantage of this protocol is the need for strict patient compliance over long periods of time. Finally, there is a risk of cervical root fracture due to weakening of the tooth following long-term calcium hydroxide application.[3, 61-63] Cvek reported the frequency of root fracture is most dependent on the stage of root development, reaching as high as 77 percent in the most immature teeth.[64]

More recently, an artificial apical barrier technique has been reported. The canal disinfection is the same procedure as described for the traditional apexification technique. Calcium hydroxide is then placed in the canal for one week to raise the acidic pH of the periapical tissues. When the tooth is asymptomatic, the calcium hydroxide is rinsed from the canal. The canal is then dried and then an apical plug of 4-mm to 5-mm thickness is condensed to the apex prior to coronal restoration.[65]

The artificial apical barrier technique provides a significant reduction in treatment time. Another advantage is the ability to permanently restore the tooth sooner, reducing

the chances of coronal leakage or cervical root fracture. Finally, this treatment avoids the detrimental effects of long-term calcium hydroxide treatment on the mechanical properties of the radicular dentin. Success rates for this procedure have been reported from 85 percent to 93.5 percent.[66,67] This technique still does not, however, allow for increased root length or thickness, leaving the tooth with thinner dentin walls and a reduced crown-to-root ratio as compared with a fully matured tooth root.

HISTORY OF REGENERATIVE PROCEDURES

In 1961 Nygaard-Østby tested whether the presence of a blood clot within a root canal promotes healing. Seventeen patients with either vital or necrotic pulps received root canal therapy followed by foraminal enlargement, medicament dressing for the necrotic teeth, evoked intracanal bleeding, and a kloroperka obturation placed coronal to the formed blood clot. After a period between 17 days and 3.5 years, the teeth were extracted and examined histologically. All teeth showed resolution of symptoms of inflammation related to foraminal enlargement, resolution of signs and symptoms of pathosis for the necrotic cases, and in some cases, radiographic evidence of apical closure.[68] This foundational study was promising because an in-growth of connective tissue was seen histologically. This tissue, however, was not identical to pulp tissue. It included undesirable cells, like cementoblasts, and lacked certain desirable cells, such as odontoblasts.[69]

In 1966, a study was published in which the investigators instrumented short of remaining vital tissue in the canals of immature teeth, followed by disinfection with interappointment medication of polyantibiotic mixes. All reported cases showed resolution of signs and symptoms of disease and continued root development.[70] This was

the first reported case to use polyantibiotic pastes to disinfect necrotic teeth and to promote root development.[69]

Recently, numerous case reports showing successful continuation of root development have regenerated interest in the use of polyantibiotic pastes to treat necrotic teeth with immature apices. Iwaya published the first contemporary regenerative endodontic procedure, using a double antibiotic paste (DAP) composed of ciprofloxacin and metronidazole to disinfect an immature necrotic tooth.[11] Treatment over six visits of chemical debridement with 5-percent sodium hypochlorite and 3-percent hydrogen peroxide and the placement of DAP as an intracanal medicament resulted in continued root development and a positive vitality test after 30 months. A case report detailing successful treatment of a necrotic immature mandibular premolar using triple antibiotic paste (TAP), composed of ciprofloxacin, minocycline, and metronidazole, was published by Banchs and Trope three years later.[10] The root canal system was chemically debrided with 5.25% sodium hypochlorite (NaOCl 5.25%) without instrumentation, followed by an interappointment TAP medication for 28 days. When the antibiotic mix was removed with saline irrigation, intracanal bleeding was evoked and a coronal restoration was placed over the blood clot. Again, the periapical radiolucency resolved, the root development continued, and the tooth tested positive to vitality tests. Key features common to all successful early case reports were a large open apex, young age of the patient, minimal instrumentation, sodium hypochlorite irrigation, calcium hydroxide or TAP interappointment medication, and the formation of a blood clot as a scaffold.[71] These case reports formed the foundation of currently recommended protocols and goals for regenerative endodontic procedures.

The goal of regenerative endodontic procedures is to replace damaged structures, including dentin, root structures, and the pulp-dentin complex.[72] For the immature tooth with pulp necrosis, this would indicate restoration of pulp function and completion of root development.[71] Regenerated pulp tissue should ideally have the following properties: vascularity, innervation, similar cell density, and architecture of the extracellular matrix to those of natural pulp, and the capacity to give rise to new odontoblasts lining against the existing dentin surface and produce new dentin.[73] To accomplish this goal, the canal must first be disinfected in order to provide an environment conducive to host tissue growth.[14] After a favorable environment has been provided, three key components are necessary for tissue engineering: stem cells, a scaffold, and growth factors.[74]

DISINFECTION

The etiologic agent of apical periodontitis is an intraradicular infection of the necrotic pulp space. It is a mixed community of mainly anaerobic gram-negative and gram-positive bacteria. The common gram-negative bacteria are Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Campylobacter and Veillonella. Gram-positive bacteria frequently identified are Parvimonas, Fillifactor, Pseudoramibacter, Olsenella, Actinomyces, Peptostreptococcus, Streptococcus, Propionibacterium and Eubacterium.[75] These bacteria colonize the root canal as biofilm communities that adhere to the dentinal walls and inside dentinal tubules.[76] Dentinal tubule infection occurs in 70 percent to 80 percent of teeth with apical periodontitis.[77]

The microflora changes during different stages of an endodontic infection due to changes in environmental conditions, such as oxygen and nutrient availability.[78] The

initial infection involves mainly facultative bacteria. After a few days to weeks, oxygen becomes limited due to metabolism of initial bacteria and reduced pulpal blood flow, creating an environment in which anaerobic bacteria dominate. This anaerobic flora is usually what endodontic treatment aims to eliminate.

The primary goal for treatment of a necrotic tooth with an open apex is the resolution of symptoms and evidence of bony healing. This is accomplished by reducing the microbial load with various antimicrobial medicaments. The most common interappointment medicament used in clinical case reports is TAP.[69] In 1996 the combination of ciprofloxacin, metronidazole, and minocycline was shown to be effective against bacteria found in infected root canals in vitro and in vivo.[16,79]

The minocycline in TAP has been shown to stain dentin.[18] To avoid this unfavorable esthetic result, recent studies suggested substituting the minocycline with another antibiotic, such as amoxicillin, cefaclor, or clindamycin.[69,80] Clindamycin has been found to be effective against various endodontic pathogens.[81,82] A modified triple antibiotic paste (MTAP) composed of metronidazole, ciprofloxacin and clindamycin was successfully used as an intracanal medicament to disinfect necrotic immature teeth during endodontic regeneration procedures.[80]

A pasty consistency of antibiotic medicaments that occurs at about 1 g/mL is commonly used in endodontic regeneration because it is easily applied into the canal.[22] This high concentration has been found to have detrimental direct and indirect effects on human stem cells of the apical papilla[22, 83] and human dental pulp cells. [84] Therefore, lower concentrations of these antibiotic medicaments ranging from 0.1 mg/mL to 2 mg/mL have recently been suggested to overcome their negative cytotoxic effects.[22,83, 84] The clinically used concentration of various antibiotic medicaments was also found to

negatively affect the mechanical[27] and chemical properties[26] of radicular dentin in vitro. Additionally, tooth fractures extending to the cemento-enamel junction was reported in two recently published cases treated with endodontic regeneration.[85,86] Although a direct causality for these results cannot be proven, when in-vitro findings are considered, it is possible that high concentrations of antibiotic pastes contributed to weakening of the dentin.

Disinfection is also accomplished with irrigation solutions. While mechanical instrumentation alone eliminates large numbers of bacteria, sodium hypochlorite has a greater effect than saline alone on intracanal microorganisms.[87] Gram-negative anaerobic rods isolated from primary apical periodontitis were killed within 15 seconds of exposure to sodium hypochlorite.[88] This experiment, however, used sodium hypochlorite in direct contact with microorganisms. Conversely, a systematic review by Estrela et al. concluded that NaOCl and chlorhexidine are not effective in completely eliminating E. faecalis within the complex anatomy of the root canal system. Regardless, sodium hypochlorite is an important endodontic irrigation solution due to its dual action as both a potent antimicrobial agent and an organic tissue solvent.

Higher concentrations of sodium hypochlorite enhance the antimicrobial and tissue dissolution properties.[89] In addition to concentration, its tissue-dissolving and antimicrobial properties are related to the duration of exposure and temperature.[90] The bactericidal rate for sodium hypochlorite is doubled for every 5- °C increase in temperature.[91] Baumgartner has found that full strength (6-percent) sodium hypochlorite is safe and efficacious for nonsurgical endodontic therapy.[92] Regenerative endodontic procedures, however, have different considerations than routine nonsurgical endodontics

on teeth with closed apices. First, the risk of extrusion of sodium hypochlorite into periapical tissues is greater in a tooth with an open apex. It is helpful to use a negative pressure irrigation system to avoid a sodium hypochlorite accident. Additionally, the toxicity to stem cells must be considered. Essner[93] found a concentration-dependent effect on dental pulp cells by demonstrating less cytotoxicity with the lowest concentrations of sodium hypochlorite. Sodium hypochlorite also effects radicular dentin, reducing its capacity to induce SCAP differentiation into an odontoblastic phenotype.[94] For these reasons, the American Association of Endodontics has recommended 1.5-percent sodium hypochlorite irrigation for regenerative endodontic procedures.[13]

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent used to remove the inorganic portion of the smear layer.[95] Smear layer consists of debris generated by mechanical instrumentation combined with remnant bacteria and their products. This debris occludes dentinal tubules and is pushed into fins and isthmuses. A systematic review by Shahravan[96] concluded that smear layer removal improves the fluid-tight seal of the root canal system. A one-minute rinse is recommended to remove the smear layer without excessive dentin demineralization.[55]

In regenerative endodontics, EDTA serves additional functions. A study by Trevino[98] investigated cell survival after irrigation with combinations of EDTA, 6-percent sodium hypochlorite, and 2-percent chlorhexidine. The results showed significantly greater cell survival in the group that was irrigated with solely 17-percent EDTA. The group with the second highest cell survival percentage was irrigated with 6-percent sodium hypochlorite, followed by 17-percent EDTA, then 6-percent sodium

hypochlorite. No viable cells were found in any of the samples rinsed with 2-percent chlorhexidine. For this reason, chlorhexidine is not recommended for regenerative endodontic procedures. Yamauchi [97] found that EDTA treatment increased adherence of the newly formed mineralized tissue to the root canal walls. EDTA is believed to promote SCAP survival in regenerative endodontic procedures by releasing dentinal growth factors that were imbedded into dentin during dentinogenesis.[72]

STEM CELLS

Adult stem cells can divide into another cell like itself, or a cell more differentiated than itself. This is defined as a multipotent stem cell, in contrast with a pluripotent embryonic stem cell that can develop into virtually any human cell. Autologous stem cells are from the person in which the cells will be used. Allogeneic stem cells are harvested from a donor of the same species. Xenogeneic stem cells originate from another species. All types are potential sources of stem cells in dental pulp tissue engineering.

Lovelace found evidence to support that when bleeding is evoked into a root canal, stem cells are delivered locally, not from distal sites.[97] Such local sources of stem cells that have the potential to develop into various pulpal cells include dental pulp stem cells (DPSCs),[98] stem cells from human exfoliated deciduous teeth (SHEDs),[99] periodontal ligament stem cells (PDLSCs),[100] dental follicle progenitor stem cells (DFPCs),[101] and stem cells from apical papilla (SCAPs).[102,103] Dental pulp stem cells can be found in the cell-rich zone of the pulp, near the odontoblastic layer.[55] These cells show promise for use in regenerative endodontic procedures because it has been demonstrated that DPSCs can differentiate into odontoblasts after carious pulp exposure

by responding to growth factors released from the demineralized dentin.[104] SCAP cells also have the potential to regenerate a functional pulp tissue. Thought to be derived from neural crest cells, SCAPs have been shown to produce dentin in vivo.[103, 105]

SCAFFOLD

The function of a scaffold is to provide an environment for cell growth, differentiation, and angiogenesis, similar to the extracellular matrix.[72] Nevins first introduced the use of a collagen gel scaffold in regenerative endodontic procedures in 1976.[106] Thibodeau[107] showed that the blood clot plays an important role in revascularization. The study compared revascularization in dogs using only a blood clot, only a collagen scaffold, and a collagen scaffold with a blood clot. It found that both groups using a blood clot had higher success rates than a collagen scaffold alone. Consequently, it appears that not only a physical scaffold is needed, but its contents are important.

Hutmacher[108] identified the following six properties of an ideal scaffold for tissue regeneration:

1. Porous structure for tissue and vascular integration.
2. Biodegradable at a rate of tissue formation.
3. Allow cellular attachment for differentiation and proliferation.
4. The mechanical properties of the site being implanted must be adequate.
5. Does not elicit any adverse reactions.
6. Easily formed into different sizes and shapes.

Traditionally, a blood clot induced from lacerating the periapical tissues has served as the scaffold for regenerative endodontic procedures,[72] but other scaffolds are

under investigation. Numerous recent case reports have used a platelet-rich plasma scaffold in an attempt to incorporate concentrated growth factors in the scaffold.[9,109,110]

GROWTH FACTORS

Growth factors have a profound impact on the composition of regenerated tissue. The same population of stem cells can differentiate into odontoblasts, chondrocytes, or adipocytes, just by changing the combination of growth factors to which they are exposed.[111] Many of these important signaling molecules are entombed in the mineralized matrix of dentin.[112] A more favorable environment for the regeneration of pulpal tissue may be created if these growth factors are released from the dentin at the appropriate time during regenerative endodontic procedures. For example, the application of EDTA to human dentin releases transforming growth factor beta (TGF-B).[113] TGF-B signals odontoblast differentiation, induces mineralization of pulp tissue, exerts anti-inflammatory effects, and promotes wound healing. Bone morphogenic protein (BMP), and vascular endothelial growth factor (VEGF) have also been identified as important to pulpal regeneration. BMP induces differentiation of odontoblasts, which is critical to the dentinogenesis involved in continued root development. VEGF regulates angiogenesis by inducing chemotaxis, proliferation, and differentiation of cells for vascular ingrowth and dental pulp cells.[112]

In addition to those already present in the dentin, other growth factors may prove useful in regenerative endodontic procedures. Patients taking long-term corticosteroids have been reported to have a reduction in the radiographic size of the pulp chamber.[114] Dexamethasone was later shown to increase the differentiation of human dental pulp cells into odontoblast-like cells.[115] This effect is further increased when dexamethasone is

combined with 1,25-dihydroxyvitamin D_3.[111] Additionally, simvastatin has shown potential in vitro and in vivo to promote differentiation of dental pulp stem cells into odontoblast-like cells.[116] While these growth factors are known to positively affect pulpal regeneration, there is much to learn about the interactions of these factors and the ideal concentrations for pulp tissue regeneration.

CLINICAL DECISION-MAKING

When deciding how to treat a necrotic tooth with an open apex, the clinician must consider the clinical outcomes of apexification and revascularization. Success rates ranging from 74 percent to 100 percent for long-term calcium hydroxide apexification and over 90 percent for apexification with an artificial MTA barrier have been reported.[2,66,117] A recent retrospective study by Jeeruphan, et al. compared outcomes of 22 calcium hydroxide apexification cases, 19 MTA apexification cases, and 20 revascularization cases.[118] It reported the following survival rates: revascularization-treated teeth (100 percent), MTA apexification treated teeth (95 percent), and calcium hydroxide apexification (77.2 percent). The revascularization group had a significantly greater increase in both root length and thickness compared with the other two groups. This is an important distinction because even if a periapical lesion heals, and if an apical barrier is successfully formed with apexification, there is still an increased risk of fracture due to thinner root walls. Additionally, long-term use of calcium hydroxide has been connected to increased risk of fracture.[3, 61] Twenty-three percent of the teeth treated with calcium hydroxide in the Jeeruphan study above failed because of root fractures.[118] Composite resin has been used to clinically strengthen the roots of immature necrotic teeth, but this eliminates the option for retreatment.[119]

The quality of evidence supporting regenerative endodontic procedures has been questioned. To date, there are no randomized clinical trials on the subject. A majority of the clinical publications on regenerative endodontic procedures are case reports. These reports lack the use of a standardized treatment protocol and include differences in disease etiology, debridement regimen, number of visits, and intracanal medicament.[69] A recent study criticizes the available evidence because most case reports use non-standardized images with foreshortening or elongation.[120] This study shows that even slight changes in angulation between preoperative and recall reviews can produce inconsistent images and inaccurate interpretations. Furthermore, the authors state that the available case reports may skew the success rates by reporting only successful cases. The modern clinician faces the problem of making treatment decisions based on evidence that is inconsistent. To address this issue, a recent review of the available literature on revascularization makes the following statement:

> Although the outcome of revascularization procedures remains somewhat unpredictable and the clinical management of these teeth is challenging, when successful, they are an improvement to treatment protocols that leave the roots short and the walls of the root canal thin and prone to fracture. They also leave the door open to other methods of treatment in addition to extraction, when they fail to achieve the desired result.[121]

VICKERS MICROHARDNESS TESTING

Hardness can be defined as a material's resistance to permanent surface indentation or penetration. Vickers microhardness uses a square-based diamond pyramid probe to make a diamond-shaped indentation on the surface of a material. The Vickers hardness number (HV) is then calculated based on the load divided by the surface area of

the indentation. Therefore, a larger HV corresponds to a harder surface. For an accurate measurement, the surface must be flat, polished, and free of defects.[122]

A common technique in dental research, microhardness testing measures the resistance of dentin to plastic deformation caused by penetration of an indenting stylus. Numerous studies have used microhardness to quantify a medicament's effect on the physical properties of dentin.[123-125] Hardness has been reported to relate to the mineral content of tooth structure.[126] Arends and Bosch[127] assessed various in-vitro techniques to measure the demineralization or remineralization of enamel or dentin, and determined that microhardness is a practical method of indirect quantitative mineral loss or gain determination. Although microhardness is not the best predictor of root fracture,[3] it can still give insight about dentin's reaction with a medicament.

Recently, microhardness tests have successfully measured the relative effects of various intracanal medicaments, including some that are commonly used in regenerative endodontics, on radicular dentin.[27] It is important to note that microhardness has been traditionally used to evaluate homogenous materials, like metals, and that less homogenous materials like dentin can lead to deviations. Pashley[128] found an inverse correlation between dentinal tubule density and microhardness. The tubule density decreases from cervical to apical dentin, and at greater distances from the pulp. Consequently, dentin microhardness decreases closer to the pulp and farther from the apex. For this reason, it was important in the design of the present study to standardize both the cross-section of the root that was measured and the distance from the pulp-dentin interface. In order to minimize the effect of variations between teeth as well as variations in the dentin between different spots on the same tooth, pretreatment and post-treatment

measurements were carefully taken adjacent to each other. Each tooth could then serve as its own control, and a percent change in microhardness could be calculated.

ATTENUATED TOTAL REFLECTION FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR)

FTIR spectra have been used in multiple previous studies to obtain information about the chemical composition of dentin.[129-132] The largest inorganic peak is created by phosphate, which absorbs infrared radiation at around 1000 cm^{-1}. The largest peak from an organic component of dentin is the Amide I peak near 1650 cm^{-1}. Yassen[26] recently used this information to gather information about the demineralization or deproteination of dentin treated with calcium hydroxide, triple antibiotic paste, or double antibiotic paste. When the area under these two peaks is calculated, a ratio of phosphate content to amide content can be obtained to determine the relative organic and inorganic content of the first few microns of dentin. When this ratio is compared with an untreated control group, it is possible to determine if a particular medicament caused a loss of organic content (deproteination) or inorganic content (demineralization). Yassen et al. discovered that calcium hydroxide caused superficial collagen degradation, whereas antibiotic medicaments caused superficial demineralization. This principle was used in the present study to determine the effects of antibiotic medicaments at different concentrations.

MATERIALS AND METHODS

MICROHARDNESS EXPERIMENT

TOOTH SELECTION AND SPECIMEN PREPARATION

Figure 1 is a flowchart of the microhardness experiment protocol from start to finish. University IRB approval (Protocol #1301010364) was obtained for collection of intact human, single-rooted permanent premolars (n = 85), which were stored in 0.1-percent thymol at 4ºC and used within six months of extraction. The inclusion criteria were absence of caries, root cracks, restorations, and previous endodontic treatment. The teeth were decoronated at the level of 0.5 mm radicular to the facial cementoenamel junction with a low-speed diamond saw (Buehler Ltd., Lake Bluff, IL) underwater cooling (Figure 4). Then, a 5-mm root cylinder was horizontally sectioned from each root. The canal in each cylinder was enlarged with EndoSequence 0.04 taper rotary instruments (Brasseler, Savannah, GA) to the largest diameter of a size 80 file (Figure 3). One mL of 5.25% sodium hypochlorite irrigation was used between files. Each canal was finally rinsed with 5-mL of sterile water.

The teeth were randomly assigned to five treatments groups. The 5-mm root cylinders were imbedded in VariDur resin blocks (Buehler, Lake Bluff, IL), with the coronal sides level to the surface of the block (Figure 5). The coronal sides of the specimens were polished using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers, Cleveland, PA) with 1200-, 2400- and 4000-grit papers (Struers), and finally a 1-µm diamond polishing suspension (Struers). After the polishing procedures, the blocks were ultra-sonically cleansed in 2-percent detergent solution (Micro 90 liquid soap,

International Product Corporation, Burlington, NJ) for 3 minutes to remove any residues of the polishing procedure and stored at 37°C in 100-percent relative humidity.

MICROHARDNESS MEASUREMENTS

Baseline microhardness values were measured for each sample. Microhardness measurements were obtained by using a Vickers Microhardness Tester (Leco, LM247, St. Joseph, MI) on the polished side of each root cylinder at 500 μm and 1000 μm from the pulp–dentin interface. At each distance from the canal, three indentations were made using a 50-g load oriented perpendicular to the indentation surface with a dwelling time of 10 seconds (Figure 3, Figure 6). The indentations were observed in an optical microscope with a digital camera and image analysis software to precisely measure their diagonals. The representative hardness value for each specimen at each distance to the pulp-dentin interface was calculated as the mean of the three indentations.

Each sample was then treated with a designated medicament. The five treatment groups were 1 g/mL TAP, 1 mg/mL TAP, 1 g/mL MTAP, 1 mg/mL MTAP, and sterile water (control). The 1 g/mL TAP was prepared by mixing 1 g of USP-grade antibiotic powders compounded of equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Pharmacy, San Antonio, TX) with 1 mL of sterile water (Braun Medical Inc., Irvine, CA). The 1 g/mL MTAP was prepared by mixing 1 g of USP-grade antibiotic powders ciprofloxacin 14 percent, metronidazole 43 percent , clindamycin 43 percent (Skywalk Pharmacy, Wauwatosa, WI) compounded with 1 mL of sterile water. To obtain a homogenous gel with a 1mg/mL concentration of TAP or MTAP, 100 mL of 1 mg/mL solution of TAP or mTAP were mixed with 8 gm of methyl cellulose powder (Methocel 60 HG, Sigma-Aldrich, St Louis, MO) under magnetic stirring for 2 hours at room

temperature. The control groups were hydrated in sterile water throughout the treatment period. The exposed coronal surface of each root cylinder was covered with adhesive unplasticized polyvinyl chloride tapes (Graphic Tape; Chartpak, Leeds, MA), leaving only the root canal orifice (Figure 7). Each root canal was dried with sterile paper points (Hygienic, Akron, Ohio) and the antibiotic paste was tamped into the canal space using sterile paper points and sterile pluggers. The acrylic blocks with the embedded samples were covered with custom vinyl material caps (Soft-Try, Ultradent Products, South Jordan, UT) to prevent condensation from dripping on the samples (Figure 8) and were kept in approximately 100-percent relative humidity at 37°C for four weeks. After four weeks, all specimens were rinsed with sterile water until no visible paste is observed. Microhardness measurements were then retaken on each sample adjacent to each of the pretreatment indentations (spaced 200 μm apart) to calculate a mean change in hardness after treatment. The percentage change in microhardness of each sample was calculated as following: (pre-treatment microhardness − post-treatment microhardness) × 100/pre-treatment microhardness.

ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR)

Sample Preparation

Figure 2 is a flowchart of the chemical composition experiment protocol from start to finish. For the measurement of changes in dentinal chemical composition, intact human permanent mandibular premolars were stored in 0.1-percent thymol at 4ºC and used within six months of extraction. The inclusion criteria were absence of caries, root cracks, restorations, and previous endodontic treatment. Each tooth was decoronated

horizontally 0.5 mm radicular to the facial cementoenamel junction and two 4-mm root dentin cylinders were obtained using a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL). Then, each cylinder was sectioned longitudinally across the maximum diameter of the root canal resulting in two specimens. Thus, four specimens were obtained from each root. The pulpal and convex sides of each specimen were leveled under continuous water-cooling using 1200-grit silicon carbide grinding paper (Buehler) so that the surfaces were flat (Figure 9). The specimens were rinsed and ultrasonicated for five minutes under sterile water to remove the smear layer.

Medicament Application

The specimens were randomly assigned to the five aforementioned treatment groups. The pastes were prepared in the same manner as described for the microhardness tests. Each specimen was placed in a small 2-mL conical sample cup (Fisher Scientific, Florence, KY) containing 0.15 mL of the treatment pastes or sterile water. This amount of paste is just enough to cover the pulpal surface of each specimen. The containers were stored in approximately 100-percent relative humidity at 37°C for four weeks. The specimens' paste were hydrated with 0.05-mL of sterile water after two weeks, and returned to the incubator. After four weeks, specimens were rinsed with sterile water until no visible paste was observed, ultrasonicated for 15 minutes under sterile water, and completely air-dried.

ATR-FTIR SPECTROSCOPY MEASUREMENT

A 4100 FTIR spectrophotometer (Jasco Inc., Tokyo, Japan) with a diamond ATR accessory was used to obtain infrared spectra for analysis of dentin specimens. Three

randomly selected spots on the pulpal surface of the specimens were positioned on a standard FTIR sample holder with a 3-mm diameter opening and spectra were obtained. The ATR-FTIR spectra of air was collected and automatically subtracted by the Spectra Manager CFR software (Jasco Inc.). Spectra were collected in triplicate from each treated dentin specimen between 800 cm^{-1} and 2000 cm^{-1} at 4 cm^{-1} resolution using 70 scans. Each obtained spectrum was then processed by smoothing, and baseline correction and normalization to the amide I peak using dedicated Spectra Manager CFR software (Jasco Inc). The effects of various treatments on collagen and apatite composition of surface dentin were evaluated using the mineral matrix ratio (the ratio of integrated areas of the phosphate v1, v3 contour to the amide I peak). The mean derived from the spectra obtained from each group was used quantitatively for statistical evaluation. Larger phosphate/amide I ratios compared with the control group correspond to a greater dentin collagen deproteinization, while smaller ratios correspond to a greater dentin demineralization.

pH MEASUREMENTS

The pH of TAP and MTAP at each concentration was measured. Given it is difficult to accurately measure the pH of a paste, a saturated solution was used to measure the pH of the 1 g/mL pastes. Solutions were made with sterile water in triplicate and measured using a digital pH meter (ATI Orion Model 330, Boston, MA). The meter was calibrated using pH standards of 4 and 7. Solutions tested were placed in direct contact with the electrode until a stable pH reading was acquired.

SEM MEASUREMENT

Two root cylinders from the microhardness experiments were randomly selected from each group for SEM analysis to observe any morphological changes in root canal dentin. Each selected root cylinder was sectioned longitudinally without touching the root canal surface. Then, each half-root cylinder was irrigated with 5 mL of de-ionized water, sonicated in de-ionized water for 5 min, and desiccated for 48 h. Then, specimens were sputter coated for 70 s with gold/palladium using a sputter coater (Polaron, Agawam, MA) and images were taken from the treated root canal surface area of the specimens with a JEOL 7800F scanning electron microscope (Peabody, MA) in secondary electron imaging mode.

STATISTICAL METHODS

All data were checked for normality using the Shapiro–Wilk test and the normality assumptions were satisfied. Paired t-tests were used to compare between the pre- and post-treatment Vickers microhardness values in each group at both depths. The effects of the type of medicaments on percentage change in microhardness and phosphate/amide I ratios were examined using one-way ANOVA followed by Fisher's Least Significant Difference. A 5-percent level of statistical significance was applied for all analyses.

SAMPLE SIZE

Based on a prior study,[27] the standard deviation for microhardness was estimated to be 5 HV. Also, the coefficient of variation for the phosphate/amide I ratio was estimated to be 0.243.[26] All sample size calculations assume two-sided tests each conducted at a 5-percent significance level. For the microhardness test, with a sample

size of 17 specimens per group, the study had 80-percent power to detect a 5-HV difference in microhardness. For the FTIR test, with a sample size of seven specimens per group the study had 80-percent power to detect a fold-change of 1.5 for the phosphate/amide I ratio.

RESULTS

MICROHARDNESS MEASUREMENTS

At 500 μm from Pulp-Dentin Interface

Normality assumptions for the ANOVA model were tested and satisfied. Figure 10 shows the percent change in Vickers microhardness at 500 μm and Figure 11 shows the percent change in microhardness at 1000 μm from the pulp-dentin interface after four weeks of treatment with each paste. The bars of these graphs are arranged in order from least to greatest percent decrease in microhardness. For each group, different letters indicate a statistically significant difference between medicaments.

Figure 12 shows the average pretreatment microhardness, post-treatment microhardness, and percent change values, along with their standard deviations. The post-treatment microhardness values were significantly lower than pre-treatment microhardness for all treatment groups ($p < 0.0001$) but not for the untreated control group ($p = 0.97$). The one-way ANOVA showed a significant effect of treatment type on the measured percentage change of microhardness ($p < 0.0001$). The percentage reduction in microhardness was significantly greater for all treated groups compared with the untreated control group ($p < 0.0001$ for 1g/mL TAP and MTAP; $p = 0.0008$ for 1 mg/mL of TAP; and $p = 0.039$ for 1 mg/mL of MTAP). The percentage reduction in microhardness was significantly greater for 1 g/mL of TAP samples compared with all other groups (1g/mL MTAP ($p = 0.0016$), both 1 mg/mL TAP and MTAP ($p < 0.0001$). Furthermore, the percentage reduction in microhardness was significantly greater for 1

g/mL MTAP compared with 1 mg/mL of TAP ($p = 0.0069$) and MTAP ($p < 0.0001$) treated roots. However, no significant difference in the reduction of microhardness was observed between the 1 mg/mL concentrations of TAP and MTAP ($p = 0.17$).

At 1000 μm from the Pulp-Dentin Interface

Figure 12 shows that the post-treatment microhardness values were significantly lower than pre-treatment microhardness values for 1 g/mL TAP, 1 g/mL MTAP ($p < 0.0001$) and 1 mg/mL of TAP ($p = 0.005$) treated roots, but not for 1 mg/mL MTAP treated roots ($p = 0.26$). The one-way ANOVA showed a significant effect of treatment type on the measured percentage change of microhardness ($p < 0.0001$). The percentage reductions in microhardness were significantly greater for 1 g/mL of TAP ($p < 0.0001$), 1 g/m MTAP- treated ($p < 0.0001$), and 1 mg/mL of TAP-treated ($p = 0.002$) roots compared with the control group. However, the percentage reductions in microhardness of 1 mg/mL of MTAP treated roots were not significantly different from the untreated control group ($p = 0.2042$). The percentage reductions in microhardness were significantly greater for 1 g/mL of both TAP- and MTAP-treated roots compared with 1 mg/mL of both TAP ($p = 0.0036$ and $p = 0.0003$, respectively) and MTAP ($p < 0.0001$) samples. However, no significant differences in percentage reduction of microhardness were observed between the 1g/mL concentrations of TAP and MTAP ($p = 0.44$), or between the 1 mg/mL concentrations of TAP and MTAP ($p = 0.06$).

ATR-FTIR Spectroscopy Measurements

A representative ATR-FTIR spectrum from the untreated control group with relevant peaks labeled can be seen in Figure 13. The ratio was calculated using the total area under the phosphate peak divided by the area under the Amide I peak. Figure 14

shows a representative ATR-FTIR spectrum of dentin from each treatment group. The one-way ANOVA showed significant effect of treatment type on phosphate/amide I ratios measured with ATR-FTIR ($p < 0.0001$). Figure 15 shows that the phosphate/amide I ratio of 1 g/mL TAP-treated group was significantly lower than all other treatment groups and the untreated control dentin ($p < 0.000$). The phosphate/amide I ratio of 1 g/mL MTAP was significantly lower than 1 mg/mL TAP ($p = 0.0004$), 1 mg/mL MTAP ($p < 0.0001$) and the untreated control dentin ($p = 0.044$). Furthermore, the phosphate/amide I ratios of 1 mg/mL MTAP-treated dentin was significantly higher than that of 1 mg/mL TAP-treated dentin and the untreated control dentin ($p < 0.0001$). However, no significant difference in phosphate/amide I ratio was observed between untreated control dentin and 1mg/mL TAP-treated dentin ($p = 0.071$). Overall, every group except dMTAP had a significant impact on the phosphate/amide I ratio compared with the control, indicating a significant change in the chemical composition of the dentin samples.

pH Measurements

Figure 16 shows the average pH of each medicament. TAP was the most acidic (3.0) followed by MTAP (4.0). The 1-mg/mL dilutions of dTAP and dMTAP measured much closer to a neutral pH (6.4 and 6.6, respectively).

Scanning Electron Microscopy

Scanning electron microscopy (SEM) images taken at various magnifications showed the presence of smear layer covering and occluding dentin tubules in instrumented root canals of the untreated control dentin (Figure 19) and the 1 mg/mL MTAP-treated dentin (Figure 23). For the other three treatment groups, no visible smear

layer was observed, and the dentin tubules were open. Additionally, the native structure of collagen fibrils was identified under higher magnification in root canals treated with 1 g/mL TAP (Figure 20), 1 g/mL MTAP (Figure 21) and 1 mg/mL TAP (Figure 22).

FIGURES AND TABLES

FIGURE 1. Flowchart of microhardness measurement protocol.

FIGURE 2. Flowchart of chemical composition protocol.

FIGURE 3. Each root was sectioned into a cylinder, 5 mm tall, and the canals enlarged. Microhardness measurements were taken before and after treatment at 500 μm (A) and 1000 μm (B) from the canal lumen.

FIGURE 4. Extracted premolar tooth to be sectioned by a low-speed diamond saw.

FIGURE 5. Premolar root sections mounted in acrylic and polished. Vickers microhardness measurements were then taken perpendicular to the exposed surface at 500 μm and 1000 μm from the lumen.

FIGURE 6. Vickers Microhardness Tester. Indentations were made in the radicular dentin to measure pretreatment and post-treatment microhardness values.

FIGURE 7. Tape placed over exposed dentin so that paste only touched canal.

FIGURE 8. Lid placed over each acrylic block to prevent condensation from dripping on the samples.

FIGURE 9. Each root (A) was sectioned into two cylinders (B). The cylinders were cut longitudinally (C), and polished flat on the pulpal side (D).

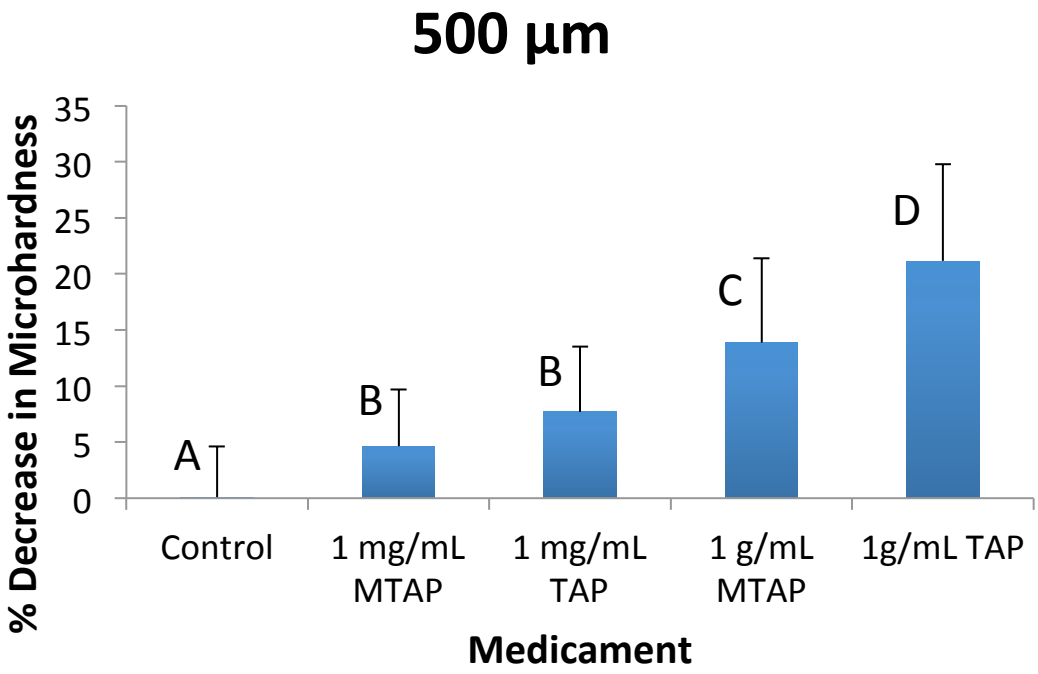


FIGURE 10. Percent change in microhardness at 500 µm from the pulp-dentin interface. Different letters indicate statistically significant difference between medicaments.

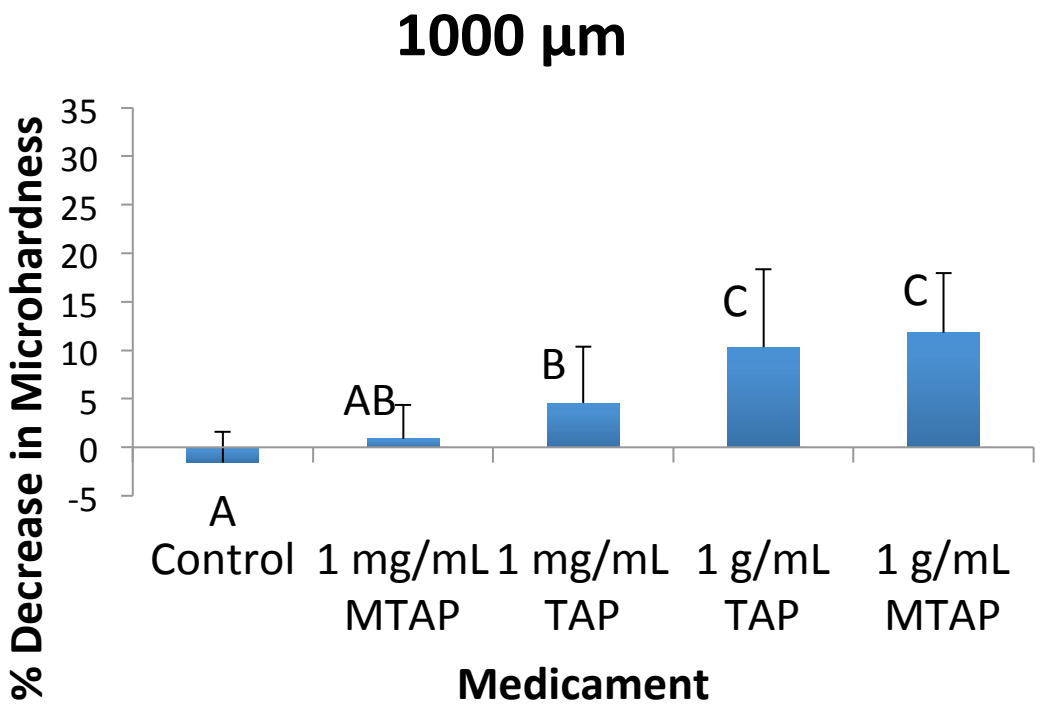


FIGURE 11. Percent change in microhardness at 1000 µm from the pulp-dentin interface. Different letters indicate statistically significant difference between medicaments.

Group	1000 μm from pulp-dentin interface∗		
	Pre-treatment	Post-treatment	% change
Control	59.1 (7.1)b	60.1 (8)a	- 1.6 (3.2) A
1 mg/mL MTAP	59.9 (6.4)a	59.4 (6.3)b	0.9 (3.5) AB
1 mg/mL TAP	61.3 (5.8)a	58.5 (6.2)b	4.5 (5.8) B
1 g/mL MTAP	60.8 (4.7)a	53.6 (5.4)b	11.8 (6.2) C
1 g/mL TAP	57.8 (6.6)a	51.4 (5.7)b	10.3 (8) C

Group	500 μm from pulp-dentin interface∗		
	Pre-treatment	Post-treatment	% change
Control	52 (7.5)a	52 (7.7)a	0 (4.6)A
1 mg/mL MTAP	52.9 (6.9)a	50.4 (6.9)b	4.6 (5)B
1 mg/mL TAP	54.6 (6.5)a	50.4 (7.2)b	7.7 (5.9)B
1 g/mL MTAP	53.5 (7.1)a	45.9 (6.6)b	13.9 (7.5)C
1 g/mL TAP	49.7 (6.9)a	39 (5.8)b	21.2 (8.6)D

∗At each distance, different lower-case letter indicates significant difference between pre- and post treatment of the same group.

∗At each distance, different upper-case letter indicates significant difference between different treatment groups.

FIGURE 12. Mean (SD) of Vickers microhardness for roots treated with various endodontic regeneration medicaments and a control group for four weeks at 500 μm and 1000 μm from the pulp-dentin interface.

FIGURE 13. A representative ATR-FTIR spectrum from the untreated control dentin.

FIGURE 14. A representative ATR-FTIR spectrum of dentin treated with each medicament.

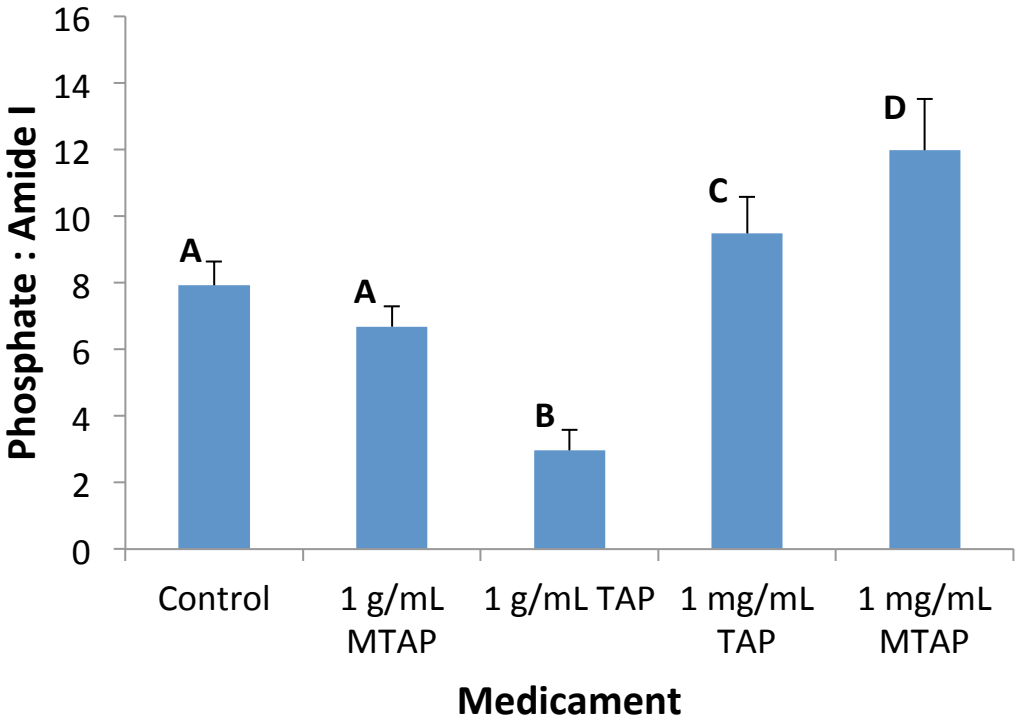


FIGURE 15. Average phosphate/amide 1 ratio after four weeks. Different letters indicate a statistically significant difference between medicaments.

Solution	1 g/mL TAP	1 mg/mL TAP	1 g/mL MTAP	1 mg/mL MTAP
Average pH	3.0	6.4	4.0	6.6

FIGURE 16. Average pH of each solution.

FIGURE 17. Post-treatment samples of each group. Top row (left to right): Control, 1 mg/mL TAP, 1 g/mL TAP. Bottom row (left to right): 1 mg/mL MTAP, 1 g/mL TAP. Note the dark staining only in the 1 g/mL TAP samples.

FIGURE 18. Darkly colored TAP paste (1g/mL) placed over dentin samples for the chemical composition experiment.

A

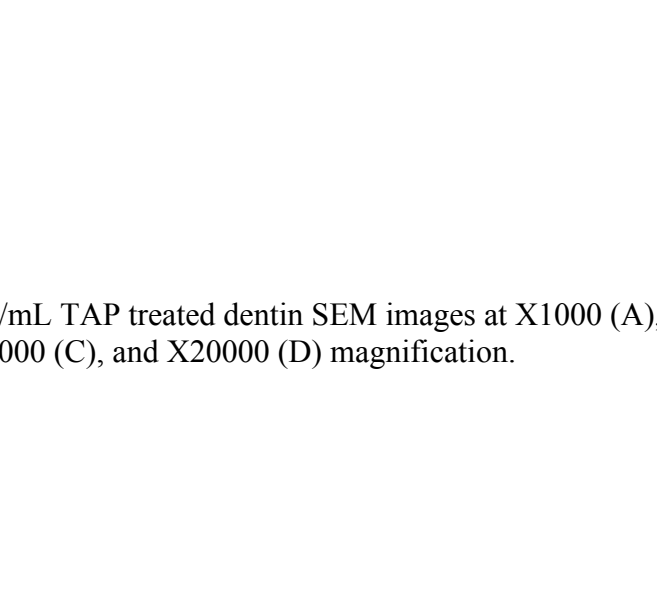
B

C

FIGURE 19. Untreated control dentin SEM images at X1000 (A), X2500 (B), and X5000 (C) magnification.

A

B



C

D

FIGURE 20. 1 g/mL TAP treated dentin SEM images at X1000 (A), X2500 (B), X5000 (C), and X20000 (D) magnification.

FIGURE 21. 1 g/mL MTAP treated dentin SEM images at X1000 (A), X2500 (B), X5000 (C), and X20000 (D) magnification.

FIGURE 22. 1 mg/mL TAP treated dentin SEM images at X1000 (A), X2500 (B), X5000 (C), and X20000 (D) magnification.

FIGURE 23. 1 mg/mL MTAP treated dentin SEM images at X1000 (A), X2500 (B), and X5000 (C) magnification.

DISCUSSION

The large size of the canals, thin root walls, and a divergent blunderbuss shape of the apical foramen of teeth with incompletely developed apices limit mechanical instrumentation in regenerative endodontic procedures. However, effective disinfection is a prerequisite to pulpal revascularization because high concentrations of endodontic pathogens do not provide an environment conducive to host tissue regeneration. Therefore, in these cases, chemical disinfection plays a crucial role in reducing the microbial load within the necrotic canal space prior to regeneration. Case studies of successful regenerative endodontic procedures have shown an increase in root thickness in the apical half, but not the coronal part of the tooth.[118,133] The cervical dentin, however, is critical because immature roots are most prone to fracture near the cementoenamel junction.[64] It is therefore imperative that the antibacterial medicaments used for regenerative endodontics have not only sufficient antimicrobial properties, but also favorable (or in the very least not unfavorable) effects on the radicular dentin.

In this study, the pasty concentrations of 1 g/mL most often used clinically caused significant reduction in the microhardness at both 500 μm and 1000 μm from the pulp-dentin interface. These results are consistent with a recently published study that showed significant decrease in microhardness of dentin treated for one month with a TAP concentration similar to the present study's 1 g/mL paste. [134] This decrease in microhardness could be explained in part by the pH measured in the present study. The pH of 1 g/mL TAP (3.0) and MTAP (4.0) were quite acidic when compared with the nearly neutral pH of the 1 mg/mL TAP (6.4) and MTAP (6.6). The present study also

showed that 1 g/mL TAP treatment caused a significant reduction in microhardness at 500 μm from the pulp-dentin interface compared with MTAP treatment of the same concentration. This could be explained by the ability of the minocycline present in TAP to demineralize radicular dentin by chelating calcium.[25]

To further understand the effect of antibiotic medicaments on radicular dentin, the change in chemical composition of superficial radicular dentin after TAP and MTAP applications was explored using ATR-FTIR. The results of these spectra further support the demineralization effect of the 1 g/mL pastes. The data showed a significantly lower phosphate/amide1 ratio for both of the higher concentration pastes when compared with the control group. This indicates a relative decrease in the inorganic content of the dentin, represented by phosphate content, when treated with the higher antibiotic concentrations. The presence of an apatite-depleted collagen layer was illustrated by the SEM images of root canals treated with 1 g/mL of TAP and MTAP (Figure 20, Figure 21). These results are also consistent with a recently published study using ATR-FTIR that reported significant demineralization of dentin treated for one month using a TAP concentration similar to the present study's 1 g/mL paste. [135]

Dentin treated with 1 g/mL of TAP had significantly lower phosphate/amide I ratio compared with dentin treated with the same concentration of MTAP. The significantly higher dentin demineralization effect of 1 g/mL TAP recorded by ATR-FTIR may substantiate the significantly higher reduction in microhardness of roots treated with 1 g/mL of TAP compared with roots treated with the same concentration of MTAP at 500 μm from pulp-dentin interface reported in this study.

The current study included a lower concentration of TAP and MTAP (1 mg/mL) as an attempt to minimize the expected unwanted effect of high concentration on the chemical and mechanical properties of radicular dentin. This 1 mg/mL concentration of TAP was found to exhibit both inhibitory and bactericidal effectiveness against Enterococcus faecalis and Porphyromonas gingivalis biofilm in vitro.[136] Additionally, 1 mg/mL of TAP has been proposed to have no direct [84] or indirect [137] cytotoxic affects on human dental pulp cells and human stem cells of the apical papilla, respectively. These dilutions alone, however, are liquids. A liquid is not clinically feasible to maintain in a canal as an interappointment medicament, so a methylcellulose vehicle was added to create a clinically applicable paste consistency. The addition of methylcellulose would not be expected to change the biocompatibility of these pastes because a commercially available methylcellulose-based calcium hydroxide medicament (UltraCal) has been found to have no cytotoxic effect against stem cells of the apical papilla.[138]

The methylcellulose-based 1 mg/mL TAP and MTAP used in the present study caused significantly higher reductions in microhardness values compared with the untreated roots at 500 μm from the pulp-dentin interface. It is uncertain if the 1 mg/mL MTAP had a lesser impact than 1 mg/mL TAP. On one hand, the diluted MTAP did not induce a significantly greater percent change in microhardness 1000 μm from the canal space compared with the control. On the other hand, the post-treatment microhardness measurements were significantly lower than the pre-treatment measurements of the same group. Although it is debatable which diluted paste has more favorable results, it is clear that both of the lower concentrations caused significantly less reduction in microhardness at the two measured depths compared with their corresponding 1 g/mL concentrations.

This indicates that the use of 1 mg/mL of TAP or MTAP in endodontic regeneration may minimize the reduction in dentin microhardness compared with the currently used concentration of antibiotic medicaments.

In the current study, a trend of higher phosphate/amide I ratio in 1 mg/mL of TAP- and MTAP-treated dentin was reported compared with untreated dentin. This is explained by the chemical overlapping in the measured spectra between the residues of organic C-O-C peak from methylcellulose and the inorganic phosphate peaks of radicular dentin. Recognizing separate organic and inorganic FTIR peaks in methylcellulose based materials was found to be challenging.[139] Furthermore, methylcellulose-based calcium hydroxide intracanal medicament was found to have significantly higher retention capacity to the radicular dentin compared with pure calcium hydroxide.[140] It is therefore feasible that methylcellulose could not be completely removed from the specimens and that the increased phosphate/amide1 ratio is due to the additive effect of the phosphate content of dentin and the organic C-O-C content of methylcellulose, both absorbing wavelengths around 1000 cm^{-1}. These results, therefore, mask the true effects of the 1 mg/mL pastes and reflect neither an increase in inorganic content nor a decrease in organic content, but a relative increase in the 1000 cm^{-1} peak due to the addition of methylcellulose.

Demineralization decreases microhardness, which has potential negative effects on a tooth's fracture resistance. Some demineralization from the antibiotic pastes, however, may also be advantageous. As seen in the SEM images, the pastes that demineralized in the FTIR experiment removed the smear layer to expose dentin tubules and collagen. The smear layer consists of organic and inorganic tissue remnants, along

with some bacteria and their byproducts. Bacteria have been shown to penetrate dentinal tubules up to half the distance between the root canal walls and the CDJ.[95] Evidence suggests removal of smear layer prior to obturation can be beneficial because it removes a potential substrate for bacterial growth, allows irrigation solution access to the dentin tubules, and promotes a better seal after obturation.[96,141] A medicament that can remove a portion of the smear layer is theoretically beneficial. The significance of these benefits for in-vivo regenerative endodontics, however, is questionable because the limited mechanical instrumentation in these procedures would not be expected to produce the extensive smear layer that was seen in the heavily instrumented samples of the present study.

Even if a thick smear layer is not produced during regenerative endodontic procedures, a small amount of demineralization may still be beneficial to the migration and survival of new pulp tissue. Demineralization may enhance a tooth's regenerative potential by releasing dentinal growth factors that were imbedded into dentin during dentinogenesis.[72,142] Yamauchi, et al. found that canal irrigation with EDTA also increases adherence of newly formed tissue to root canal walls.[142] EDTA-treated samples showed projections from the newly formed tissue that interlocked with the demineralized dentin wall. New odontoblasts were not seen, however, partly because the demineralization from EDTA was too mild to fully expose the dentin matrix. Perhaps exposure of greater amounts of dentin matrix due to antibiotic medicaments would further enhance attachment.

The MTAP formulation has been suggested as an alternative to TAP in order to avoid some deleterious effects of minocycline. As previously mentioned, minocycline

chelates calcium ions[25] and in this study was found at the 1 g/mL concentration to both demineralize more significantly and decrease microhardness more significantly than any other medicament near the pulp-dentin interface. A previous study used the same protocol as the present study to take microhardness measurements and found a correlation between decrease in microhardness and decrease in fracture resistance.[27] Therefore, strong demineralization from high concentrations of TAP may translate to a reduced fracture resistance compared with a tooth treated with MTAP.

Another potential advantage of MTAP is that it does not discolor dentin, whereas the TAP paste turns black when it sets (Figure 18). This dark paste severely discolors dentin at high concentrations.[18,19] No significant discoloration of dentin was noted in this study from either the diluted TAP group, either concentration of MTAP, or the control group (Figure 17). This suggests that any of these pastes may render a more esthetic result than the 1 g/mL TAP, especially when used in anterior teeth.

The current study adds information to the body of knowledge about intracanal medicaments and their concentration dependent effects on radicular dentin. Further studies in this area are needed to determine the best disinfection protocol for immature teeth with necrotic pulps. The ideal intracanal medicament would be inexpensive, easy to place in the canal, effective against pathogenic endodontic microbes, and would have no detrimental effects on the treated tooth structure or host cells that repopulate the canal space. Future studies could investigate the effects on mechanical properties of radicular dentin using other antibiotics alone or in combination, the addition of EDTA, different concentrations of medicaments, or multiple time points. Further ATR-FTIR studies could include groups with and without methylcellulose to determine the extent of its

effect on the spectra of dentin. Fracture resistance studies could be performed to correlate the extent of demineralization to its effect on the mechanical properties of dentin. Finally, animal studies and prospective randomized clinical trials are needed to determine the relevance to in-vivo success rates.

Current clinical protocols are based on limited evidence from in-vitro studies, as well as the historical precedence set by case reports of previously successful cases. The key question underlying the discussion of demineralization is to determine the best treatment time, for the best medicament(s), at the best concentration, to allow for the advantages of demineralization without compromising the physical properties of the tooth. Thus far the perfect answer to this question is unknown, but with a growing body of data, decision-making for regenerative endodontics becomes more evidence-based and, hopefully, more predictably successful.

SUMMARY AND CONCLUSIONS

The first null hypothesis that TAP and MTAP have no significant effect on the microhardness of radicular dentin at either concentration tested was rejected. The 1 g/mL TAP, 1 g/mL MTAP, and 1 mg/mL TAP caused significant reduction in microhardness of roots compared with untreated control roots at 500 μm and 1000 μm from the pulp-dentin interface. The 1 mg/mL concentrations of both medicaments caused significantly less reduction in microhardness than the corresponding 1 g/mL pastes.

The second null hypothesis that TAP and MTAP have no significant effect at either 1 g/mL or 1 mg/mL concentrations on the chemical structure of radicular dentin was also rejected. The dentin samples treated with 1 g/mL concentrations of either medicament had a significantly lower phosphate:amide1 ratio than the untreated control samples, indicating a demineralization effect. Furthermore, the 1 g/mL TAP caused significantly greater demineralization than the 1 g/mL MTAP. The effect of the 1 mg/mL pastes on radicular dentin is unclear because the methylcellulose added to these medicaments in this study has an ATR-FTIR absorbance peak that overlaps the inorganic phosphate peak of dentin. This skews the phosphate:amide1 ratio of samples treated with the lower concentration pastes.

MTAP should be considered as a substitute for TAP in regenerative endodontic procedures. It was found that MTAP does not demineralize dentin as extensively as TAP, which led to a smaller decrease in microhardness. MTAP also did not stain dentin, whereas higher concentrations of TAP cause severe discoloration.

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ABSTRACT

EFFECT OF ANTIBIOTIC PASTES ON CHEMICAL STRUCTURE AND MICROHARDNESS OF RADICULAR DENTIN

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Introduction: Regenerative endodontic therapy in immature teeth with necrotic pulps triggers continued root development, thereby improving the prognosis of these teeth.[8-12] Disinfection of the canal is accomplished with an intracanal medicament, such as triple antibiotic paste (TAP) composed of metronidazole, ciprofloxacin, and minocycline.[13] A modified triple antibiotic paste (MTAP) that replaces minocycline with clindamycin has recently been suggested to avoid the tooth discoloration and potential demineralization from minocycline. The effect these pastes have on radicular dentin is unknown.

Objectives: The aim of this study was to investigate the effects of two intracanal medicaments used during endodontic regeneration, TAP and MTAP, at concentrations of 1 g/mL and 1 mg/mL, on the microhardness and chemical structure of radicular dentin.

Materials and Methods: Roots from extracted, unrestored, non-carious human premolar teeth were sectioned. An antibiotic paste (MTAP or TAP) or sterile water (control) was applied to treatment groups and stored for four weeks in 80-percent humidity at 37 °C. The effect of each paste on the microhardness of radicular dentin was measured using a Vickers Microhardness Tester (n = 17) to take three pretreatment and post-treatment measurements at both 500 μm and 1000 μm from the pulp-dentin interface. The chemical structure was assessed from dentin specimens treated with the same medicaments or sterile water for four weeks. After treatment, three measurements were taken on each specimen using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy to measure the phosphate/amide I ratios of dentin (n = 7).

Results: The 1 g/mL of TAP or MTAP and the 1 mg/mL methylcellulose-based TAP caused significant reduction in microhardness of roots compared with untreated control roots at 500 μm and 1000 μm from the pulp-dentin interface. Furthermore, the methylcellulose-based 1 mg/mL TAP and MTAP caused significantly less reduction in microhardness compared with 1 g/mL TAP and MTAP. The 1 g/mL of TAP and DAP caused significantly lower phosphate/amide I ratios compared with other groups.

Conclusion: The use of methylcellulose based 1 mg/mL of TAP and MTAP may minimize the reduction in microhardness of roots compared with the currently used 1 g/mL concentration of these antibiotics.