EFFECT OF PLACING A GLASS IONOMER RESTORATION BEFORE SETTING OF A GREY MTA FURCATION PERFORATION REPAIR USING MICRO-COMPUTED TOMOGRAPHY

A THESIS SUBMITTED TO THE
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Dedication

This thesis is dedicated to my wife, Tamara Brown, for your love and support throughout this adventure. I will never forget the sacrifices you made to ensure that I could pursue my dreams. To my children, Clara, Andrew and Matthew Brown, whose energy and love bring me endless happiness and drive. To my parents, Andrew and Diane Brown, for their love and support, and for instilling a passion for knowledge and ambition to accomplish new things.

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INTRODUCTION

Endodontic pathology is caused by bacteria and bacterial byproducts present in the root canal system. Bacterial membrane components, metabolic byproducts and enzymes can act as stimulators of the host inflammatory response that can result in periapical bone loss and symptoms (1,2). Successful therapy relies upon chemomechanical debridement and disinfection of the root canal system; however complete disinfection of the system is difficult if not impossible to achieve (3). After root canal treatment, areas that harbor residual bacteria may provide a persistent source of irritation (4). Thus, endodontic success relies upon reducing the bioburden within the tooth to a level that allows periapical health to be maintained. If pre-existing or novel bacteria are permitted to repopulate the area, pathology and symptoms may return or worsen. Any unfilled canal spaces may have a reduced capacity to discourage recontamination as the ability of the treatment to resist fluid movement between radicular and periradicular spaces is dependent upon the degree to which the system has been sealed or filled (Wu, Fan, & Wesselink, 2000). To achieve long lasting successful endodontic treatments, adequate cleaning and filling of the system is paramount.

In the quest for successful endodontic therapy, iatrogenic errors can occur. One such error is a perforation, which is defined by the AAE Glossary of Endodontic Terms as "the mechanical or pathologic communication between the root canal system and the external tooth surface" (6). This can occur during access, cleaning and shaping, or restoration of the tooth. Once a perforation occurs, it presents an additional area which must be disinfected and sealed to achieve healing. The degree of difficulty in obtaining healing

after a perforation is dependent upon a multitude of factors including size, location, degree of bacterial contamination and the restorative material used (7). These factors all relate to our ability to clean and seal the new communication.

In regards to location of the perforation, furcal perforations have historically been demonstrated to be the most challenging to treat successfully (8,9). This is likely due to their proximity to the gingival sulcus which can more readily allow for exposure to bacteria as well as ingrowth of epithelial tissue, both of which complicate cleaning and sealing the perforation.

The material used to repair the perforation can influence the success of treatment in two ways; its ability to form and maintain a seal and its biocompatibility. Materials that are cytotoxic can result in a chronic proinflammatory reaction in the adjacent periodontal tissues even in the absence of bacteria (8). Many materials been used historically including gutta-percha, amalgam, calcium hydroxide, and glass ionomer. These materials have variable effectiveness in forming a seal and biocompatibility, and unfortunately none of them have excelled in both. Mineral trioxide aggregate (MTA) was introduced to endodontics in 1993 (13). This was the first material that could provide both an excellent seal and excellent biocompatibility (11). Since then, the principle components of MTA have since given rise to many other bioceramic restorative materials. These materials are ideally suited to perforation repairs and as such have been adopted by most clinicians as the material of choice for this clinical challenge (12).

The original formulation of grey MTA is still used by many clinicians for perforation repairs due to its clinical success in outcomes studies. However, one of the downsides to

the material is the long time required for a complete setting of the material. Although the material begins to harden relatively quickly full setting is estimated to take nearly three hours (13). Additionally, the setting requires hydration thus it is recommended to leave a moist cotton pellet adjacent to the material to ensure full setting. Clinically, this means that to follow recommendations the MTA can be placed first appointment, but the final restoration cannot be placed on top of the MTA immediately. The tooth must be temporarily restored and the patient must return for a second appointment to place the definitive restoration. To circumvent this inconvenience, many clinicians and some researchers have advocated placing a light-cured resin liner on top of unset MTA to allow for a definitive restoration to be placed without disturbing the setting MTA. A full understanding of what impact this approach has on the performance of the materials is lacking.

There are several qualities that are considered important in the evaluation of a restorative material. Arguably, one of the most important qualities for a furcation repair material is its ability to form and maintain a seal that resists the movement of bacteria and bacterial byproducts. Historically, this quality has been tested in the endodontic literature by leakage studies. This type of study has recently fallen out of favor due to several short falls including: the qualitative nature of the results, an inability to draw a correlation between leakage and clinical outcomes, and the fact that seemingly everything leaks. Thus, there is a need to develop a standardized, quantitative methodology for testing leakage.

LITERATURE REVIEW

Perforations

Tsesis *et al.* found that root perforations are present in approximately 2% of endodontically treated teeth using a retrospective analysis of over two thousand dental school patients (14). It is important to note that this may be lower than the true occurrence as it was based upon radiographic surveys which are approximately 70% sensitive for the detection of root perforations (15) and does not include perforated teeth that may have been extracted after endodontic treatment but before the radiographic survey had been taken. A study from the University of Toronto found approximately 7% of teeth presenting for retreatment had a perforation (16). This study however only includes patients who presented for retreatment and does not represent a random selection of the entire population of endodontic patients.

Perforations can occur iatrogenically at any phase of cleaning, shaping or subsequent restoration of the tooth, or pathologically as a result of root caries or resorptive lesions (17). Kvinnsland *et al.* found that 47% of root perforations occurred during endodontic treatment and 53% occurred during post preparation (Kvinnsland, Oswald, Halse, & Gronningsaeter, 1989). A perforation can also occur as the result of pathological internal or external root resorption if the process is given adequate time to progress (19).

Regardless of the cause, once a perforation has occurred the adjacent periodontal ligament can be injured and undergo an inflammatory reaction which can cause periodontal bone and attachment loss in the area. More severe consequences can develop

if bacteria from within the root canal, the periodontal ligament, or both colonize the area. These include serous or purulent exudate, percussion sensitivity, and periodontal furcation involvement and even tooth loss (20).

The location, size, and timing of the repair all influence the prognosis of a perforated tooth. The location of a perforation can be categorized as coronal, crestal or apical. Crestal perforations have been associated with a significantly poorer prognosis (7,14,20,21). Studies that further categorized crestal perforations as either lateral or furcal found that furcation perforations are associated with a poorer prognosis (8,9). In regards to size, any perforation smaller than 0.2mm diameter can be classified as a small perforation and has a better prognosis than larger perforations (7,22). Finally, the more immediate the repair the better the prognosis (7,8). This is likely the result of increased time for bacterial colonization and epithelial infiltration of the site. Since bacteria and bacterial byproducts cause progressive periodontal damage (2), it is reasonable to presume that the prognosis of perforation repair will also be affected by the bioburden associated with the defect.

The mandibular molar furcation perforation provides a very clinically relevant model to study perforations as 54% of all perforations occur in mandibular molars (14) and furcation perforations have the poorest prognosis (8,9). The difficulty in restoring furcation perforations is likely due to the crestal location facilitating communication with bacteria of the oral cavity and the furcal location making accessibility for cleaning and restoration a challenge.

Perforation Repair

Management of perforations has changed dramatically over time. Initial attempts at repairing perforations were performed both surgically and non-surgically and used materials like chloro-percha, amalgam and zinc phosphate cement. Histological examination demonstrated that sealed perforations responded favorably when compared unsealed ones and that chloro-percha repairs resulted in less inflammation adjacent to the repair then other materials (23). However, outcomes studies by Benenati *et al.* and Kvinnsland *et al.* found that the long term prognosis for non-surgical repairs with these materials is limited (18,24). Interestingly, both studies found that these materials had a 92-100% success rate when a combined non-surgical/surgical approach was taken.

To improve healing of perforation repairs and possibly invoke hard tissue development, Beavers *et al.* tested the use of a hard setting calcium hydroxide to fill the defect. However, no difference was seen in the pattern of healing when compared to controls (25).

Resin restorations have also been tested as furcation repair materials. Dye leakage studies by Alhadainy and Himel found that light-cured materials performed better than self-curing resin materials and found that acid etching further improved resistance to dye leakage (26,27). The major issue with resin materials however is their inherent cytotoxicity as the potential exists for continuous release of cytotoxic substances could prevent healing (28).

As mentioned above, surgery provides a possible avenue for improving the contours and thus potentially the healing potential of perforation repairs. Another means of achieving the same goal is to limited extrusion of the material during restoration using an internal matrix. An internal matrix is a restoration that fills the bony defect adjacent to a perforation allowing condensation or compaction of the repair material while limiting extrusion beyond the confines of the root. A review article by Lemon described an internal matrix technique using hydroxyapatite (29). A number of other materials have been tested for use as a matrix including amalgam (30), collagen (31), calcium hydroxide (30), decalcified freeze-dried bone (32), and plaster of Paris (26,33). These studies demonstrated that an internal matrix resulted in better adaptation and improved healing when tested, regardless of material placed. The improved outcome is likely due to improved adaptation of the repair material which may limit the inflammation caused when using a less biocompatible material.

MTA

According to Kratchman, there are three restorative factors that determine the effectiveness of a perforation repair; seal, adaptation and biocompatibility (34). MTA is known for being both highly biocompatible and forming an excellent seal. This led to its introduction as a perforation repair material in 1995 (12).

Biocompatibility

An *in vivo* dog study found that MTA produced a significantly milder inflammatory response compared to amalgam (12). When studying the response of specific cells, MTA

has also performed very well. Camilleri *et al.* found that MTA supported human osteosarcoma cell proliferation significantly more after undergoing a hydration reaction during setting in which calcium hydroxide is produced (Camilleri, Montesin, Di Silvio, & Pitt Ford, 2005). Using scanning electron microscopy (SEM), Balto demonstrated that set MTA supported human periodontal ligament cell attachment, which the authors argue is an important measure of biocompatibility for endodontic materials (36). Al-Rabeah *et al.* used SEM to demonstrate that human alveolar bone cells will attach to MTA and proliferate forming an intimately associated matrix (37). D'Anto *et al.* found that human bone marrow-derived mesenchymal stem cells responded to MTA with increased adhesion, cell proliferation and migration (38). The authors argue that this evidences the ability of MTA to form an environment which is conductive to wound healing. Periodontal ligament fibroblasts also respond favorably to MTA with increased cell adhesion and growth (39). Guven *et al.* demonstrated that MTA stimulated expression human growth factor, and subsequently transforming growth factor beta-1 and bone morphogenic protein 2 in human fibroblasts (40). The ability of MTA to stimulate growth factor expression may explain its ability to induce proliferation and function in a wide variety of cells.

The biocompatibility of MTA is a result of its composition. Raw MTA is primarily made up of calcium oxide and silicon dioxide. Upon mixing, tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite are formed. Adding water to initiate setting results in a hydration reaction that forms a silicate hydrate gel (11). As part of the setting reaction calcium hydroxide is produced resulting in a release of

calcium ions. Additional calcium ions are released gradually as calcium silicate hydrate decomposes (41). Camilleri suggests that it is the high amount of calcium that leaches out from MTA that accounts for its biocompatibility.

Interestingly, in the presence of phosphate, crystals form upon the surface of the MTA which chemically and structurally resemble hydroxyapatite (42). This was confirmed by Gandolfini *et al.* who found that apatite formation begins as quickly as five hours after immersion in a phosphate-containing solution and within seven days a uniform layer of apatite crystals formed over the MTA (43). The layer of hydroxyapatite is osteogenic and likely the reason for MTA's osteogenic potential and biocompatibility. The hydroxyapatite may also stimulate cementum deposition at the repair site. An *in vivo* dog study by Holland *et al.* found that MTA repairs resulted in consistent cementum deposition during 180 days of healing (44).

Seal of MTA

Shortly after its introduction, Torabinejad *et al.* demonstrated the superior seal of MTA when compared to amalgam, Super-EBA and IRM in a root end filling dye leakage study (10). This finding was confirmed by a second dye leakage study that used a lateral root perforation model (45). Leakage studies which have compared grey and white MTA have found no difference in performance between the two iterations (46,47) and when leakage did occur it was more likely to occur in an orthograde rather than retrograde direction (47). The excellent seal associated with MTA may be due in part to its tendency to expand during setting. Storm *et al.* demonstrated that grey MTA undergoes a 1.02% hydroscopic linear expansion while setting (48).

Adaptation

Bargholz presented a modified matrix technique that was used in conjunction with MTA to repair perforations in a case series. Collagen was used to displace granulation tissue and act as a barrier against which MTA was condensed. This method resulted in excellent adaptation and nearly complete healing of the defect at one year (49). However, it is reasonable to expect that extrusion of extremely biocompatible materials would be less impactful to healing outcomes. This was the case in a study by Mente *et al.* which demonstrated that extrusion of MTA in open apex orthograde treatments did not significantly impact long term healing (50) suggesting that adaptation may be less of an issue when more biocompatible materials are used for the repair.

Set time

One disadvantage of MTA is its lengthy set time. Torabinejad *et al.* first advised a set time of up to two hours and forty-five minutes for grey MTA (13). However, Charland *et al.* found that all samples of MTA were not set until 36 hours after placement when in the presence of human blood (51). Both studies however suggest that to ensure full setting of the material a second visit is required, which has led to several interesting alternatives being studied that could allow for a single visit procedure.

Ha *et al.* demonstrated a strong positive correlation between particle size and setting times. The authors speculate that the larger particles react more slowly due to a decrease in relative surface area and propose that developing a MTA formulation with a smaller mean particle size could decrease setting times (52). Camilleri found that bismuth oxide

caused an increase in setting time and therefore excluding it from the formulation could improve setting time (53). Various additives have also been used in attempts to accelerate setting. Kogan *et al.* tested a several additives and measured their effect on setting time and compressive strength. The authors found that when sodium hypochlorite gel, KY jelly or calcium chloride were added to MTA the setting time was reduced to 25 to 30 minutes. However, compressive strength decreased significantly (54). Another study demonstrated that calcium chloride increased the setting pH and calcium release, both suggestive of an increase in biocompatibility (55). This was supported in a later study which found that the pulpal response to direct pulp cap using MTA accelerated with calcium chloride was similar to that of plain MTA (56). Wiltbank *et al.* tested several other calcium based additives and found that calcium nitrite/nitrate and calcium formate decreased the setting time of MTA to below ten minutes (57). To the satisfaction of dental providers and patients alike, this setting time would allow for a single visit repair. Although the results of these studies are promising, further study of how these additives may affect biocompatibility are recommended by all authors.

Possibly the simplest solution to overcoming the two-visit procedure is to simply restore the tooth before the MTA is set. Using spectral analysis and a glass mold model, Nandini *et al.* demonstrated that the setting of MTA was unchanged when an adjacent glass ionomer was placed after only 45 minutes of set time (58). A subsequent study by Ballal *et al.* found that there was no difference in glass ionomer setting time or appearance of craze lines in the glass ionomer if MTA was completely set or after only 45 minutes of setting time (59). Together these results suggest that the MTA repair can be completed

followed by a resin-based material to cover the unset MTA and a definitive restoration. Eid *et al.* tested the effectiveness of this approach using glass ionomer placed over the MTA repair either immediately, after one day or after seven days. The authors used a plastic cylinder model and found that the immediate restoration resulted in a transient decrease in microhardness which was not significantly different from delayed restoration groups after eight days. The MTA-glass ionomer interface was also studied using SEM and the authors found that the adaptation of MTA appeared to improve with the increased time between placement of MTA and glass ionomer. This was evidenced by a higher incidence of interfacial porosity and cracking in the immediately restored group (60). This corroborated the findings of another study by Camilleri which demonstrated that glass ionomer cements can absorb the water from setting MTA resulting in increased porosity (61).

Yesilyurt *et al.* tested the shear strength of glass ionomer restorations which were placed over the either fully set MTA or after 45 minutes of setting. The authors found no significant difference between the two groups (62). This is contradicted by Atabek *et al.* who examined the effect of setting of MTA on the shear force strength of an adjacent composite restoration in acrylic blocks using a variety of bonding systems. The authors of this study found that shear force strength significantly improved with increased setting time of MTA before restoration (63). The difference in results is likely due to the inherent differences in hydrophilicity of glass ionomer and resin composites. Tsujimoto *et al.* evaluated surface integrity qualitatively using SEM and found no difference in gap formation between groups restored with composite resin within ten minutes, 24 hours or

seven days of MTA placement (64). The results of most studies support the assertion that restoration in a single visit is feasible, however there have been no studies that have examined how the tooth-restoration interface may be influenced.

Leakage Studies

One of the factors that has been demonstrated to impact the success of root canal treatment is the quality of obturation (65–68). Any gaps in obturation of the root canal system offer a potential space for bacteria to persist or move from the oral environment to periradicular spaces. Subsequent exposure of the peridontium to persistent or secondary bacterial contamination can result in persistent disease (69). Thus, the goals of obturation include providing a dense filling of the root canal system as well as a fluid tight seal. The relationship between outcomes and quality of obturation justifies maintaining the concept of leakage as important area of study in endodontics. The correlation between incomplete obturation and leakage was established in the 1950's by Dow and Ingle (70). This combined with the relative ease and cost effectiveness of leakage studies led to an explosion in their popularity in endodontic literature. In the early 1970's leakage studies comprised approximately 3% of the published studies in the Journal of Endodontics, however by 1990 this number increased to approximately 25% (71).

A wide variety experimental designs have been used to study leakage in endodontics. Historically, the most popular method was a semi-quantitative technique that involved exposing the sample to a tracer and taking a linear measurement of the maximum depth of tracer penetration. This is considered semi-quantitative as it is dependent upon the manner in which the sample is prepared for measurement (horizontal sectioning,

longitudinal sectioning or clearing) and does not measure a volume of leakage (71). Camps and Pashley illustrated the ineffectiveness of this measurement technique by demonstrating that linear dye penetration was not correlated to relative volume of dye in a spectrophotometric study (72). These techniques are fraught with many other issues. Matloff *et al.* compared the most popular tracers of the time and derived several important conclusions: 1) despite rigorous standardization of sample preparation there was still great variability was seen in amount of leakage, 2) no teeth showed perfect sealing, 3) the relative volume of tracer penetration was very small and may not be clinically relevant, and 4) there was no correlation between the leakage of the dye tracers and radioisotope tracers included in the experiment (73).

These findings have led to a moratorium on the publication of leakage studies in many endodontic journals and prompted a list of recommendations for future leakage research from Wu and Wesselink. The authors suggest that: 1) the length/size and anatomy of all samples should be similar, 2) measurements should produce quantitative volumetric data, 3) the pH of the tracer should be known, and 4) the quantitative relationship between tracer leakage and bacterial leakage should be established (71).

This has prompted researchers to develop novel methods to measure leakage. Garip *et al.* developed a fluid filtration technique using a computerized fluid filtration meter with a laser system and a digital air pressure regulator (74). Camps and Pashley introduced a dye extraction and spectrophotometric technique (72). These techniques produce quantitative data, however we lose the ability to visualize where the leakage is occurring. Bacterial (75) and lipopolysaccharide (76) leakage studies have also been explored

however these studies rely upon time to turbidity and do not produce quantitative volumetric data. A quantitative glucose leakage model was developed (77), however the system is sensitive to microbial consumption of glucose which could artificially lower measurements. Radioisotopes have been used as tracers, however these methods provide only qualitative data (78). Finally, correlates of leakage like radiographic or microscopic assessment of voids could be used, however these are indirect measures of leakage potential.

A promising solution may lie in a technique that has been pioneered at the University of Minnesota and recently applied in the study of marginal adaptation in coronal restorations. Carrera *et al.* used silver nitrate infiltration and micro-computed tomography to quantitatively evaluate tracer penetration at the tooth-restoration interface of composite restorations (79). The study demonstrated that the technique could be effective to in evaluating the marginal integrity of coronal restorations.

SPECIFIC AIMS

1. To determine if restoration with glass ionomer over unset MTA affects the amount of silver nitrate penetration in a furcation perforation repair.
2. To evaluate micro-computed tomography as a technique by which to quantitatively measure marginal integrity in endodontic research.

HYPOTHESIS

Covering a furcation perforation repair with glass ionomer over unset grey MTA will differ in penetration of silver nitrate as measured by micro-computed tomography compared to set grey MTA.

NULL HYPOTHESIS

Covering a furcation perforation repair with glass ionomer over unset grey MTA will not differ in penetration of silver nitrate as measured by micro-computed tomography compared to set grey MTA.

MATERIALS AND METHODS

1. Sample Preparation

Twenty-two previously extracted human mandibular molars were selected for this study. Teeth with fused roots, inaccessible furcation areas, radicular caries or restorations, previous root canal treatment or visible root fractures were excluded. The use of human molars was approved by the University of Minnesota Institutional Review Board. The teeth had been stored in 10% formalin. The teeth were cleaned by removing the residual soft tissues and hard deposits attached to the surface. A furcation region section was obtained by sectioning approximately one millimeter below the cementoenamel junction and again approximately one millimeter below the roof of the furcation using an Isomet™ diamond saw (Buehler, Lake Bluff, IL, USA). Any pulp tissue remaining was removed with an endodontic explorer. The sections were then transferred to 5.25% sodium hypochlorite for 24 hours for disinfection. Canal orifices and radicular openings were blocked with Krazy Glue™ (Elmer's Products, Columbus, OH, USA) and all surfaces of the samples were coated with two layers of nail polish to limit silver nitrate penetration outside the area of interest.

Each sample had a perforation induced from the middle of the pulpal floor to the height of the furcation using a 556 bur in a highspeed handpiece. An approximately half millimeter radius of nail polish was removed from the pulpal floor using a #6 round bur and a slow speed handpiece. Samples were then randomly assigned to one of three groups. The ten samples in group 1 would be restored with grey ProRoot MTA (Dentsply Sirona Inc., York, PA, USA) followed immediately by Vitrebond (3M ESPE, St. Paul,

MN, USA), while the ten samples in group 2 would not have the glass ionomer placed for approximately 72 hours after the MTA was placed. 2 samples were used as negative controls. One was restored in the same manner as group 1 and the other in the same manner as group 2. The negative control samples would not be exposed to silver nitrate prior to scanning to measure the volume that could be accounted for by radiopaque elements of the restoration itself. Samples were mounted by group into one of two blocks of green floral foam which had been moistened with sterile saline. Perforation repairs were performed by one endodontic resident under 7.5x magnification using a dental operating microscope (OPMI 1-FC, Carl Zeiss Meditec Inc., Jena, Germany). MTA was mixed on a glass slab with sterile water using a dental spatula according to manufacturer's instructions (80). Mixed MTA was delivered to the perforation using a 0.99mm disposable MTA carrier (Inter-Med/Vista Dental Products, Racine, WI, USA) and condensed using a #60 nickel titanium condenser. At approximately ten minutes after the MTA was placed, group 1 samples had a layer of glass ionomer placed. The Vitrebond™ was dispensed onto a mixing pad and mixed according to manufacturer's instructions (81) and placed over the repair site using the condenser such that the MTA and any exposed dentin were covered. The Vitrebond™ was then light cured for twenty seconds. All samples were left in the foam blocks. The blocks were wrapped in moist paper towel and sealed in a plastic storage bag. 72 hours later group 2 had Vitrebond™ placed in the same manner described above. Figure 1 illustrates the sample design.

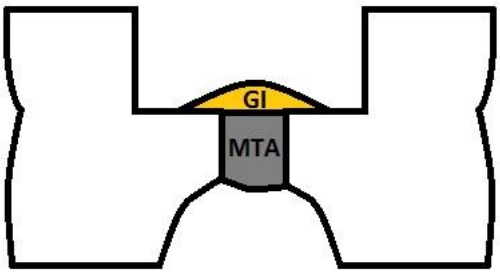

Figure 1. Sample restorative strategy. Furcal perforations were first restored with grey ProRoot MTA™ (MTA) and subsequently with Vitrebond™ glass ionomer liner (GI).

Samples were then stacked in columns of four such that the furcation area and pulp chamber floor were still accessible to fluid and stabilized with Krazy Glue™. This was done to reduce the number of micro-computed tomography scans required.

2. Silver nitrate exposure and scanning

To reveal any interfacial defects between the restoration and the tooth structure, the specimens were submerged in a radiopaque silver nitrate solution (25% w/w) for six hours. The pH of the silver nitrate solution was measured to be approximately 3.0. After the silver nitrate exposure samples were rinsed with deionized water to remove any silver nitrate that may be adhered to the surface of the nail polish. The negative control samples were submerged in deionized water for six hours instead of the silver nitrate solution. Scanning of the specimens was performed using a micro-computed tomography machine (XT H 225, Nikon Metrology Inc., Brighton, MI, USA). The scanning parameters used were 90 kV, 90 µA, 708ms of exposure, 720 projections and 4 frames per projection. The total scanning time was approximately 30 minutes for each stack of samples.

3. Image Processing

Three-dimensional reconstructions were completed using CT Pro 3D (Nikon metrology, Inc., Brighton, MI, USA). Visualization and 3D rendering was performed using VGStudio MAX 2.1 (Volume Graphics GmbH, Heidelberg, Germany). The reconstructed images were examined in all planes for silver nitrate penetration, as indicated by a bright area produced by the radiopaque dye along the interface between the tooth and restoration as well as within the restoration.

The radiodensity at the peak volume for root dentin was determined for each scan using the Volume Analyzer tool and the threshold for silver nitrate was set to 3.95 times this measurement. This threshold had been determined by relative radiopacity of the examiner's selection of what appeared to be silver nitrate in several samples. The volume above this threshold was then used to determine the volume of silver nitrate solution.

4. Stats

Due to the low number of samples, a Mann-Whitney U-test (Microsoft® Excel® 2016) was carried out to determine if there was a statistically significant difference in the mean volume of silver nitrate penetration between the two groups. The level of statistical significance was set at $p < 0.05$.

RESULTS

Upon initial inspection, it was apparent that all samples in groups 1 and 2 had silver nitrate penetration, which was demonstrated by a bright radiopacity along the MTA-dentin interface and/or within the MTA itself. Figure 2 demonstrates a representative scan prior to analysis, selection of an area and radiodensity of interest, and a three-dimensional representation of the volume of silver nitrate penetration.

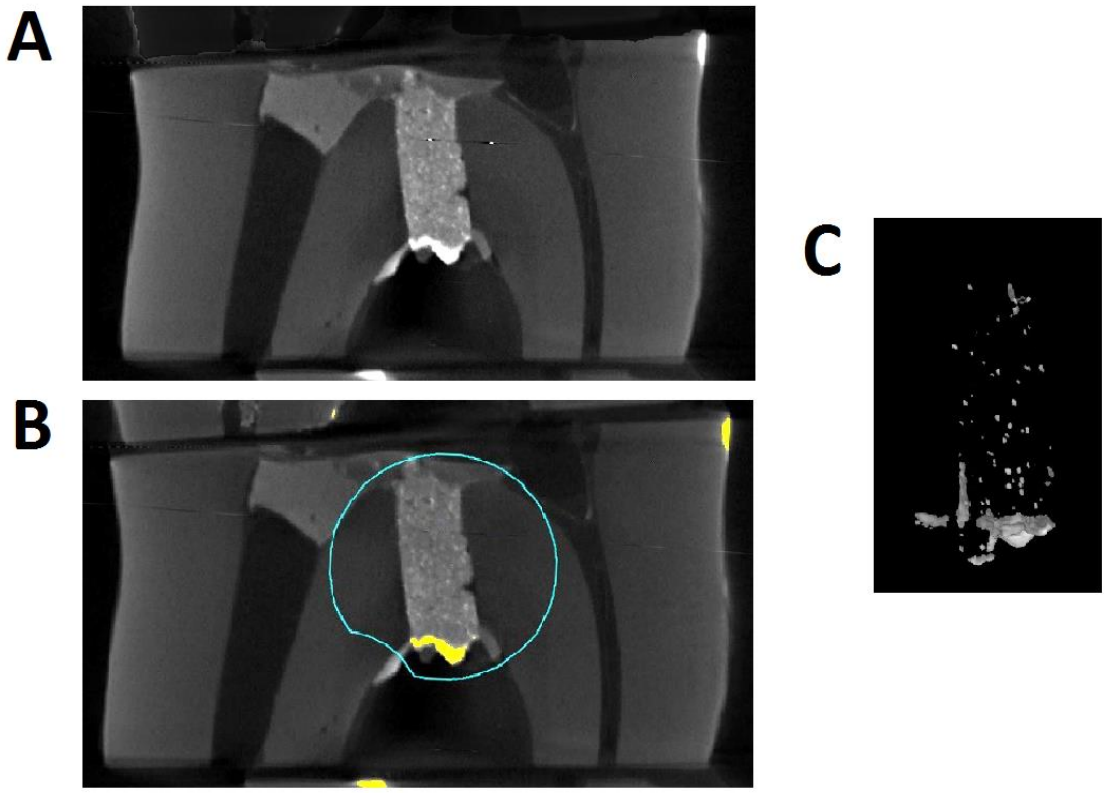

Figure 2. Representative sample analysis. A) Micro-computed tomography cross section prior to analysis. B) Selection of radiodensity of interest highlighted in yellow and area of interest contained within the light blue line. C) Three-dimensional volume obtained by the selection.

Three samples had excessive silver nitrate penetration and were all associated with restorations that had inadequate adaptation. Figure 3 demonstrates a sample with excessive silver nitrate penetration and obvious voids in the restoration.

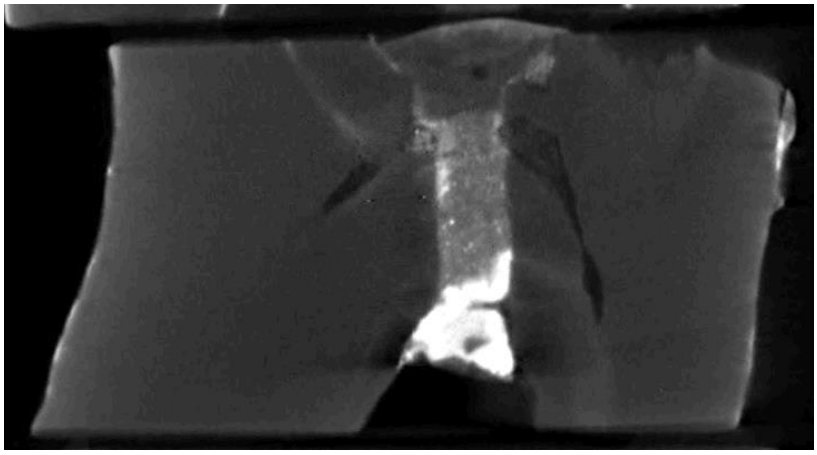

Figure 3. Inadequate restoration. Excessive silver nitrate penetration and large voids in the MTA restoration.

All samples had silver nitrate solution penetrating from the furcal surface and only 2/20 samples also had penetration from the pulp chamber surface. Both were from group 1. Figure 4 demonstrates a sample that had silver nitrate penetration from the pulp chamber surface.

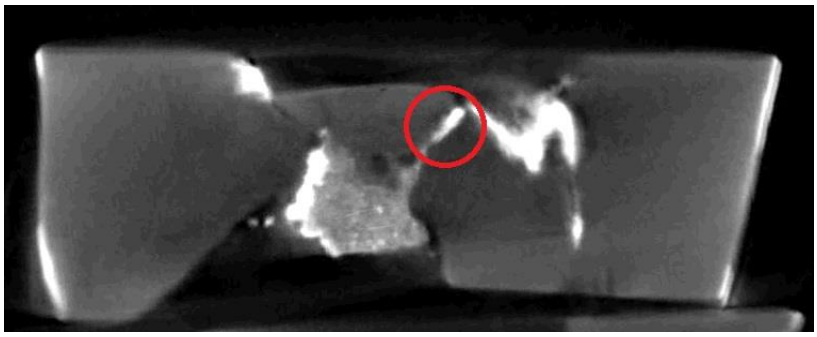

Figure 4. Pulpal silver nitrate penetration. Red circle highlights area of pulpal silver nitrate penetration.

11/20 samples also had silver nitrate solution penetrating into the MTA restoration itself.

Figure 5 demonstrates a sample that has silver nitrate solution penetration into the MTA.

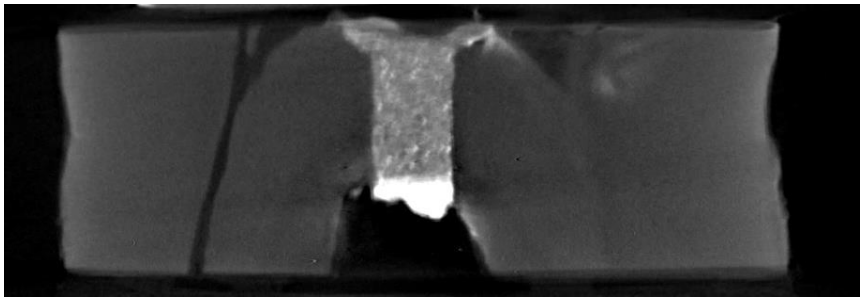

Figure 5. Silver nitrate penetrating restoration. Many samples demonstrated a permeation of silver nitrate into the MTA restoration. This presents as a radiopacity within the restoration itself.

9/20 samples also had excessive MTA extending beyond the confines of the radicular tooth structure. Figure 6 demonstrates a sample that has extruded MTA.

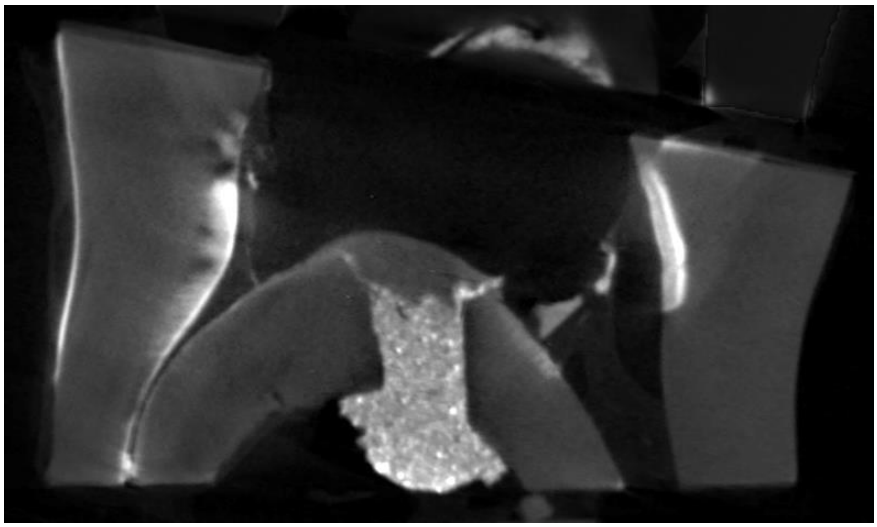

Figure 6. MTA extrusion. Many samples had relatively dramatic extrusion of MTA into the extraradicular space.

Most samples had very little noise. Table 1 details the incidence of each of the conditions described above. Control samples had no apparent silver nitrate penetration. Figure 7 demonstrates one of the control samples.

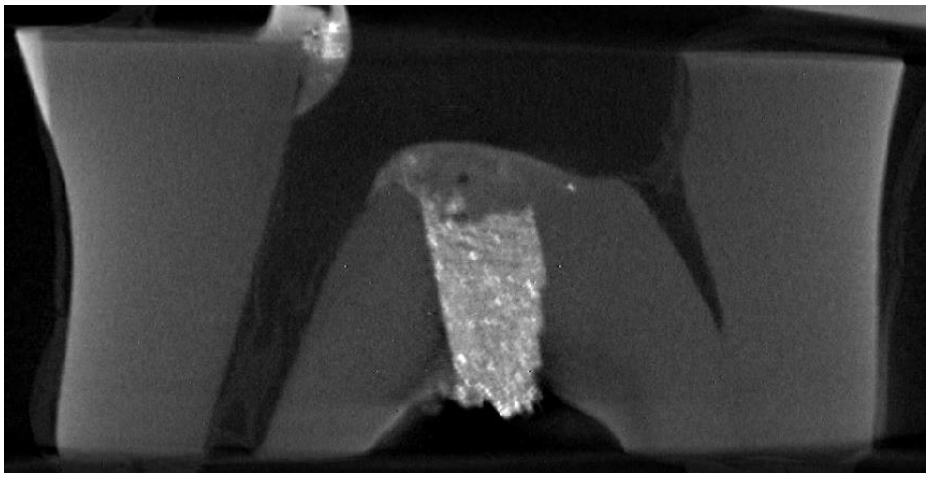

Figure 7. Representative control sample. Although the restoration has some radiodense inclusions, there is no apparent silver nitrate penetration into or around the restoration.

Calculation of the volume of silver nitrate penetration showed that the samples that were immediately covered with Vitrebond^{TM} (group 1) demonstrated less silver nitrate penetration than those that had been covered after full setting of the MTA. Table 2 demonstrates the calculated volume of silver nitrate penetration. The mean volumes above the radiographic threshold for group 1 and group 2 were 0.24 ± 0.27 and 0.54 ± 0.25 mm^3, respectively. The difference between the two groups was statistically significant ($p < 0.05$). Although the control samples had no apparent silver nitrate penetration upon initial inspection, they had volumes measuring 0.01 and 0.02 mm^3 that exceeded the threshold.

Condition	Group 1	Group 2	Total
Inadequate Restoration	1/10	2/10	3/20
Pulpal Penetration	2/10	0/10	2/20
Penetration into MTA	3/10	8/10	11/20
MTA Extrusion	3/10	6/10	9/20

Table 1. Incidence of observed conditions. The incidence of an inadequate restoration, pulpal penetration, penetration into the MTA and MTA extrusion by group and totaled.

	Group 1	Group 2
	0.02	0.10
	0.95	0.39
	0.04	0.55
	0.03	0.37
	0.39	0.99
	0.27	0.58
	0.09	0.51
	0.14	0.50
	0.35	0.95
	0.09	0.41
Mean	0.24[a]	0.54[b]
Standard Deviation	0.27	0.25

Table 2. Silver nitrate penetration. Volume of silver nitrate penetration per sample as well as mean and standard deviation displayed in cubic millimeters. A significant difference exists between groups 1 and 2.

DISCUSSION

Restoring on top of set or unset MTA

Grey MTA was the first bioceramic on the market and is still used by many clinicians today due to its numerous beneficial properties, most notably excellent biocompatibility and sealing. Although other bioceramics have emerged on the dental market, none have the litany of publications, including long term outcome studies, that MTA has. One of the drawbacks of MTA is the recommendation that full setting be achieved prior to restoration. The extended setting time of two hours and 45 minutes essentially necessitates that all procedures using MTA be done in at least two visits. The manufacturer recommends that at the first visit, a moist cotton pellet is placed over the unset MTA followed by temporary restoration. At the second visit, the cotton pellet can be removed and a definitive restoration can be placed against the set MTA (80). Many solutions have been attempted to get around the issue including altering the formulation and physical characteristics of the restoration itself. However, whenever any change from the recommendations is made it is important to ensure that none of the fundamental properties of the material have been affected. This is especially true of the attributes that support the materials use in the first place. For MTA, these are biocompatibility and seal. Without ensuring that the MTA restoration itself has not changed, we cannot rely on the results of previous studies that followed recommended protocols.

One of the simplest solutions has been to simply restore on top of unset MTA with a resin liner. This option has the benefit being unlikely to affect the biocompatibility as nothing has been added or removed from the formulation. Multiple studies have confirmed that

setting of the MTA and the resin does not appear to be compromised (58,59). As a correlate for all properties of MTA, Eid *et al.* tested microhardness and determined that the change in procedure had no effect (60). Although a consistency in microhardness is a positive sign that the material has not changed, it is not one of the principle reasons that a biocermic is recommended over other materials in a perforation repair. Similarly, many studies have attempted to indirectly measure seal using microscopy, radiography or shear strength and found at most a small increase in voids between the MTA and the glass ionomer (60–62,64). To date there have been no attempts to directly measure changes in seal when a single visit protocol is used. Additionally, these studies were all performed in glass or ceramic cylinders, which can reduce variability among samples but may not accurately reflect the conditions *in vivo*. Finally, with the exception of Tsujimoto *et al.* (64), these studies used a 45 minute set time as the test condition. If the goal is to validate using the technique in a single visit, 45 minutes may not reflect a timepoint that is clinically meaningful.

The current study uses an extracted tooth model which is more reflective of an *in vivo* scenario. It also uses a ten-minute time for the unset group. This more accurately reflects the clinical demands if the goal is to complete the procedure in a single appointment. Finally, the ability to quantitatively measure leakage allows the direct testing of one of MTA's most important qualities, the formation of an excellent seal.

The finding that every sample had some silver nitrate penetration is one that is common to many similar studies. This lessens the discriminatory ability of the test and complicates interpretation of the results. We know that clinically not all MTA perforation repairs fail

and in fact the success rate is extremely high (12,82). Yet all samples demonstrated silver nitrate penetration, therefore we cannot make a direct connection between tracer penetration and failure. We must however except that if bacteria or bacterial byproducts can move from the peridontium to the root canal system or vice versa the result is an increased likelihood for persistent or recurrent disease. Therefore, while silver nitrate penetration may not be correlated directly to successful outcomes, it can serve as point of comparison of restoration quality, which in itself is related to successful outcomes (66,67). The link between tracer penetration and quality of restoration is demonstrated in the findings of this study by the observation that the three samples which had inadequate adaptation of the restoration to the defect walls had dramatically more silver nitrate penetration than all other samples regardless of which group the sample came from.

When comparing the two groups, the immediately restored group (group 1) leaked significantly less than the two-visit group (group 2), allowing rejection of the null hypothesis. This finding was unexpected and suggests that a single visit protocol improves the seal produced by grey MTA. One possible explanation for this result can be proposed based on the findings of Storm *et al.* (48) who found that as MTA sets it undergoes linear expansion, particularly grey MTA. This expansion occurs over the first 24 hours and if the pulp chamber surface is sealed by a restoration this may force the MTA to expand toward the defect walls more than it would have otherwise. The consequences of this would be an improved adaptation to the defect walls and an improved seal. Regardless of the explanation, these results do support the findings of others and justify immediately restoring on top of unset MTA (58–60,64). In fact, these

findings suggest that certain characteristics of MTA may improve when used in this fashion.

This study found that while all samples had silver nitrate penetration from the furcal side, only two had penetration from the pulp chamber side. This is in contrast to the findings of another MTA study, however the experimental models are vastly different and glass ionomer was not used to cover the coronal surface of the MTA in the other study (47). The propensity for furcal penetration may be due to one or both of the following explanations; a good seal is more readily achieved at the pulpal aspect of the defect as it is easier to visualize and firmly condense, or glass ionomer provides a better seal than MTA. None of the samples had complete silver nitrate penetration from the root canal system to the extraradicular area, which may be a sign of clinical success of all the restorations.

Perhaps one of the most interesting findings of the study was the penetration of silver nitrate into the MTA itself. This was first noted in a single sample pilot study and was thought to be due to the sample having been allowed to desiccate prior to submersion in silver nitrate. In this study, samples were always stored in the moistened floral foam, wrapped in a moistened paper towel and sealed in a plastic storage bag. Still, 11/20 samples demonstrated a front of silver nitrate penetration extending from the furcal surface into the MTA. Two other explanations have come to mind to explain this finding. First, it is possible that the silver ions are small enough, at 0.059nm diameter, to penetrate the MTA itself and if given enough time eventually the entire length of MTA would be infiltrated by the silver nitrate solution. Secondly, perhaps the furcal portion of the MTA

restoration was inadequately condensed leaving gaps within the restoration that allowed penetration. In the present experiment, an internal matrix was not used as it was assumed that the floral foam would provide sufficient resistance to allow adequate condensation of the MTA. This assumption was shown to be incorrect as many of the samples had MTA extrusion into the extraradicular space. If the second explanation is true it would suggest that use of an internal matrix could not only limit extrusion of the restorative material but also improve the density of the restoration.

The control samples did have a small volume of radiopacity that was above the chosen threshold. This is a result of some of the components of MTA, likely tetracalcium aluminoferrite, having a radiodensity that overlaps with silver nitrate. In future studies, this could be accounted for using the subtraction method suggested by Carrera *et al.* (79), however this would double the number of scans required and complicate analysis. The volumes detected in the control samples are small enough that they should be considered negligible.

Silver nitrate exposure and micro-computed tomography

The second aim of this study was to determine if silver nitrate immersion and micro-computed tomography could be useful in the study of marginal integrity in endodontics. To evaluate this one can reflect upon the criteria proposed by Wu and Wesselink (Wu & Wesselink, 1993). Although the use of tooth sections may be a more clinically relevant model, it certainly increased the variability among samples. If techniques like this are to be accepted and incorporated into the literature a large sample size or more stringent

sample selection criteria may be required to account for the inherent variability of tissue samples.

The technique produces quantitative volumetric data but this can be skewed by the radiopacity of adjacent structures and restorations. Certain endodontic materials, like grey MTA, may allow for relatively simple analysis of the resulting scans, however more radiopaque materials may require volume subtraction (79) or may not be amenable to this technique.

The silver nitrate solution is acidic. This may have led to erosion of the restoration or dentin and resulted in artificially higher penetration. Acceptance of the technique may be enhanced if the solution can be made more neutral or if a more neutral radiopaque tracer could be used.

The most concerning aspect of this technique is that the quantitative relationship between the silver nitrate penetration and bacterial penetration has not been established. In fact, since the average diameter of bacteria is approximately 0.5-1.0 μm (83), silver nitrate likely dramatically overestimates the amount of leakage that would be clinically relevant.

Leakage studies form the basis of many didactic discussions and impact many clinical decisions. Although there is a need for a standardized technique to evaluate leakage, a total ban on leakage studies will not persist indefinitely. With the emergence of new materials, particularly bioceramics, there is a need to develop at least some type of understanding of the seal that they provide. The technique used in this study provides a viable option to study leakage in endodontics.

Limitations

The study has several limitations most notably the small sample size. The cost of micro-computed tomography scans limited the number of samples that were included in the study and although efficiency was quadrupled by stacking samples, a larger sample size would have strengthened the results. The acidity and small size of the tracer, which were mentioned earlier, have likely overemphasized the amount of clinically relevant leakage that has occurred. Finally, although a sectioned tooth model is more clinically relevant than glass tubes, it does not account for the myriad of variables that are at play *in vivo*.

Future directions

Several facets of this study warrant further investigation. Repeating the experiment with different materials, particularly bioceramics, would provide useful information about how these materials compare in regards to seal in a furcation perforation repair. Another interesting study would be to compare multiple delivery techniques. Hand condensation, direct ultrasonic condensation, and indirect ultrasonic activation have been explored previously (Aminoshariae, Hartwell, & Moon, 2003; El-ma'aita, Qualtrough, & Watts, 2012; Yeung, Liewehr, & Moon, 2006), but not in a furcation perforation model. It would also be worthwhile to repeat the experiment using a firm internal matrix to allow for better condensation of the MTA. It would be interesting to see what effect if any this would have on the amount of silver nitrate penetrating the MTA. Finally, future research is required to improve and validate the technique for use in endodontic research. This could include finding a tracer that is less acidic and more size appropriate, as well as establishing the nature of the link between penetration of the tracer and bacteria.

CONCLUSIONS

1. In a furcation perforation repair model, silver nitrate solution penetration is lower in grey MTA restorations that are immediately covered by glass ionomer on the pulp chamber floor side than those that were covered after 72 hours.
2. Certain properties of MTA, namely seal, may be enhanced in single-visit procedures.
3. Silver nitrate penetration and micro-computed tomography is a feasible option for the study of leakage in endodontics and with continued research may fulfill all the requirements of an appropriate endodontic leakage methodology.

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APPENDIX: Statistical Analysis

Group	Volume AgNO3	Rank
1	0.02	1
1	0.95	18.5
1	0.04	3
1	0.03	2
1	0.39	11.5
1	0.27	8
1	0.09	4.5
1	0.14	7
1	0.35	9
1	0.09	4.5
2	0.1	6
2	0.39	11.5
2	0.55	16
2	0.37	10
2	0.99	20
2	0.58	17
2	0.51	15
2	0.5	14
2	0.95	18.5
2	0.41	13

Group	Sum of Ranks	Count	U Stat
1	69	10	14
2	141	10	86

Difference in U Stat between treatment 1 and 2 (72) is greater than corresponding critical value for $p \leq 0.05$ (23), therefore there is a statistically significant difference between groups 1 and 2.