AN IN VITRO STUDY OF PH CHANGES WITH ENDOSEQUENCE® BC ROOT REPAIR MATERIAL FAST SET PUTTY IN SIMULATED RESORPTIVE DEFECTS

A THESIS

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DEDICATION

To my co-residents and life-long friends: Drs. Regan Anderson, Bienias, Brown, Divine, Penaz and Schuurmans

To my best friend and partner in life, Daniel Vishnevetsky for his love, support and patience

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

The goal of endodontic therapy is to prevent and treat apical periodontitis. This is accomplished via the biological and mechanical objectives outlined by Schilder in 1967 and 1974. These objectives include the following: preparing the canal system as a continuously tapering funnel with a cross-sectional diameter narrower at every point moving apically and wider at every point approaching the coronal aspect, occupying as many planes of space and following the original shape of the canal, maintaining the apical foramen in relation to the root and bone, keeping the apical shape as small as possible, avoiding extrusion of necrotic debris, removing all tissue within the canal, leaving adequate space for irrigation and use of intracanal medicaments, and a three-dimensional obturation of the canal system.

One of the challenges to endodontic therapy is resorption, where parts of the tooth are destroyed internally, externally or both. Causes of resorption include the following: trauma, orthodontics, excessive occlusal forces, tumors or cysts, bleaching of pulpless teeth (Andreason, 1985) or idiopathic (Bakland, 1992). Systemic diseases including herpes zoster (Soloman, 1986) and Paget's disease (Barnett, 1985) have also been implicated in root resorption.

Particularly detrimental are cases of external inflammatory resorption, in which the outer layer of cementum is damaged and infection in the canal system follows, which is frequently as a sequelae of traumatic injuries. External inflammatory resorption involves the resorption of adjacent bone as well as the root (Levin & Trope, 2002). External resorption is further classified into four categories described below: external surface resorption, replacement resorption, ankylosis and external inflammatory root resorption (Ne, 1999).

External surface resorption occurs when there is minor damage to the outer root surface or periodontal ligament (PDL), such as in orthodontic treatment. There is a lack of continued stimulation by inflammatory mediators or bacteria, and the process is self-limiting. No treatment is indicated (Figure 1).

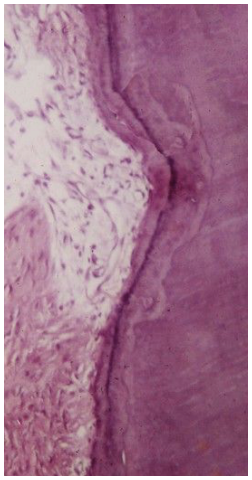

Figure 1. External Repair Related or Surface Root Resorption Diagram (Pathways of the Pulp, 10th ed., Fig. 17-26))

Ankylosis related or replacement resorption occurs when there are larger areas of damage to the root and PDL. This type is typically seen with trauma (luxation and avulsion injuries). The tooth attempts to heal itself but gradually becomes replaced by bone in the normal physiologic bone turnover process. The cells are unable to differentiate between bone and tooth and has been described as a "mistake" (Tronstad, 1988). There is a layer of inflamed connective tissue between the tooth and bone and loss of PDL is seen radiographically (Figure 2). Ankylosis appears similar radiographically but no layer of inflamed connective tissue is present. There is no treatment for these types of resorption, and often results in tooth loss.

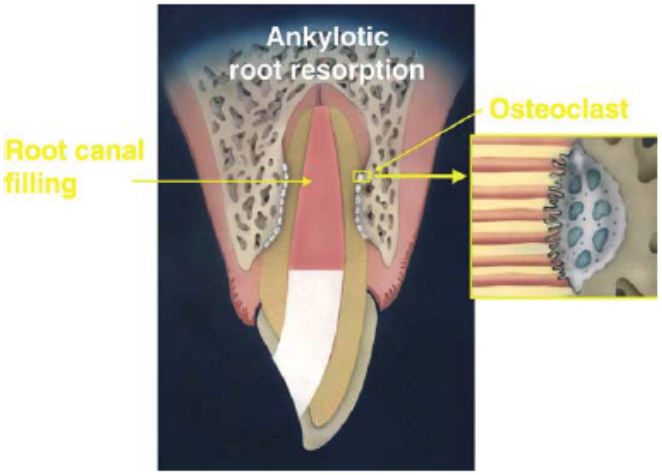

Figure 2. Ankylosis Related Root Resorption Diagram

(Fuss, 2003)

The most common form of external resorption is external inflammatory root resorption (EIRR) or infection related resorption. It is characterized by defects in dentin which appear semi-circular in shape. In cases of external

resorption mineralized portions of the tooth becomes resorbed when under pathologic states (Figure 3). Normally, the radicular structures are protected by the predentin layer, precementum layer and the cementum (Tronstad, 1988). If these layers are damaged or scraped off, osteoclastic cells can to adhere to these surfaces and initiate resorption of the tooth structure. This leads to osteoclastic activity which destroys the radicular portion of the tooth and may result in tooth loss (Andereason, 1981). Osteoclasts are not able to adhere to unmineralized structures of intact predentin and cementum (Levin & Trope, 2002). Osteoclasts are able to bind to extracellular proteins which contain the arginine-glycine-aspartic acid sequence (RGD). These are bound to mineralized surfaces via calcium salts. A lack of RGD proteins on predentin and cementum surfaces thereby protects the radicular surface from osteoclast binding and resorption (Hammarstrom & Lindskog, 1985).

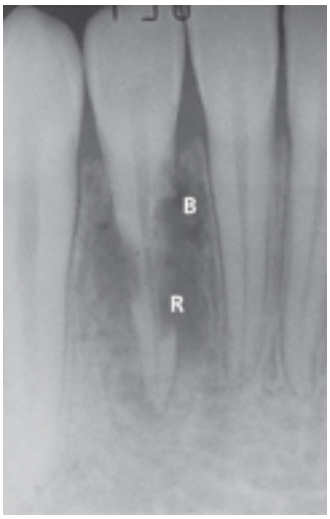

Figure 3. External Inflammatory Root Resorption

(Levin & Trope, 2002)

Treatment of EIRR has historically been via use an intracanal medicament of calcium hydroxide ($Ca(OH)_2$). Calcium hydroxide works via dissociation in an aqueous vehicle into calcium and hydroxyl ions, which create free radicals capable of destroying bacterial cell membranes. It displays antimicrobial properties, the ability to dissolve tissue, inhibit resorption and induce hard tissue repair. Calcium hydroxide's alkaline pH (12.5) alters bacterial enzymes and disrupts cellular metabolism. It can diffuse across dentin and can act as a physical barrier to residual microbes (Siqueira, 1999) and effectively eliminates bacteria that can survive instrumentation within seven days (Sjogren, 1991). Calcium hydroxide has demonstrated a positive effect on inhibiting osteoclasts involved in resorbing bone and the progression of apical pathosis. Calcium hydroxide disrupts the ruffled border, where osteoclasts reside, but osteoblasts can withstand the elevated calcium levels, preventing further bone resorption (Haga & Stern, 1993). These properties make calcium hydroxide the standard choice as an intracanal medicament prior to obturation of the root canal system. In cases of EIRR, $Ca(OH)_2$ has demonstrated an ability to down-regulate osteoclasts adjacent to the radicular surface by altering pH levels to more alkaline levels (Tronstad, 1981; Saif, 2008; Chamberlain, 2009; Heward, 2011). Disadvantages to using $Ca(OH)_2$ include the potential for tooth fracture (Andreason, 2002; Sahebi, 2010; Hawkins, 2015) as well as the need for multiple appointments; patient cooperation and compliance is thereby required (Trope, 1995).

Bioactive endodontic cements (BECs) have gained popularity in recent years for their many positive attributes and biocompatibility. Uses include vital pulp therapy, root end filling material, apical barrier placement, guided endodontic repair, perforation repair and in treatment of resorptive lesions. The most notable material with the most literature behind it is mineral trioxide aggregate (MTA). It is composed of calcium and silicate cements and was introduced by Torabinejad in the 1990s, with use in the U.S. starting in 1997 (Parirokh, 2017). Benefits include a high pH which may induce hard tissue formation, biocompatibility, better sealing ability for retro-filling compared to traditional endodontic microsurgery materials, and minimally affected by blood contamination (Johnson, 2008). Disadvantages to MTA include the long set time of 2 hours and 45 minutes (Torabinejad, 1995) and difficult handling and manipulation properties (Johnson, 2008). The interaction between the bismuth oxide in MTA and intracanal irrigants results in severe discoloration and staining (Kohli, 2015).

Numerous other BECs have been introduced claiming the same benefits as MTA without the drawbacks. One such material is EndoSequence® BC RRM™ Fast Set Putty (Brasseler, Savannah, GA), which is a ready-to-use, premixed bioactive cement (Ma, 2011). It is composed of zirconium oxide,

calcium silicates, tantalum oxide, calcium phosphate monobasic and filling and thickening agents (Parirokh, 2017). The manufacturer claims EndoSequence® BC RRM™ Fast Set Putty can be used in many of the same procedures as MTA, including as a root end filling material, apical barrier and in vital pulp therapy, but with a shorter set time of 20 minutes and better handling properties without potential for staining (Kohli, 2015).

BECs have an alkaline pH and have been shown to be antibacterial. The pH of MTA is initially 10.2, but rises to 12.5 after three hours (Torabinejad, 1995). Brasseler states the pH of EndoSequence® BC RRM™ Fast Set Putty is greater than 12; both materials have comparable pH levels to calcium hydroxide. MTA has been shown to allow for diffusion of calcium ions from the canal space to the radicular dentin onto simulated external root surface cavities (George, 2009). This generates the discussion of use of BECs, rather than calcium hydroxide, as a treatment modality for external inflammatory resorptive lesions. In an *in vitro* study comparing calcium hydroxide with MTA for potential treatment of external inflammatory resorptive lesions, MTA was shown to exhibit a small but significantly higher pH at four weeks compared to calcium hydroxide (Heward, 2011). To date, no studies thus far have compared $Ca(OH)_2$ to EndoSequence® BC RRM™ Fast Set Putty in a similar methodology as Heward et. al., bringing forth the concept of using EndoSequence® BC RRM™ Fast Set

Putty as an alternative treatment option to external inflammatory resorptive lesions.

The purpose of this study was to compare *in vitro* the effects of intracanal placement of EndoSequence® BC RRM™ Fast Set Putty and $Ca(OH)_2$ on hydroxol ion diffusion through dentin of teeth with simulated root surface cavities, mimicking external resorptive lesions. The goal was to determine if EndoSequence® BC RRM™ Fast Set Putty is a conceivable alternative to $Ca(OH)_2$ in cases of external inflammatory resorptive lesions.

REVIEW OF THE LITERATURE

A review of the literature focused on the use of $Ca(OH)_2$ and bioactive endodontic cements, specifically EndoSequence® BC RRM™ Fast Set Putty.

Calcium hydroxide:

The first use of $Ca(OH)_2$ was reported in 1920 by Hermann (Dammaschke, 2008). It is formulated from limestone and the mode of action is via dissociation into an aqueous solution of calcium and hydroxyl ions (Fava, 1999). Key properties include its antimicrobial properties, tissue dissolving ability, resorption inhibition and hard tissue induction capabilities.

Calcium hydroxide is delivered as a paste and the vehicle used affects its properties and duration of action (Fava, 1999):

1. Aqueous vehicles provide a rapid release and maintain the high pH. Examples include Calasept® (52% $Ca(OH)_2$, 8% calcium chloride, 4% sodium bicarbonate, 8% potassium chloride, 0.35% sodium chloride and 16% water), Pulpdent® (53.5% $Ca(OH)_2$, methylcellulose), and UltraCal® (35% $Ca(OH)_2$, water, hydroxyapetite and a "water-soluble thickener").

2. Viscous vehicles provide a slower rate of release over longer time periods. Examples include Calen® (53% $Ca(OH)_2$, 10% zinc oxide, 37% polyethyleneglycol) and Calen+® (Calen® and camphor CMCP).
3. Oily vehicles display the lowest solubility and slowest rate of diffusion. Examples include Endoapex® ($Ca(OH)_2$, silicone oil and iodoform) and Vitapex® (30% $Ca(OH)_2$, 40% iodoform, 22% silicone oil and 8% other ingredients).

Mixing $Ca(OH)_2$ in an aqueous vehicle allows dissolution of the hydroxol ions. Mixtures with non-aqueous vehicles, such as glycerin or propylene glycol, may hinder the efficacy of $Ca(OH)_2$ (Safavi & Nakayama, 2000). A final irrigation protocol of 3mL 17% EDTA and full-strength sodium hypochlorite (NaOCl) prior to placement of $Ca(OH)_2$ allows for the optimal diffusion of hydroxol ions (Saif, 2008).

An important property of $Ca(OH)_2$ is its antimicrobial effect. Hydroxyl ions from the diffusion of the medicament can destroy bacterial cell membranes and interact with bacterial DNA replication (Siqueira, 1999). Calcium hydroxide hydrolyzes the lipid A moiety of lipopolysaccharide (LPS), displaying the ability to destroy Gram negative bacteria often implicated in endodontic infections (Safavi & Nichols, 1993). Similar conclusions were found in a dog model;

$Ca(OH)_2$ was shown to be capable of detoxification of lipopolysaccharides and thereby, minimized inflammation and resorption (Silva, 2002). Calcium hydroxide is also capable of detoxifying lipoteichoic acid (LTA) from gram positive microbes, specifically *E. faecalis* at low concentrations and short time periods (Baik, 2008). The use of $Ca(OH)_2$ to effectively kill microbes can be predictably achieved with a one-week dressing inside the canal (Sjogren, 1991). However, $Ca(OH)_2$ is not effective against all bacterial species and microbes can survive and persist after its use as an intracanal medicament (Siqueira, 1999). Calcium hydroxide has been found to decrease bacterial levels in isthmuses and lateral canals when used as an intracanal medicament (Vera, 2012). It has also been demonstrated to need up to 60 days to kill cultures of common endodontic microbiota (*S. aureus, E. faecalis, P. aeruginosa, B. subtilis, C. albicans*) with saline as an irrigant (Estrela, 2003). Candida species have displayed more resistance to $Ca(OH)_2$ than *E. faecalis* species (Waltimo, 1999).

Calcium hydroxide is effective at dissolution of necrotic tissue (Hasselgren, 1988). It has demonstrated the ability to induce swelling in pulpal tissue, thereby increasing the tissue dissolving ability of sodium hypochlorite (Turkun, 1997). Both ultrasonics and $Ca(OH)_2$ use are effective in debridement of the canal and are better at dissolving tissue than instrumented canals and an irrigation of 2.6% NaOCl alone (Metzler & Montgomery, 1989). There is, however, no technique which fully removes all $Ca(OH)_2$. Use of irrigation alone

leaves the most remaining calcium hydroxide (Phillips, 2015), while using rotary instruments and/or ultrasonics combined with irrigation and hand files has been showed to produce cleaner canal (Kenee, 2006).

Historically, $Ca(OH)_2$ has been the medicament of choice in vital pulp therapy (Dumsha, 1985). In direct pulp capping procedures, the $Ca(OH)_2$ is placed in direct contact with the vital pulp, and a lower pH formulation is ideal, as this limits the potential for coagulative necrosis (Stanley, 1989). Studies in monkeys demonstrated that Dycal® (Dentsply Sirona, York, PA) was the preferred medium and induced differentiation of odontoblasts and a dentin bridge of good quality (Tronstad, 1974). Later research via SEM evaluation determined that the dentin bridge formed with $Ca(OH)_2$ was porous and leaked extensively (Goldberg, 1984). In a ten-year retrospective study, direct pulp capping with $Ca(OH)_2$ had an 80% failure rate (Barthel, 2000); thus, BECs have emerged as a better option with greater success rates and faster and more predictable dentin bridge formation with virtually no microleakage (Paranjpe, 2011).

More recent endodontic literature points toward the use of $Ca(OH)_2$ as a viable treatment option in guided endodontic repair (GER). In a rat model, triple antibiotic paste (TAP) and $Ca(OH)_2$ were compared to assess biocompatibility in non-vital permanent immature teeth. Findings suggested both are biocompatible

and exhibited moderate reactions at days 7 and 15, but mild reactions from day 30 onward (Gomes-Filho, 2012). Calcium hydroxide has been shown to have no detrimental effect on stem cells of the apical papilla (SCAP), of which the preservation of is critical in GER cases, while higher concentrations of triple and double antibiotic paste (TAP and DAP) demonstrated detrimental effects on SCAP (Ruparel, 2012). Concentrations of TAP or DAP of 1000mg/mL resulted in no viable SCAP; doses at 1mg/mL displayed no adverse effects on cell viability, but $Ca(OH)_2$ was shown to significantly increase SCAP survival and proliferation (Althumairy, 2014). The survivability and proliferation of SCAP, as well as no risk of discoloration compared to TAP and DAP, seen with $Ca(OH)_2$ makes it the current best choice in GER cases (Nagata, 2014).

After traumatic injuries, specifically avulsions, the use of $Ca(OH)_2$ acts as a temporary obturation material to be placed in the canal until confirmation of an intact periodontal ligament presents. Time of usage may be longer if the traumatic injury occurred more than two weeks prior to treatment and has been shown to reduce the risk of ankylosis in traumatized teeth with a damaged periodontal membrane (Lengheden, 1990). In a dog model, short-term (1 week) and long-term (8 week) treatment with $Ca(OH)_2$ displayed similar healing patterns, provided endodontic treatment is initiated two weeks after replantation of an avulsed tooth (Trope, 1992). In the same model it was later found that long-term use and "refreshing" the $Ca(OH)_2$ allows for better cemental repair (Trope, 1995). Similar

findings suggest pulp extirpation 7-10 days after avulsion followed by $Ca(OH)_2$ dressing for one month prior to obturation reduced the risk of ankylosis without increasing the risk of root resorption (Gregoriou, 1994). Extra-oral dry time factors into reducing the risk of external root resorption. If the extra-oral time is 25 minutes or more, any advantage of a $Ca(OH)_2$ dressing is minimized provided the tooth is debrided 14-28 days after replantation (Dumsha, 1995). Therefore, the duration of its use in avulsion injuries is controversial and is a clinical decision of the endodontist.

Use of an intracanal medicament of $Ca(OH)_2$ affects dentin as well. In a sheep model, long-term (two months) use of $Ca(OH)_2$ in immature apices displayed a reduction in root fracture strength due to its ability to denature some components of the organic matrix (Andreason, 2002). Similar findings were described in a bovine model with five weeks of $Ca(OH)_2$ intracanal medicament (White, 2002). While in another sheep study, conclusions suggest that six months of $Ca(OH)_2$ did not affect the dentin fracture resistance (Hawkins, 2015).

Changes in pH of radicular dentin occur as a result of $Ca(OH)_2$ as an intracanal medicament (Esberard, 1996). Over the first week, pH starts to increase in outer radicular dentin, with peak pH levels and antimicrobial effects achieved after 2-3 weeks (Nerwich, 1993). This was further confirmed by Chamberlain, et.

al. in which teeth were prepared with artificial radicular cavities at varying lengths and filled fully with $Ca(OH)_2$. The pH peaked at 14 days then gradually dropped off, verifying that $Ca(OH)_2$ provides hydroxol ions capable of diffusion through dentin tubules to the root surface (Chamberlain, 2009).

EndoSequence® BC RRM™ Fast Set Putty:

The literature is replete with articles discussing MTA, and less so with other BECs (Torabinejad, 2018). As previously mentioned, EndoSequence® BC RRM™ Fast Set Putty relatively new to the marketplace and is advertised for use as an alternative to MTA for many procedures including root end filling material, as an apical barrier and in vital pulp therapy. It is hydrophilic, insoluble, radiopaque, aluminum-free and has an alkaline pH (Ma, 2011). The cytotoxicity is low (Alanezi, 2010) and displays similar *in vitro* biocompatibility to MTA (Ma, 2011). EndoSequence® BC RRM™ Fast Set Putty exerts both antimicrobial (Lovato, 2011) and antifungal effects (Alsalleeh, 2014) like that of MTA. In a rat model, both materials induced inflammatory reactions after implantation in subcutaneous tissues but EndoSequence® BC RRM™ Fast Set Putty was less injurious than MTA (Khalil, 2015).

The use of EndoSequence® BC RRM™ Fast Set Putty as a root end filling material has become more prevalent in recent years. It meets the characteristics of an ideal root end filling material as outlined by Kim: tolerated by periapical tissues, displays antimicrobial properties, adheres to the tooth, dimensionally stable, easy to handle and use, non-corrosive, does not stain tissue or the tooth, dissolution resistant, electrochemically inactive, promotes cementogenesis, and is radiopaque (Kim, 2001). Surgical outcomes are similar when comparing MTA to EndoSequence® BC RRM™ Fast Set Putty. In a canine study, EndoSequence® BC RRM™ Fast Set Putty displayed superior healing to MTA; however, the authors noted this was only appreciable via CBCT and micro-CT imaging modalities (Chen, 2015). The overall success rate has been reported at 92% in a four-year retrospective study (Shinbori, 2015), and can be considered a suitable root end filling material in apical surgery.

Apical barrier placement is indicated for teeth with necrotic pulps, open apices and thin radicular dentin walls (Trope, 2016). The goal is to create an apical stop as a means to contain the obturation material. MTA is frequently used and has demonstrated success in achieving apical closure (Shabahang, 1999; Mente, 2009), and at a higher rate than traditional calcium hydroxide apexification procedures (Witherspoon, 2008). EndoSequence® BC RRM™ Fast Set Putty is a possible option without the potential for staining and easier handling

properties; however, only low-level of evidence studies consisting of case reports and case series exists concerning the use of other BECs as apical barriers (Torabinejad, 2018).

Similarly, MTA has become the dressing of choice for vital pulp therapy. It has a higher success rate, greater and more predictable dentin bridge formation and less inflammation to the pulp than $Ca(OH)_2$ in direct pulp capping procedures (Li, 2015). EndoSequence® BC RRM™ Fast Set Putty displays similar capabilities as MTA but without the same extent of coronal discoloration (Shi, 2016). Coronal color stability was maintained for as long as two months post-treatment in a comparison with various forms of MTA (Marconyak, 2016). In an *in vivo* study comparing materials for direct pulp capping procedures, MTA and EndoSequence® BC RRM™ Fast Set Putty induced dentin bridge formation without the pulpal inflammation seen with $Ca(OH)_2$, but no discoloration was observed with EndoSequence® BC RRM™ Fast Set Putty (Shi, 2016). The cytotoxicity of MTA and EndoSequence® BC RRM™ Fast Set Putty display similar levels, while Dycal® (Dentsply Sirona, York, PA) has shown to increase cytotoxic effects (Hirschman, 2012). Success rates in the endodontic literature for EndoSequence® BC RRM™ Fast Set Putty is minimal, but long-term outcomes for MTA have been reported at 80.5%, while only 59% were successful with

calcium hydroxide, with a 2.5 times greater failure rate versus MTA (Mente, 2014).

Treatment for external inflammatory resorption has traditionally been calcium hydroxide as an intracanal medicament with fewer studies in the endodontic literature evaluating BECs, including EndoSequence® BC RRM™ Fast Set Putty. Most published literature using BECs as treatment options in resorption are case reports and case series (Torabinejad, 2018). MTA has demonstrated better outcomes when compared to $Ca(OH)_2$ when used to treat inflammatory resorption in animal models (Maráo, 2012).

There is a lack of alternative treatment options for external inflammatory resorption in our literature. The alkaline pH and biocompatibility of BECs may offer the endodontist options aside from $Ca(OH)_2$. In an *in vitro* model, MTA showed small but statistically significantly higher pH levels in simulated resorptive defects as compared to $Ca(OH)_2$ over a four-week period (Heward, 2011). The potential clinical implications could mean the diffusion of the hydroxol ions from MTA may result in an arrest in the progression of external inflammatory resorptive lesions. Similar studies are needed to determine if the same is true with other BECs. This leads to the current study, which follows methodology from previous literature (Tronstad, 1981; Saif, 2008; Chamberlain

2009; Heward, 2011) to to evaluate pH levels in simulated resorptive defects over a four-week period using EndoSequence® BC RRM™ Fast Set Putty.

HYPOTHESIS AND SPECIFIC AIM

The null hypothesis was as follows:

There is no difference in hydroxyl ion diffusion through dentin in teeth with simulated root surface resorption defects filled with intracanal EndoSequence® BC RRM™ Fast Set Putty as compared to $Ca(OH)_2$ as determined by measuring pH over time.

The specific aim was to determine pH changes in teeth with simulated resorptive defects by comparing pH values of $Ca(OH)_2$ with EndoSequence® BC RRM™ Fast Set Putty over set time frames as a means of an alternative treatment option to external inflammatory resorptive lesions.

MATERIALS AND METHODS

An Institutional Review Board (IRB) form was submitted with the IRB office at the University of Minnesota. The IRB reviewed the submission and determined the study was not human research; no further action was required.

A review of the literature indicated the sample size needed for the current comparison study (Tronstad, 1981; Chamberlain 2009; Heward, 2011). It was determined that a sample size of twenty-one per group and seven control teeth would be able to detect at a power or 80% a difference at the 0.05 level of significance using repeated-measures analysis of variance (ANOVA) to evaluate changes in pH over time in the control and experimental ($Ca(OH)_2$ and EndoSequence® BC RRM™ Fast Set Putty) groups, and paired *t* tests to compare between the experimental groups.

Single-rooted, single-canaled extracted teeth were collected from a sample repository managed by the Division of Oral Surgery and Division of Endodontics at the University of Minnesota. The teeth were stored in thymol until use for the study. After screening the specimens were stored in 0.9% sodium chloride (NaCl) (Baxter, Deerfield, IL; LOT # G132340) until use.

Tooth Selection and Preparation:

Forty-nine maxillary and mandibular anterior teeth total were selected for the study, with an additional three used for a pilot study. The teeth were paired by tooth type. Periapical radiographs were taken of all specimens to determine canal anatomy prior to preparation. Twenty-one matched paired teeth were selected per treatment group; 21 assigned to the $Ca(OH)_2$ group and 21 assigned to the EndoSequence® BC RRM™ Fast Set Putty group, with 7 teeth as controls to be filled with 0.9% NaCl. The teeth were stored in labeled glass 1.5-ounce vials of the same batch of 0.9% NaCl (pH 5.5) until use and between preparations.

A pilot study using three teeth, one each filled with $Ca(OH)_2$, EndoSequence® BC RRM™ Fast Set Putty group and 0.9% NaCl was completed using the protocol outlined below. pH readings were taken and recorded at 3 hours and 24 hours to ensure the proper methodology and functioning equipment.

For preparation of the specimens, any soft tissue remnants were removed carefully with an explorer and teeth were soaked in 5.25% NaOCl for 30 minutes, then rinsed with distilled water. Decoronation was performed using a serrated diamond disk (Brasseler, Savannah, GA) and to a standard length of 14mm. The

working lengths were set at 14mm, and a glide path was confirmed with #8 or #10 K-file depending on canal anatomy. Initial flaring of the canal was completed using a Gates-Glidden #2. Instrumentation of the root canal system was completed using nickel-titanium rotary files of K3XF (Kerr, Orange, CA) in a crown-down method to a final apical preparation of 50.04. This canal size was standardized to allow for similar needle irrigation fit and depth and in accordance with the prior studies from Heward, et. al. and Chamberlain, et. al. Canals were irrigated using ProRinse tips (Dentsply Sirona, York, PA) with 3mL of 5.25% NaOCl for 30 seconds between each file. Final irrigation was completed with 3mL of 17% ethylenediaminetetraacedic acid (EDTA) to remove the smear layer, followed by 3mL 5.25% NaOCl and 3mL distilled water.

External cavities were prepared on each tooth on the buccal surface according to the protocols of Heward, et. al. and Chamberlain, et. al. A high-speed #4 round bur (Brasseler, Savannah, GA) was used to prepare a cavity 0.7mm deep and 1.4mm in diameter on the root surface at 5mm from the apex. The cavities were rinsed with 3mL 17% EDTA for smear layer removal for 60 seconds followed by 3mL distilled water.

Intracanal Placement of Calcium Hydroxide and EndoSequence® BC RRM™ Fast Set Putty:

The teeth from the 21 matched pairs were randomly assigned to one of two groups (Figure 4), $Ca(OH)_2$ placement using UltraCal® XS (Ultradent, South Jordan, UT; LOT #BF JMY) and Endosequence® BC RRM™ Fast Set Putty (Brasseler, Savannah, GA; LOT #1801FSPS). Prior to placement of filling materials, the teeth were removed from their vials of 0.9% NaCl and gently blotted with a paper towel. The canals were dried with paper points (course and medium). Calcium hydroxide was delivered into the canals using a Navi-Tip® (Ultradent, South Jordan, UT) placed at the apical stop (14mm) and slowly backed out up to a depth of 10mm from the apex. EndoSequence® BC RRM™ Fast Set Putty was placed on a sterile glass slab, transferred into the canals using the flat end of a sterile instrument and using a sterile Dovgan plugger size 35-45.04 and 60-80.04 (Integra Miltex, York, PA), condensed to fill the canal to the level of 10mm from the apex. Placement was evaluated radiographically (Figure 5). Control teeth were filled with 0.9% NaCl. Coronal access openings were filled with Cavit™ G (3M ESPE, Seefeld, Germany, LOT #664890) to a depth of approximately 3.5mm. The apical 3mm of the root was covered with yellow sticky wax to seal the apical foramen. The coronal portion of the tooth was attached to the internal surface lid of a 1.5-ounce clear glass vial (Figure 6). The lids with attached teeth were placed on the vials and filled with 0.9% NaCl (same lot number as used prior), to fully submerge the root.

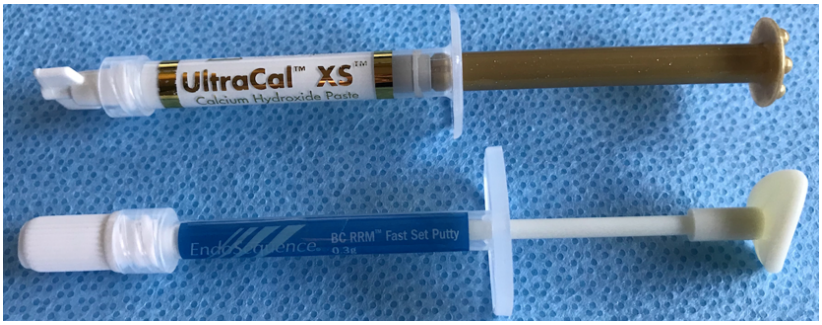

Figure 4. UltraCal® XS and EndoSequence® BC RRM™ Fast Set Putty

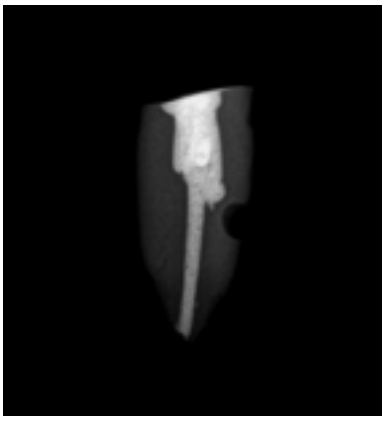

Figure 5. Radiograph of sample filled with EndoSequence® BC RRM™ Fast Set Putty

Figure 6. Vial lid with mounted root

pH Measurements:

pH measurements were recorded at 3 hours, 24 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks. Each vial was stirred for 10 seconds and pH was recorded using the Thermo Scientific Orion Star A211 pH benchtop meter (Figure 7) (Sigma-Aldrich, St. Louis, MO). The tip of the meter was wiped with a Kimwipe® (Sigma-Aldrich, St. Louis, MO) between readings. All pH readings were recorded and vial lids replaced immediately after recording. All specimens were stored in their labeled vials in the same solution of un-buffered isotonic 0.9% NaCl in an incubator (Thermo Scientific Heratherm™ Incubator; Waltham, MA) at 37°C between pH readings. The pH meter was calibrated to pH values of 4.01, 7 and 10.01 at the start of each session.

Figure 7. Thermo Scientific Orion Star A211 pH benchtop meter

RESULTS

Pilot Study:

pH readings from the pilot study are shown in the table below:

Pilot Data	Control	$Ca(OH)_2$	EndoSequence® BC RRM™ Fast Set Putty
pH 3hr	7.50	7.71	7.78
pH 24hr	7.51	7.73	7.81

Table 1. pH readings from pilot study

Statistical analysis was completed using Minitab® software (State College, PA). A paired *t* test was used to evaluate the experimental groups ($Ca(OH)_2$and EndoSequence® BC RRM™ Fast Set Putty) by comparing pH values at 3 hours and 24 hours; significance was set at $P < 0.05$. The results showed a $P > 0.05$, which is not statistically significant for this time period.

Repeated-measures analysis of variance (ANOVA) was used to evaluate the changes in pH over 3 hours ad 24 hours in the control group and experimental

groups. Results showed no significant difference in pH in the groups at these time intervals ($P > 0.05$).

The pilot study compared pH levels at only two time periods, thus requiring readings at longer time intervals in order to determine if statistically different changes in pH might exist between the groups.

Final Experiment:

pH readings from the final experiment are located in Appendices A and B.

Statistical analysis was completed using Minitab® software (State College, PA). Paired *t* tests were used to evaluate effects of the experimental groups ($Ca(OH)_2$and EndoSequence® BC RRM™ Fast Set Putty) by comparing pH values at three hours, twenty-four hours, one week, two weeks, three weeks and four weeks. Repeated-measures analysis of variance (ANOVA) was used to evaluate the changes in pH values at three hours, twenty-four hours, one week, two weeks, three weeks and four weeks within the control and experimental groups; significance was set at $P < 0.05$.

The mean pH values for the control group and experimental groups were calculated and are graphically represented in Figures 8 through 11.

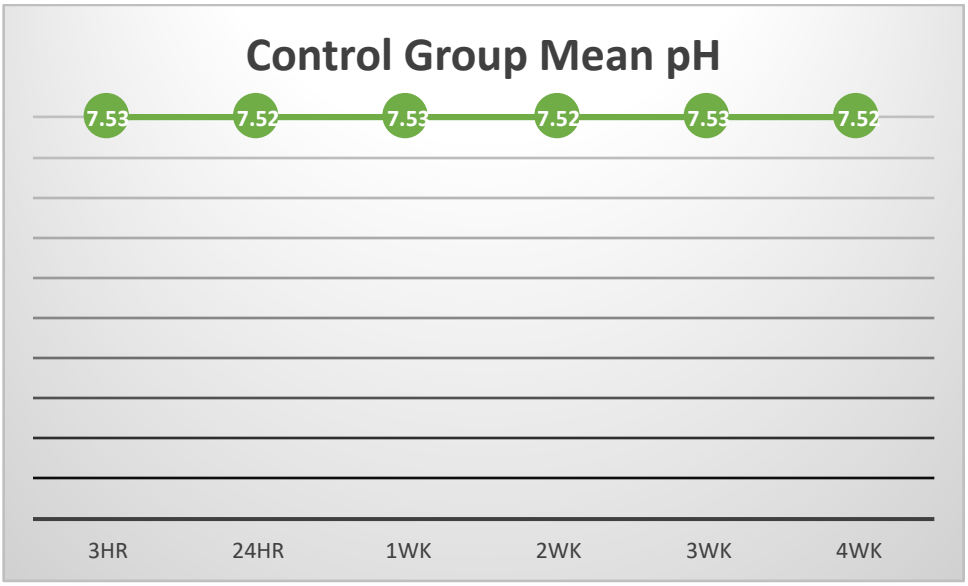

Figure 8. Control group mean pH values

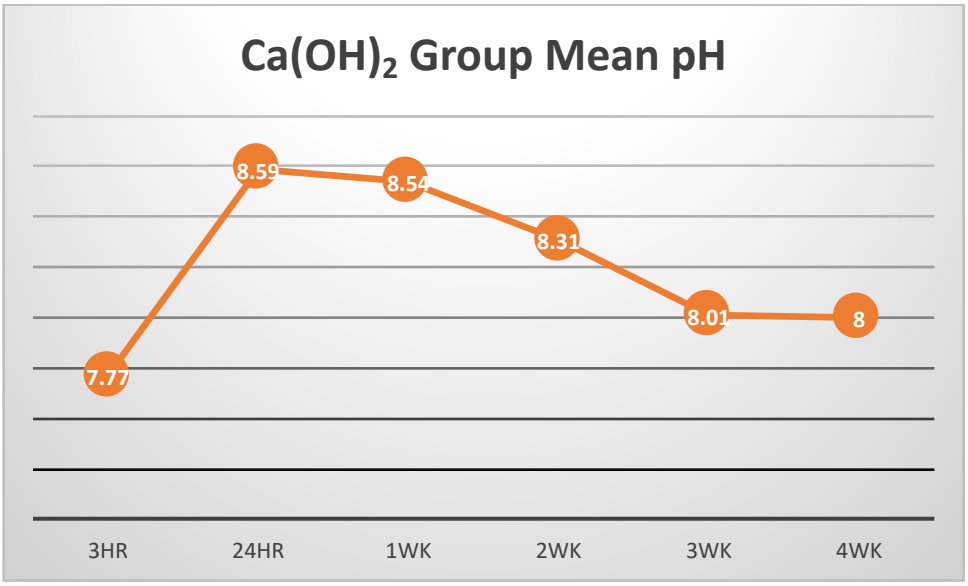

Figure 9. Calcium hydroxide group mean pH values

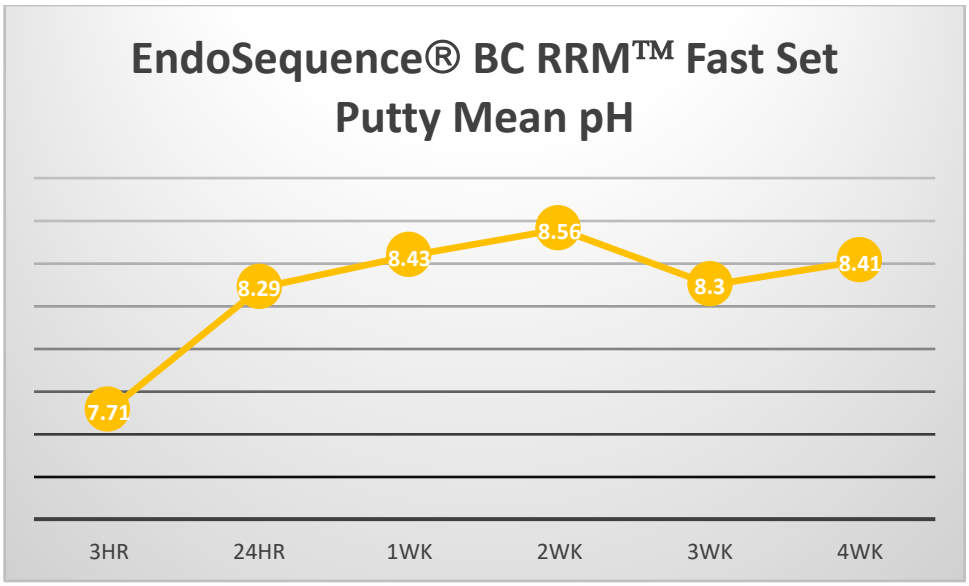

Figure 10. EndoSequence® BC RRM™ Fast Set Putty group mean pH values

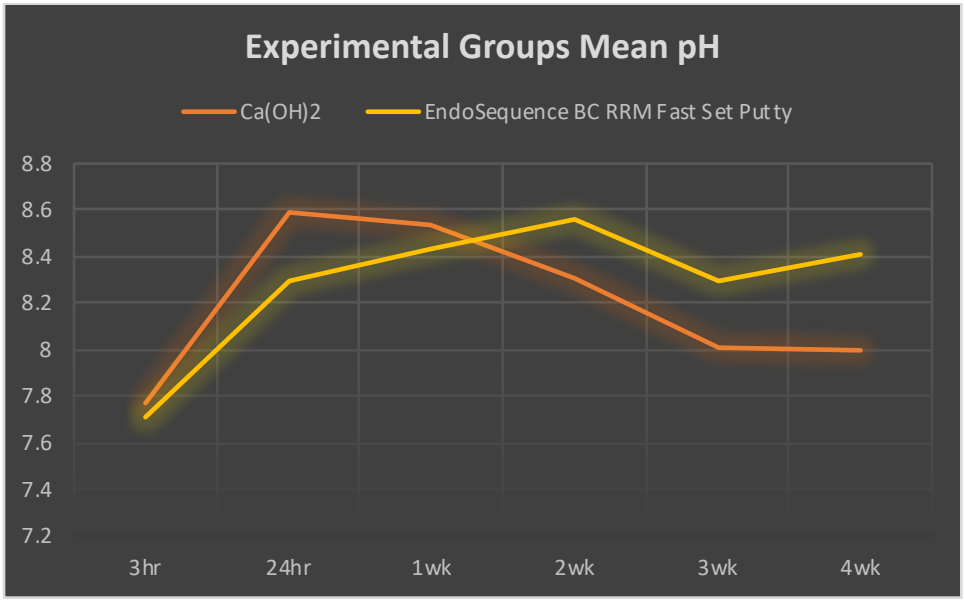

Figure 11. Calcium hydroxide and EndoSequence® BC RRM™ Fast Set Putty groups mean pH values

Control Group:

The pH readings for the control group did not differ significantly during the experimental period of four weeks ($P > 0.05$) using repeated-measures ANOVA.

Experimental Groups:

Intragroup Analysis:

Significant changes in pH occurred over time within the EndoSequence® BC RRM™ Fast Set Putty group ($P < 0.001$) and the $Ca(OH)_2$ group ($P < 0.001$) using repeated-measures ANOVA. In the EndoSequence® BC RRM™ Fast Set Putty, pH differed significantly between 3 hours and 24 hours, 3 hours and 1 week, 3 hours and 2 weeks, and between 3 hours and 4 weeks (all $P < 0.001$). In the $Ca(OH)_2$ group, pH differed significantly between 3 hours and 24 hours and between 3 hours and 1 week (both $P < 0.001$).

Intergroup Analysis:

During the experimental time period of four weeks, the overall mean pH of the EndoSequence® BC RRM™ Fast Set Putty (8.28) was slightly higher than that of the $Ca(OH)_2$ group (8.20). A paired *t* test for overall means showed no significant difference between the groups ($P = 0.498$).

Additional paired *t* tests compared the EndoSequence® BC RRM™ Fast Set Putty with the $Ca(OH)_2$ group at each time point (see Table 2). At the 24-hour mark, the mean pH was significantly higher in the $Ca(OH)_2$ group (8.59) compared to the EndoSequence® BC RRM™ Fast Set Putty (8.29; $P = 0.019$). All other time points intergroup mean pH differences were not significantly different.

3hr	***P* = 0.508**
24 hr	***P* = 0.019**
1 wk	***P* = 0.398**
2 wk	***P* = 0.267**
3 wk	***P* = 0.238**
4 wk	***P* = 0.101**

Table 2. Results of paired *t* tests for intergroup analysis

DISCUSSION

The study showed that over the four-week time period significant pH changes occurred within both experimental groups. By comparison, only at the twenty-four-hour reading was the pH significantly higher in the $Ca(OH)_2$ group. All other time points displayed no significant differences in pH between experimental groups. The overall mean pH readings of the $Ca(OH)_2$ group (8.20) and the EndoSequence® BC RRM™ Fast Set Putty group (8.28) were similar for the four-week duration of the study.

One of the first studies to examine diffusion of hydroxol ions in teeth filled with $Ca(OH)_2$ was performed on extracted and replanted monkey teeth (Tronstad, 1981). A color indicator strip was placed over the roots and the pH was recorded by comparing color to corresponding pH. Readings in the peripheral dentin were found to be in the pH range of 7.4-9.6, comparable to present methodology using an electronic pH meter.

In similar models, calcium ion diffusion through dentin of teeth filled with MTA or $Ca(OH)_2$ was measured in simulated external root resorption lesions (George, 2009; Heward, 2011). This is the first study that evaluated hydroxol ion

diffusion from intracanal placement of EndoSequence® BC RRM™ Fast Set Putty by measuring pH levels over a four-week period.

In this study, pH readings were taken in the immersion media holding the samples, similar to studies from Saif, et. al. and George, et. al. As in the Saif, et. al. study, the vials were stirred for ten seconds prior to taking a pH reading. This aspect of the methodology differed from Chamberlain, et. al. and Heward, et. al. which used a microelectrode to read the pH in the simulated resorptive lesion. In these two studies, the authors rinsed the simulated defects with distilled water prior to taking a pH reading. In the study by Heward, et. al., the authors found that the size of the simulated resorptive defect was problematic, as fluid evaporation occurred prior to stabilization of the pH microelectrode probe. In this study, the simulated defect was made larger as a means to address the issue of evaporation when using the microelectrode attachment. However, findings were comparable in both methodologies, that is, use of a microelectrode versus measuring the pH directly in the immersion fluid.

All materials used in this study (UltraCal® XS calcium hydroxide, EndoSequence® BC RRM™ Fast Set Putty, Cavit™ G and 0.9% NaCl) were from the same lot numbers. This was done to standardize the experiment. The Cavit™ G

was placed to a depth of approximately 3.5mm, as this is the minimum thickness of Cavit™ G needed to prevent leakage (Beach, 1996).

In the present study, 0.9% NaCl was placed in the vials containing the samples. The samples were stored in an incubator at 37°C between all readings to simulate the normal temperature of the oral cavity and approximate clinical conditions for this *in vitro* study. The saline was not changed throughout the experiment in concordance with the methodology from Chamberlain, et. al. and Heward, et. al. It is possible that by not changing the solution, a potential for equilibration in a static solution may occur. As noted by Heward, et. al., future studies may elect to change the solution more frequently to simulate the normal circulation or oral fluids; however, this may not result in comparable pH readings to past studies using previously described methodology.

The ability of hydroxol ions to diffuse through dentin is hampered by the buffering capability of the dentin (Wang & Hume, 1988), especially if the cementum is intact (Nerwich, 1993). Previously published studies have shown conflicting evidence in support of the removal of the smear layer as a method to enhance hydroxol ion diffusion (Foster, 1993; Deardorf, 1994; Saif, 2008). In this study, as well as that of Chamberlain, et. al. and Heward, et. al., the smear layer was removed from the canal space as well as the simulated external root resorptive

cavities with 17% EDTA. The objective for removal of the smear layer from the cavities was to allow for optimal hydroxol ion diffusion through dentin, though it is unknown *in vivo* if the smear layer is present in external inflammatory root resorption.

After damage to the cementum and the subsequent infection of the pulpal tissue, external inflammatory root resorption may occur (Andreason, 1981). Traditionally, $Ca(OH)_2$ has been used to stop the resorption process by inhibiting osteoclastic activity (Haga & Stern, 1993). Its antimicrobial abilities (Siqueira, 1999) address microbial infection within the canal. Calcium hydroxide can reduce the penetration of microbes via the dentinal tubules to the resorptive defect. If cementum can regenerate over dentin exposed to resorption, the process of external inflammatory resorption will cease (Andreason, 1981). In the 1980s it was first suggested that $Ca(OH)_2$ possesses the ability to influence localized root resportion by increasing pH (Tronstad, 1981). The findings of this study demonstrated, like that of Chamberlain, et. al. and Heward, et. al., that the peak pH in the experimental groups was observed in the first half of the experiment. The EndoSequence® BC RRM™ Fast Set Putty group peak pH occurred at the two-week mark and decreased by week four. The $Ca(OH)_2$ group peaked earlier than the two-week mark in this study. This finding differed than that of Chamberlain, et. al. and Heward, et. al., but the trend of pH decrease in the $Ca(OH)_2$ group was similarly noted in this study.

The findings are in accordance with the recommendation of four-weeks of $Ca(OH)_2$ to be used as an intracanal medicament (Chamberlain, 2011). As stated previously, earlier research found peak pH readings for $Ca(OH)_2$ are observed at the two-week mark; future research could be directed toward shorter time frames for $Ca(OH)_2$ intracanal medicament usage as a means of harnessing optimal hydroxol ion diffusion, as was found in this study.

Further research could be focused on determining a baseline of the diffusion of hydroxol ions without teeth in vials. Similarly, future research methodology could address possible hydroxol ion diffusion from other areas of the root. This would be accomplished by covering the root with varnish or nail polish. A standardized volume of saline solution per vial may provide further consistency in data collection. SEM imaging of the dentin of the sample teeth before and after treatment would be an useful adjunct to analyzing depth of dentin penetration.

EndoSequence® BC RRM™ Fast Set Putty is a BEC used in many of the same procedures with which MTA is employed. EndoSequence® BC RRM™ Fast Set Putty has been shown to be minimally cytotoxic (Alanezi, 2010), antimicrobial (Lovato, 2011), and displays an alkaline pH which may be useful in treating resorptive lesions.

In the *in vitro* model from Heward, et. al., MTA displayed significantly higher pH levels in simulated resorptive defects when compared to $Ca(OH)_2$ over a four-week period. Their findings suggested potential use of MTA in cases of external inflammatory root resorption due to its hydroxol ion diffusion and good sealing ability. Based on the results of this study, EndoSequence® BC RRM™ Fast Set Putty displayed comparable hydroxol ion diffusion as that of $Ca(OH)_2$. The overall mean pH for the experimental period was similar in both groups. EndosSequence® BC RRM™ Fast Set Putty displayed a higher overall mean pH, but this was not statistically significant ($Ca(OH)_2$= 8.20; EndoSequence® BC RRM™ Fast Set Putty = 8.28; $P = 0.498$). The only statistically significant pH difference in the root surface cavities filled with Endosequence® BC RRM™ Fast Set Putty compared to the root surface cavities filled with $Ca(OH)_2$ occurred at the twenty-four-hour reading, with the $Ca(OH)_2$ group significantly higher ($Ca(OH)_2$ = 8.59; EndoSequence® BC RRM™ Fast Set Putty = 8.29; $P = 0.019$). This could be due to potentially quicker dissociation of hydroxol ions in the aqueous vehicle used in this study as compared to other vehicles for $Ca(OH)_2$. EndoSequence® BC RRM™ Fast Set Putty requires moisture to set. The amount required is naturally present in dentin per manufacturer literature. The moisture requirement may impact on the rate of hydroxol ion diffusion. The experimental comparison group in both this study and that of Heward, et. al. was $Ca(OH)_2$. Both studies concluded that the BEC used displayed similar hydroxol ion diffusion to the gold

standard $Ca(OH)_2$. Future studies could compare $Ca(OH)_2$ with experimental groups of EndoSequence® BC RRM™ Fast Set Putty and MTA.

EndoSequence® BC RRM™ Fast Set Putty is a BEC with good handling properties, an alkaline pH, and faster set time and less staining potential when compared to MTA. If used to treat cases of external inflammatory root resorption, EndoSequence® BC RRM™ Fast Set Putty would be placed as an obturation material, as opposed to the multiple rounds of intracanal medicament placed prior to obturation with traditional $Ca(OH)_2$ therapy. Filling the canal with EndoSequence® BC RRM™ Fast Set Putty may reduce the potential risk of root fracture as seen with both long- and short-term use of $Ca(OH)_2$ (Andreason, 2002; Sahebi, 2010). Further studies are needed to determine if EndoSequence® BC RRM™ Fast Set Putty is a viable treatment modality for external inflammatory root resorption *in vivo*.

CONCLUSIONS

The null hypothesis was rejected because the samples containing $Ca(OH)_2$ displayed significantly higher pH values in simulated root surface cavities as compared to EndoSequence® BC RRM™ Fast Set Putty samples over a twenty-four-hour time frame ($Ca(OH)_2$ = 8.59; EndoSequence® BC RRM™ Fast Set Putty = 8.29; P = 0.019) and no other significant differences were noted between the experimental groups for during the study's duration. Since there were no statistically significant differences with EndoSequence® BC RRM™ Fast Set Putty and $Ca(OH)_2$, the EndoSequence® BC RRM™ Fast Set Putty can be considered an alternative to the gold standard calcium hydroxide in the clinical management of external inflammatory resorption.

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APPENDIX A

UNIVERSITY OF MINNESOTA ORAL AND FACIAL SURGERY

515 Delaware St. SE
7th Floor-Maos Tower
Minneapolis, MN 55455
(612) 624-8600

7/10/17

To Whom It May Concern:

The Department of Oral Surgery has given permission for Dr. Katie Kickertz to use extracted teeth specimens for research purposes within the Division of Endodontics.

Sincerely,

Dr. Robert Nadeau

APPENDIX B

CONTROL	1	2	3	4	5	6	7	Mean
3hr	7.55	7.54	7.56	7.55	7.52	7.51	7.51	7.53428571
24hr	7.5	7.53	7.55	7.54	7.54	7.52	7.5	7.52571429
1wk	7.52	7.55	7.54	7.53	7.57	7.52	7.5	7.53285714
2wk	7.51	7.54	7.54	7.52	7.54	7.51	7.5	7.52285714
3wk	7.53	7.54	7.54	7.55	7.52	7.51	7.5	7.52714286
4wk	7.5	7.52	7.55	7.55	7.52	7.51	7.49	7.52

APPENDIX C

CALCIUM HYDROXIDE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean
3hr	7.69	7.67	7.63	8.01	8.04	7.96	7.85	8.14	7.89	8.23	7.36	8.01	7.84	8.04	7.67	7.73	7.24	7.36	7.29	7.51	7.92	7.76571429
24hr	7.51	7.76	7.64	10.11	8.82	7.82	7.98	9.04	8.29	8.4	8.95	8.71	8.91	8.76	8.86	8.96	8.8	8.92	8.8	8.51	8.76	8.58619048
1wk	7.75	7.23	8.13	10.18	8.19	8.3	7.01	8.84	8.3	8.22	9.17	8.63	8.45	8.78	8.89	9.13	9.02	8.92	8.88	8.47	8.79	8.53714286
2wk	7.28	6.94	7.11	10.01	8.33	7.79	6.73	7.29	7.42	7.08	9.28	8.68	8.67	8.77	8.96	9.36	9.16	9.19	8.98	8.54	9.02	8.31380952
3wk	7.07	6.63	6.52	9.22	8.22	7.65	6.7	6.91	6.83	6.61	9.12	8.46	8.47	8.58	7.54	9.21	9.01	9.08	8.97	8.51	9	8.0147619
4wk	7.16	6.75	6.7	9.1	8.4	7.07	6.77	6.99	7.06	6.81	9.23	8.49	8.75	7.76	6.98	9.29	9.02	9.1	8.95	8.75	9.04	8.00809524

ESRRM PUTTY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean
3hr	7.21	7.71	7.65	7.78	7.79	7.59	7.48	7.64	7.59	7.72	7.21	8.2	7.68	8.01	7.89	7.54	8.03	7.65	7.82	7.92	7.88	7.71380952
24hr	7.88	7.69	7.76	8.06	7.95	7.77	7.62	8.09	7.76	8.6	8.87	8.65	8.81	8.57	8.71	8.58	8.38	8.68	8.76	8.78	8.18	8.29285714
1wk	8.39	8.03	8.01	8.77	8.17	7.89	6.95	8.68	7.72	9.08	9.09	8.78	8.82	8.61	9.11	8.64	8.09	8.56	9.14	8.59	7.92	8.43047619
2wk	8.96	8.16	7.13	8.89	8.48	8.35	6.92	9.56	7.04	9.42	9.24	9.19	9.19	8.64	9.44	8.76	8.18	8.91	9.3	8.62	7.46	8.56380952
3wk	8.85	8.32	6.75	8.8	8.34	7.63	6.24	9.25	6.32	9.05	8.92	8.91	9	8.4	9.07	8.71	8.07	8.86	9.03	8.37	7.41	8.3
4wk	8.45	8.31	6.93	8.94	8.49	7.84	6.51	9.52	6.23	9.12	9.01	9.01	9.02	8.48	9.18	8.88	8.53	8.91	9.11	8.44	7.6	8.4052381