An In Vitro Comparison of the Setting of MTA With and Without the Application of a Moist Cotton Pellet

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AN IN VITRO COMPARISON OF THE SETTING OF MTA WITH AND WITHOUT THE APPLICATION OF A MOIST COTTON PELLET

by

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ABSTRACT
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Introduction: The purpose of this study was to compare the setting of MTA with and without the application of a moist cotton pellet. Methods: Fifty samples of MTA were mixed according to manufacturer's instructions, placed with an amalgam carrier, and condensed with a plugger into a custom metal block with a well depth of 3.5 mm. Twenty-five of the MTA samples had a moist cotton pellet placed in contact with MTA mixture and twenty-five MTA samples had no moist cotton pellet in contact with the MTA mixture. The samples were placed in 100% humidity at 37°C and evaluated for penetration resistance with a Vickers microhardness tester every 30 minutes for 6 hours and again at 1 and 3 days. A Gillmore needle was used to test the setting time of the MTA. Statistical analysis was completed using repeated measures ANOVA. Results: MTA without the application of a moist cotton pellet displayed significantly greater penetration resistance for 6 hours and set significantly ($P<0.05$) faster than MTA with the moist pellet. At 1 and 3 days, however, the MTA with the moist pellet was significantly ($P<0.05$) harder than the MTA without the moist pellet. Conclusions: Application of a moist cotton pellet on MTA affects setting time and the hardness of the cement.

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CHAPTER I

INTRODUCTION

Mineral trioxide aggregate (MTA) was first introduced into the endodontic literature in 1993 by Lee et al. (1) as a repair material of lateral root perforations. Since then the use of MTA has expanded to other clinical applications that include root end filling, direct pulp capping, apical retrofills, furcation repair, treatment of root resorption, and apexification (2-4). The ideal material for many of these applications should provide a superior seal in order to prevent leakage. Additionally, the material should be biocompatible in order to prevent tissue irritability, toxicity, or other reactions. Many studies have shown that MTA falls under this "ideal material" category (5-24).

The main constituent of MTA is Portland cement (75 wt %) with bismuth oxide (20 wt %) and gypsum (5 wt %). Portland cement itself is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite (25). One gram of this powder component is mixed with 0.35 grams of water to form a creamy mixture with a working time of about 5 minutes. When the material is hydrated it becomes a colloidal gel and sets within 4 hours (25). Because of its hydrophilic characteristic, moisture in the surrounding tissue acts as an activator of the chemical reaction in this material (1). The initial pH of MTA when hydrated is 10.2 and the set pH is 12.5, which is comparable to that of calcium hydroxide. Arens and Torabinejad (26) have recommended covering MTA with a wet cotton pellet and a layer of IRM to get a better setting of the material. Sluyk et al. (27), who tested the material for retention characteristics when used as a furcation perforation repair material, found no statistical difference when a dry cotton pellet was used compared to a wet cotton pellet. However, they placed the MTA against a moist matrix which could be a possible explanation why it did not matter if a moist or dry cotton pellet was placed. Walker et al. (28) tested the

flexural strength of MTA and concluded that the materials strength at 24 hours was greatest when placing moisture on both sides of the material while setting compared to placing it on just one side of the material with dry gauze on the other side. There are certain procedures, such as apical retrofills and true one-step apexification, when a wet cotton pellet cannot be placed. In these situations, it is important to know whether the material is setting properly without the wet cotton pellet and whether the amount of moisture in the surrounding tissues with 100% humidity is adequate.

A review of the endodontic literature has indicated there is no general consensus if the application of a moist cotton pellet should be used to enhance the setting ability of MTA in various endodontic procedures. The purpose of this study was to compare the setting and resistance to penetration of MTA with and without the application of a moist cotton pellet.

CHAPTER II

LITERATURE REVIEW

In-Vitro Studies/ Dye-Leakage

Lee et al. (1) compared the sealing ability of Amalgam and IRM against the relatively new material MTA. They experimentally induced lateral perforations in extracted human teeth. They used 50 extracted teeth, made a standard coronal access, located the mesial canals and made a perforation from one of the mesial canal orifices toward the mesial surface of the root. The perforation site was enlarged with a #80 file until the tip extruded beyond the surface. Perforation sites were irrigated with saline, and then placed in a saline-soaked Oasis. Teeth were randomly divided into 4 groups and the perforations were repaired with either amalgam, IRM, MTA, or left alone as a positive control. After repair of the perforations, the access cavity in each tooth was filled with the same materials as had been used to seal the perforations. The teeth were kept in Oasis for 4 weeks. The entire surface except the perforation site was covered with nail varnish. The teeth were then placed in methylene blue for 48 hours. To determine the depth of dye penetration, each tooth was ground parallel to its long axis to expose the filled perforation site. All sections were evaluated under a dissecting microscope. The lengths of dye penetration were measured using an ocular micrometer. Linear dye penetration was measured independently by two observers. A two-way analysis of variance was used to determine the statistical difference between the three groups. They found that the dye penetrated the entire length of the perforation sites in the positive controls. Both IRM and amalgam demonstrated a considerable amount of dye penetration. The average linear penetration for IRM was 1.30 mm and 1.52 mm for amalgam. The MTA showed the least degree of dye penetration averaging 0.28 mm. There was no statistical difference

between IRM and amalgam while the MTA leaked significantly less than the other two materials ($p<0.05$).

Torabinejad et al. (5) looked at the sealing ability of MTA, amalgam, and Super-EBA as root end filling materials. He used a Tandem Scanning Reflected Light microscope (TSM) in conjunction with Rhoda mine B fluorescent dye to determine leakage and compare the various materials. Thirty extracted human single-rooted teeth were used in this experiment. Preoperative radiographs showed an absence of multiple canals, calcifications, or severe apical curvature. The clinical crown was removed at the CEJ, working length was determined and enlarged to a #40 K file, and NaOCl was used as the irrigant. Canals were dried with paper points and obturated laterally with gutta-percha and Grossman sealer. Access cavities were closed with Cavit. The teeth were wrapped in moist gauze and stored in a closed glass bottle at room temperature and 100% humidity for 1 week. Two coats of nail polish were applied to the external surface of each root. Apical roots were sectioned. In order to reduce the amount of dye penetration through the exposed dentinal tubules, the resected surface was sealed with a layer of Scotchbond multipurpose adhesive. The dentin was also etched, primed, and followed by a thin layer of adhesive. Apical cavities were made with a #1 round through the resin into the gutta-purcha filling material. The cavities were enlarged and deepened to approximately 3 mm. Then the same size diamond bur was used to standardize the preparation to a diameter of 1.5mm and a depth of 3mm. The roots were then randomly divided into three groups of 10 roots each. In group 1 the apical preps were filled with zinc-free amalgam. In group 2, the preparations were filled with super-EBA cement. In group 3, the preparations were filled with MTA. Two roots without sealer were used as

positive controls. Another 2 roots were covered entirely with two coats of nail polish and were used as negative controls. All roots were wrapped in wet pieces of gauze and stored in 100% humidity for 24 hours. The roots were then totally immersed in an aqueous solution of rhodamine B fluorescent dye for 24 hours. The root was sectioned into halves and placed on a glass slab for examination. Statistical analysis showed that the mineral trioxide aggregate leaked significantly less than amalgam and super EBA.

Torabinejad et al. (6) looked at the marginal adaptation of mineral trioxide aggregate (MTA) as a root-end filling material, compared with other commonly used root-end filling materials. They used a scanning electron microscope (SEM) to compare the adaptation of each material used. They used eighty-eight single rooted freshly extracted human teeth. The teeth were prepared for study by cleaning, shaping, and obturating the canals with gutta-purcha and sealer. The teeth were then sectioned at the apex and a cavity prep was made. The root end preparations were filled with amalgam, Super-EBA, IRM, or MTA. They used a slow-speed diamond saw to longitudinally section 40 roots into halves. Then a resin replica of resected root ends of the remaining nonsectioned roots were also prepared for study. They mounted the roots halves and resin replicas of resected roots on aluminum stubs and measured at four points under SEM the distance between the test root-end filling materials and surrounding dentin. Their findings showed that the original samples showed numerous artifacts in the longitudinal sections of the materials and their surrounding dentin shows that MTA had better adaptation compared with amalgam, Super-EBA, and IRM.

Fischer et al. (7) examined the bacterial leakage of MTA compared with commonly used root-end filling materials. Fifty-six single-rooted, extracted, human teeth

with straight canals were used for this experiment. Teeth were stored in 10% formalin before and throughout the experiment. Standard access cavities were prepared; canals were prepared in a crown down fashion with .04 taper Proseries 29 files. A standard diameter of the apical foramen was obtained; NaOCl was used as an irrigant to remove debris. The apical 3 mm of each root was sectioned at 90 degrees. A 3 mm deep root-end cavity was prepared with ultrasonic tips. The teeth were steam sterilized for 30 min. Zinc-free amalgam, IRM, Super-EBA, and MTA were mixed. The teeth were divided into groups of 10, and each group was root-end filled with a different material. For controls, eight other teeth were divided into two equal groups of four. Four were filled with gutta-percha with no sealer and the other four were filled with sticky wax. Nail polish was applied to external surfaces to prevent bacterial leakage through the root surfaces. The teeth were suspended in vials to the level of the cemento-enamel junction. After placing sterile phenol red broth in the vials, the caps were snapped into place and then sealed. The vials were inoculated overnight with a broth of Serratia marcescens into the root canal of each tooth via the coronal access cavity preparation. The entire apparatus was placed into an incubator maintained at a constant 37°C. The number of days required for S. marcescens to penetrate the four root-end filling materials and grow in the phenol red broth was recorded and analyzed. Most of the samples filled with zinc-free amalgam leaked bacteria in 10 to 63 days. IRM began leaking in 28 to 91 days. Super-EBA began leaking in 42 to 101 days. MTA did not begin leaking until day 49. At the end of the study, four of the MTA samples had not exhibited any leakage. Statistical analysis of the data indicated MTA to be the most effective root-end filling material against penetration of S. marcescens.

Torabinejad et al. (8) evaluated the ability of MTA as a root-end filling material to prevent bacterial leakage compared to amalgam, IRM, or Super-EBA. Fifty-six single-rooted extracted, human teeth were used, access cavities prepared, and the coronal portions of the canals were enlarged with Gates Glidden drills. The apical foramens were enlarged; NaOCl was used to remove debris. The apical 3 mm of each root was removed at 90 degrees. A root-end cavity preparation with 3 mm depth was made with a 330 bur. Forty-eight teeth were divided into four equal groups of 12 teeth each. Zinc-free amalgam, IRM, Super-EBA, and MTA were prepared. Ten root-end preparations were filled with each of the four root-end filling materials. As controls, eight teeth were divided into two equal control groups of four each. To prevent bacterial leakage through the root surfaces, two layers of nail polish were applied except for the resected ends. In the control teeth, four root-end cavities were filled with thermoplasticized gutta-percha without sealer, and the other four were filled with sticky wax covered with nail polish. Forty-six root canals were filled with trypticase soy broth contaminated with Staphylococcus epidermidis. A special apparatus set-up was constructed to perform this study. The number of days required for the test bacteria to penetrate various root-end filling materials was determined. Most samples whose apical 3 mm were filled with amalgam, Super-EBA, or IRM began leaking at 6 to 57 days. In contrast, the majority of samples whose root ends were filled with MTA did not show any leakage throughout the experimental period. Statistical analysis of the data showed no significant difference between the leakage of amalgam, Super-EBA, and IRM. However, MTA leaked significantly less than other root-end filling materials.

Tang et al. (9) compared the ability of amalgam, IRM, Super-EBA, and MTA to prevent endotoxin leakage when used as root end filling materials. One hundred four single-rooted extracted human teeth were used in this experiment. The teeth were cleaned; the apical 3 mm of each root was removed perpendicular to the long axis of the tooth with a diamond bur. The coronal portion of each tooth was resected at a point that provided a root with a total length of 15 mm. Each root canal was cleaned and shaped. The apical opening was enlarged to a #50. Canals were irrigated with 5.25% NaOCl. The roots were placed in 10 ml glass test tubes filled with 5 ml of 5.25% NaOCl solution, dried, and filled with Obtura gutta-percha without sealer. The apparatus consisted of upper and lower reservoirs. The root was inserted into the vial with the apex of the root protruding out of the vial. Except for the resected surface of the root, the rest of each root was coated with two layers of sticky wax. The root-end preparation was performed with a #557 carbide bur 3 mm deep and 1 mm in diameter. Two sets of four teeth served as controls with the first four having gutta-percha with no sealer as a positive control and the second four filled with sticky wax and served as a negative control. A remaining 92 roots were randomly divided into four equal experimental groups of 23 samples each. The root cavities were filled with either Super-EBA, amalgam, IRM, or MTA. One week after filling the top and the lower reservoirs with nonpyogenic water, a sample from the lower reservoir was examined for the presence of endotoxin. Then the lower was filled with the same water. They used a modified Limulus Amebocyte Lysate test for the presence of endotoxin as a tracer and compared the sealing ability of the materials listed above. They found that the MTA permitted less endotoxin leakage than

IRM and amalgam at 1, 2, 6, and 12 weeks, and leaked less than Super-EBA at 2 and 12 week periods.

Andelin et al. (10) evaluated the effect of resection on the microleakage of MTA. They used forty-six extracted human single rooted teeth that had to be at least 12 mm with no history of previous root canal therapy. Each tooth was accessed, instrumented to an ISO size 50 at 0.5 mm from the apex, and 5.25% NaOCl was used as an irrigant. The teeth were randomly divided into four groups. In Group 1, twenty canals were obturated orthograde with MTA. In Group 2, twenty more canals were obturated with gutta-percha using warm vertical compaction. Three canals were also filled the same as group 2 and used for negative controls. Three additional canals were filled with gutta-percha and no sealer and served as positive controls. All teeth were placed in a humidor for 48 hours. The apical 3 mm of the root apex of each tooth from Group 1 was resected at approximately 45 degrees. The coronal and lateral aspects of these teeth were coated with two coats of nail polish. The root ends were then submerged in India ink for 48 hours. After root-end resection, root-end cavities were prepared with a 330 carbon-steel bur to a depth of 3 mm in Group 2. MTA was placed in these preparations as a root-end filling material. All teeth were placed in a humidor for an additional 48 hours. The coronal and lateral aspects of these teeth were also coated with nail varnish. The root-ends were then submerged in India ink for 48 hours, after which the roots of all groups were then grooved on the buccal and lingual surfaces and split into two sections. The amount of dye penetration was examined with a surgical microscope at 16 X magnification. There was no discernible leakage in teeth with resected MTA or those with MTA placed as a retrograde root-end filling material. They also found no significant

difference in dye leakage between resected MTA (Group 1) and non-resected MTA (Group 2). Based on these results it appears that the resection of set MTA does not affect its sealing ability.

Valois and Costa (32) compared the ability of different thicknesses of MTA to prevent leakage through the use of a protein-dye complex with Coomassie Brilliant Blue G. The protein solution used in this experiment was bovine serum albumin at 22%. Sixty-four single-rooted, caries-free, human maxillary teeth with straight canals were selected for this experiment. Sixty-two teeth were prepared as standard access cavities with coronal portions of the canals flared with Gates-Glidden burs. The apical foramen was enlarged with a # 30 file to standardize the diameter. The preparation was completed by using a step-back of 1 mm increments. The apical 3 mm of roots were cut at 90 degrees to the long axis of the teeth. After root-end resection, sixty teeth were randomly distributed into 4 equal test groups containing 15 teeth each. Class I cavities were then prepared in root ends. In group I, the cavities were prepared to a depth of 1 mm; group II, the cavities were prepared to a depth of 2 mm; group III, the cavities were prepared to a depth of 3 mm; group IV, the cavities were prepared to a depth of 4 mm. The root-end cavities were dried and filled with MTA. An apparatus was prepared to evaluate protein leakage. The 1 mm thick MTA was the least effective in preventing apical leakage. No significance difference was found between 2 and 3 mm thick MTA. Four mm thick MTA was significantly more effective than the other thicknesses tested. The results of this study suggest that the thickness of 4 mm is most adequate for the use of MTA as a root-end filling material.

In-Vitro Studies/ Tissue Reaction

Kettering and Torabinejad (13) examined IRM, Super-EBA, and MTA for mutagenic potential by the Ames Test. IRM and Super-EBA were mixed according to the manufacturer's instructions. To prevent hardening of these substances and to keep them in a finely dispersed particulate suspension, a sufficient amount of alcohol was added to the mixture immediately following preparation of each material. MTA was mixed in a 1:1 powder to liquid ratio. A medium E was used and 15 g of agar and 20 g of glucose were added to 985 ml of medium E. The medium was autoclaved, and 20 ml was poured into plates and stored at 4°C until use. Nutrient Oxoid #2 broth and Bactoagar were obtained, as was Liver microsomal preparation S-9 which was prepared according to published methods. A plate incorporation assay was conducted using Salmonella typhimurium strains T-98 (R-factor) and T-1535 (non-R-factor) as the test strains. Organisms were streaked across the master plate and stored at 4°C after 24 hour incubation at 37°C. Positive controls (S9 protein and benzo-(α)-pyrene and N-methyl-N'-nitro-N-nitrosoguanidine) operated properly. No increase in revertant bacteria colony counts occurred with any of the test materials. Based on these results, it seems that IRM, Super-EBA, and MTA are not mutagenic as measured by the Ames Test.

Keiser et al. (14) investigated the cytotoxicity of MTA as compared with Super-EBA and amalgam, using human PDL fibroblasts, and an assay that assessed the metabolic activity of cells after exposure to extracts of the test material. PDL fibroblasts were obtained from the roots of impacted human maxillary third molar teeth extracted in the Oral Surgery Clinic at the University of Tennessee College Of Dentistry.

Immediately after extraction, the teeth were placed in Dulbecco's Modified Eagle's Medium (DMEM) at 4 °C. PDL tissues attached to the middle third of the roots were gently curetted off and placed in DMEM containing streptomycin, gentamycin, and amphotericin-B to prevent contamination. DMEM supplemented with 20% fetal bovine serum and antibiotics were added to the flasks followed by incubation at 37 °C in a humidified atmosphere of 5% carbon dioxide-95% air until fibroblast-like cells had grown to confluency. After reaching confluency, first passage cells were trypsinized, collected by centrifugation, placed in a freeze medium and stored. Test materials were MTA, Super-EBA, and a dispersed phase amalgam. Methyl methalcrylate (MMA) 2% was used as a positive control. They were placed into the bottom of 48-well tissue culture plates to achieve a thickness of approximately 5 mm and divided into two groups. The first group of all materials was freshly mixed and the second group was allowed to set for 24 hr at 37°C at 100% relative humidity. Extracts of test material were made and placed over each sample. Differences in mean cell viability values were assessed by ANOVA. In the freshly mixed state, the sequence of toxicity was amalgam>Super-EBA>MTA. In the 24 hr set state the sequence of toxicity at a low extract concentration was Super EBA>MTA, amalgam, and Super-EBA>amalgam>MTA at a higher extract concentration. This study supports the use of MTA in the root-end environment due to its low toxicity.

Koh et al. (15) aimed to find out why cementogenesis appears to be induced by MTA by investigating a cell capable of producing matrix, which in turn can be calcified. The selected cell line, MG-63 cells derived from human osteosarcoma, were grown in Ham's F12 medium with Dulbecco's modified Eagles medium(1:1) in a tissue incubator

with a humidified atmosphere of 5% CO_2; 95% air at 37°C until confluence was observed. Two cements were examined, MTA and IRM. Twelve samples of IRM were prepared and culture medium was added to each dish and left for 72 hours to age the cement. After this time, fresh samples of IRM and MTA were prepared. Fresh solutions of cells were prepared, placed in Petri dishes, medium was added, and then the cover slips with material were introduced. Petri dishes with glass cover slips, but no cement, were used as controls. After 1, 3, and 7 days, four Petri dishes from each group were removed from the incubator and prefixed in 1% osmium tetroxide. A total of 48 cover slips from the Petri dishes were examined. The specimens were dehydrated and critically point-dried before being sputter-coated with gold to a thickness of 15 nm. To determine cytokine production from cells in contact with MTA and fresh IRM, six specimens in each group at each time interval were used. The specimens were viewed by scanning electron microscopy. For cytokine evaluation, cells were grown either alone or in other dishes containing the test materials for 1 to 144 hours. Media were removed for ELISA analysis of interleukin (IL)-1α, IL-1β, IL-6, and macrophage colony-stimulating factor. The SEM revealed healthy cells in contact with MTA at 1 and 3 days; in contrast, cells in the presence of IRM appeared rounded. The ELISA assays revealed raised levels of all ILs at all periods when cells were grown in the presence of MTA; in contrast, cells grown alone or with IRM produced undetectable amounts. The macrophage colony-stimulating factor was produced by cells irrespective of the group. It appears that MTA offers a biologically active substrate for bone cells and stimulates IL production.

Koh et al. (16) investigated the possible role of MTA in orthopedic work and examined its cellular effects in terms of gross morphology of the cells and with respect to

its possible stimulatory effects on cellular activation. The MTA was mixed with 5 different proportions of water and powder and allowed to set. The material was analyzed in the absence of and within the environment of the cultured cells. To fix the cells the medium was removed and then fixed with 2.5 % glutaraldehyde in phosphate buffer. The cells were then prepared for histological examination. The cells were collected and assayed using the Sigma protocol. Examination of the MTA shows specific phases throughout the material. The MTA material appeared to be divided into calcium oxide and calcium phosphate. When examining the behavior of the cells with respect to the MTA, cells were seen in close proximity and growing over the amorphous noncrystalline structures that are phosphate. Areas of calcium oxide alone typically showed very little ingress of cells, and, in addition, it was found that upon setting, and in the absence of cells, the material formed a calcium oxide shell. The change in pH levels during the setting of MTA may induce changes in cellular behavior. Cells without MTA served as controls. In all dishes containing MTA, cells were seen adhering to the base at 6 hours and had decreased to confluence at 144 hours. Osteocalcin production also definitely was enhanced in the presence of MTA. There was no statistical difference between the control levels of alkaline phosphatase activity in the presence of MTA when compared to the control cells.

Zhu et al. (17) observed the adhesion of human osteoblasts on commonly used root-end filling materials with scanning electron microscopy. Human osteoblast-like Saos-2 cells were grown in RPMI medium supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic cocktail under standard cell culture conditions. Root-end filling materials used were MTA, IRM, amalgam, and composite resin. These materials

were mixed and placed in 96-well flat-bottom plates and condensed to disks of 1 mm thickness. Cell culture medium was put into the wells with the material and incubated. Then the material disks were removed from the plates for the cell adhesion test. Human osteoblasts were seeded into the wells and then incubated for 24 hours. The disks of materials along with the growing cells were washed. The samples were dehydrated in ascending grades of ethanol, immersed in hexamethyldisilazane for 30 minutes, air-dried, and sputter-coated with gold palladium. Specimens were examined in a scanning electron microscope at an accelerating voltage of 8 kV. The results showed that osteoblasts attached and spread on MTA and composite by forming a monolayer. Osteoblasts also attached on amalgam, but with few cells spreading. In the presence of IRM, osteoblasts appeared rounded with no spreading. These results indicate that osteoblasts have a favorable response to MTA and composite when compared with IRM and amalgam.

Thomson et al. (24) aimed to evaluate cell growth and morphology of cementoblast-like cells on MTA by scanning electron microscopy and to determine if MTA allows the production of gene and protein markers consistent with expression of mineralized cell phenotype in tissue culture. Murine cementoblastic cells used in this study were isolated and characterized as described previously. Cells were cultured in Eagle's medium and evaluated for cell attachment and growth in short-term assays using scanning electron microscopy. Materials tested in this assay included MTA, amalgam, and IRM. The cementoblasts were seeded on prepared materials. The results suggest that mineral trioxide aggregate permits cementoblast attachment and growth and the

production of mineralized matrix gene and protein expression, indicating that mineral trioxide aggregate can be considered cementoconductive.

In-Vitro Studies/ Chemical Effects

Sluyk et al. (27) evaluated the effect of time and moisture on the setting, retention, and readaptation characteristics of MTA when used to repair furcation perforations. Thirty-two freshly extracted maxillary and mandibular molars were used in this study. The crowns were removed at the level just above the floor of the pulp chamber, and the roots were moved just below the furcations. A perforation was made using a #2 round bur and the opening was enlarged to a #5 Gates Glidden bur to create a perforation 1.4 mm in diameter. The 32 teeth were randomly divided into four equal groups. MTA was then mixed and placed into the perforation followed by a moist cotton pellet. Setting characteristics were varied by placing a wet or dry cotton pellet in contact with the MTA for 24 or 72 hours. Group 1 was with moist cotton for the 24 hour setting time. Group 2 had the moist pellet placed on MTA for 72 hours. Group 3 was the same as group 1 except that a dry pellet was used, and group 4 received a dry cotton pellet for 72 hours. Instron testing was used to measure the force required to displace the material from the perforation. The force measurements showed that MTA resisted displacement at 72 hours to a significantly greater level than at 24 hours. When slight displacement occurred at 24 hours the material demonstrated the ability to re-establish resistance to dislodgement from the dentin wall. The presence of some moisture in the perforation during placement was advantageous in aiding adaptation of MTA to the walls of the

perforation, but there was no significant difference in MTA retention when a wet or dry cotton pellet was placed in the pulp chamber within the setting time.

Walker et al. (28) compared the flexural strength of MTA as a function of setting time and different hydration conditions. A split mold was machined from stainless steel to accommodate beam specimens. The mold was open on both the upper and lower surface. MTA was mixed according to the manufacturer's recommendation and condensed into the split mold. Then it was transferred to a container lined with gauze saturated with saline. To simulate MTA setting with external tissue moisture in combination with a dry, intracanal cotton pellet, the lower surface of the split mold/MTA specimen was in direct contact with the saline-saturated gauze and the upper specimen surface was covered with dry gauze. To simulate MTA setting with external tissue moisture in combination with an intracanal moistened cotton pellet, both the lower and upper surface of the MTA specimens were covered with saline-saturated gauze. The closed containers with specimens were stored at 37°C for either 24 or 72 hours. Thus, there were four experimental conditions, (1) 24-hour set time, 2-surface moisture; (2) 24-hour set, 1-surface moisture; (3) 72-hour set, 2-surface moisture; (4) 72-hour set, 1-surface moisture. The specimens were removed and the flexural properties measured using a three-point bend test. They found that the flexural strength of the 24h/moist/2-sided specimens (14.27 MPa) was significantly higher than the flexural strength values associated with conditions two, three, and four respectively (10.77, 11.16, and 11.18 MPa). The authors concluded that a moist cotton pellet should be placed on the intracanal MTA surface under a temporary restoration; and if possible, the moist cotton should only remain for 24 hours.

Huang et al. (29) attempted to reduce the setting time of WMTA without adverse diametral tensile strength (DTS) was pursued. The ProRoot WMTA was obtained and a liquid/powder ratio of 0.3 mL/g was used. In addition to water (the control) as a liquid phase, 0.9 % sodium chloride (NaCl), 1 mol/L tris (hydroxymethyl) aminomethane-hydrochloride (Tri-HCl) buffer, 15 % sodium hydroxide (NaOH), and 15 % calcium hydroxide (CaOH) were used to harden the WMTA powder. Specifically, the liquid accelerator was an aqueous solution of 5 % , 10 %, and 15 % Na_2HPO_4. In addition, 15 % sodium phosphate monobasic (NaH_2PO_4) was used as the liquid phase. After mixing the MTA, the cement was placed into a mold and stored in an incubator at 100 % humidity and 37°C for measurements of the final setting time, pH value, and DTS. The setting time of the phosphate solutions significantly reduced the setting time. There was no significant difference in the pH value of WMTA when mixed with the different liquids. The DTS values were significantly different from each other. When using 15 % Na_2HPO_4, after just 30 minutes, WMTA could achieve a higher DTS than that obtained for the 3-hour aged specimens at the other three conditions.

Ding et al. (31) evaluated the physicochemical and cytologic properties of mineral trioxide aggregate mixed with distilled water and sodium phosphate dibasic buffer solution. The MTA setting time and pH value were evaluated. MTA micrographs with scanning electron microscopy were also observed. Mouse fibroblasts were used to test the toxicity of MTA after the first and seventh day of treatment by a mitochondrial colorimetric assay. The results show the sodium phosphate dibasic buffer group reduced the MTA setting time, and the pH value in the distilled water group is similar with the sodium phosphate dibasic buffer group. The setting time decreased as the concentrations

of sodium phosphate dibasic buffer group increased. The results of the X-ray diffraction XRD produced similar peaks of the distilled water and sodium phosphate dibasic buffer solution groups. The initial pH of both the MTA mixed with distilled water group and MTA mixed with 15 % disodium hydrogen orthophosphate groups was approximately a pH of 11. The L929 cells were growing over the MTA surface on both groups. The survival rate of distilled water and sodium phosphate dibasic buffer solution groups did not exhibit any significant difference. There are differences in SEM observations both of the MTA surface and of the cells in culture on the surface of the MTA with sodium phosphate dibasic versus distilled water. The results suggest that 15% sodium phosphate dibasic buffer can be successfully used as an accelerator of MTA

Nandini et al. (33) assessed the dissolving ability of carbonic acid, 2 % chlorhexidine gluconate, 17% EDTA, and saline on set WMTA. The materials used were WMTA, freshly prepared carbonic acid, 2% chlorhexidine gluconate solution, 17% EDTA solution and saline. Eighty hollow cylindrical stainless steel ring molds of 5 mm height and 5 mm internal diameter were made. Surgical gel foam was placed on one end and moistened to simulate the clinical situation. WMTA was mixed to a thick creamy consistency and immediately condensed into the molds to a height of 4 mm. A moist cotton pellet was kept on top of the condensed WMTA and was stored in a humidor. Forty specimens were tested for hardness after day 1 of setting by using a Vickers microhardness testing machine. The specimens were randomly divided into 4 groups and were exposed to various chemical treatments. The surface hardness was measured before and after 5-, 10-, 15-, and 20-minute intervals. The samples were probed after 20 minutes of chemical exposure and hardness testing. They found that carbonic acid

significantly reduced the surface hardness of set WMTA at 1 and 21 days, whereas 2% chlorhexidine gluconate reduced the surface hardness of set WMTA significantly on only the first day. The authors conclude that carbonic acid can be effectively used to dissolve set WMTA even after 21 days but 2% chlorhexidine gluconate showed significant surface dissolution only within 24 hours of WMTA placement and EDTA has no effect on the dissolution of WMTA.

In-Vivo Studies/ Tissue Reaction

Pitt Ford et al. (11) investigated the histologic response to intentional perforation in the furcations of mandibular premolars in seven dogs. Twenty eight teeth were used in this study. In fourteen of the teeth, the perforations were repaired immediately with either amalgam or mineral trioxide aggregate; in the other 14 teeth, the perforations were not repaired immediately and were left open to salivary contamination. The 14 teeth that were repaired immediately were left for 4 months before histologic examination. In the immediately repaired group, inflammation was observed for all of the amalgam specimens. Contrastingly, only one of the six with mineral trioxide aggregate was associated with inflammation. The five noninflamed mineral trioxide aggregate specimens had some cementum over the repair material. In the delayed group, i.e. those left open to salivary contamination, all of the amalgam specimens/teeth showed inflammation, whereas only four of seven filled with MTA were inflamed. Based upon this study, it appears that mineral trioxide aggregate is a far more suitable material than amalgam for perforation repair preferably immediately after perforations.

Torabinejad et al. (12) investigated the response of periradicular tissues of monkeys to MTA and amalgam when used as root-end filling materials in teeth in which bacterial contamination of the root canals was avoided. The left and right maxillary central and lateral incisors of three healthy 4-year old Cynomolgus monkeys were used in this experiment. Anesthesia was provided. The teeth were isolated with rubber dam and the pulps exposed through a standard occlusal access opening. The root canals were debrided and enlarged to a #40 size master apical file. The root canals were obturated with laterally condensed gutta-purcha and Roth root canal sealer before restoration of their access cavities with amalgam. Periradicular surgery was carried out 1 week after root canal obturation. Root end cavities were prepared to a depth of 2 mm using a #2 round bur in a high speed hand piece. The root end cavities on one side were randomly selected to be filled with zinc-free amalgam and on the other side with MTA. The animals were reanesthetized 5 months after surgery and perfused. Block sections containing incisor teeth and their surrounding tissues were then removed and placed in formalin for 2 weeks. The specimens were demineralized in EDTA, embedded in paraffin, and sectioned buccolingually at a specified thickness and stained with hematoxylin and eosin, Masson's trichrome, or by the Brown and Brenn method. Concentration, extent of inflammation, and predominant inflammatory cell type in the periradicular tissue adjacent to the root-end filling materials were recorded. The severity of the inflammations was recorded as none, mild, moderate, and severe. The extent of inflammation from the surface of root-end filling material was recorded. Presence or absence of bacteria, a fibrous capsule, cementation deposition on the root end and root-end filling materials, and new bone formation were also recorded. The results showed no

periradicular inflammation adjacent to five of six root ends filled with MTA; also five of six root ends filled with MTA had a complete layer of cementum over the filling. In contrast, all root ends filled with amalgam showed periradicular inflammation, and cementum had not formed over the root end filling material, although it was present over the cut root end. Based on these results and previous investigations, MTA is recommended as a root-end filling material in man.

Torabinejad et al. (18) seem to think that MTA has similar or better properties as a root-end filling material than existing compounds, and warrants a usage test in experimental animals. The purpose of this study was to investigate the response of periradicular tissues of dogs to amalgam and MTA when used as root-end filling materials. The right and left mandibular 3rd and 4th premolars of six healthy 2-year old beagle dogs were used in this experiment. After anesthesia was obtained, the root canals of the teeth were contaminated by exposing the pulp through an occlusal access opening, and debriding and enlarging root canals of each tooth to a #40. The root canals were left open to the oral flora for 2 weeks and then closed with Cavit for 4 weeks. The teeth were then randomly divided into two groups of 12 teeth each. Group 1 was cleaned, shaped, and obturated with gutta-percha and Roth sealer. Their access cavities were sealed with MTA. Group 2 was the same as Group 1 without the Roth's sealer, and the access cavities were left open. One to two weeks after obturation, each animal was scheduled for surgery. Apical surgery was performed with roots resected at a 45 degree angle. Root end cavities were prepared to a depth of 2 mm using a #35 inverted cone bur. One of the root-end cavities in each premolar was randomly selected to be filled with zinc-free amalgam and the other with MTA. Three of the animals were killed 2 to 5 weeks post

surgery and the remaining three 10 to 18 weeks post surgery. Mandibular block sections containing the premolar teeth and tissues were removed and placed in formalin. After, they were demineralized, sectioned, and stained with hematoxylin and eosin, Masson's trichrome, and Brown and Brenn stain. Severity, extent of inflammation, and predominant inflammatory cell type were recorded. Severity of the inflammation was recorded as: none, mild, moderate, and severe. Also the extent of inflammation from the surface of root-end filling material was recorded. The presence or absence of a fibrous capsule and cementum deposition was also noted. A total of 25 amalgam and 21 MTA samples were available for histological studies. Statistical analysis of the results showed less periradicular inflammation and more fibrous capsules adjacent to MTA, compared with amalgam. In addition, the presence of cementum on the surface of MTA was a frequent finding. The results show that MTA can be used as a root-end filling material.

Pitt Ford et al. (19) compared the dental pulp responses in monkeys after MTA or a calcium hydroxide preparation (Dycal) was applied as a pulp-capping material. Twelve mandibular incisors in four healthy 4-year old cynomolgus monkeys were used in this experiment. Anesthesia was achieved, a rubber dam placed, and pulps were accessed 1 mm in diameter. The pulp capping materials were placed. Five months later the teeth were surgically removed along with their surrounding tissue and processed for histological examination. They found that all of the pulps capped with MTA showed dentin bridge formation, and all but one were free of inflammation. The bridge formed was thick and continuous with the original dentin. In contrast, only two dental pulps capped with the calcium hydroxide preparation had dentin bridges, and all six had pulpal inflammation.

A case study was presented by Koh et al. (20) in which they were looking for an effective pulp capping material that is biocompatible, promotes hard tissue formation, is insoluble, and has good sealing properties. They mention mineral trioxide aggregate appears to have most of these desired characteristics. The purpose of this case report was to present the use of MTA instead of calcium hydroxide as the pulp capping material for prophylactic treatment of dens evaginatus in two patients. Each patient had a mandibular second premolar affected with a prominent tubercle. Partial pulpotomy was conducted. MTA was mixed and packed directly onto the pulp and back-filled to cover the whole access cavity. The patients were seen 2 days later when MTA was trimmed back and a composite placed. Teeth were then x-rayed and extracted after 6 months. Both teeth showed the deposition of an apparently continuous dentin bridge under the MTA. There was no pulpal inflammation. MTA can be used as a pulpotomy material in the prophylactic management of dens evaginatus as an alternative to existing materials, such as calcium hydroxide.

Holland et al. (21) observed the reaction of apical tissues of dog teeth after root canal filling either with MTA or Ketac-Endo. Thirty root canals of two mongrel dogs were used. After anesthesia and placement of rubber dam, the pulp chamber of each tooth was opened. The pulp was removed at the radiographic apex with a #30. After the final irrigation, all the canals were carefully dried with paper points, dressed with a corticoid-antibiotic solution, and sealed for one week with cotton pellets and a temporary filling of zinc oxide eugenol cement. During the second treatment, root canals were irrigated again with saline, dried with paper points, and filled with gutta-percha and MTA or with Ketac-Endo. One-hundred eighty days after treatment, the animals were killed

and areas prepared for histological examination. Closure of the main canal by new cementum deposition was observed in all the specimens studied. The results also showed no inflammatory reaction of apical tissue and total closure of the apical foramen of all the teeth sealed with MTA. The teeth sealed with Ketac-Endo showed two cases of partial closure and different degrees of chronic inflammatory reaction. In conclusion, MTA exhibited better biological properties than Ketac-Endo.

Apaydin et al. (22) examined hard-tissue healing adjacent to fresh or set MTA as root-end-filling material in dogs. A total of 24 roots of mandibular second, third, and fourth premolars from four beagle dogs were used in this experiment. The mesial and distal roots were assigned to have either retrograde MTA root-end fillings or MTA previously placed in an orthograde manner. After gaining occlusal access to the pulp chambers of each tooth, the pulps were cleaned and shaped. Half of the roots of the mandibular second, third, and fourth premolars were obturated entirely with MTA. The remaining roots were obturated to 5 mm from the apical stop with warm vertical compaction of gutta-percha. The coronal portion of each root in this group was obturated with MTA. The gutta-percha was placed in the apical segment of the canal in this group to facilitate easy ultrasonic apical preparation during planned periradicular surgery. Two weeks after the root canals, the dogs underwent periradicular surgery on the mandibular right or left quadrants. After anesthesia, a flap was reflected. The root ends in both groups were resected 3 mm from the apex. The root-end preparations were made to a depth of 3 mm with an ultrasonic unit. The cavities were filled with MTA in the roots filled with gutta-percha. In the remaining roots, after root-end resection to the level of set MTA, no root-end cavity preparations were induced. The results indicated that although

freshly placed MTA resulted in a significantly higher incidence of cementum formation, there is no significant difference in the quantity of cementum or osseous healing associated with freshly placed or set MTA when used as root-end filling material.

Tziafas et al. (23) investigated the early pulpal cell response and the onset of reparative dentine formation after pulp capping with MTA. They used thirty-three teeth from three dogs. They exposed the pulps and placed MTA as a pulp cap. The pulpal tissue reactions were assessed by light and electron microscopy after healing 1, 2, or 3 weeks. They found a zone of crystalline structures along the pulp-MTA interface initially. They also observed pulpal cells showing changes in their cytological and functional state were arranged in close proximity to the crystals. Deposition of hard tissue of osteotypic form was found in all teeth in direct contact with the capping material. Formation of reparative dentine was consistently related to a firm osteodentinal zone. MTA is an effective pulp capping material according to this study.

Physical/ Chemical Properties

Mahmoud Torabinejad (2) from Loma Linda University and others conducted this study to determine the chemical composition, pH of the setting cement, and radiopacity of MTA, and second to compare the setting time, compressive strength, and solubility of this material with those of three commonly used root-end filling materials, amalgam, Super-EBA, and IRM. They studied the chemical composition of MTA by using the KVEX Delta 4460 X-ray Energy dispersive spectrometer, modified with Micro EDS software, in conjunction with a Hitachi S520 scanning electron microscope. MTA was

mixed with sterilized distilled water and allowed to set in a 37°C incubator with 5% carbon dioxide and moisture. The material was set on glass. Five set specimens with different proportions of water and powder were examined. The specimens were carbon-coated to a thickness of 100 nm and again mounted in the S520 using the quantum DVEX system. Accelerating voltage for standard examination was 15 kV and that for X-ray analysis was 10 kV. The pH of MTA as it set was measured with a pH meter using a temperature-compressed electrode. The radiopacity of MTA was determined according to the method described by the International Organization for Standardization. After mixing, MTA was packed into a 10 mm diameter stainless steel ring mold with a 1 mm depth and then covered with a glass slide and allowed to set for 3 hours. Radiographs were taken to give a radiographic density reading. A total of 5 films were taken for each specimen. The photographic densitometer was used to take readings of the radiographic image of the specimens. Three reading were taken for each film and the mean calculated. The net radiographic density was derived. The setting time was determined for MTA, amalgam, SEBA, and IRM by using the indenter needle (Gillmore). Compressive strength was measured using an Instron 1185 Testing Machine. The degree of solubility of test materials was determined by a modified method of the ADA specification where the materials were weighed after being immersed in water. The pH of MTA after mixing was 10.2 and rose to 12.5 at 3 hrs. MTA was more radiopaque than SEBA and IRM. The mean radiopacity for MTA was 7.17 mm of equivalent thickness of aluminum. The mean setting time for amalgam was 4 min; Super EBA was 9 min; IRM was 6 min; and MTA was 2 h 45 min. Amalgam had the highest compressive strength. SEBA was

significantly higher than that of IRM. None of the materials tested showed any solubility under the conditions of this study.

Islam et al. (30) compared the physical properties, namely, the pH, radiopacity, setting time, solubility, dimensional change, and compressive strength of ProRoot MTA (PMTA), ProRoot MTA (tooth colored formula) (WMTA), white Portland cement (WP), and ordinary Portland cement (OP). PMTA and WMTA were mixed according to manufacturer's instructions. OP and WP were mixed with a ratio of 3.5 ml of water to 1 g of cement powder. The pH of the materials was measured as they set. The radiopacity, solubility, and dimensional change after cements were determined by the ISO for dental root canal sealing materials. The compressive strengths of the test materials were determined by modifying the method recommended by the BSI. The results showed that the pH of WP and OP was found to be higher than PMTA and WMTA. WP and OP also reached the peak pH values earlier than PMTA and WMTA. The radiopacity of WMTA was 6.74 mm while that of PMTA was 6.47 mm. WP and OP had much lower radiopacity. WP and WMTA showed significantly faster setting time than OP and PMTA. WMTA also showed significantly greater solubility than the other cements. WMTA and PMTA also showed significantly lesser dimensional change than WP and OP. The compressive strength values of PMTA and WMTA were also greater than the Portland cements at 28 days. The major constituent of PMTA is Portland cement. Given the low cost of Portland cement and similar properties when compared to PMTA, the authors concluded it is reasonable to consider Portland cement as a possible substitute for PMTA in endodontic applications.

Clinical Applications/ Case Studies

Torabinejad and Chivian (3) outline the various advantages of MTA which they state has been investigated as a potential compound to seal off the pathways of communication between the root canal system and the external surface of the tooth. Among the background and properties mentioned, based upon earlier studies are: MTA is hydrophilic, setting in the presence of moisture, and achieves a pH of 12.5. A drawback is its long setting time of 4 hours. Its compressive strength is comparable to IRM and SEBA, but MTA's sealing ability has been shown in dye and bacterial leakage studies to be superior to that of amalgam and to be equal or better than SEBA, while displaying less cytotoxicity compared to IRM or SEBA. Next, the authors describe the indications and procedures for various clinical applications of MTA. Vital pulp therapy such as direct pulp caps and pulpotomies are one clinical application for MTA due to its capacity to prevent bacterial leakage and high level of biocompatibility. MTA can also be used as a apical plug with necrotic pulps and open apexes. Another indication for the use of MTA is repair of root perforations. This is because of its sealing ability being greater than most all other materials.

Schwartz et al. (4) presented 5 cases in which MTA was used to manage clinical problems. These included vertical root fracture, apexification, perforation repair and repair of resorptive defects. Other materials have been used in the past to repair root defects, but their use resulted in the formation of fibrous connective tissue adjacent to the bone. Because MTA allows the overgrowth of cementum and periodontal ligament, MTA may be an ideal material for certain endodontic procedures.

Schmitt et al. (34) reviewed MTA and described it as the preferred material of choice when it comes to direct pulp capping. When pulp exposures are encountered, sodium hypochlorite has been shown to be an effective agent for disinfection, dentinal chip removal, and hemostasis. It is imperative that the material used to protect the pulp have an enhanced seal to compensate for potential marginal leakage of the restoration. MTA also has been shown to have superior sealing ability to amalgam, ZOE, or IRM. MTA is made primarily of fine hydrophilic particles of tricalcium aluminate, tricalcium silicate, silicate oxide, and tricalcium oxide. When the material is hydrated it becomes a colloidal gel. The main components of MTA are calcium phosphate and calcium oxide, according to these authors. The material sets in approximately 3-4 hours, and for radiopacity, bismuth oxide powder has been added. The initial pH of MTA when hydrated is 10.2 and the set pH is 12.5, which is comparable to that of calcium hydroxide. The biocompatibility of MTA has been found to be equal or superior to amalgam, IRM, and ZOE. Cementum has shown to grow over the MTA along with new bone formation and no periradicular inflammation. MTA stimulated the release of cytokines and the production of interleukin. The setting ability of MTA is uninhibited by blood or water. It is recommended that MTA have a wet cotton pellet placed over it to gain better setting. MTA has been demonstrated to have diverse applications. These include direct pulp capping, repair of internal resorption, root end filling, apexification, and repair of root perforations.

Arens and Torabinejad (26) reviewed the use of MTA as a furcal perforation repair material and presented two case studies. A furcal perforation is an unfortunate incident that can occur during the search of the chamber floor for canal orifices or in the

process of post-space preparation. Investigators have shown that perforating the furca predisposes the periradicular tissues to chronic inflammation. In these two case studies, the doctor chose to use MTA due to it's biocompatibility as well as other favorable characteristics. Both cases had been previously perforated some years prior to the repair with MTA and still experienced positive results. If the positive results of these "worst case scenarios" in humans is this good, there would seem to be promise for the use of MTA in a more timely manner with "recent" perforations. More cases and studies are needed to substantiate the effectiveness of MTA for repair. Early indications are promising enough to suggest its use.

CHAPTER III

MATERIALS AND METHODS

White MTA (Figure 1; ProRoot MTA; Dentsply Tulsa Dental Specialties, Tulsa, OK) was mixed, three parts powder to one part water, on a glass slab with a cement spatula according to the manufacturer's instructions. The MTA was placed in a custom metal block tray with an amalgam carrier and condensed with Schilder pluggers. A mixing spatula was used to smooth the surface of the sample to allow easier reading of the Vickers and Gillmore needle indentations. A Vickers microhardness tester (Kentron; Torsion Balance Co., Clifton, NJ, USA), which measures the indentation resistance of any given material, was used for quantitative assessment of setting for this study (Figures 2 and 3). A Gillmore needle (Figure 4), with a weight of 453.6 grams and diameter of 1.06 mm, was used for the traditional assessment of MTA setting time. The well had a depth of 3.5 mm and was wide and long enough to accommodate several tests with the Vickers indenter and Gillmore needle. Fifty samples were randomly divided into two groups of twenty-five with one group having a moist cotton pellet placed on top of the MTA mixture and the other group had no moist cotton pellet on the MTA mixture. The samples were then placed in an incubator with 100% humidity at 37°C. All the MTA samples were indented with the Vickers hardness tester every 30 minutes for six hours and again at 1 and 3 days. Concurrently, the MTA was assessed with a Gillmore needle to determine setting time where the time at failure to make a complete circular indentation in the cement represents setting time. At each testing period, a different portion of the MTA sample was tested. Once the testing was complete, the diagonals of the square indentations of the Vickers hardness test were measured under a microscope with a calibrated measurement grid built into the eyepiece. This was accomplished with a Spencer optical stereomicroscope (Figure 5; American Optical Corp., Buffalo, NY)

with external illumination for larger indents or with the microscope attached to the Vickers indenter for smaller indents. Penetration resistance is given as indentation load divided by indentation area. The data was statistically analyzed using repeated measures analysis of variance to examine the relationships between a) indentation resistance and the two groups (with/without moist cotton) and b) indentation resistance and time. All analyses were conducted using SAS software (SAS Institute Inc., Cary, NC).

Figure 1. ProRoot MTA Used in This Study

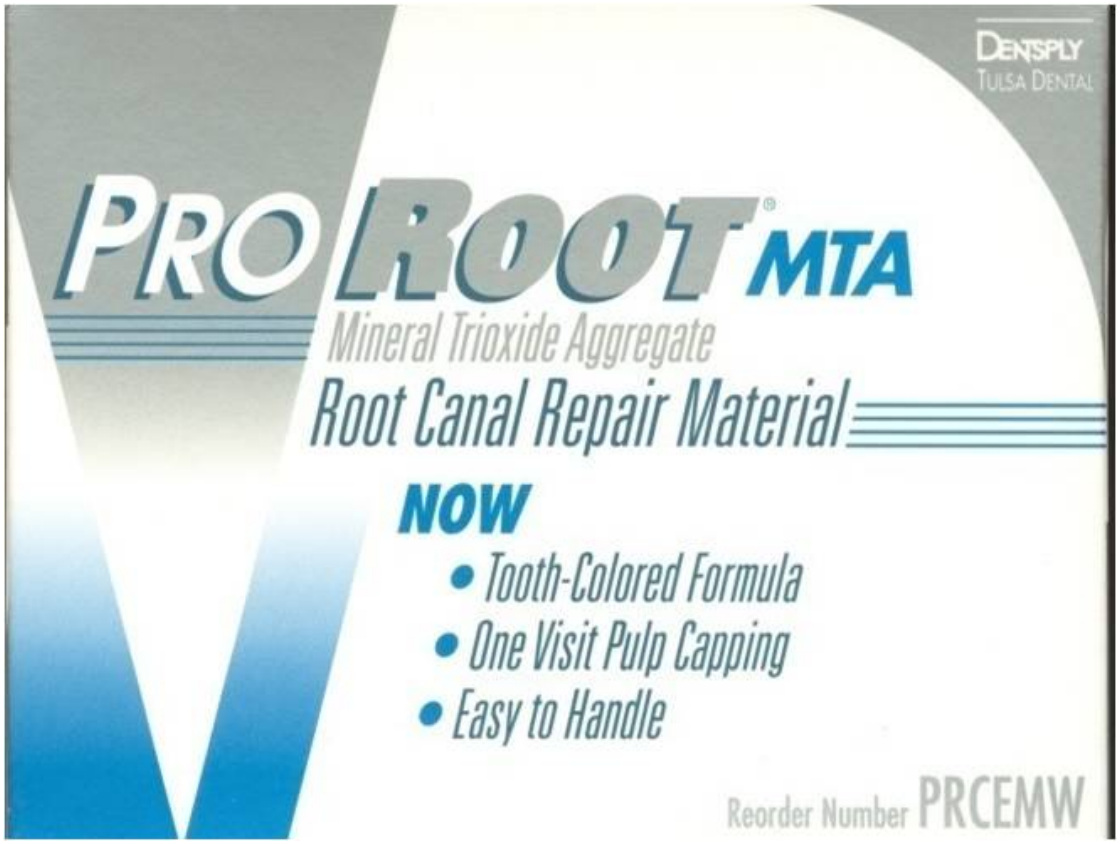


Figure 2. Vickers Microhardness Tester Used in This Study

Figure 3. Close-up View of Metal Block with MTA in Vickers Tester

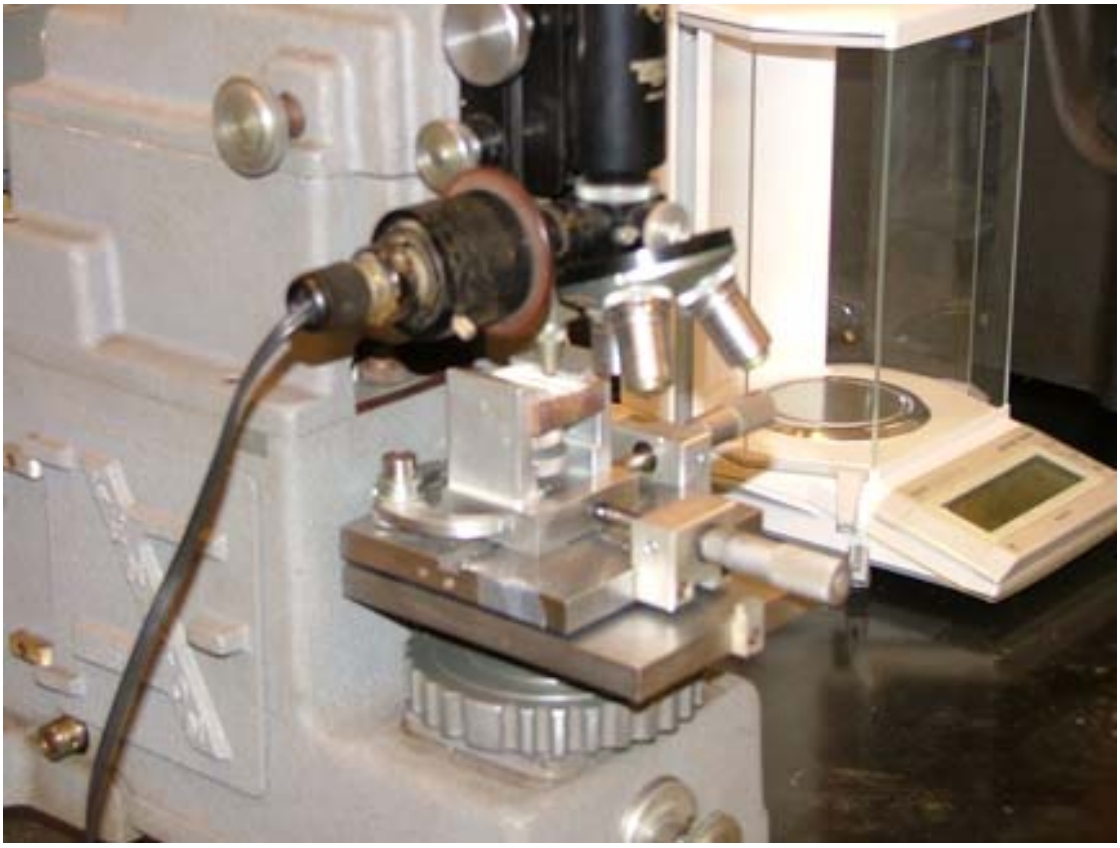


Figure 4. Gillmore Needle

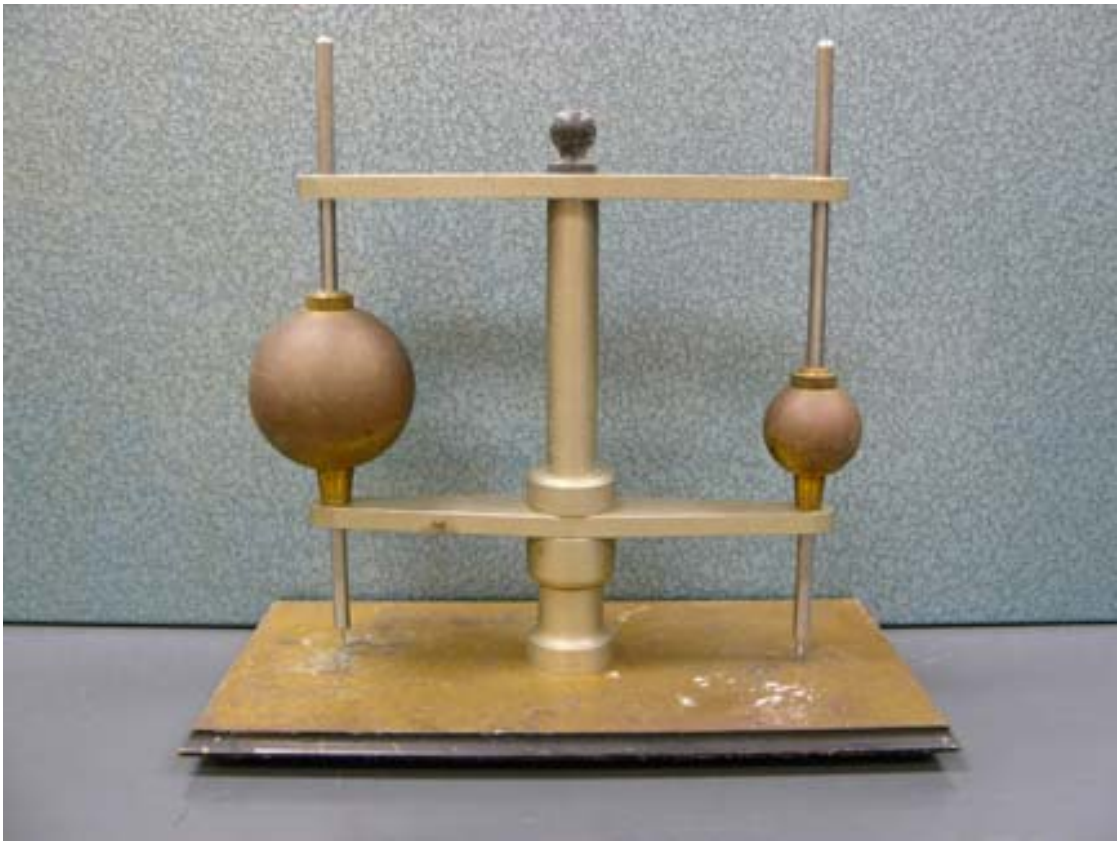


Figure 5. Optical Stereomicroscope Used to Measure Larger Indents

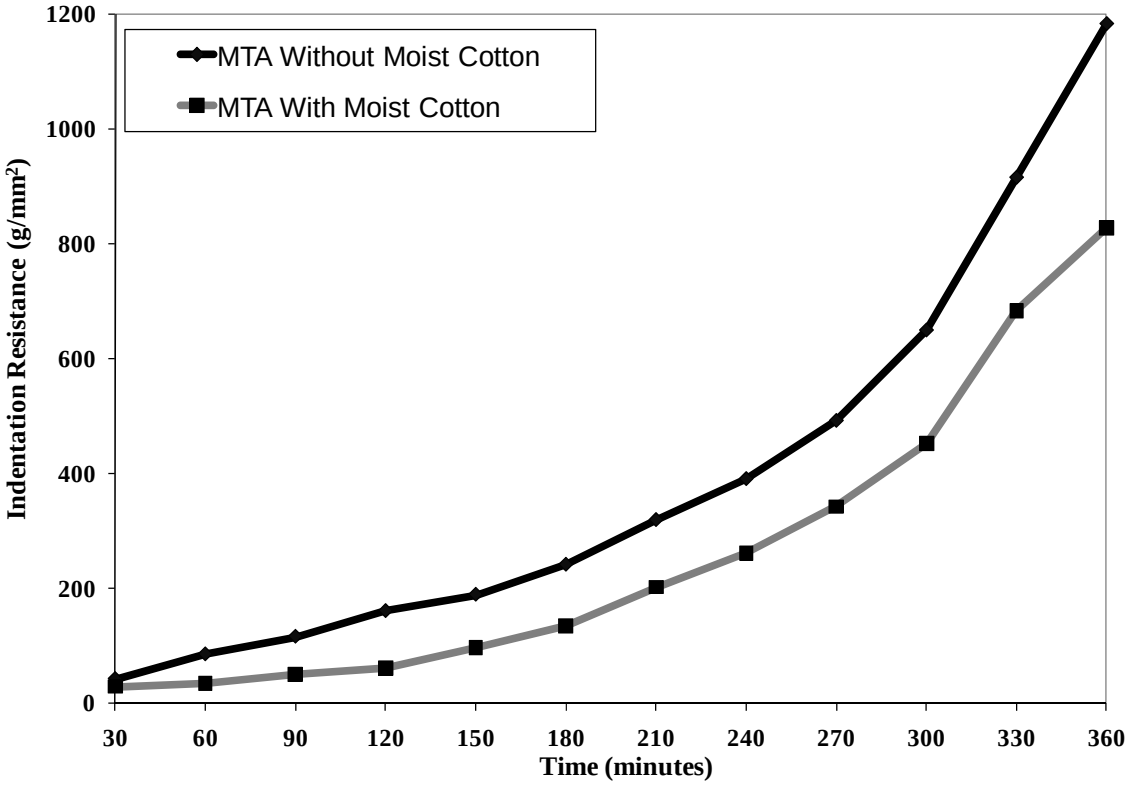
CHAPTER IV

RESULTS

Using a Gillmore needle for assessment, MTA without the application of a moist cotton pellet set faster than MTA with a moist cotton pellet (4.5 and 5.5-6.0 hours, respectively). Figure 6 shows an example of the Vickers indents in the MTA contained in the metal block. Repeated measures ANOVA demonstrated a significant difference in indentation resistance between MTA groups ($p<0.0001$) and by time elapsed ($p<0.0001$). The increase in indentation resistance at each time interval was significant ($p< 0.01$), meaning the MTA became harder over time (Figure 7). At each time interval up to 6 hours, the MTA without cotton had a greater resistance to indentation when compared to the MTA group with the moist cotton pellet up to 6 hours. However, at 1 day and 3 days, the MTA group with the moist cotton pellet had significantly greater resistance to penetration as evidenced by Vickers hardness numbers (kg/mm^2) of 17.2±6.7 and 84.1±16.4, respectively, whereas the other group (no moist cotton pellet) exhibited Vickers hardness numbers of 10.0±3.1 and 67.3±28.4, respectively.

Figure 6. Example of Vickers Indents in MTA

Figure 7. Penetration Resistance of MTA Over Time



CHAPTER V

DISCUSSION

MTA without a moist cotton pellet placed over the material set faster by approximately 1 hour as determined by the Gillmore needle testing of setting time. The added moisture from the moist cotton pellet perhaps effectively increased the water: powder ratio, which would lead to an increase in setting time. The setting time determined in this study was generally longer than that found in previous studies,[2,29,30] although it compares favorably to that of Ding et al. (31). Dissimilarities between various setting time determinations could be ascribed to differences in methodology and storage conditions. Nevertheless, examination of the penetration resistance to the Vickers indenter showed continual development of the material since the hardness of the cement increased with all time periods evaluated. Thus, similar to other dental materials such as glass ionomers, and also as shown in previous studies evaluating compressive strength associated with MTA,[2] MTA continues to mature beyond its clinical setting time.

As the Gillmore needle setting time data would suggest, during the first six hours after mixing, the MTA without the moist cotton pellet on top exhibited greater Vickers indenter penetration resistance. However, at 1 and 3 days, the MTA group with the moist cotton pellet had significantly greater Vickers hardness numbers. Thus, the added moisture available from the moist cotton pellet resulted in MTA with superior properties, at least with regard to microhardness. This is not surprising considering the setting reactions of MTA are predominantly due to the hydration of the tricalcium silicate and dicalcium silicate powder components. Still, care should be taken with regard to how moist the cotton pellet is since before MTA is set, washout of the cement may occur if it is too moist. Once set, though, MTA has shown to possess very low solubility.[2]

Although the endodontic literature reports that a moist cotton pellet should be placed on the MTA following placement to encourage setting (26), there are times that placing a moist cotton pellet cannot be accomplished or is not convenient to the patient or restoring clinician. Examples are when MTA is used as a retrofill or as an obturation material, when performing apexification in a young, non-compliant patient, for a patient unable to schedule a second or third appointment, for direct pulp caps in a young patient, or when placement of a permanent restoration following treatment is critical. Clinicians should be aware that although the material sets faster in these conditions, optimal surface hardness and material development may not be attained. This generally agrees with the flexural strength results from Walker et al. (28) mentioned earlier. On the other hand, Sluyk et al. (27) found no statistical difference in the furcal repair retention of MTA when a wet or dry cotton pellet was placed in the pulp chamber during the setting time. They suggested the amount of moisture in the environment is adequate to keep the hydrophilic powder moist, and that the condition of the pellet in direct contact with the material probably makes little difference.

The metal trays in the current study were prepared with a depth of 3.5 mm to simulate a clinically realistic MTA thickness. A depth of 3.5 mm was chosen because the ultrasonic tips used for retropreparations are usually 3-3.5 mm in length. Valois and Costa (32) compared the ability of different thicknesses of MTA to prevent apical leakage through the use of a protein-dye complex and showed that MTA of 4 mm sealed significantly better than a 3 mm thickness. The use of the metal trays does not replicate the conditions of clinically-placed MTA which would be exposed to moisture from surrounding tissue. Nevertheless, it was the exposed outer surface of the MTA which

was tested with the indenters and differentially exposed to either 100% humidity or the moist cotton pellet, as would occur in the clinical setting.

In the literature pertaining to the application of a moist cotton pellet, there is no indication as to the source of the water to moisten the cotton pellet. In this study, water from an air-water syringe was used to moisten the cotton pellets as this would be most likely match clinical procedure. Contrastingly, Walker et al. (28) used saline. It is likely that the surface of MTA exposed to either distilled water or saline would have little effect on the long-term surface hardness of the set material (33). Overall, the results presented in this study suggest a moistened cotton pellet should be placed in contact with MTA before placement of the permanent restoration if possible. Still, clinical studies examining the outcome of treatment are desired to conclusively determine whether the increase in surface hardness and maturation of MTA associated with exposure to a moist cotton pellet yield greater clinical success.

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