HISTOLOGY OF CALCIUM ALUMINATE VS. TRICALCIUM SILICATE IN PULPAL AND PERIRADICULAR CONTACT

A Thesis

by

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ABSTRACT

NeoMTA Plus (Avalon Biomed Inc., Bradenton, FL) is a tricalcium silicate material similar to the first MTA product, ProRoot MTA (Dentsply, Tulsa Dental, Tulsa, OK) but with improvements such as decreased setting time, increased ion release, increased water sorption and decreased porosity. Quick-Set2 (Avalon Biomed Inc., Bradenton, FL) is a newly formulated calcium aluminosilicate material that has a faster setting time, increased acid resistance and is non-discoloring. The purpose of this study was to compare healing of pulpal and periapical tissues after exposure to NeoMTA Plus and Quick-Set2 following pulpotomy, root canal and root-end surgery procedures.

One hundred eight teeth (36 for each procedure) in 6 Beagle dogs were isolated, accessed and treated with pulpotomy, root canal or root-end surgery. The materials for pulpotomy and root-end surgery were mixed to a putty-like consistency and the materials for root canals were mixed to a sealer consistency. The dogs were sacrificed at 90 days and the teeth and surrounding tissues were prepared for histological evaluation. Eighty-five teeth were evaluated and scored histologically. Specific for each procedure, specimens were scored for inflammation, quality and thickness of dentin bridging, pulp tissue response, cementum and PDL formation and apical bone healing.

Both materials displayed favorable healing at 90 days. The only significant difference was higher quality of dentin bridge formation in pulpotomies exposed to NeoMTA Plus. Quick-Set2 and NeoMTA Plus had similar effects on inflammation, dentin bridge formation, pulp response, PDL and cementum formation and apical tissue healing in dogs.

DEDICATION

This thesis is dedicated to beautiful wife, Lindsey, whose love and support has been unwavering. To my Mother and Father who have instilled in me the importance of education. My Mother, who herself returned to pursue her Master's degree, showed me learning is a life-long journey. I will forever be grateful for the guidance of Dr. Karl Woodmansey; without him this endeavor would have never left the ground. I would like to thank my committee chair Dr. Lynne Opperman and my committee members, Dr. Kathy Svoboda, Dr. Jenny He and Dr. Karl Woodmansey, for their guidance and support throughout this project.

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This work was supervised by a dissertation committee consisting of Dr. Lynne A. Opperman (committee chair) and Dr. Kathy Svoboda of the Department of Biomedical Sciences, Dr. Jianing He of the Departments of Endodontics and Biomedical Sciences and Dr. Karl F. Woodmansey of the Center for Advanced Dental Education at Saint Louis University.

The procedures performed in Chapter III were completed by Dr. Ryan M. Walsh and Dr. Karl F. Woodmansey. Data analyzed for Chapter III was provided by Dr. Ryan M. Walsh and Dr. Lynne A. Opperman.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND SPECIFIC AIMS

I.1 Introduction

A hydraulic calcium silicate cement is a material commonly composed of tricalcium silicate and tricalcium silicate, although other minor phases may be present. These cements set when mixed with water by a hydration reaction yielding an insoluble cement product. When mixed with a radiopaque powder, such cements are suitable for dentistry. The hydraulic cements are ideal for use in a high moisture environment, such as the mouth. The prerequisites for a material being bioactive are calcium ion release and high pH (>10) for the formation of calcium phosphate compounds on their surface in contact with body fluids. Bioceramic cements possess these properties and are bioactive, which can stimulate tissue regeneration.

The original and gold-standard bioceramic cement used in dentistry is ProRoot MTA (Dentsply, Tulsa Dental Specialties, Tulsa, Oklahoma). Several disadvantages have been identified including, poor handling, long setting time and tooth discoloration. Numerous newer bioceramic cements have been developed to provide easier handling, faster setting, anti-washout properties and a lower material cost than ProRoot MTA. Many of these newer hydraulic cements are based on material compositions similar to ProRoot MTA, but with reformulations to alter the handling, setting or biocompatibility.

Materials such as MTA Angelus (AMTA) (OdontoLogika, Ind. Prod. Odont. Ltda, Londrina, Parana, Brazil), Ortho- and Retro MTA (OMTA) (RMTA) (BioMTA,

Seoul, Republic of Korea), MTA Plus and NeoMTA Plus (MTAP) (Avalon Biomed Inc., Bradenton, FL), Biodentine (Septodont, Lancaster, PA), EndoSequence products (BrasselerUSA, Savannah, GA) have attempted to reduce setting time, reduce washout, improve handling and prevent tooth staining all while maintaining a similar biocompatibility. These materials maintain a similar basic composition, but vary in the amounts and physical characteristics of constituent components. In addition to these calcium silicate-based products, newly developed calcium aluminosilicate products have been introduced to the dental community as alternatives. Calcium aluminosilicates, such as Quick-Set (QS) and Quick-Set2 (QS2) (Avalon Biomed Inc., Bradenton, FL) and EndoBinder (Binderware, Sao Carlos, SP, Brazil) have a different composition and attempt to achieve the same goals of providing improved clinical and chemical biocompatibility.

There is a very small volume of literature exploring the potential benefits of calcium aluminosilicate materials for dental use and no study evaluating the newly reformulated Quick-Set2. Quick-Set2 contains tantalum oxide, rather than iron or bismuth oxides, as a radiopacifier to prevent stained or grayed teeth. The new reformulation also contains less free alumina particles than the original Quick-Set1 formulation. Based on the review of the literature, there is currently no study to evaluate Quick-Set2 and NeoMTA Plus in three distinct treatment modalities including pulpotomies, root canal treatments and root-end fillings. The null hypothesis is that Quick-Set2 will perform as favorably as NeoMTA Plus and, thus, they are both suitable for clinical use under the previously described procedural applications.

I.2 Specific Aims

Aim 1: Evaluate the pulpal healing and repair mechanisms after exposure to the experimental materials in a pulpotomy.

Pulpotomy procedures will simulate identical procedures performed in human permanent and primary teeth. These procedures will be done on the six posterior maxillary premolars.

Aim 2: Evaluate the periapical tissue healing and biological response after exposure to the experimental materials used as sealers in non-surgical root canal treatment.

A single gutta-percha cone endodontic obturation technique will be used to simulate identical procedures in human teeth using the materials mixed as a sealer. This procedure will be accomplished in the mesial roots of the six posterior mandibular premolars and in all six maxillary anterior teeth.

Aim 3: Evaluate the periapical tissue healing and biological responses after these tissues are exposed to the experimental materials following root-end resection

Canal obturation followed by root-end resection (apicoectomy) will assess periapical healing, simulating endodontic surgical procedures performed in humans. The procedures will be accomplished on the distal roots of the six posterior mandibular premolars.

CHAPTER II

LITERATURE REVIEW

II.1 ProRoot MTA

Mineral Trioxide Aggregate (MTA), now marketed as ProRoot MTA (MTA) (ProRoot MTA, Dentsply Tulsa Dental Specialties, Tulsa, Oklahoma), was developed in 1993 by Torabinejad for use as a root-end filling material and was one of the first calcium silicate materials to obtain widespread notoriety as a dental material. The development of MTA was stimulated by a need for a more ideal endodontic material. As one of the first bioactive endodontic hydraulic silicate cements MTA offers some distinct advantages compared to older root-end filling materials such as amalgam, IRM or SuperEBA (Torabinejad Hong et al. 1995; Torabinejad & Pitt Ford 1996). MTA is advocated for use during pulp capping, pulpotomy, apexogenesis, apexification, perforation repair and as a root canal filling material. MTA was initially marketed as gray MTA (GMTA), however, due to concern for discoloration, it was reformulated and white MTA (WMTA) was introduced to the marketplace alongside GMTA (Kratchman 2004; Parirokh & Torabinejad 2010).

According to Torabinejad, an ideal ortho- or retrograde filling material should be non-toxic, non-carcinogenic, biocompatible, insoluble in tissue fluids, dimensionally stable and prevent the ingress or egress of microorganisms into the root canal system (Torabinejad & Pitt Ford 1996; Parirokh & Torabinejad 2010). Clinically, the material should be easy to handle and sufficiently radiopaque to visualize on radiographs (Torabinejad & Pitt Ford 1996; Parirokh & Torabinejad 2010). The setting time of MTA

is 165 minutes with a pH starting at 10.2 and increasing to 12.5 after three hours (Torabinejad Hong et al. 1995).

The chemical composition of MTA has been extensively evaluated. The patent states it is composed of type I Portland cement (PC) in a 4:1 mixture with bismuth oxide to add radiopacity. The type I Portland cement included in the patent contains calcium oxide and silicon oxide. These findings were confirmed independently using both energy dispersive analysis and electron probe microanalysis (Camilleri Montesin et al. 2005; Camilleri 2007). Additionally, WMTA contains gypsum and a small amount of aluminate normally found in Portland cement. As previously mentioned, MTA (Asgary Parirokh et al. 2005)is marketed in two different forms, GMTA and WMTA. Both forms are comprised mainly of di-calcium and tri-calcium silicates. GMTA has a higher mix of the di- and tri-calcium silicates along with the radiopacifier, whereas WMTA mainly contains tri-calcium silicate and the radiopacifier (Camilleri Montesin et al. 2005; Oliveira Xavier et al. 2007). Additionally, GMTA has higher amounts of elements aluminum, iron and magnesium (Asgary Parirokh et al. 2005; Camilleri Montesin et al. 2005).

Clinically the composition of MTA has raised concerns about discoloration. Ex-vivo studies have shown WMTA can cause discoloration in extracted teeth (Felman & Parashos 2013). Contamination with blood in the canal adjacent to the setting WMTA exacerbated the discoloration (Felman & Parashos 2013). Initially, it was hypothesized that iron oxide in WMTA caused the discoloration, although the content is less than gray MTA, it may still be sufficient to discolor teeth (Asgary Parirokh et al. 2005; Felman &

Parashos 2013). Studies comparing staining in extracted teeth filled with MTA, AMTA, ENDOCEM Zr and RetroMTA determined MTA and AMTA use bismuth oxide as a radiopacifier while ENDOCEM Zr and RetroMTA use zirconium oxide to add radiopacity. Consequently, both MTA and AMTA displayed increased discoloration compared to the other experimental materials (Kang Shin et al. 2015). Materials containing zirconium oxide for radiopacifier displayed more color stability and discolored less over time. Based on these findings it was determined the main component responsible for discoloration is the bismuth oxide present in MTA and AMTA (Kang Shin et al. 2015).

When comparing other types of calcium silicates in vitro, MTA has remained the gold standard for comparison. Due to the high pH during and after setting, tissues adjacent to MTA, and like materials, initially undergo necrosis (De Deus Ximenes et al. 2005). The initial changes in pH are attributed to the cell death at early stages of exposure. After the material sets, the pH changes and the cytotoxicity decreased and the cell culture was able to repair resulting in favorable post-operative healing (De Deus Ximenes et al. 2005).

To assess MTA's biocompatibility, it was compared to other root-end filling materials such as IRM, Super EBA (SEBA) and amalgam. Compared to other non-calcium silicate-based materials MTA had superior biocompatibility and performed favorable when exposed to in vitro human tissues (Torabinejad Hong et al. 1995; Keiser Johnson et al. 2000). MTA has been studied for bioactivity among dental pulp stem cell populations for mineralization potential, odontoblastic differentiation, cytotoxicity and

levels of inflammation when cell cultures were exposed to the materials. MTA and similar calcium silicate-based materials all performed favorably and showed signs of alkaline phosphatase activity, formation of mineralized nodules, expression of odontoblastic markers and cell proliferation (Chang Lee et al. 2014).

In vivo animal studies have also supported using MTA for multiple dental uses. Favorable healing is observed when MTA is used as a pulp capping agent in rats and dogs (Kuratate Yoshiba et al. 2008; Kramer Woodmansey et al. 2014). A common finding is adjacent tissue necrosis as a result of the high pH with subsequent deposition of dentin matrix and reorganization of pulp tissue. As early as 14 days after exposure to MTA, dental pulp began showing odontoblast-like morphology with a dentin bridge immediately adjacent to the MTA (Kuratate Yoshiba et al. 2008). Similar findings were observed in a canine model and at 70-days following exposure of pulp tissue to MTA, a thick, cellular and partially organized dentin bridge was noted immediately adjacent to the material. Additionally, the underlying pulp tissue formed odontoblast-like cells and remained organized (Woodmansey KF 2015). Using a primate model to evaluate apexification, MTA induced less periapical inflammation and elicited more hard tissue formation than did calcium hydroxide (Ham Witherspoon et al. 2005). Additionally, MTA has proven beneficial for use as a root-end filling materials in dogs, resulting in superior healing with increased apical bone deposition, minimal inflammation and reformation of apical periodontal structures compared to similarly formulated calcium aluminate cements (Kohout He et al. 2015).

MTA has also shown favorable healing results in humans. 60-days after pulpotomy with MTA or calcium hydroxide, the MTA group showed complete dentin bridge formation in all specimens and there was evidence of odontoblast like cells and pulp fibroblasts formed from the existing pulp tissue (Min Park et al. 2008). Human case series studies have found MTA to have a success rate around 95% when used for both direct pulp capping and pulpotomies (Witherspoon Small et al. 2006; Bogen Kim et al. 2008). In a five year longitudinal study of apical surgeries retrofilled with MTA, Super EBA or Retroplast, treatment with MTA had a significant impact on the success of the teeth (von Arx Jensen et al. 2012). Compared to the other root-end filling materials 86% of teeth filled with MTA were successful after 5 years (von Arx Jensen et al. 2012). A recent meta-analysis concluded the use of MTA is a positive predictor of success in root-end surgeries using modern surgical techniques (Tsesis Faivishevsky et al. 2009).

II.2 MTA Plus

MTA Plus (MTAP) (Avalon Biomed Inc., Bradenton, FL) is a calcium silicate based material that was developed to overcome the previously mentioned drawbacks of MTA. Similar to MTA, MTAP can be purchased in both gray and white versions. MTAP is a tricalcium and dicalcium silicate-based powder containing bismuth oxide that can be mixed with a liquid or a gel (Formosa Mallia et al. 2013; Guven Tuna et al. 2014). The gel improves handling properties and washout resistance of the material (Formosa Mallia et al. 2013; Gandolfi Siboni et al. 2014). When mixed with the gel it can be used as sealer or repair putty and when mixed with liquid it handles similarly to MTA. The calcium silicate powder of MTAP is a finer particle size than WMTA

(Formosa Mallia et al. 2013; DeLong He et al. 2015). The decreased particle size may contribute to a decreased setting time, increased ion release, water sorption and porosity compared to WMTA (Camilleri Formosa et al. 2013; Gandolfi Siboni et al. 2014).

Part of the bioactivity of calcium silicate cements depends on the hydration setting reaction. The setting reaction produces byproducts including calcium hydroxide and water (Camilleri 2007). MTAP has prolonged reactivity, thus, sustaining calcium ion release resulting in a higher pH (Gandolfi Siboni et al. 2014). The extended release of the calcium ions and the thick calcium-phosphate layer aid in biocompatibility *in vitro,* similar to MTA. Calcium ion release, formation of calcium hydroxide and high pH are important in apatite crystal formation and, subsequently, bone and dentin mineralization (Rashid Shiba et al. 2003; Gandolfi Siboni et al. 2014; Camilleri 2015). MTAP has demonstrated equivalent biocompatibility with WMTA in vitro as well as in vivo (Eid Niu et al. 2013; Kramer Woodmansey et al. 2014). In a rat pulpotomy model, teeth treated with MTAP showed evidence of dentin bridge formation within 30 days (Kramer Woodmansey et al. 2014).

A new reformulation of MTA Plus is NeoMTA Plus. The single difference between the materials is the radiopacifier (Primus 2016). NeoMTA Plus contains tantalum oxide rather than bismuth oxide as the radiopacifier and claims to be non-staining (Avalon Biomed 2015). In vitro studies have demonstrated the color stability of NeoMTA Plus after exposure to both water and sodium hypochlorite, a common endodontic irrigant (Camilleri 2015).

The dentinal tubule penetration and apical sealing ability of NeoMTA Plus have also been evaluated. NeoMTA Plus performed comparably to ProRoot MTA and EndoSequence products in an open apex tooth model to evaluate material adaptation and apical seal (Tran He et al. 2016). Additionally, NeoMTA Plus had similar dentin tubule penetration compared to other hydraulic silicate cements such as EndoSequence and Quick-Set2 (McMichael Primus et al. 2016).

II.3 MTA Angelus

MTA Angelus (OdontoLogika, Ind. Prod. Odont. Ltda, Londrina, Parana, Brazil) is a calcium silicate cement indicated for perforation repair, internal resorption, root-end filling, pulp capping, pulpotomy, apexification and apexogenesis (Odontológicos 2015). The constituent components are similar to MTA and consist of tri- and dicalcium silicates, tricalcium aluminate, tetracalcium aluminoferrate and dehydrated calcium sulfate with minor variations in trace elements (Estrela Bammann et al. 2000; Song Mante et al. 2006; Oliveira Xavier et al. 2007; Camilleri Kralj et al. 2012). The manufacturer claims a decreased setting time compared to traditional MTA due to decreased calcium sulfate (gypsum) content, which is a feature exclusive to AMTA (Odontológicos 2015). Similarly to MTA, bismuth oxide serves as the radiopacifier (Camilleri Formosa et al. 2013). AMTA has a less homogenous particle size mixture than MTA, but it contains more numerous small particles throughout ranging from 6-160μm (Saidon He et al. 2003; Komabayashi & Spangberg 2008).

Despite differences in composition, in vitro analysis have compared AMTA with other calcium silicate-based cements and have found similar biocompatibility (Duarte

Demarchi et al. 2003; Saidon He et al. 2003; De Deus Ximenes et al. 2005). The initially high pH of MTA-like cements may account for the transient cytotoxicity observed immediately following contact of the material with body tissues. (Estrela Bammann et al. 2000; De Deus Ximenes et al. 2005). Compared to MTA, AMTA has a high calcium ion release with prolonged activity up to 168-hours (Duarte Demarchi et al. 2003). The increased calcium release is due to the higher content of Portland cement and other calcium releasing substances in AMTA (Duarte Demarchi et al. 2003). Increased calcium release and mineralized tissue biomarkers allows AMTA to promote desirable healing such as increased dentin and bone deposition in vivo.

Both animal and human studies comparing MTA and AMTA have shown comparable and favorable results for use as a pulp-capping and root filling materials. Without contact to periapical tissue, AMTA has demonstrated stimulation of mineralized tissue in the subcutaneous tissue of rats (Gomes-Filho Watanabe et al. 2009). In canines, AMTA has been shown to be as effective as MTA at repairing root perforations and specimens exposed to both MTA and AMTA demonstrated cementum reformation on the root surface (Juarez Broon Bramante et al. 2006). In humans, AMTA used as a pulp capping material can induce hard tissue formation over the exposed pulp tissue (Accorinte Loguercio et al. 2009). Initially, as seen with other calcium silicate materials, inflammation was present in the circumpulpal region, however after 60 days the inflammation had subsided and given way to a reparative dentin bridge (Accorinte Loguercio et al. 2009).

II.4 Biodentine

Biodentine is a calcium silicate-based material that has been commercially available for since 2009 (Malkondu Karapinar Kazandag et al. 2014). The main feature of Biodentine is that it attempts to overcome the well-known shortcomings of MTA; namely improved handling, faster setting time and use as a direct restoration. Biodentine, unlike many hydraulic silicate cements, is advocated for direct dental restorations. The coronal applications include temporary enamel and permanent dentin restorations (cavity base or liner), large carious lesions or deep cervical and radicular lesions in addition to pulpotomy and pulp capping (Septodont). The material can also be used for perforation and resorption repair as well as apexification and root-end fillings (Septodont).

One reported advantage of Biodentine is the rapid setting time with an initial set of approximately 9-12 minutes (Septodont). This is achieved by the addition of calcium chloride to the mixing liquid (Ber Hatton et al. 2007; Wang Sun et al. 2008). It contains zirconium as a radiopacifier which differs from the bismuth oxide present in MTA and AMTA (Camilleri Kralj et al. 2012). The main silicate component of Biodentine is tricalcium silicate (Camilleri Kralj et al. 2012). Biodentine is triturated in a pre-dosed capsule which provides thorough mixing.

Initial in vitro testing of Biodentine disclosed similar cell viability compared to MTA in culture and demonstrated an ability to stimulate pulp cells to begin producing collagen and DSP. Thus, Biodentine is not only biocompatible, but bioactive as well (Laurent Camps et al. 2008; Luo Li et al. 2014). Biodentine has superior calcium release

compared to EndoSequence BC Putty and is capable of forming calcium and phosphate deposits within the dentinal tubules (Han & Okiji 2013).

Similarly to MTA, Biodentine and is able to induce odontoblast-like cell differentiation in the areas of pulp capping (Laurent Camps et al. 2012). In rats, dentin bridges were seen in both WMTA and Biodentine with formation of mature dentinal tubules (Tran Gorin et al. 2012). In humans, Biodentine is as effective as MTA at inducing dentin bridging over pulpotomies with an organized odontoblast layer adjacent to the dentin bridges (Nowicka Lipski et al. 2013).

II.5 BioAggregate

BioAggregate (Innovative BioCeramix, Inc., Vancouver, British Colombia, Canada) is a calcium silicate-based dental material developed for use as a root canal filling material, retrograde root filling, perforation repair and vital pulp therapy (Innovative BioCeramix ; Park Hong et al. 2010). It is also available by the trade name DiaRoot Root Canal Repair Filling Material and is a white ceramic powder mixed with deionized water to initiate the hydration setting reaction (Innovative BioCeramix). BioAggregate is an aluminum-free hydraulic cement that contains tantalum oxide as a radiopacifier (Camilleri Kralj et al. 2012; Kum Kim et al. 2014).

In vitro studies have demonstrated similar biocompatibility to MTA (Chang Lee et al. 2014; Jang Lee et al. 2014). When exposed to BioAggregate, human dental pulp stem cells exhibited cell growth, spreading and attachment to the material in culture media (Chang Lee et al. 2014). Additionally, it is capable of inducing mineralized nodule formation and increased levels of biomarkers such as ALP and DSPP (Chang Lee

et al. 2014). Bioaggregate exposed to rat tissues, again, showed similar biocompatibility to MTA. Initially, an inflammatory response is noted, however, at longer periods of 60 and 90 days, the inflammation subsides (Batur Acar et al. 2013). Although the inflammatory reaction decreases over time, BioAggregate demonstrates more inflammation compared to AMTA and WMTA (Saghiri Tanideh et al. 2013). Although multiple rat studies have been conducted, no further in vivo studies could be located using larger animal models such as dogs or humans.

II.6 EndoSequence

EndoSequence Root Repair Material (ERRM) and EndoSequence BC Sealer (BC Sealer) (BrasselerUSA, Savannah, GA) are pre-mixed materials consisting of calcium silicates, zirconium oxide, tantalum oxide, calcium phosphate monobasic and filler agents (BrasselerUSA 2009). Both the ERRM and BC Sealer are available outside the United States as iRoot BP Plus (Innovative BioCeramix, Vancouver, BC, Canada). The primary difference between the ERRM and BC sealer other than consistency and fillers is calcium hydroxide content in the ERRM (BrasselerUSA 2009). The materials are collectively recommended for use as a retrofilling material, perforation repair, resorption repair, apexification and pulp capping and as a sealer (BrasselerUSA 2009). These pre-mixed materials contain proprietary thickening agents to allow the material to be delivered in a paste-like consistency which take 72 hours to reach an initial set and up to 240 hours to achieve a final set (Loushine Bryan et al. 2011).

Mouse fibroblast cells exposed to both set and freshly mixed materials showed there was no significant difference in cell viability for MTA and ERRM (Alanezi Jiang

et al. 2010). Both BC Sealer and AH Plus sealer (Dentsply Tulsa Dental Specialties, Tulsa, Oklahoma) were shown to be cytotoxic when initially exposed to mouse fibroblasts, however, the initial toxicity decreased for both materials over time allowing cell proliferation (Loushine Bryan et al. 2011). Moreover, in vitro studies have demonstrated ERRM and BC Sealer have similar cytotoxicity to MTA (Ma Shen et al. 2011).

Rat maxillary molars pulp capped with iRoot BP Plus or WMTA displayed early hard tissue deposition adjacent to the material and at 4 weeks post-op the hard tissue was more developed and could be identified as organized dentin in both experimental groups (Liu Wang et al. 2015). These findings support the use of iRoot BP Plus as a suitable pulpotomy and pulp capping agent. Similar results were found when pulp caps were observed in dogs (Shi Bao et al. 2015). The pulp caps displayed favorable results with most specimens of both test materials showing complete dentin bridge formation beneath the material and a lack of inflammation at 90 days post-op for EndoSequence (Shi Bao et al. 2015). A more recent retrospective analysis of EndoSequence materials used for root-end surgeries in humans has highlighted the favorable healing of EndoSequence materials with 92% successful treatment at 1-year post-operative recalls (Shinbori Grama et al. 2015).

II.7 Quick-Set

Quick-Set and newly reformulated Quick-Set2 (Avalon Biomed Inc., Bradenton, FL) is a relatively new material composed of calcium aluminosilicate powder and other proprietary components mixed with a water-based gel. The predecessor of Quick-Set

was a similar experimental material named Capasio (Primus Consulting, Bradenton, FL). The main difference is the water-based gel of Capasio had a cationic surfactant in the liquid, whereas Quick-Set does not. The cationic surfactant was removed because it was thought to interfere with the biocompatibility of the material. Quick-Set has a short setting time of 9 minutes and a final pH of 10.9 (Porter Berto et al. 2010). Additionally, Quick-Set has improved tubule penetration, acid resistance and washout resistance compared to MTA (Porter Berto et al. 2010; Bird Komabayashi et al. 2012). Quick-Set has been developed with tantalum oxide as a radiopacifier to avoid the adverse clinical findings of stained or grayed teeth due to the presence of iron and bismuth oxides (Kang Shin et al. 2015).

In addition to the material properties, Quick-Set has been shown to display excellent biocompatibility and bioactivity and has been suggested as a possible replacement for calcium silicate materials. Capasio has been shown to allow formation of apatite crystals on the surface of the material similar to WMTA (Wei Qi et al. 2012; Eid Niu et al. 2013). In vitro, Quick-Set demonstrated increased expression of alkaline phosphatase (ALP) and dentin sialophosphoprotein (DSPP) which are molecules important for bone and dentin mineralization (Eid Niu et al. 2013). The cytotoxicity of Capasio/Quick-Set has been shown to be similar to that of WMTA (Wei Qi et al. 2012).

As previously mentioned, calcium aluminate cements have a variety of potential clinical uses including pulp capping, pulpotomies, sealers and root-end surgeries. Quick-Set performed as favorably as MTA in rat pulpotomies (Kramer Woodmansey et al. 2014). Dogs treated with Quick-Set pulpotomies had significantly more

inflammation and less organized reparative tissue compared to the WMTA specimens, however, there was no difference in the quality of reparative dentin formed (Woodmansey KF 2015). No difference was noted when Quick-Set was compared to WMTA for use as a retrofilling material (Kohout He et al. 2015). The observed increase in inflammation in some Quick-Set specimens has led to a reformulation reducing the amount of free alumina in the powder, however, there is currently no available research on the new material Quick-Set2.

II.8 EndoBinder

Another calcium aluminate cement, EndoBinder, (Binderware, Sao Carlos, SP, Brazil) has been recently introduced to the dental marketplace. EndoBinder has been advocated for use in root repairs, perforations and root-end fillings (Silva Herrera et al. 2014). EndoBinder is composed primarily of aluminum oxide, calcium oxide, silicon, magnesium and iron oxides (Silva Herrera et al. 2014). Unlike Quick-Set which uses tantalum oxide, EndoBinder uses bismuth oxide as a radiopacifier (Aguilar Roberti Garcia et al. 2012). Similarly to white MTA, the iron oxide has been reduced in EndoBinder to prevent potential tooth discoloration (Aguilar Roberti Garcia et al. 2012). The set pH of EndoBinder, like Quick-Set, is lower than calcium silicate materials (Silva Herrera et al. 2014).

In in vitro studies, EndoBinder has performed similarly to AMTA. Mouse fibroblast cells exposed to EndoBinder and AMTA demonstrated EndoBinder was not cytotoxic and performed as well as AMTA. Comparing AMTA to EndoBinder, the

bioactivity of EndoBinder is superior and had higher levels of alkaline phosphatase expression compared to the AMTA exposed cells (Castro-Raucci Oliveira et al. 2011).

A limited volume of research has been conducted on EndoBinder in vivo. Polyethylene tubes filled with EndoBinder, AMTA or calcium hydroxide were implanted into the backs of rats (Garcia Lda Huck et al. 2014). As seen with other hydraulic silicate cements materials, there was a moderate inflammatory response at the 7-day interval, which decreased over time and resulted in all three materials showing no inflammatory response at the long-term intervals (Garcia Lda Huck et al. 2014). Currently, no further in vivo studies have been conducted on larger mammals.

II.9 Ortho MTA / Retro MTA

Ortho MTA and its sister materials Retro MTA (BioMTA, Seoul, Republic of Korea) were developed to improve handling and decrease working time compared to MTA (Alibaba.com 2015). According to the manufacturer, Ortho MTA is indicated for use as an orthograde root canal filling, repair of perforation and resorption, root-end fillings, apexification and pulp capping (Alibaba.com 2015). They claim that it is heavy metal-free and is more biocompatible than other comparable materials. Comparing heavy metal composition, Ortho MTA contains less arsenic and chromium than MTA, however, both metal levels were within the ISO specification 9917 regarding the safety limits of heavy metals (Chang Baek et al. 2011; Kum Zhu et al. 2013). Ortho MTA is supplied in centrifuge tubes that are mixed with the provided water in a centrifuge for 15 seconds to provide a thorough mix. Retro MTA is mixed chairside by manually combining the powder and liquid.

Although it has improved handling properties, in vitro testing suggests Ortho MTA may be more cytotoxic that MTA (Lee Son et al. 2012). Comparing Ortho MTA to other calcium silicate materials MTA and Biodentine in vitro, all materials increased alkaline phosphatase activity, formation of mineralized nodules, expression of odontoblastic markers and cell proliferation (Chang Lee et al. 2014).

Retro MTA (BioMTA, Seoul, Republic of Korea) is a relatively new material with few published studies. It is reported to have similar composition to Ortho MTA (BioMTA, Seoul, Republic of Korea) which is manufactured by the same company (Chung Kim et al. 2015). Retro MTA (BioMTA, Seoul, Republic of Korea) is comprised mainly of calcium carbonate, silicate, aluminate and a calcium-zirconium complex (Alibaba.com 2015). The manufacturer claims a setting time of 180 seconds.

Compared to MTA, Retro MTA has similar in vitro cell viability and promotes cell adhesion to the material surface (Chung Kim et al. 2015). Retro MTA, however, has demonstrated an inferior ability to promote growth factor production, such as VEGF, compared to MTA. The calcium ion release during setting of Retro MTA is similar to MTA (Chung Kim et al. 2015). Moreover, both Ortho- and Retro MTA have less favorable biocompatibility compared to the gold standard ProRoot MTA and may require further evaluation or reformulation.

II.10 Endocem

Endocem (Endocem, Maruchi, Wonju, Korea) is a newly developed MTA-derived pozzolan cement. Pozzolan cement, in comparison to calcium silicate cement, has a higher silica content and is a weaker cement than Portland cement. Endocem

attempts to improve the slow setting time of MTA without the use of a chemical accelerator (Choi Park et al. 2013). The faster setting time is achieved by using small particle pozzolan cement, although Endocem and MTA have similar components with the largest constituents being calcium, oxygen and silica (Park Heo et al. 2014). The set pH of Endocem is 11.5 with a set time of only 4 minutes (Park Heo et al. 2014).

To evaluate Endocem and WMTA for biocompatibility and bioactivity a pulp-capping model with human dental pulp cells and Wistar rats was developed (Park Heo et al. 2014). Both Endocem and WMTA induced mineralization and increased levels of bone and tooth mineralization markers (Choi Park et al. 2013; Park Heo et al. 2014). Endocem was capable inducing dentin bridge formation with minimal inflammation following pulp capping in rats (Park Heo et al. 2014).

Clinical trials have demonstrated comparable results for pulp capping with EndoCem and MTA (Jang Song et al. 2015; Song Kang et al. 2015). A 3-month evaluation compared direct pulp caps of MTA with those of EndoCem and both materials had greater than 90% success rates (Song Kang et al. 2015). A randomized clinical control trial over 1-year compared MTA and Endocem as pulp capping materials (Jang Song et al. 2015). After 1-year, MTA had an 87% success rate and Endocem 83%, which were not significantly different from each other (Jang Song et al. 2015). The same study determined the only predictable factors for success versus failure were age and cavity type, not the type of material used (Jang Song et al. 2015).

II.11 EndoCPM

EndoCPM (EGEO SRL Bajo licencia MTM Argentina SA, Buenos Aires, Argentina) is a newly developed calcium silicate root canal sealer that is based on the material science of MTA. According to the manufacturer EndoCPM contains calcium chloride to aid in setting and bismuth oxide as a radiopacifier (Gomes-Filho Watanabe et al. 2009). Additionally, the manufacturers claim the addition of calcium carbonate helps to reduce the initial spike in pH typically associated with MTA and make EndoCPM more biocompatible at earlier times (Gomes-Filho Watanabe et al. 2009).

To evaluate the initial cytotoxicity and cell viability, mouse fibroblasts were exposed to EndoCPM and it did not inhibit cell viability compared to controls (Gomes-Filho Watanabe et al. 2009). EndoCPM has been shown to have an extended release of calcium ions (Tanomaru-Filho Chaves Faleiros et al. 2009). The calcium ion release followed a similar time-dependent decrease with EndoCPM releasing more calcium ions compared to AMTA (Tanomaru-Filho Chaves Faleiros et al. 2009).

Multiple studies have evaluated the biocompatibility of EndoCPM by implanting polyethylene tubes in the dorsum of rats. (Scarparo Haddad et al. 2010; Gomes-Filho Watanabe et al. 2013). As in previous studies, they demonstrated an initial inflammatory infiltrate present at 7 and 15 day intervals, however at longer intervals both AMTA and EndoCPM had minimal inflammatory cells present (Gomes-Filho Watanabe et al. 2009; Scarparo Haddad et al. 2010). In addition, EndoCPM was capable of stimulating mineralized tissue in the connective tissue of rats via calcium carbonate formation (Gomes-Filho Watanabe et al. 2009).

Molar root perforations in rats were sealed with EndoCPM, AMTA or Zinc Oxide Eugenol (ZOE) cement (da Silva Guerreiro-Tanomaru et al. 2011). EndoCPM produced less bone destruction compared to AMTA, however the consistency of EndoCPM lead to more material being extruded beyond the confines of the tooth (da Silva Guerreiro-Tanomaru et al. 2011).

To compare healing of periapical lesions in dogs EndoCPM, MTA Fillapex and Sealapex were used as root canal sealers (Gomes-Filho Watanabe et al. 2013). In the EndoCPM group less than half of specimens showed any cemental healing, no specimens showed complete closure of the apical delta canals and only a single specimen had closure of the main canal with newly formed cementum (Gomes-Filho Watanabe et al. 2013). Severe inflammatory infiltrate was observed in all but one specimen. Compared to the EndoCPM group, MTA Fillapex only had moderate inflammation present in most specimens with some areas of mild inflammatory infiltrate (Gomes-Filho Watanabe et al. 2013).

II.12 MTA Fillapex

MTA Fillapex (Angelus, Londrina, PR, Brazil) is a resin-based sealer mixed with tricalcium silicate cement that is marketed as a biocompatible MTA-based root canal sealer (Bin Valera et al. 2012) (Angelus 2015). MTA Fillapex is composed of Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, silica, zirconium oxide, iron oxide pigments. Paste B: dibenzyldiamine, aminoadamantane, tricyclodecanediamine, calcium tungstate, zirconium oxide, silica, silicone oil (Bin Valera et al. 2012).

MTA Fillapex was compared to BC Sealer, an epoxy resin sealer and a silicone-based sealer and had superior flow than the other materials (Zhou Shen et al. 2013). Additionally, MTA Fillapex had dimensional stability but higher film-thicknesses than the other test materials. Both of the calcium silicate materials had an alkaline pH, however, MTA Fillapex has a lower pH than other calcium silicate materials without the resin component (Kuga MC 2011; Zhou Shen et al. 2013). MTA Fillapex was particularly cytotoxic in a concentration dependent manner and was more cytotoxic than AMTA and AH Plus in hamster fibroblasts (Bin Valera et al. 2012). It is speculated the resin component of MTA Fillapex contributed to the higher levels of cytotoxicity, however, contrasting results have indicated decreased cell cytotoxicity after 7-days post-exposure to MTA Fillapex (Bin Valera et al. 2012; Salles Gomes-Cornelio et al. 2012). Moreover, despite the resin component, MTA Fillapex is a bioactive material capable of releasing calcium hydroxide into adjacent tissues (Salles Gomes-Cornelio et al. 2012).

MTA Fillapex, EndoCPM and Sealapex were compared to evaluate the periapical healing response in dogs (Gomes-Filho Watanabe et al. 2013). MTA Fillapex group had new cementum formed in 2/3 of the specimens, but the repair was less than 1/3 of the total missing cementum compared with less than half of the specimens in the EndoCPM group. Apical delta closure was observed in 2/3 of the MTA Fillapex specimens, but only one main canal was repaired with new cementum, versus no delta or main canal closure in the EndoCPM group (Gomes-Filho Watanabe et al. 2013). Lastly, MTA Fillapex had moderate inflammation with some areas of mild inflammatory infiltrate. In this in vivo dog study, MTA Fillapex had superior biocompatibility and bioactivity

responses than EndoCPM, however, both experimental materials had at least a mild inflammatory response (Gomes-Filho Watanabe et al. 2013).

II.13 ProRoot ES

ProRoot ES (ProRoot ES, Dentsply Tulsa Dental Specialties, Tulsa, Oklahoma) is a recently launched calcium silicate-based endodontic sealer. It is a calcium silicate powder that is extremely similar to MTA. The powder is mixed with a proprietary gel to obtain a sealer consistency. With an extremely limited literature presence, the only study identified was an in vivo canine study that evaluated the histological response to ProRoot ES and Kerr pulp canal sealer. The study concluded ProRoot ES was biocompatible and bioinductive and is suitable for use as an endodontic sealer, however, further investigation is warranted to compare ProRoot ES to other calcium silicate sealers (Gutmann JL 2015).

CHAPTER III

RESEARCH MANUSCRIPT

III.1 Introduction

The original hydraulic tricalcium silicate cement used in dentistry has been ProRoot® MTA (Dentsply, Tulsa Dental, Tulsa, OK), for the past 2 decades. However, ProRoot MTA has suffered from criticism about its poor handling, long setting time, tooth discoloration, and high cost (Torabinejad Hong et al. 1995; Bortoluzzi Araujo et al. 2007; Felman & Parashos 2013). Several newer hydraulic tricalcium silicate cements have been developed since the introduction of ProRoot MTA, including MTA Angelus (OdontoLogika, Ind. Prod. Odont. Ltda, Londrina, Parana, Brazil) , Bioaggregate (DiaRoot) (Innovative BioCeramix, Inc., Vancouver, British Colombia, Canada), Biodentine (Septodont, Lancaster, PA), Endosequence bioceramics (BrasselerUSA, Savannah, GA) and the MTA Plus group of materials (Avalon Biomed Inc., Bradenton, FL). These newer hydraulic tricalcium silicate cements have been developed with easier handling, faster setting, improved washout resistance and lower material costs (Formosa Mallia et al. 2013; Gandolfi Siboni et al. 2014).

When considering bioceramic cements for dental uses, two primary categories have been tested: tricalcium silicates (MTA-like materials) and calcium aluminosilicates (Capasio/Quick-Set/Quick-Set2 & Endobinder). NeoMTA Plus (Avalon Biomed Inc., Bradenton, FL) is a tricalcium silicate-based material that overcomes the previously mentioned drawbacks of ProRoot MTA. NeoMTA Plus kits contain a cement powder and gel that when mixed has easier handling and washout resistance (Formosa Mallia et

al. 2013; Gandolfi Siboni et al. 2015). The powder of NeoMTA Plus has a finer particle size than ProRoot MTA, which may contribute to its decreased setting time, increased ion release, increased water sorption and decreased porosity compared to ProRoot MTA (Camilleri Formosa et al. 2013; Gandolfi Siboni et al. 2014). NeoMTA Plus contains tantalum oxide as a radiopacifier, rather than bismuth oxide, to prevent post-procedural tooth discoloration. MTA Plus has been used in patients since 2011, NeoMTA Plus has been marketed for clinical use since 2013 and both materials are considered to be equivalent to ProRoot MTA in biological response (Camilleri 2015; Gandolfi Siboni et al. 2015).

Much less scientific literature is available regarding the calcium aluminate-based biomaterials. The Endobinder calcium aluminate material (Binderware, Sao Carlos, Brazil) has been successfully tested for repair of bony defects (Garcia Lda Huck et al. 2015). Subcutaneous implantation showed its biocompatibility in rats (Aguilar Roberti Garcia et al. 2012). The physical properties and sealing ability of Endobinder are similar to other tricalcium silicate materials (Garcia Lda Chinelatti et al. 2014).

Like Quick-Set and Capasio, Quick-Set2 has similar short setting time, final pH, tubule penetration, acid resistance, and washout resistance (Porter Berto et al. 2010; Bird Komabayashi et al. 2012; Niu Watson et al. 2015). Both Quick-Set and Quick-Set2 have been shown to be as biocompatible as ProRoot MTA in vitro, and Quick-Set has demonstrated favorable healing and osteogenic/dentinogenic properties in in vivo animal models (Eid Niu et al. 2013; Kramer Woodmansey et al. 2014; Cornelio Rodrigues et al.

2015; Niu L Accepted 2016). Also, Quick-Set has similar osteogenic/dentinogenic properties to ProRoot MTA in vitro (Eid Niu et al. 2013).

Quick-Set2 (Avalon Biomed Inc., Bradenton, FL) is another calcium aluminate material composed of a calcium aluminosilicate powder, a radiopaque powder and other proprietary components mixed with a unique water-based gel. Quick-Set2 contains tantalum oxide as a radiopacifier to avoid the adverse clinical findings of discolored teeth caused by the color of calciumaluminoferrite or bismuth oxide, both of which are present in the ProRoot MTA and some other MTA-type materials (Marciano Costa et al. 2014). Additionally, Quick-Set2 contains fewer free alumina particles than the predecessor materials Quick-Set and Capasio (Avalon Biomed Inc., Bradenton, FL). The free alumina particles in Quick-Set were hypothesized to cause histological evidence of inflammation in the periapical region following root-end surgeries in canines (Marciano Costa et al. 2014; Avalon Biomed 2015; Camilleri 2015; Woodmansey Kohout et al. 2015). However, no in vivo animal studies have been performed on Quick-Set2.

The purpose of this study was to histologically evaluate the pulpal and periapical healing of Quick-Set2 compared to NeoMTA Plus in pulpotomies, root canal obturation and root-end fillings in a canine model.

III.2 Materials and Methods

The study was approved by the Institutional Animal Care and Use Committee, Texas A&M University College of Dentistry. One hundred eight teeth were treated in six beagle dogs to evaluate healing of pulpal tissues following endodontic procedures with either Quick-Set2 or NeoMTA Plus (Table 1). Thirty-six maxillary premolar teeth

received pulpotomy procedures. Thirty-six maxillary incisor teeth received root canal treatment with a single gutta percha cone and sealer obturation. The mesial roots of thirty-six mandibular premolar teeth received root canal treatment with a single gutta percha cone and sealer. The distal roots of these premolars were instrumented and obturated with the same sealer material used in the mesial root. Immediately after the orthograde treatment, apicoectomy was performed on the distal root. This procedure simulated root canal treatment followed by root-end resection, which may be performed after root canal treatment failure. This minimized the animal's trauma. The material assigned to each tooth was randomized by a combination of coin flipping for the material and random drawing for the tooth. Both materials were mixed with their corresponding gel. To achieve a sealer consistency, the powders were mixed in an approximately 1:1 powder to gel ratio to achieve a thin stringy consistency. For the pulpotomy and root-end filling procedures each powder was mixed at approximately a 3:1 powder to gel ratio to achieve a putty-like consistency.

Clinical procedures were similar to those reported previously (Kohout He et al. 2015; Woodmansey Kohout et al. 2015). Prior to every procedure, 11 mg/kg of clindamycin was injected intramuscularly 1 hour pre-operatively, then 2.2 mg/kg

Table 1: Procedures and teeth for testing

Teeth	Procedure	# Teeth/Roots Treated		# Teeth/Roots Scored/Analyzed		
		Expt'l (QS2)	Control (NMTA)	Expt'l (QS2)	Control (NMTA)	Total Teeth Analyzed
Maxillary Premolars	Pulpotomy	6x4=24	6x2=12	19	10	29
Maxillary Incisors	Single-cone obturation (sealer)	6x4=24	6x2=12	0	0	0
Mandibular Premolars	Single-cone obturation (sealer)	6x4=24	6x12	18	9	27
Mandibular Premolars	Obturation & root-end resection*	6x4=24	6x2=12	21	10	31
*Simulates root canal followed by root-end filling.				58	29	87

ketamine and 0.22 mg/kg xylazine-100 were delivered intramuscularly to induce general anesthesia. The dogs were intubated and 1 L/min of 1-2% Isoflurane in oxygen was used as an inhalational anesthetic throughout the procedure. Local anesthesia with 3.6 ml 2%

Lidocaine with 1:100,000 epinephrine (Novocol Pharmaceutical, Cambridge, Ontario, Canada) was achieved. Pre-operative digital radiographs of the teeth were obtained. Then the teeth were cleaned of debris using an ultrasonic scaler (NSK Dental, Chicago, IL) and disinfected with 0.12% chlorhexidine (Patterson Dental, Southlake, TX).

Pulpotomy

The teeth were isolated with a dental dam for the pulpotomy procedures. The pulpotomy procedures followed the protocol of Dominguez et al. (Dominguez Witherspoon et al. 2003). The access preparations and coronal pulp removal were made using 3 to 3.5x magnification and high-speed #4 carbide round burs. The pulp chambers were irrigated with 6% sodium hypochlorite until hemostasis was achieved. Each material was mixed according to manufacturer's directions, then the material was gently placed over the pulp tissues and the chamber floor to a depth of approximately 3 mm. The access cavities were restored with Ketac Nano Light-Curing Glass Ionomer (3M ESPE, St. Paul, MN), and the occlusion was adjusted to ensure no occlusal trauma. Post-treatment radiographs were obtained following all the other procedures.

Root Canal Treatment

The non-surgical root canal and surgical root canal therapy followed the protocol of Kohout et al. (Kohout He et al. 2015). The teeth were isolated with a dental dam. Non-surgical root canal treatment was initiated with access to the coronal pulp chamber using high-speed carbide round burs. Gates Glidden drills were used for orifice shaping, and EndoSequence .06 taper nickel-titanium instruments (Brasseler USA Dental,

Savannah, GA) were used for preparation of the canal. Cleaning and shaping procedures were accompanied by irrigation with 6% sodium hypochlorite. Cleaning and shaping of the canal was completed to the apical delta. The working lengths were determined by tactile sense of the apical delta. The final irrigant was 6% sodium hypochlorite.

After drying with paper points, the canals of the anterior teeth and the mesial root of the mandibular premolars were obturated with corresponding .06 tapered gutta percha cones. The gutta percha cones were coated with the materials mixed, according to the manufacturer instructions, to a sealer-like consistency. The cones were placed to length and seared using a Touch-N-Heat (Kavo Kerr Group, Charlotte, NC,) at the level of the CEJ and compacted. The distal canals of the mandibular premolars were completely obturated with either Quick-Set2 or NeoMTA Plus using a lentulo spiral (Dentsply Tulsa Dental Specialties, Tulsa, OK) and indirect ultrasonic compaction with pluggers (Miltex, York, PA) using the NSK Varios 350 (NSK Dental, Chicago, IL). The access cavity was restored with Ketac Nano Light-Curing Glass Ionomer and the occlusion was adjusted.

Root-end Surgery

The surgical phase of the study was performed immediately following the nonsurgical root canal treatment of the mandibular premolars. Additional 1.8–3.6 mL 2% Lidocaine with 1:50,000 epinephrine (Novocol Pharmaceutical, Cambridge, ON Canada) was injected for hemostasis adjacent to the apices of teeth planned for resection. A buccal, full-thickness, mucoperiosteal flap was reflected. Osteotomies approximately 5 mm in diameter were made using a Lindemann bone bur (Hu-Friedy, Chicago, IL) at the apex of each distal root. Approximately 3-mm was resected from the distal roots to

expose the root filling materials to the periapical tissues. Saline irrigation was used continuously during the osteotomy and root-end resection. Flaps were reapproximated and closed with 4-0 Vicryl sutures (Ethicon, Somerville, NJ).

The dogs were restricted to a soft diet for 90 days post-operative. Post-operative care included intramuscular injection of 2.0 mg/kg ketoprofen immediately following the procedures to control inflammation. For pain control, 2 mg/kg nalbuphine was administered subcutaneously immediately postoperatively and every 12 hours for 1 week postoperatively. The dogs were sacrificed 90 days after surgery with methods in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association using 2.2 mg/kg ketamine intramuscularly, 0.22 mg/kg xylazine-100 intramuscularly, and 2 mL Beuthanasia-D (Merck Animal Health, Millsboro, MI) (Association. 2013). One liter of normal saline was used to flush the blood from the head followed by perfusion with 1 L 70% ethanol. Block sections of bones containing the treated teeth were dissected at sacrifice, and stored in a container of 70% ethanol waiting fixation.

Histology

The resected blocks were gradually demineralized in 0.5 mol/L ethylenediamine tetraacetic acid. When demineralized, the blocks were embedded in paraffin and 5 μm serial sections were cut and stained with hematoxylin and eosin. Histologic samples were prepared from all teeth treated, with 1 to 7 sections per tooth. Sections that were damaged, distorted or did not contain the necessary anatomy for scoring were excluded.

The histologic sections were evaluated using transmission light microscopy by

two calibrated examiners (RW and LO). The examiners were blinded to the type of material used in each sample. If a discrepancy in scoring a section occurred, the examiners conferred to reach consensus for the scores. Each tooth and each procedure within the same tooth were scored independently. Between two and eight sections were available for each tooth. When multiple sections were available for each tooth, the scores were averaged to provide a single score.

The scoring criteria were adapted from Stanley, Dominguez et al. and Kohout et al. (Stanley 1998; Dominguez Witherspoon et al. 2003; Kohout He et al. 2015). The criteria are described in Table 2 for apical histology and Table 3 for pulpotomy or orthograde root canal histology. The root-end surgery sections were scored for inflammation, cementum deposition on the root canal aperture, apical periodontal ligament formation, and bone quality. The non-surgical root canal therapy sections were scored for presence of inflammation and reactionary dentin formation in the apical delta. The pulpotomy sections were scored for inflammation, pulp tissue organization, reactionary dentin formation and dentinogenesis. To maintain consistency, lower scores represent desirable healing responses for all categories, whereas higher numeric scores represent undesirable histological results. Statistical analysis was performed using the Mann-Whitney U test with a significance level of $P \leq 0.05$.

Table 2: Grading scale for apical histological samples (*% functionally oriented collagen fibers insertion in the new cementum & bone)

Inflammation	**Bone Quality, apical resorption**
0 = None	0 = Normal bone formation; no resorption
1 = mild	1 = Lack of bone formation; no resorption
2 = moderate	2 = Normal bone formation; concomitant resorption
3 = severe	3 = Lack of bone formation; resorption
Cementum deposition on root canal aperture	**Apical periodontal ligament (PDL) formation***
0 = Cementum observed on >75%	0 = FOCF >75%
1 = Cementum covering >50<75%	1 = FOCF >50<75%
2 = Cementum covering >25<50%	2 = FOCF >25<50%
3 = Cementum covering <25%	3 = FOCF <25%

Table 3: Grading of pulpotomy and root canal treatment histological samples

Inflammation	**Pulp tissue Organization**
0= None or a few scattered inflammatory cells	0= Normal tissue
1= Slight inflammatory cell infiltrate with polymorphonuclear or mononuclear leukocytes.	1= Odontoblastic layer disorganization, but central pulp normal
2= Moderate inflammatory cell infiltrate involving the coronal pulp.	2= Total disorganization of the pulp tissue morphology.
3=Severe inflammatory cell infiltrate involving the coronal pulp or abscess present	3= Pulp necrosis.
Reactional Dentin Formation	**Quality of Dentinogenesis**
0= Intense hard tissue deposition beneath the exposed area appearing as 75 – 100% complete	0= Highly organized dentinogenesis – greater than 75% up to 100% normal tubular dentin formation
1= Moderate hard tissue deposition beneath the exposed area; bridge up to 50% complete	1= Mixture of organized (tubular) and irregular, dystrophic dentinogenesis 25% - 50%
2= Modest hard tissue deposition beneath the exposed area; bridge up to 25% complete	2= Minimal cells and matrix; up to 25% organized
3= No bridge	3= None

III.3 Results

Pulpotomy

Pre-sacrifice radiographs show material confined to the pulp chamber region with minimal extension into the root canal space. For all specimens, no evidence of periapical pathosis is noted (Figure 1).

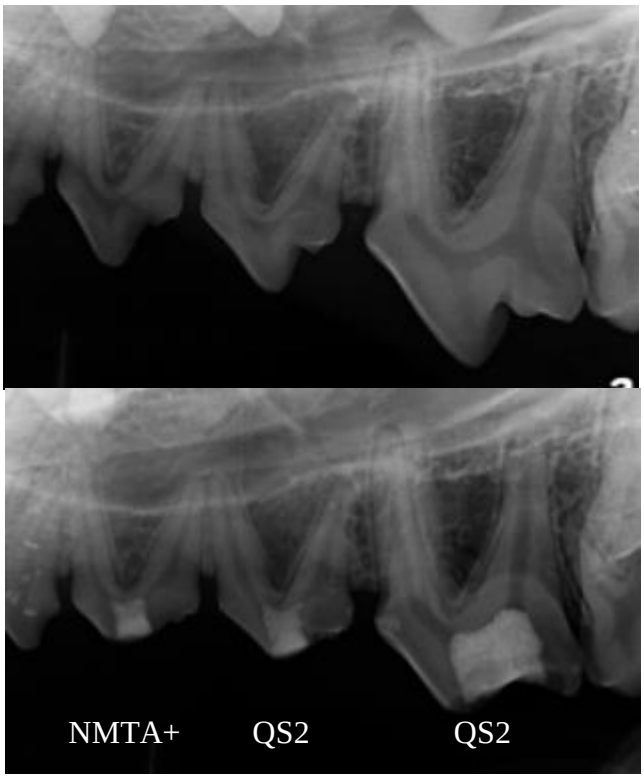

Figure 1: Pre-opeative and pre-sacrifice radiographs depicting 90-day healing of pulpotomy procedures.

Twenty-five of the 36 teeth could be scored. Dentin with well-defined tubules was visible in the dentin bridge adjacent to the experimental and control materials (Figure 2). Thick layers of dentin were routinely visible separating the materials from the underlying pulp tissue. The dentin was more organized in the presence of NeoMTA Plus, with some dystrophic dentin present in sections with Quick-Set2. Odontoblasts were located adjacent to the secondary dentin along the canal walls. The pulp tissue was normal in appearance with organized cells and fibroblasts. Occasionally, pulp tissue tags were contained completely within the dentin bridge.

No significant differences were noted for inflammation, pulp tissue organization or dentin bridge formation between the experimental and control materials (Figure 3). Significant differences between materials were only noted for the quality of dentin formation ($P < 0.001$), with NeoMTA Plus showing better results.

Moderate inflammation was noted in 2 teeth, and mild inflammation was observed in 2 additional teeth, both in the Quick-Set2 group. Two of the sections with inflammation were from the same animal (dog F). No inflammation was observed in the NeoMTA Plus group in this animal. The differences in pulp tissue organization and dentin bridge formation trended toward, but did not show, a significant difference ($P=0.052$).

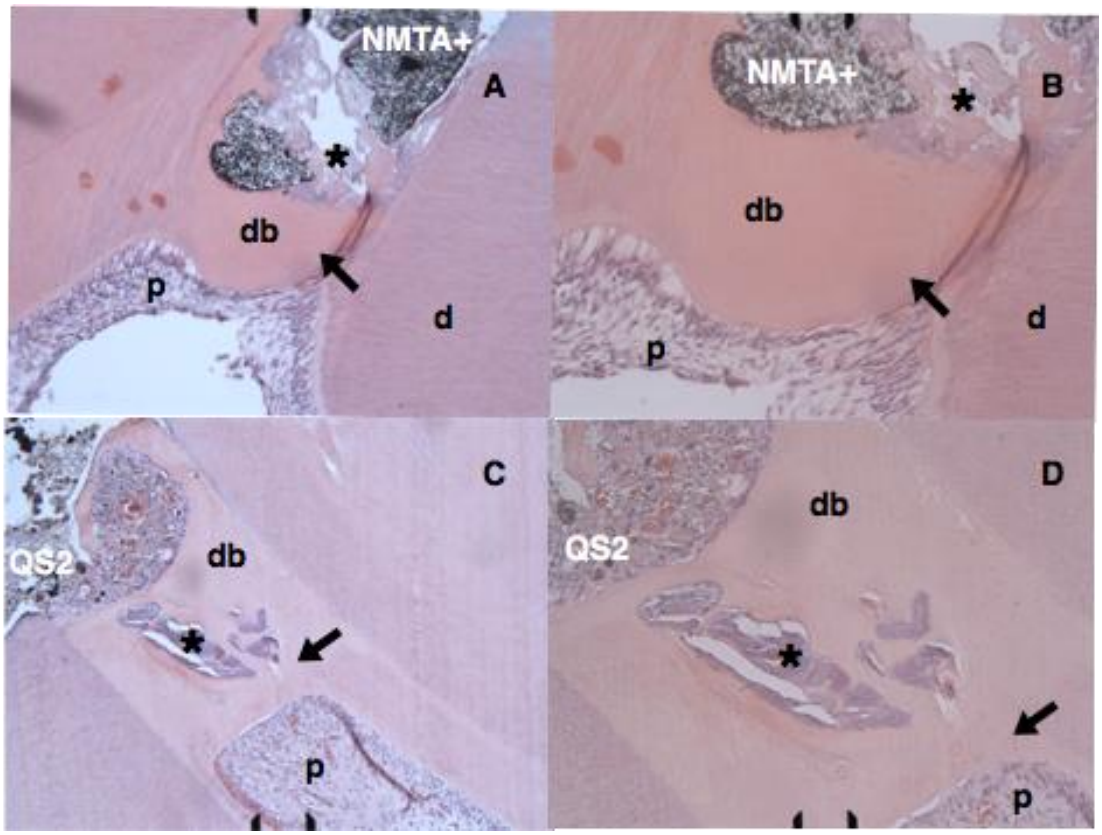

Figure 2: Micrographs showing hematoxylin-eosin-stained histologic sections of pulp tissue exposed to NeoMTA Plus A (10x), B (20x) and Quick-Set2 C (10x), D (20x). Asterisk (*) indicates pulp tissues inclusion and solid arrows indicate dentin bridging. (db, dentin bridge; d, dentin; p, pulp; QS2, Quick-Set2; NMTA+, NeoMTA Plus).

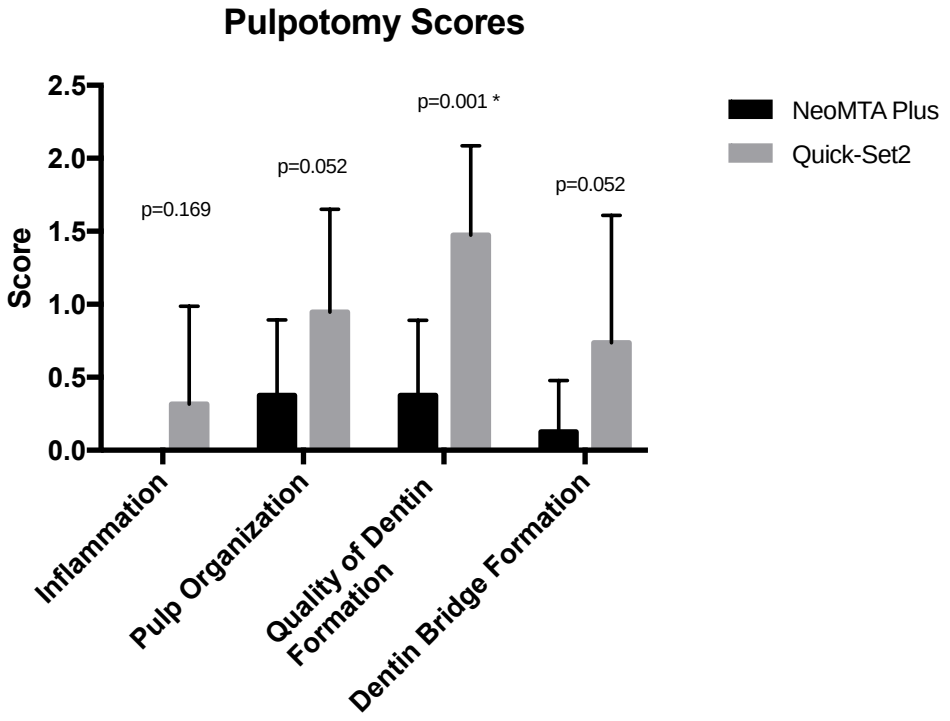

Figure 3: Mean histological scores with standard deviation. Significant difference in quality of dentin formation between NeoMTA Plus and Quick-Set2 (* indicates significance). (NMTA N=8; QS2 N=19)

Root Canal Treatment

Post-operative and pre-sacrifice radiographs show mesial root canal treatment of adequate length, density and taper. For all specimens, no evidence of periapical pathosis is noted (Figure 4).

Twenty-five of 72 root canal treatments were able to be scored. Many of the maxillary anterior teeth with single cone obturations were damaged during processing, which resulted in fewer sections suitable for examination and scoring.

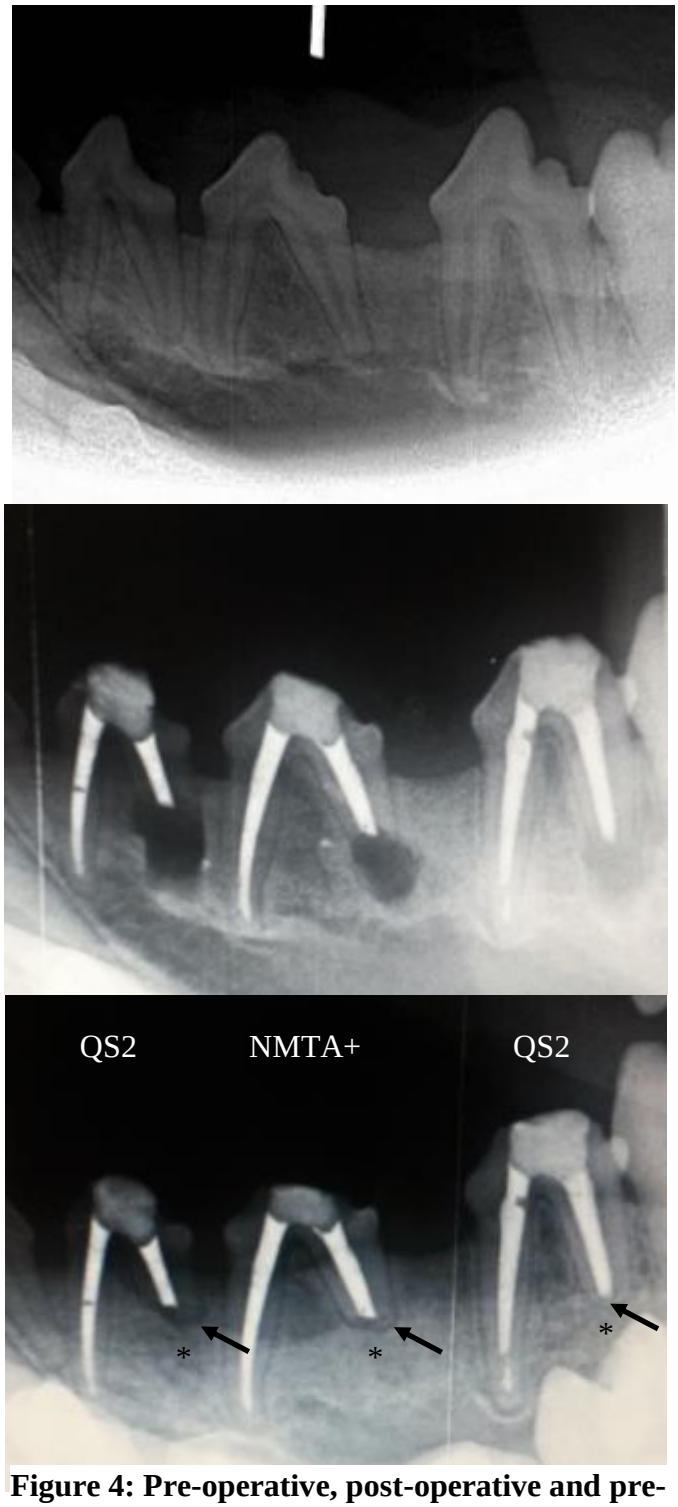

Figure 4: Pre-operative, post-operative and pre-sacrifice radiographs depicting 90-day complete bone healing (*) with PDL reformation (solid arrows).

For the scorable samples, intracanal debris was noted apically, often adjacent to the junction of the apical delta and the main canal terminus (Figure 5). When present in the sections, the canals of the apical delta adjacent to the experimental and control materials were seen to be occluded by dentin, which appeared translucent and sclerotic in nature.

Figure 6 shows the histological scores for the roots that received non-surgical root canal treatment. Sections through teeth with either NeoMTA Plus or Quick-Set 2 had minimal inflammation at the apical delta and periapical region. Both materials had dentin occluding the delta canals. Inflammation (1 mild and 1 severe) was noted in two roots in the NeoMTA Plus group, both in the same animal (dog F). Five roots in the Quick-Set2 group had signs of inflammation (1 mild, 1 moderate and 3 severe). Of the 7 total roots with inflammation, 4 of them were from the same animal (dog F). For these sections, the inflammation in the periodontal ligament was immediately adjacent to the root apex. Dentin was observed occluding the apical delta more frequently in the NeoMTA Plus group than the Quick-Set2 group. However, no significant differences were observed in inflammation or dentinal occlusion between the control and experimental groups ($P>0.05$).

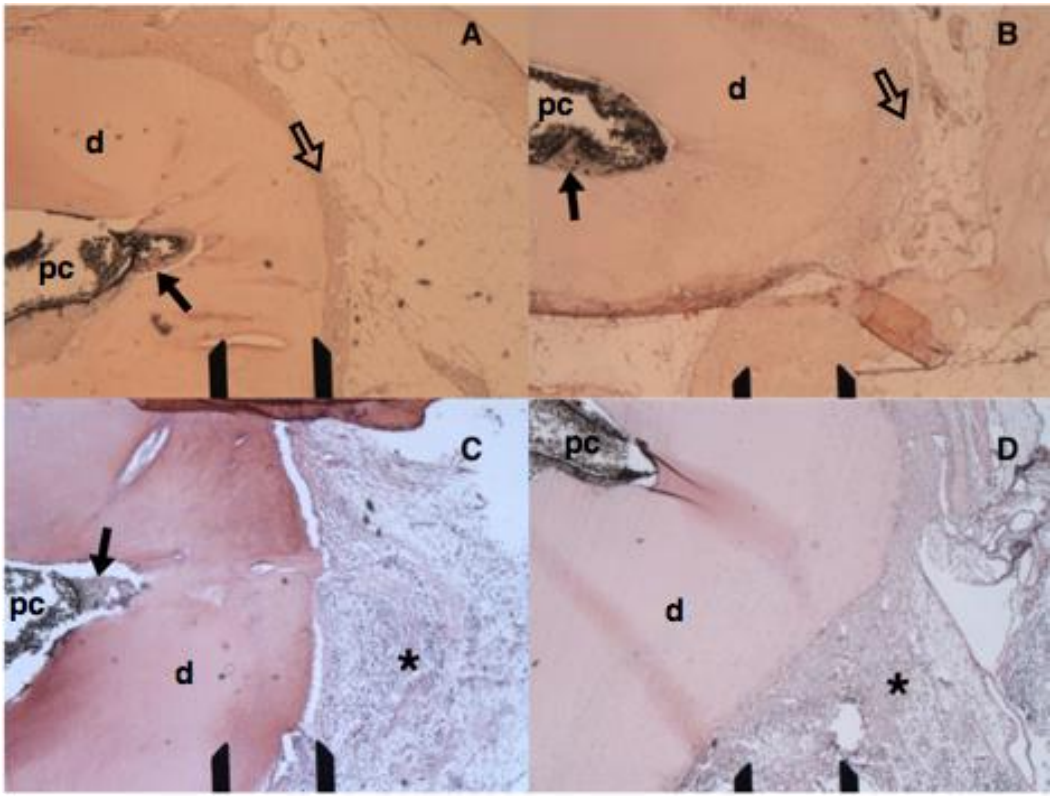

Figure 5: Micrographs showing hematoxylin-eosin-stained histologic sections of teeth obturated with NeoMTA Plus (5x)(A) and Quick-Set2 (5x)(B). Root canals with NeoMTA Plus (5x) (C) and Quick-Set2 (5x) (D) with inflammation (*). Solid arrows indicate intracanal

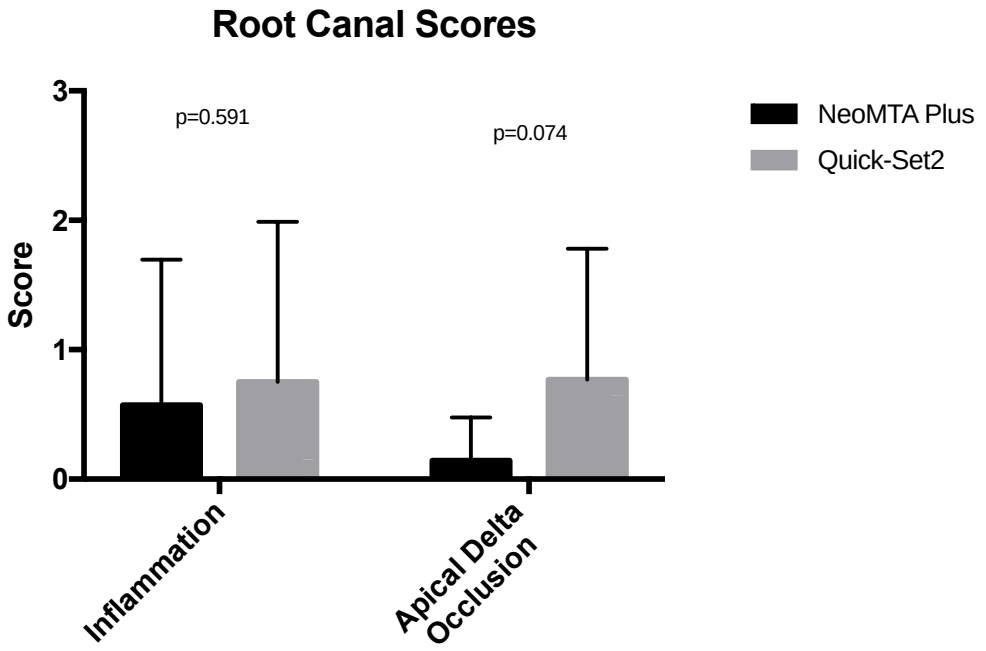

Figure 6: Mean histological scores with standard deviation. No significant difference between groups. (NMTA N=9; QS2 N=16)

Root-End Resection

Post-operative and pre-sacrifice radiographs show the distal root obturation of adequate length, density and taper (Figure 4). The osteotomies of the root apices of the distal roots are visible radiographically in the post-operative radiograph. Pre-sacrifice radiographs show the osteotomy sites with bone healing and PDL formation (Figure 4). For all specimens, no evidence of periapical pathosis is noted.

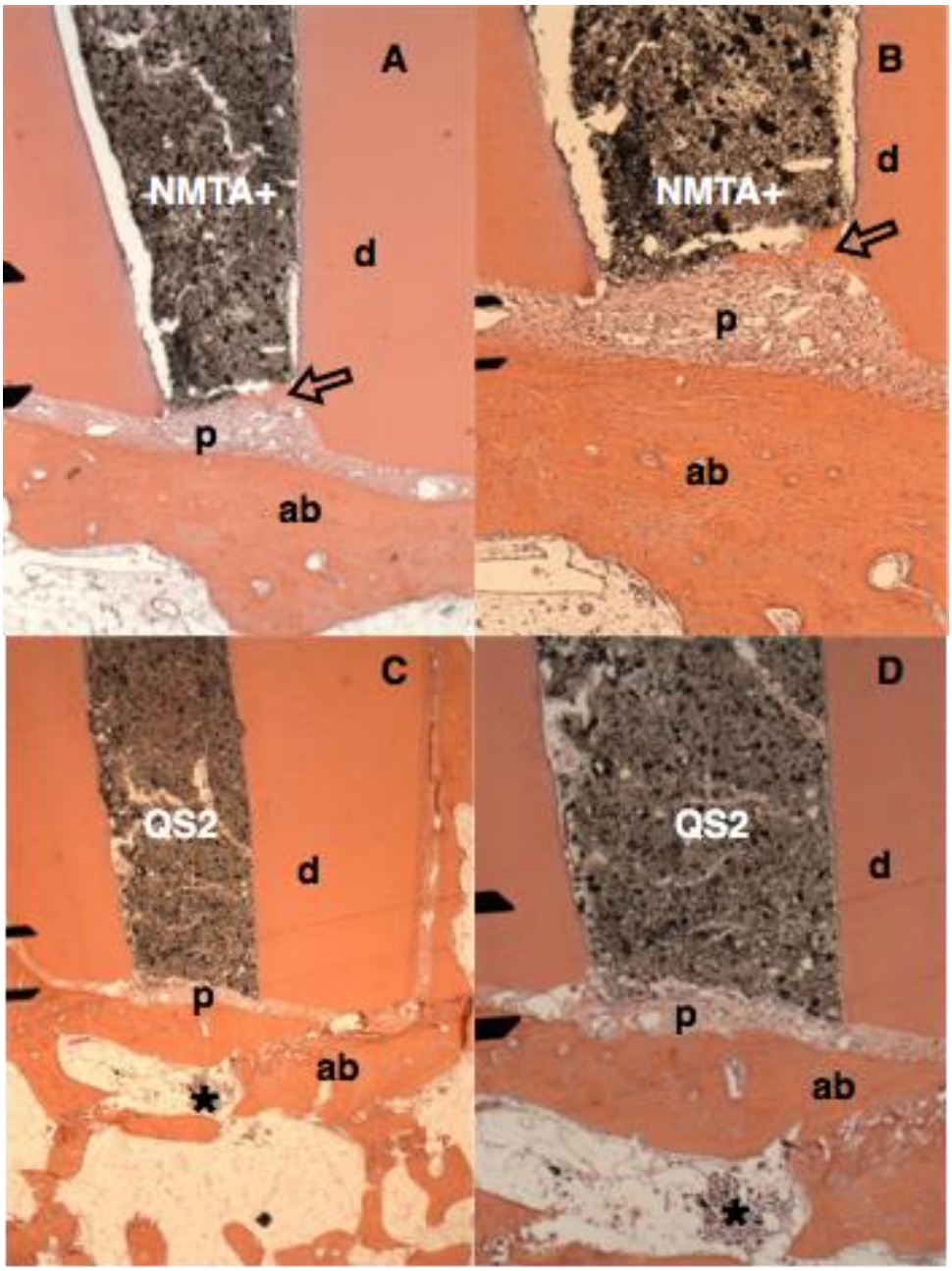

Figure 7: Micrographs showing hematoxylin-eosin-stained histologic sections of root-end resections exposed to NeoMTA Plus (A-5x/B-10x) and Quick-Set2 (C-5x/D-10x). Dense newly formed bone present in all samples. Closed arrows indicate newly formed PDL. Open arrows indicate new cementum formation. Asterisk indicates experimental material particles contained within the newly formed bone in the Quick-Set2 sample. (ab, alveolar bone; d, dentin; p, PDL; NMTA+, NeoMTA Plus; QS2, Quick-Set2)

Thirty-one of the 36 teeth could be evaluated histologically and scored. The majority of specimens had some calcified cementum present immediately adjacent to the materials (Figure 7). The calcified cementum extended from the lateral resected surface toward the center of the canal space and in some specimens spanned the entire resected root surface. Functionally oriented periodontal ligament fibers are noted at the periphery of the root-end resection and continued across resected surface. The fibers nearest the center of the resected surface were often not completely functionally oriented but were clearly ligamentous fibers. Dense and highly mineralized bone is present throughout the apical crypt. Some specimens displayed experimental material particles contained within the newly formed bone (see asterisks in Figure 7 C/D). However, the majority of the material was clearly contained within the root canal space.

Inflammation was noted in one of the NeoMTA Plus specimens and in three of the Quick-Set2-treated roots (Figure 8). Both groups had a low score (desirable healing) for inflammation and reparative bone formation, and generally displayed cementum and PDL reformation. No significant differences were found in inflammation, cementum deposition, bone formation or PDL formation between the two materials ($P>0.4$)

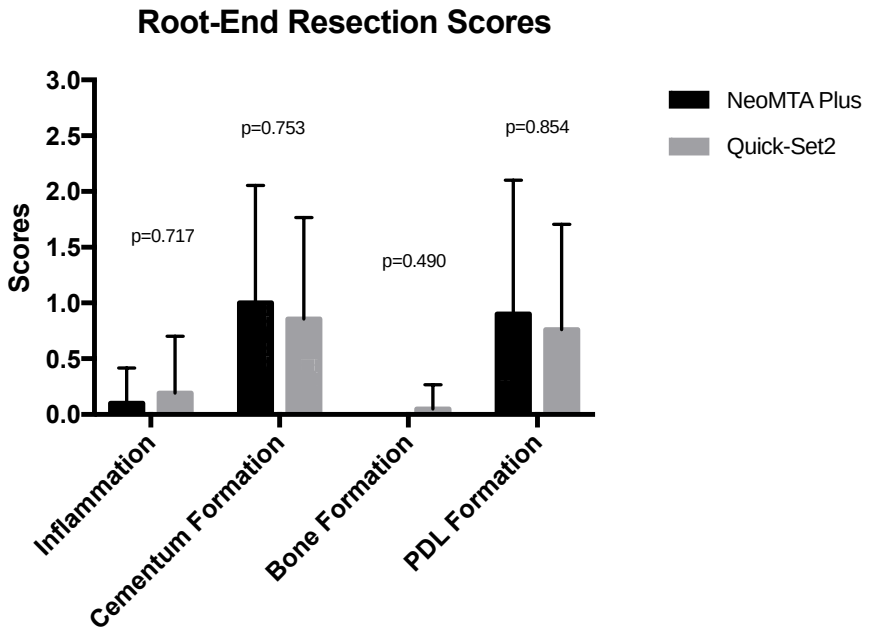

Figure 8: Mean histologic scores with standard deviation. No significant difference between groups. (NMTA N=10; QS2 N=21)

III.4 Discussion

Both surgical and nonsurgical procedures are employed to achieve the classical goal of endodontic treatment: to prevent or cure periapical pathosis. (Gutmann & Harrison 1998; Trope 2003; Kim & Kratchman 2006). A more modern goal of endodontics is to regenerate or restore the native tissues to their original form and function. The current study evaluated the pulpal and periapical tissue healing response after exposure to Quick-Set2 and NeoMTA Plus. These bioceramic materials were shown to induce healing both in the pulp and periapical tissues in this canine model after 90 days. This is the first report on the utility of Quick-Set2 for procedures related to pulpotomy, root-end resection and sealing in vivo.

Early studies by Torabinejad demonstrated success of an experimental MTA for various endodontic applications (Torabinejad & Chivian 1999). In animal studies, the biocompatibility of MTA has been highlighted for use in root-end surgeries (Torabinejad Pitt Ford et al. 2009; Bernabe Gomes-Filho et al. 2010). Until recently, tricalcium silicate cements have primarily been used for pulpotomy, perforation repair or root-end fillings. Completely obturating canals with MTA-like materials has proven challenging and is only feasible when the entire canal can be accessed with pluggers or spreaders to introduce the materials throughout the length of the canal system (Yeung Liewehr et al. 2006; Holland Mazuqueli et al. 2007). The challenge of creating an MTA-like sealer was limited by the high film thickness, poor handling properties of the original product, ProRoot MTA, and unavailability of a vehicle to sufficiently introduce the tricalcium silicate throughout the small confines of the canal (Holland Mazuqueli et al. 2007). (Torabinejad & Pitt Ford 1996; Torabinejad & Chivian 1999; Holland Bisco Ferreira et al. 2007). Combining the bioinductive properties of bioceramic cements with a sealer consistency has modernized the clinical use of these bioactive materials (Holland Mazuqueli et al. 2007).

Historically, teeth have been obturated with gutta percha and a zinc oxide-eugenol or epoxy-resin type sealers. Tricalcium silicate sealers are expanding the types of sealer materials available. Some newer materials, such as NeoMTA Plus and Quick-Set2, can be used in a variety of mixtures including putty and sealer. The predecessor of NeoMTA Plus, MTA Plus was evaluated for biocompatibility as a sealer (Mestieri Gomes-Cornelio et al. 2015). Compared to a calcium silicate and resin-based sealer,

MTA Plus showed favorable biocompatibility and was advocated for clinical use (Mestieri Gomes-Cornelio et al. 2015). Other tricalcium silicate sealers have also demonstrated favorable biocompatibility. Endo-CPM Sealer (CPM Sealer; EGEO S.R.L., Buenos Aires, Argentina) is an MTA-based product capable of inducing mineralization and is equivalently biocompatible to Angelus MTA (Angelus, Londrina, Brazil) (Gomes-Filho Watanabe et al. 2009; Scarparo Haddad et al. 2010).

Holland demonstrated that MTA paste mixed with either distilled water or propylene glycol resulted in materials with similar biocompatibility. Tricalcium silicate cements like NeoMTA Plus and calcium aluminosilicates like Quick-Set2 use their unique water-based gels to allow for variations in viscosity. By varying the powder to liquid ratio, the clinician can achieve a putty-like consistency or a more sealer-like texture. As previously demonstrated by Holland with "MTA pastes" and Kohout et al. and Woodmansey et al. using Quick-Set, the biocompatibility of these materials remained unchanged when mixed in thin or thick consistencies (Holland Mazuqueli et al. 2007; Kohout He et al. 2015; Woodmansey Kohout et al. 2015). Additionally, the findings by Kohout et al. and Woodmansey et al. demonstrated Quick-Set had comparable pulpal and periapical tissue healing compared to White ProRoot MTA (Kohout He et al. 2015; Woodmansey Kohout et al. 2015). The histological results of this study showed equivalent healing with Quick-Set2 to the earlier studies using experimental MTA or ProRoot MTA(Torabinejad & Pitt Ford 1996; Torabinejad & Chivian 1999; Holland Bisco Ferreira et al. 2007).

The only significant difference between the two materials in the current study was the quality of the dentin bridge after pulpotomy. The dentin bridge formed in response to NeoMTA Plus appeared more organized with less cell or matrix inclusion compared to Quick-Set2. An ideal dentin bridge is formed of organized tubules produced by underlying odontoblasts (Ricucci Loghin et al. 2014; Woodmansey Kohout et al. 2015). These organized dentinal tubules may provide a superior barrier compared to amorphous calcified "dentin-like" tissue seen in pulp tissue underlying rapidly progressing caries lesions (Ricucci Loghin et al. 2014; Woodmansey Kohout et al. 2015). However, the clinical implications of the quality of dentin bridging are currently unknown because it can only be assessed histologically.

The difference in the quality of bridge formation may be attributed to the chemical differences between the materials. The free alumina particles present in the previous formulation of Quick-Set may have been responsible for increased inflammation, and while inflammation was still present in some sections in the present study, the degree of inflammation and number of teeth with inflammation was much reduced in the modified formula of Quick-Set2 (Kohout He et al. 2015; Woodmansey Kohout et al. 2015). The maximum pH for NeoMTA Plus is approximately 12 and 10 for Quick-Set2. Decreasing the pH results in fewer calcium and hydroxyl ions at the material-tissue interface, which may lead to poorer bridge quality during the healing process (Woodmansey Kohout et al. 2015).

Both Quick-Set2 and NeoMTA Plus are mixed with a gel to form a putty-like consistency for pulpotomies. Since both materials were mixed to a similar consistency,

the handling properties, placement and material adaptation against dentin and pulp surfaces were nearly identical. Therefore, any differences in the regeneration of pulpal tissues may be attributed to differences in the material's biocompatibility.

Both materials demonstrated good healing in non-surgical root canal treatment and root-end resection procedures. In root canal procedures, occlusion in the delta was observed. The occlusions were likely an accumulation of both dentin and pulp tissue debris resulting from canal instrumentation. Since the canine tooth anatomy does not allow for direct contact of the intracanal material and the periapical tissues, it is unknown whether there was any newly formed reactionary dentin in the apical deltas as a direct response to material contact. The ion release and pH changes created by the bioceramic materials, however, may have promoted apical healing in the surrounding tissues resulting in favorable healing results observed in the root canal treatments. Apical healing, including cementum and periodontal ligament formation, and physical occlusion of the apical delta will help form a biological seal of the root canal system.

NeoMTA Plus and Quick-Set2 are advocated for multipurpose use by varying the mixing consistency. Endosequence products are premixed and come in consistencies of sealer or root repair putty. Endosequence bioceramics are calcium phosphate silicate-based with added zirconium oxide for radiopacity and water-free liquid thickening agents (Loushine Bryan et al. 2011). Recent studies have found favorable pulpal and periapical tissue responses after exposure to Endosequence bioceramics (Shi Bao et al. 2015; Shinbori Grama et al. 2015). Although Endosequence materials are generally similar to NeoMTA Plus and Quick-Set2, they contrast by being premixed. The current

study demonstrates favorable healing with operator mixed bioceramic materials as has previously been demonstrated with premixed bioceramic materials advocated for similar use (Shi Bao et al. 2015; Shinbori Grama et al. 2015).

A difference was observed in histologic appearance of the materials when used for pulpotomies versus obturation. When the materials were mixed for a (thin) consistency of sealer the materials appeared uniform in the mesial root canals. In the pulpotomy or root-end resection sections, the material had a uniformly dense gray background interspersed with dense areas approximately 50 μm in diameter (compare Figures 2 and 7 with Figure 5). The dense areas are black because transmission light microscopy was used.

These dense areas were universally associated with the materials when mixed to a thicker consistency and there are a couple alterative explanations. The areas could be small foci of incompletely wetted cement powder where the materials set quickly enough that the internal particles were not completed wet on the interior. Alternatively, these dense areas may be small aggregations of calcium hydroxide reaction products formed during the hydration setting reaction as described by Camilleri et al (Camilleri Formosa et al. 2013). Since no significant differences in healing were determined between procedures or materials, these aggregations may be clinically insignificant. The composition of these aggregations is unknown and warrants further investigation.

Of the total inflammation observed across all procedures, the level of inflammation was disproportionately high in one animal having one third of all incidences. This single outlier had increased inflammation for unknown reasons.

When evaluating only the clinically relevant factors, inflammation, pulp tissue organization and presence of dentin bridge formation, both materials performed similarly. However, this study may be underpowered and the availability of all the treated teeth may have been able to discern a statistical difference. Comparable favorable healing results for both NeoMTA Plus and Quick-Set2 were observed for this study.

III.5 Conclusions

Both Quick-Set2 and NeoMTA Plus demonstrated favorable histological response in the pulp and periapical tissue. Within the limitations of this study, both materials appeared to be suitable for clinical use in pulpotomy, non-surgical root canal treatment, and root-end filling.

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