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The Outcome of MTA as a Root End Filling Material: A Long Term Evaluation

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by

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the requirements for the degree of
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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree of Master of Science in Endodontics.

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ABSTRACT OF THE THESIS

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Master of Science, Graduate Program in Endodontics
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Dr. Mahmoud Torabinejad, Chairperson

Periradicular surgery is a viable option to save natural teeth when non-surgical treatment fails or when endodontic retreatment is not feasible or contraindicated. Laboratory and animal studies have demonstrated that MTA is biocompatible, provides an excellent seal against penetrating bacteria, and promotes hard tissue healing. The purpose of this study was to provide long term (>3 years) clinical evidence for its use as a root-end filling material in endodontics. The clinical records of 294 patients who had MTA used during endodontic treatment from 1996 to 2001 were reviewed. From these, 75 patients whose root end cavities had been filled with MTA were identified for recall. Twenty five patients responded for clinical and radiographic evaluations providing a total of 27 cases. Clinical and radiographic exams were completed on these patients. Three independent examiners evaluated the radiographs for the presence or absence of pathological changes adjacent to the MTA restored site utilizing a Surgical Healing Index. Overall, twenty-two (81%) were considered healed, four (15%) were not healed, and one (4%) was classified as healing. The healed vs. not-healed outcome was statistically significant ($p<0.0001$). Twenty-five (93%) of the recalled cases were

functional and asymptomatic. Based on the results, it appears that the use of MTA should promote healing in a majority of surgical endodontic cases.

INTRODUCTION

Periradicular surgery is a viable treatment option to save natural teeth when non-surgical treatment fails or when endodontic retreatment is not feasible or contraindicated. The surgical intervention should address extra-radicular etiology through apical curettage procedures, and should also address intra-radicular etiology through root end modification and sealing procedures. Studies have recommended placement of root end fillings in teeth that require root end resection to prevent the egress of antigens into the periradicular tissues (Hirsch et al. 1979; Reit et al. 1986; Friedman et al. 1991). Obtaining an apical seal with root end filling materials is likely the most important factor in achieving success in surgical endodontics (Harty et al. 1970).

In the early 1990's, Torabinejad and associates developed Mineral Trioxide Aggregate (MTA). The primary goals with this material were to satisfy the requirements of an ideal root end filling material (Gartner et al. 1992) and to provide a more predictable pattern of healing with endodontic surgery.

Prior to FDA approval in 1996, MTA had been extensively investigated for its marginal adaptation, sealing ability, biocompatibility, and dimensional stability (Torabinejad et al. 1993, 1994, 1995a-f; Kettering et al. 1995). These initial studies are now being reproduced and expounded upon to more fully understand the complex physical properties, biologic interactions, and inductive potential of MTA.

Data suggests that MTA exhibits superior marginal adaptation under SEM than does amalgam when used as root-end filling materials (Torabinejad et al. 1995a; Shipper et al. 2004). MTA was originally reported to have the best adaptation to the dentinal walls and least dye leakage compared to other materials (Torabinejad et al. 1993), and recent data supports this notion and indicates that this adaptation is not adversely affected by resection of the material after setting (Andelin et al. 2002; Lamb et al. 2003). MTA has also been shown to seal significantly better than amalgam against fluid filtration, especially during the first 2-4 weeks following placement (Bates et al. 1996; Yatsushiro et al. 1998; Wu et al. 1998).

A minimum thickness of 3mm has been recommended for MTA to seal well against a fluid filtration model (Lamb et al. 2003). However, a 4mm thickness may be more adequate considering the results of Valois et al. (2004) evaluating various thicknesses of MTA against a protein-dye complex.

MTA has been shown to seal very well against bacterial penetration when compared to other materials (Torabinejad et al. 1995b; Fischer et al. 1998), and this sealing ability appears to prevent the penetration of endotoxin as well (Tang et al. 2002).

Studies that tested the cytotoxicity, mutagenicity, and biocompatibility of MTA found it to be significantly better than other root end filling materials such as Super-EBA, IRM, and amalgam (Osorio et al. 1992; Torabinejad et al. 1995c, 1995d, 1998; Kettering et al. 1995; Sousa et al. 2004). The response of various animal and human cell lines have been tested with MTA and other root end filling materials, with MTA promoting the most cellular attachment and normal morphologic behavior than any other material (Thomson et al. 2003; Asrari et al. 2003; Zhu et al. 2003; Balto 2004). The response of the periradicular tissues to MTA has also been evaluated. MTA has been shown to promote hard tissue formation and cementum deposition (Torabinejad et al. 1995e; Regan et al. 2002; Economides et al. 2003; Baek et al. 2005). Additionally, the local inflammatory response to MTA is generally mild as compared to other materials (Torabinejad et al. 1995e, 1997; Shabahang et al. 1999; Baek et al. 2005).

Based on the above review, it appears that MTA is the most biocompatible and well tolerated root end filling material available today. Its use in clinical endodontics has been extensive, but few outcome studies have been completed to provide long-term clinical evidence for its use. A search of the literature showed the presence of only two controlled outcome studies that evaluated MTA as a root end filling material (Chong et al. 2003; Wang et al. 2004). The purpose of this study was to evaluate the clinical and radiographic healing observed using MTA as a root-end filling material followed for a minimum of 3 years.

LITERATURE REVIEW

Role of Bacteria

The role of bacteria in the pathogenesis of periradicular lesions has been clearly demonstrated by Kakehashi et al. (1965) as well as Moller and associates (1981). Their findings in animal models have been corroborated by many other investigators confirming that the etiology of pulpal and/or periradicular diseases is microbial (Korzen et al. 1974; Bergenholtz et al. 1974; Bystrom et al. 1987; Sjogren et al. 1997). The infected root canal has the capacity to harbor numerous species of bacteria, including their toxins and by products. Accordingly, many investigators have attempted to categorize or identify the major endodontic pathogens and determine their impact on disease progression (Baumgartner et al. 1991; Molven et al. 1991; Sunqvist 1992; Baumgartner et al. 2003; Rocas et al. 2004). Newer detection techniques such as PCR have enabled the detection of bacterial species that are difficult or even impossible to culture as well as cultivable bacterial strains showing a phenotypically divergent or convergent behavior (Siqueira et al. 2003). The microbial flora recovered from untreated root canals with necrotic pulps and periradicular lesions is typically polymicrobial, with anaerobic bacteria dominating in approximately equal proportions of Gram-positive and Gram-negative species (Bystrom et al. 1981; Sundqvist 1992). The influence of combinations of bacteria has been demonstrated, with specific mixed infections demonstrating the most pronounced apical destruction (Fabricius et al. 1982). However, in treated root canals with persistent periradicular 1esions only one or a few bacterial species are present, primarily belonging to facultative Gram-positive species. The frequent recovery of *Enterococcus faecalis* from teeth with persistent lesions following root canal treatment is noteworthy (Fukushima et al. 1990; Molander et al. 1998; Pinheiro et al. 2003), and this bacterial strain has proven itself quite resistant to current treatment modalities in endodontics (Sundqvist et al. 1998; Evans et al. 2002; Radcliffe et al. 2004).

Route of Infection

Microorganisms may remain in the root canal system due to incomplete cleaning and shaping (Bystrom et al. 1981; Fukushima et al. 1990; Sjogren et al. 1997) or they may contaminate the root canal space during or after treatment from inadequate aseptic techniques (Siren et al. 1997). They may also invade the root canal system through defective or inadequate coronal restorations (Torabinejad et al. 1990; Magura et al. 1991; Ray et al. 1995).

Extraradicular Infection

Bacteria can also prevent healing of periradicular lesions by establishing themselves in the periradicular tissues. These extraradicular infections are predominately caused by *Actinomyces israelii* and *Propionibacterium propionicum* (Nair et al. 1984). In cases of long standing lesions, a bacterial plaque or biofilm may be established on the external root surface which limits the effectiveness of non-surgical treatment (Tronstad et al. 1990). Yeasts have also been implicated in cases of apical periodontitis persisting after conventional treatment, especially *Candida albicans* (Waltimo et al. 1997). The presence of infected dentin or cementum chips in the periradicular tissues has also been shown to be associated with impaired healing (Holland et al. 1980; Yoshida et al. 1987).

Alternative Etiology of Periradicular Lesions

Occasionally, no microorganisms can be detected, and treatment failures can be attributed to a foreign body reaction in response to remnants of excess root filling materials (Nair et al. 1990). A foreign body response has also been demonstrated against remnants of paper points utilized during root canal treatment (Koppang et al. 1992). Compounding factors such as a true cyst, sometimes containing cholesterol crystals, can affect the healing process following root canal therapy, resulting in a refractory lesion (Nair et al. 1993). In addition, unresolved periradicular radiolucencies may occasionally be due to healing by scar tissue, which may be mistaken as a sign of failed root canal treatment (Nair et al. 1999).

Surgical Considerations

In the presence of persistent pathosis, when a recurrent lesion develops after non-surgical treatment, or when orthograde endodontic retreatment is not feasible or contraindicated, periradicular surgery is considered. The surgical intervention should address extraradicular etiology through apical curettage procedures, but should also address intraradicular etiology through root end modification and sealing procedures. Studies have recommended the placement of root end fillings in teeth that require root end resection to prevent the egress of antigens into the periradicular tissues (Hirsch et al. 1979; Reit et al. 1986; Friedman et al. 1991). The achievement of an apical seal with these root end fillings is likely the most important factor in achieving success in surgical endodontics (Harty et al. 1970). The ideal requirement of such a root end filling material is close adaptation and adherence to the dentinal walls of the root end preparation. In addition, the material should prevent egress of noxious irritants into the periradicular tissues and it should be biocompatible. Furthermore, it should be insoluble in tissue fluids, dimensionally stable, and should not be adversely affected by the presence of moisture (Torabinejad et al. 1993).

Numerous materials have been suggested as root end fillings, including amalgam, gold foil, gutta-percha, ZOE, Cavit, Super-EBA, composite resin, and glass ionomer cement (Gutmann et al. 1991). None of these materials have demonstrated all of the properties of an ideal root end filling material (Torabinejad et al. 1993), which prompted the development of Mineral Trioxide Aggregate (MTA) in the early 1990's. The primary goals with this material were to satisfy the requirements of an ideal root end filling material and to provide a more predictable pattern of healing with endodontic surgery.

MTA in Endodontic Surgery

Prior to FDA approval in 1996, MTA had been extensively investigated for its marginal adaptation, sealing ability, biocompatibility, and dimensional stability. These initial studies have since been substantiated by many other investigators, and broad areas of research are now being employed to more fully understand the biologic interactions and inductive potential of MTA.

Marginal Adaptation

In 1995, Torabinejad and associates evaluated the marginal adaptation of several root end filling materials by scanning electron microscopy (SEM). They found that MTA demonstrated significantly smaller gap sizes between the material and surrounding dentin than the other materials tested. More recently, Shipper et al. (2004) repeated this study, but subjected the specimens to low and high-vacuum SEM. They evaluated MTA and amalgam under dry and wet conditions and found that MTA produced a superior marginal adaptation than amalgam, especially at low-vacuum wet conditions.

Sealing Ability

Sealing ability is likely considered the most important characteristic of a root end filling material. Since the etiology of post-surgical disease is typically microbial in nature, a material that resists penetration or leakage should provide the best long term outcome. MTA has been extensively studied for its ability to seal against dye leakage, fluid filtration, bacterial leakage, protein-dye complex leakage, and endotoxin penetration.

In 1993, Torabinejad and associates compared MTA to Super-EBA and amalgam in their ability to prevent dye leakage. Following exposure to rhodamine B fluorescent dye for 24 hours, MTA leaked significantly less than amalgam and Super-EBA. Remarkably, the effect of moisture and blood contamination has been shown to be inconsequential in the setting and sealing ability of MTA against dye penetration (Torabinejad et al. 1994).

In 2001, Roy and Jeansonne subjected several materials to different acidic environments at different time intervals following root end filling procedures. Their findings revealed that an acidic environment did not affect any of the materials tested against dye leakage, but that low pH enhanced the sealing ability of MTA and Geristore.

In 2002, Andelin et al. evaluated the effect of resection procedures on the ability of MTA to prevent dye leakage compared to freshly mixed MTA. Following exposure to India ink for 48 hours, there was no significant difference between the orthograde placed MTA (resected) versus the retrograde placed MTA (freshly mixed).

In 1996, Bates et al. evaluated amalgam, Super-EBA, and MTA in a fluid filtration measurement system at different time intervals ranging from 24 hours to 12 weeks. MTA demonstrated an excellent sealing ability throughout 12 weeks of fluid immersion, comparable to that of Super-EBA, and superior to amalgam.

In 1998, Wu et al. utilized 3mm bovine dentin cylinders and tested five commonly used root end filling materials against fluid filtration for up to 12 months. During the first three months, the percentage of gross leakage increased noticeably for amalgam and Super-EBA, whereas it decreased noticeably for MTA. This improved seal of MTA was maintained up to the end of the 12 month experimental observation period.

Lamb et al. (2003) determined the minimum depth of MTA required to maintain an apical seal following root resection. MTA was used to obturate the apical 6mm of the root canal and was allowed to set for 48 hours. Following 3, 4, 5 and 6 mm resections, the teeth were subjected to a fluid filtration test. Fluid leakage was shown to increase after each incremental resection, but did not reach statistical significance until 4 mm of the apex had been removed. This outcome corroborates the findings of Andelin et al. who showed that resected MTA can adequately seal the root end against dye leakage and fluid filtration models, especially if a 3mm thickness is maintained.

The first article to evaluate the bacterial leakage of MTA was completed by Torabinejad et al in 1995. This study determined the time needed for *Staphylococcus epidermidis* to penetrate a 3mm thickness of amalgam, Super-EBA, IRM, or MTA. Most samples of amalgam, Super-EBA, and IRM leaked within 6-57 days, but most MTA samples prevented leakage completely up to 90 days. Overall, MTA leaked significantly less than the other materials tested.

In 1998, Fischer and co-workers challenged the same root end filling materials to a different bacterial strain. In essentially the same study design, *Serratia marcescens* was placed in the root canals of teeth which had been retrofilled with amalgam, IRM, Super-EBA, and MTA. The bacterial challenge continued for 120 days. MTA samples did not begin to leak until 49 days, and many did not leak at all. Statistical analysis indicated that MTA was most effective against bacterial penetration throughout the study.

Several studies have demonstrated no significant difference between the tested materials relative to bacterial leakage. Most notably, Mangin et al. in 2003 found no difference between hydroxyapatite cement, MTA, or Super-EBA against radioactively labeled *Enterococcus faecalis*. Also, in 2001, Scheerer et al. found no significant difference between Geristore, MTA, or Super-EBA against *Prevotella nigrescens*. In 1999, Adamo et al. compared MTA, Super-EBA, TPH composite, and amalgam for bacterial leakage against *Streptococcus salivarius*. At 4, 8, and 12 weeks, no statistically significant difference in the rate of microleakage was seen. These studies utilize different bacterial strains and filling materials and none have shown a single material to be superior to MTA against bacterial leakage.

In 2004, de Leimburg and Berutti utilized Polymerase Chain Reaction (PCR) followed by reverse dot blot to detect *Enterococcus faecalis* leakage through MTA apical obturations of pulpless teeth with open apices. MTA was placed at 1, 2, and 3mm thicknesses and 50 days were allowed for bacterial penetration. At the end of the experiment, 17% of the specimens were contaminated, and no difference could be established between the 1, 2, and 3 mm thickness groups.

In an effort to overcome apparent limitations in dye and bacterial leakage studies, Valois and Costa (2004) set out to compare the ability of various thicknesses of MTA to prevent leakage through the use of a protein-dye complex with Coomassie Brilliant Blue G. MTA was placed at depths of 1, 2, 3, or 4 mm and the teeth were challenged to the protein solution for 60 days. The 1 mm thick MTA was the least effective. No difference could be seen between the 2 and 3 mm samples, and the 4 mm samples were significantly more effective than the other thickness. The authors recommend that a 4 mm MTA root end filling is most adequate for sealing purposes.

Endotoxin is a component of the cell wall of Gram negative bacteria, and has been shown to play a role in the pathogenesis of periradicular lesions (Dwyer et al. 1981; Yamasaki et al. 1992; Pitts et al. 1982; Shonfeld et al. 1982). In 2002, Tang and associates used a modified Limulus Amebocyte Lysate test as a tracer for endotoxin to evaluate the sealing ability of Super-EBA, IRM, amalgam, and MTA. Their results showed that MTA permitted significantly less *Escherichia coli* endotoxin leakage compared to the other materials for up to 12 weeks.

Biocompatibility and Tissue Response

Root end filling materials are placed in direct contact with the periradicular tissues, and thus, their biocompatibility is of primary importance. Numerous studies have been completed testing the biocompatibility of MTA. These studies have evaluated cytotoxicity against several established cell lines, mutagenicity potential, and the histologic response of the periradicular tissues in animals. More recently, investigators have gone beyond simply determining whether a material is capable of allowing normal cellular growth, but have evaluated the inductive potential of materials and their ability to upregulate or suppress certain differentiation mediators.

In 1995, Torabinejad and associates utilized an agar overlay and radiochromium method to evaluate the cytotoxicity of amalgam, Super-EBA, IRM, and MTA to mouse fibroblasts. MTA was the least toxic material to this cell line, even when the material was not set. This initial test was intended to validate the use of MTA as a potential root end filling material. Additional studies using murine cell lines, particularly cementoblasts and neural cells, have revealed that MTA is well tolerated (Thomson et al. 2003; Asrari et al. 2003).

Human cytotoxicity tests have primarily been done using either an MTT assay for cellular viability via mitochondrial enzymatic activity or SEM for direct visualization of cellular topography and morphology. The benefit of SEM studies is that the tested materials are actually in direct contact with the established cell lines, whereas MTT assay studies utilize the soluble products of the materials. Nonetheless, MTA has performed well against numerous human cell lines regardless of the model chosen (Osorio et al. 1998; Keiser et al. 2000; Zhu et al. 2003; Balto 2004).

In 1998, Osorio et al. evaluated the cytotoxicity of MTA, other root end filling materials, and several root canal sealers to human gingival fibroblasts. Utilizing the MTT assay, they found that MTA significantly outperformed all other root end filling materials and root canal sealers. The MTT assay was later used using human periodontal ligament fibroblasts and MTA outperformed amalgam and Super-EBA in either a fresh or set condition (Keiser et al. 2000).

Zhu and associates (2003) studied the adhesion of human osteoblasts to MTA, IRM, composite, and amalgam using SEM. The results showed that osteoblasts attached and spread on MTA and composite by forming a monolayer, but cellular attachment to amalgam and IRM was sparse with the osteoblasts appearing rounded without spreading.

In 2004, Balto evaluated the attachment and morphological behavior of human periodontal ligament fibroblasts to MTA with SEM. Interestingly, the fibroblasts in direct contact with freshly mixed MTA demonstrated rounding with rough surfaces and very few cells were attached to the substratum. In the 24 hour set MTA samples, the fibroblasts appeared flattened with smooth surfaces and appeared to be tightly attached to MTA with filopodia. The author concluded that the cytotoxicity of fresh samples could be caused by the presence of leachable and toxic components. These results were still within the parameters of Torabinejad's findings in 1995 comparing fresh and set MTA to other root end filling materials.

The in vitro cytotoxicity tests mentioned above cannot examine the complex interactions between materials and host tissues. Because of this limitation, in vivo subcutaneous and intraosseous implantation techniques in small animals have been recommended (Torabinejad et al. 1995d).

In 1995, Torabinejad and associates in a pilot study examined the tissue reaction of implanted Super-EBA and MTA in the mandible of guinea pigs. The test materials were placed in Teflon cups and implanted into the mandibles for 2 months. The block sections were evaluated histologically for the presence of inflammation, predominant cell type, and thickness of fibrous connective tissue adjacent to each implant. The tissue reaction to MTA was slightly milder than Super-EBA, but both materials were considered biocompatible.

In a follow-up study, Torabinejad and associates (1998) added several materials to the study design and added a second osseous implant site to draw more information about bone quality and material selection. MTA, IRM, Super-EBA, and amalgam were placed in Teflon cups and implanted in guinea pig mandibles and tibias. After 80 days, histologic evaluation revealed that the tissue reaction to MTA is most favorable at both implantation sites. In every specimen, no inflammation could be seen adjacent to the MTA and the tibia sites were most often seen with direct bone apposition. The results of these studies have been replicated by Sousa and associates in 2004, finding that MTA and composite resin are biocompatible in the intraosseous implant technique.

In 1995, Kettering and Torabinejad investigated the mutagenicity of several root end filling materials, including IRM, Super-EBA, and MTA. The Ames mutagenicity test was conducted using *Salmonella typhimurium* strains on a histidine-free medium. The results of this study showed that no revertant bacterial colony counts occur with any of the test materials and that these materials are not mutagenic as measured by the Ames test.

Considering the results of cytotoxicity and mutagenicity studies, scientists then began to evaluate the response of periradicular tissues to MTA. In 1995, Torabinejad and co-workers completed a study evaluating the periradicular tissue response of dogs to MTA and amalgam. Periapical lesions were developed in beagle dog teeth and root canal treatment was completed with or without sealer. The group completed without sealer was deliberately left open to the oral environment to promote coronal leakage. Half of the specimens received amalgam retrofills and half received MTA. Histologic evaluation revealed that after 10-18 weeks, 100% of the MTA samples demonstrated some cementum deposition over the resected root ends and the degree of inflammation was significantly less than amalgam. Cementum deposition was found to occur even in the MTA group left open to the oral environment demonstrating its ability to seal against bacterial penetration. A thick cementum deposition has also been observed over MTA and Diaket root end fillings of dogs by Regan et al. in 2002.

Following the study in beagle dogs, Torabinejad and associates set out to determine the periradicular response to MTA in monkeys. This time, the root canal treatment was completed on teeth without lesions and endodontic surgery was carried out 1 week later. Amalgam and MTA were used as root end filling materials in six teeth each and histologic evaluation was completed after five months. The periradicular tissues of all roots with amalgam as the root-end filling material had moderate to severe inflammation, but only one MTA sample displayed tissue inflammation. No cementum was ever seen on the amalgam root end fillings, but a thick layer of cementum was seen over five of six MTA samples (Torabinejad et al. 1997).

In 2003, Economides and associates evaluated the short-term healing response of periradicular tissues to MTA or IRM when used as a root end filling material in dogs. Histologic evaluation was completed at 1 and 5 weeks. Hard tissue formation was evaluated by scanning electron microscopy. The most characteristic reaction to MTA was the presence of connective tissue after the first postoperative week. Hard tissue formation was noted progressing from the peripheral root walls along the MTA-soft tissue interface. In contrast, no hard tissue formation was noted with the IRM samples.

In 2005, Baek and associates again tested the periapical tissue response of beagle dogs to three widely used root end filling materials. Amalgam, Super-EBA, and MTA were placed as root end fillings using modern microsurgical techniques on endodontically treated premolars and molars. After 5 months, the cell and tissue reactions of surface-stained un-decalcified ground sections were evaluated by light microscopy. MTA samples exhibited the least amount of inflammatory cell infiltrate, which were primarily plasma cells, lymphocytes, and some macrophages. Cementum deposition on the resected root-ends and over the material itself occurred statistically significantly more in the MTA group than Super-EBA and amalgam. There was no significant difference between Super-EBA and amalgam relative to cementum deposition, but Super-EBA performed better than amalgam relative to inflammatory infiltrate.

Physical properties and Inductive Potential

Based on the reports of many investigators regarding hard tissue formation and cementum overgrowth adjacent to MTA, many researchers have attempted to determine or propose a mechanism for the inductive nature of this material. In order to build a report on this subject, the physical properties of MTA must first be outlined.

In 1995, Torabinejad and associates found that MTA powder consists of fine hydrophilic particles which set in the presence of moisture and result in a colloidal gel which solidifies in approximately 2 hours and 45 minutes. It is comprised mostly of calcium and phosphorous ions, and has a pH of 10.2 initially, which rises to 12.5 three hours after mixing. The material is non-soluble and is more radiopaque than IRM. MTA sets with a low compressive strength initially, but after 21 days increases to one fifth that of amalgam (67 MPa).

According to Lee and co-workers (1993), the principle compounds present in MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and tricalcium oxide. In addition to the trioxides, there are a few other mineral oxides that are responsible for the chemical and physical properties of MTA. Interestingly, this original formulation is different than the product marketed by Tulsa Dental®. According to the MSDS for ProRoot MTA, the current formulation contains tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, tetracalcium aluminoferrite, and calcium sulfate dehydrate.

In 2003, Fridland and Rosado evaluated the solubility and porosity of ProRoot MTA with respect to different water/powder ratios. They found that solubility and porosity significantly increase when more water is used. A secondary finding of this study identified calcium hydroxide as the main compound released by MTA in water.

Lee and associates (2003) determined the effects of various physiologic environments on the hydration behavior and physical properties of MTA. ProRoot MTA was mixed per manufacturer's instructions and then exposed to various solutions including distilled water, normal saline, pH 7, and pH 5. After seven days of exposure, the specimens were evaluated by SEM, X-ray diffraction (XRD), and a microhardness test. Results showed that hydrated MTA stored in distilled water possessed a microstructure consisting of cubic and needle-like crystals. No needle-like crystals were observed in the pH 5 specimens, and erosion of the cubic crystal surfaces was noted. XRD indicated a peak corresponding to Portlandite, a hydration product of MTA, and the peak decreased noticeably in the pH 5 group. The pH 5 specimens' microhardness was also significantly weaker compared to the other three groups. These findings suggest that physiological environmental effects on MTA crystal formation are influenced by environmental pH and the presence of ions. The authors conclude that an acidic environment of pH 5 adversely affects both the physical properties and the hydration behavior of MTA. However, the clinical significance of this phenomenon is not clear. In 2001, Roy et al. found that the sealing ability of MTA is not affected by pH, and that MTA seal against dye leakage is enhanced at lower pH values.

In 2003, Duarte and associates evaluated the pH and calcium release of ProRoot MTA at different time intervals. Their model in test tubes revealed lower pH values than that reported by Torabinejad et al. in 1995, but they did demonstrate a rise in pH following 72 hours. The pH rise following setting is likely due to the release of calcium hydroxide from the material, which occurs when calcium oxide contacts tissue fluid or water. They also evaluated calcium release from MTA, and concluded that the released calcium hydroxide dissociates into calcium and hydroxide ions thus resulting in greater calcium release following the final set of the material.

The above findings serve to support the observations made by Holland and associates in 1999. They studied the rat subcutaneous connective tissue reaction to implanted dentin tubes filled with calcium hydroxide or MTA. The connective tissue adjacent to the materials was evaluated for the presence of calcium by the Von Kossa technique and polarized light. For both calcium hydroxide and MTA groups, there were Von Kossa-positive granules that were birefringent to polarized light. Next to these granulations, there was an irregular tissue like a bridge that was Von Kossa-positive. The authors concluded that the mechanism of action for hard tissue formation may be similar for both calcium hydroxide and MTA. These findings are supported by Yaltirik and associates in 2004, as they found notable dystrophic calcification in rat connective tissue adjacent to implanted MTA tubes.

Even if the mechanism of action is similar, there may be differences relative to the inductive capacity of MTA and calcium hydroxide. In 1999, Shabahang and associates placed orthograde root end barriers in beagle dog teeth consisting of either MTA, OP-1, or $Ca(OH)_2$. The degree of inflammation, hard tissue formation, and induced apical barrier formation were evaluated histologically after 12 weeks. Histological examination of the samples treated with MTA showed that 13 of 14 roots had apical closure with a calcific barrier, while only 5 of 13 treated with calcium hydroxide exhibited apical closure. Furthermore, almost twice the thickness of hard tissue formation was seen adjacent to the MTA group than the calcium hydroxide group. These findings do not rule out similar mechanisms of action, but certainly point to a different degree of inductive capacity between the materials.

In 2004, Apaydin and associates compared the effect of fresh MTA with set MTA on hard-tissue healing after periradicular surgery. The root canals of 24 mandibular premolars in four beagle dogs were filled with MTA. Two weeks later, the root ends of half of the samples were surgically exposed and resected to the set MTA within the canals. After exposing and resecting the other 12 root ends, they were prepared with ultrasonic instrumentation and preparations were filled with fresh MTA. Hard tissue healing was analyzed histomorphometrically after 4 months. Results indicated that although freshly placed MTA resulted in a significantly higher incidence of cementum formation (12 of 12 versus 8 of 12), there is no significant difference in the quantity of cementum or osseous healing. These findings point to an increased inductive capacity with freshly mixed MTA that may be related to the initial setting process of the material.

Numerous investigators have begun to evaluate local physiologic responses to MTA and the response of several cell lines to differentiation mediators. Certainly, many more studies are needed to fully understand the mechanism of action, but several key mediators of osteoinduction and cementoconduction have been identified.

In 1998, Koh and associates studied the cytomorphology of osteoblasts in the presence of MTA and examined cytokine production. MTA and IRM were placed in separate Petri dishes and osteoblasts were seeded into the dishes and incubated for 1-7 days. The specimens were viewed by SEM and the concentration of IL-1α, IL-1β, IL-6, and M-CSF were assessed via a solid phase ELISA assay. The results of SEM evaluation revealed that the cells cultured in the presence of MTA were flat and adhered to the material whereas osteoblasts cultured in the presence of IRM were rounded and sparse. The ELISA assay revealed that IL-1α, IL-1β, and IL-6 were upregulated in the presence of MTA compared to the control group of osteoblasts cultured in the absence of the tested materials.

In 2003, Thomson and co-workers investigated the effects of MTA on cementoblast growth and osteocalcin production in tissue culture. For cellular morphology comparisons, cementoblasts were cultured in the presence of MTA, IRM, and amalgam for 48 hours and then fixed for SEM analysis. Gene expression of mineralized matrix markers was evaluated by reverse transcriptase polymerase chain reaction (RT-PCR) analysis to determine if cementoblasts cultured on the tested materials supported expression of mRNA associated with mineralization. Immunohistochemical staining was performed to detect osteocalcin expression by cementoblasts cultured on MTA or glass coverslips. Osteocalcin is a noncollagenous protein that is expressed in mineralizing tissue during biomineralization. Results of the morphology comparisons were in agreement with Koh et al. (1998), revealing that cementoblasts could attach and grow well adjacent to MTA similar to osteoblasts. RNA expression of all genes investigated (Control, COL-1, ALP, BSP, and OCN) was found to occur on both MTA and tissue culture plastic (positive control). Confocal images indicated the production of osteocalcin by cementoblasts at 7-12 days on both glass coverslips and MTA disks. The results suggest that MTA permits cementoblast attachment and growth and permits the local production of mineralized matrix genes and protein expression. The authors concluded that MTA should be considered cementoconductive.

In 2003, Pistorius et al. determined the influence of root-end filling materials on specific cellular responses of gingival fibroblasts. Samples of ProRoot MTA, amalgam, and titanium alloy were incubated with human gingival fibroblasts for up to 9 days. PGE_2 production from the cultured cells was determined by a highly sensitive and specific competitive enzyme immunoassay using PGE_2 monoclonal antibodies. Also, the influence of the test materials on cellular protein synthesis and lactate concentrations was evaluated via spectophotometry and UV testing. There was a significant decrease in PGE_2 synthesis potential when cells were in contact with amalgam. In comparison, MTA and titanium resulted in an elevated level of cellular PGE_2 synthesis. The fibroblasts demonstrated a normal rate of protein synthesis in the presence of MTA or titanium, but a significant decrease was noted in the presence of amalgam. The rate of cell proliferation in contact with MTA and titanium was only slightly influenced and showed similar values to that of the controls after 96 hours.

Recently, Bonson and associates (2004) assessed the effect of several root end filling materials on gingival and periodontal ligament fibroblast differentiation. MTA, Hybrid Ionomer Composite Resin (HICR), Super-EBA, and amalgam were exposed to fibroblast lines to determine cell survival and proliferation, cell differentiation, and alkaline phosphatase activity. After incubation in the presence of the materials for 11-13 days, the relative number of cells in each sample was determined by a fluorescent cell attachment assay. Fibroblast differentiation was determined by polymerase chain reaction with many primers used to target known differentiation mediators. Results indicated that PDL fibroblasts displayed enhanced proliferation on MTA as compared to gingival fibroblasts. MTA preferentially induced alkaline phosphatase expression and activity in both PDL and gingival fibroblasts. In contrast, HICR inhibited alkaline phosphatase expression and activity. In addition, MTA and HICR repressed pleiotrophin in PDL fibroblasts, while HICR repressed periostin in both fibroblasts.

In 2004, Su Jung Shin completed a Masters Thesis addressing the cellular mechanisms underlying the bone and dentin inductive properties of MTA. In the first series of experiments, osteogenic and odontogenic cells were cultured on plates coated with MTA and compared with control cells with respect to cell proliferation, survival, gene expression profile, extracellular calcium ion concentrations, and the level of phosphor-rk and Akt. Results of this experiment showed that MTA stimulates osteogenic and odontogenic cell proliferation and survival, while maintaining the differentiated state of the cells. Also, cell cultures grown on MTA have significantly higher levels of extracellular calcium ion, phosphor-Erk and phosphor-Akt. In the second series of experiments, cell cultures were treated with specific chemical inhibitors to either block intracellular calcium ion mobilization or phosphorylation (activation) of ErK/Akt, or to reduce extracellular calcium ion concentration. Results suggested that extracellular calcium ion released from MTA induces an intracellular mobilization of calcium ion and activation of phosphor-Erk which induces cell proliferation.

Clinical Outcome

The use of MTA as a root end filling has been shown to be effective in numerous case reports, but only two controlled outcome studies have been completed evaluating MTA against other materials. No studies exist strictly evaluating the long term outcome of endodontic surgery cases completed with MTA, which leaves endodontists without clinical evidence for predicting the overall surgical prognosis. This concern is based on studies done by several investigators that have followed surgical cases for an extended period of time and these groups have realized that early success and late failures may occur in endodontic surgery.

In 2003, Chong and Pitt Ford completed a prospective randomized clinical trial evaluating the outcome of endodontic surgery with MTA or IRM as a root end filling material. A standardized surgical technique was employed: the root end was resected perpendicularly and a root-end cavity was prepared ultrasonically and filled with one of the two materials. A radiograph taken immediately following surgery was compared with those taken at 12 and 24 months post operatively. The results from 122 cases revealed that the highest number of teeth with complete healing at both times was observed when MTA was used. When the numbers of teeth with complete and incomplete healing, and those with uncertain and unsatisfactory healing were combined, the success rate for MTA was higher (84% after 12 months, 92% after 24 months) compared with IRM (76% after 12 months, 87% after 24 months). However, statistical analysis showed no significant difference in success between materials ($P > 0.05$) at both 12 and 24 months. The authors concluded that the use of MTA as a root-end filling material resulted in a high success rate that was not significantly better than that obtained using IRM.

In 2004, Wang and associates completed phase 1 and 2 trials of the Toronto study evaluating the outcome of apical surgery. This report of 155 teeth in 138 patients evaluated the outcome of endodontic surgery utilizing a variety of root end filling materials including amalgam, composite resin, Super-EBA, IRM, and MTA. Root end cavities were prepared by various graduate endodontic residents with ultrasonic tips and surgical loupes. Follow-up periods ranged from 4-8 years and a recall rate of 85% was obtained. The overall healed rate was 74%, and the healed rate was significantly higher for teeth with small (≤5mm) than larger preoperative lesions. The use of MTA was reported in an "other" category with amalgam, composite resin, and IRM. Unfortunately, detailed information regarding the outcome of MTA retrofilled roots was not published.

In 1999, Kvist and Reit found a significant decrease in healing at 4 years when compared to a 1 yr recall. Also, Rubenstein and Kim demonstrated a 94% success rate at 12 months, but found only an 87% success rate at 6-8 years when using Super-EBA as a root end filling material (Rubenstein et al. 1999, 2002). These studies support the recommendations of Rud and Andreasen in 1972. They conducted a follow-up study on 1000 cases after endodontic surgery and reported that a standard follow-up be made one year after surgery. If at that time it was determined that, the healing was uncertain or incomplete, a four year time period would be necessary for re-evaluation before initiation of further treatment.

METHODS

The research protocol for this investigation was approved by the Loma Linda University Institutional Review Board for human subject research.

Two hundred ninety four records from the Graduate Endodontic Clinic at Loma Linda University were identified utilizing an MTA procedure code which had been in use since 1996. Of these charts, 75 involved the use of MTA as a root end filling material. A database was generated identifying the patients name, chart number, age, gender, pertinent health information, telephone number, and specific information relative to the surgical procedure and tooth number. The patients were contacted utilizing information from their chart. If the telephone numbers on record were not valid, then a second attempt was made using public telephone directories.

Requirements for inclusion in the study included informed consent for a follow-up examination, consent to use their protected health information as research data, and the involved tooth had a permanent restoration.

Clinical Assessment

All patients completed a questionnaire relative to the surgical site including current medical history, pain reported on a ten point visual analogue scale, and symptom history. Vital signs were taken and two periapical radiographs were exposed utilizing Kodak E-speed film. A RIN paralleling device was used and care was taken to expose a direct straight on image and a twenty degree off angle image. After an oral cancer screening, a soft tissue examination was completed to determine the presence of a sinus tract, presence of swelling, and gingival health of the tooth in question. If a sinus tract was present, a gutta-percha point was used to trace the source of drainage. Clinical tests included assessment of tenderness to percussion, palpation, and biting as well as determining periodontal probing depths and furcation involvement. Control measurements were obtained from adjacent and contra-lateral teeth. Only the presence or absence of palpation tenderness over the involved root end or sinus tract development traceable to the involved root end was considered in the clinical outcome data.

Radiographic Assessment

The two periapical radiographs obtained during the clinical examination were digitized by photographing the films with a 6.3 MP Canon Digital Rebel camera (Canon) with a Tamron 90mm F/2.8 Macro 1:1 lens mounted on a Quantary QSX 8001 tripod (Sunpak). The radiographs were mounted on a light view box before photographing. The images were then imported into Adobe Photoshop 7.0 to be cropped and desaturated. No other manipulations were introduced. The images were then imported into a Microsoft Powerpoint presentation allowing both digital radiographs to be viewed on the same powerpoint slide. The radiographic evaluation was completed on a 19" ViewSonic flat panel monitor (VX900) and each powerpoint slide had a black background with the digital radiographs overlaid.

The digital radiographs were evaluated independently by three examiners that were appropriately blinded to the study purpose and materials. Prior to evaluating the study material, the examiners were calibrated with a series of similar, but unrelated radiographs to ensure appropriate reliability and training. The examiners were asked to report periapical status as healed, not-healed, or uncertain. In order to define these three categories, a new Surgical Healing Index (SHI) was established through modification of the Periapical Index (PAI) Scoring System (Orstavik et al. 1986). The SHI system incorporates a set of five drawings that are intended to represent a correlation between periapical radiographic appearances and presumed histologic associations (Table 1, Figure 1).

Table 1. Surgical Healing Index

Category	Radiographic Appearance adjacent to the Root End
I	Complete osseous regeneration. Normal PDL width and intact lamina dura.
II	Complete osseous regeneration. Slightly widened PDL width and intact lamina dura.
III	Complete osseous regeneration. Unable to discern a distinct PDL and lamina dura.
IV	Incomplete osseous regeneration. Normal PDL width and intact lamina dura. Radiolucent lesion must be distinctly separated from the root end (Residual cyst or scar).
V	Incomplete osseous regeneration. Unable to discern a distinct PDL and lamina dura. Radiolucent lesion must communicate with the root end.

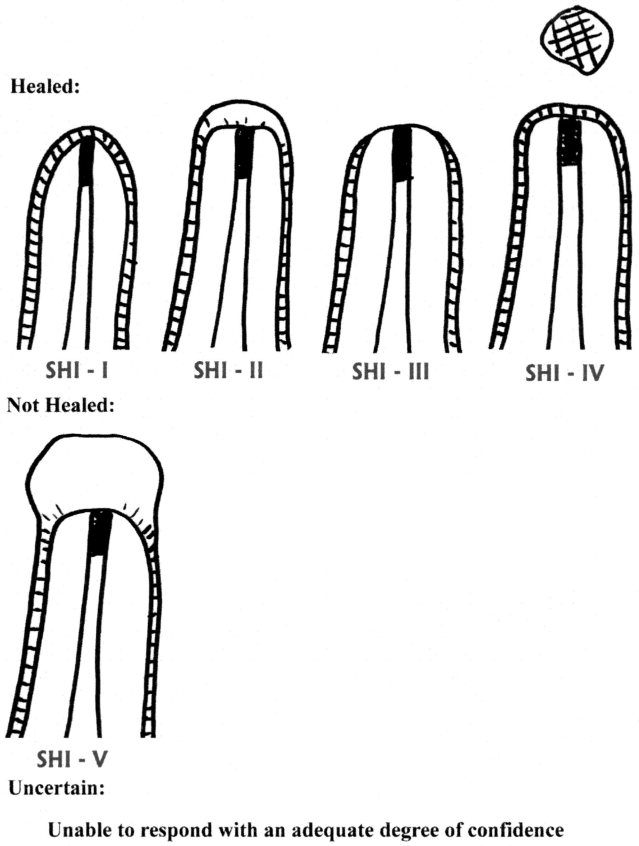

Figure 1. Diagrammatic representation of the surgical healing index.

Examiners were instructed to report the periapical status as *healed* when the SHI reflected category 1-4. A *not-healed* response was expected for SHI category 5, and an *uncertain* response should be reserved for those cases lacking an adequate degree of confidence in the radiographic appearance relative to the SHI categorization. An overall result was obtained by a majority rule between the three examiners. If a majority was not obtained, then a radiographic outcome of uncertain was registered for that particular case.

Overall Healing Criteria

The overall radiographic and clinical assessment criteria for treatment outcome following the placement of MTA as a root-end filling material were as follows:

1. <u>Healed:</u> A radiographic interpretation of *healed* must be obtained reflecting an SHI score ≤ 4. Clinical signs and symptoms related to sinus tract development must be within normal limits and no trans-mucosal tenderness to palpation over the root end is allowed.
2. <u>Not-Healed:</u> A radiographic interpretation of *not-healed* must be obtained reflecting an SHI score = 5. There may be trans-mucosal palpation sensitivity over the root end. Teeth with a sinus tract traceable to the apex of the involved root(s) will not be considered healed.
3. <u>Uncertain:</u> Strictly a radiographic interpretation category reflecting an inadequate degree of confidence related to the Surgical Healing Index. Clinical signs and symptoms related to sinus tract development must be within normal limits and no trans-mucosal palpation tenderness over the root end is allowed.

Data Analysis

Inter-Correlation Coefficient was used to judge agreement between the independent examiners. Hypothesis testing was completed by the 2-sample binomial test at $\alpha = 0.05$, which accounted for sample size and potential random error.

RESULTS

Twenty-five patients (33%) were available for follow-up providing 27 cases to evaluate healing ranging from 35 to 98 months post treatment. The age of the patients ranged from 18 to 84, 9 (36%) being male and 16 (64%) being female. Hypertension, asthma, and hypothyroidism were the most significant health conditions noted. Of the patients recalled, random samples of maxillary and mandibular teeth were evaluated. Six teeth were maxillary molars, four were maxillary premolars, one was a maxillary canine, and eight were maxillary incisors. Five teeth were mandibular molars, two were mandibular premolars, and one was a mandibular canine (Table 2).

Table 2. Clinical Evaluation

Case	Tooth	Age	Gender	Recall Term (months)	Findings
1	19	39	M	37	Functional and Asymptomatic
2	3	64	M	97	Functional and Asymptomatic
3	19	73	M	89	Functional and Asymptomatic
4	4	80	F	48	Functional and Asymptomatic
5	5			48	Functional and Asymptomatic
6	12	52	M	41	Functional and Asymptomatic
7	7	57	F	78	Functional and Asymptomatic
8	3	65	F	40	Functional and Asymptomatic
9	10	61	F	53	Functional and Asymptomatic
10	7	34	F	50	Functional and Asymptomatic
11	10			50	Functional and Asymptomatic
12	6	81	F	59	Functional and Asymptomatic
13	9	43	F	38	Functional and Asymptomatic
14	30	56	F	75	Functional and Asymptomatic
15	3	53	F	49	Functional and Asymptomatic
16	27	82	F	92	Functional and Asymptomatic
17	3	66	F	75	Functional and Asymptomatic
18	28	45	M	89	Functional and Asymptomatic
19	19	18	F	52	Functional and Asymptomatic
20	2	71	F	65	Functional and Asymptomatic
21	14	57	M	72	MB Root Dehiscence and Palpation Sensitive
22	10	48	F	35	Functional and Asymptomatic
23	7	69	M	49	Functional and Asymptomatic
24	21	50	M	53	Functional and Asymptomatic
25	5	76	M	98	Percussion, Bite, and Palpation Sensitive
26	30	70	F	54	Functional and Asymptomatic
27	7	61	F	49	Functional and Asymptomatic

The clinical recall results showed that 25 of 27 cases (93%) were functional and asymptomatic. Two cases (7%) were clinically not-healed based on the presence of trans-mucosal palpation tenderness over an involved root end (Table 2). No sinus tracts were identified in any of the recalled patients.

The radiographic evaluation demonstrated that 23 of 27 cases (85%) were healed, three cases (11%) were not-healed, and one case (4%) was uncertain (Table 3, Figure 2-10). A unanimous response between the three examiners was noted in 22 of the 27 cases (81%). A 2/3 majority was noted in 4 of 27 cases (15%), and no consensus was reached in one case (4%). The Inter-Correlation Coefficient was 0.654.

Table 3. Radiographic Evaluation

Case	Examiner 1	Examiner 2	Examiner 3	Result
1	Not-Healed	Healed	Uncertain	Uncertain
2	Healed	Healed	Healed	Healed
3	Healed	Uncertain	Healed	Healed
4	Healed	Healed	Uncertain	Healed
5	Not-Healed	Not-Healed	Not-Healed	Not-Healed
6	Healed	Healed	Healed	Healed
7	Not-Healed	Healed	Healed	Healed
8	Healed	Healed	Healed	Healed
9	Healed	Healed	Healed	Healed
10	Healed	Healed	Healed	Healed
11	Healed	Healed	Healed	Healed
12	Healed	Healed	Healed	Healed
13	Healed	Healed	Healed	Healed
14	Healed	Healed	Healed	Healed
15	Healed	Healed	Healed	Healed
16	Healed	Healed	Healed	Healed
17	Healed	Healed	Healed	Healed
18	Healed	Healed	Healed	Healed
19	Healed	Healed	Healed	Healed
20	Healed	Healed	Healed	Healed
21	Not-Healed	Not-Healed	Not-Healed	Not-Healed
22	Healed	Healed	Healed	Healed
23	Not-Healed	Healed	Not-Healed	Not-Healed
24	Healed	Healed	Healed	Healed
25	Healed	Healed	Healed	Healed
26	Healed	Healed	Healed	Healed
27	Healed	Healed	Healed	Healed

Overall, after combining the clinical and radiographic data, 22 of 27 (81%) were considered healed, four (15%) were not-healed, and one (4%) was uncertain (Table 4). Only one case (Case #21) was clinically and radiographically not-healed. The healed vs. not-healed outcome was statistically significant (p<0.0001). The four cases that were not-healed demonstrated recall terms of between 4 and 8 years.

Table 4. Overall Clinical and Radiographic Result

Case	Radiographic	Clinical	Overall
1	Uncertain	Healed	Uncertain
2	Healed	Healed	Healed
3	Healed	Healed	Healed
4	Healed	Healed	Healed
5	Not-Healed	Healed	Not-Healed
6	Healed	Healed	Healed
7	Healed	Healed	Healed
8	Healed	Healed	Healed
9	Healed	Healed	Healed
10	Healed	Healed	Healed
11	Healed	Healed	Healed
12	Healed	Healed	Healed
13	Healed	Healed	Healed
14	Healed	Healed	Healed
15	Healed	Healed	Healed
16	Healed	Healed	Healed
17	Healed	Healed	Healed
18	Healed	Healed	Healed
19	Healed	Healed	Healed
20	Healed	Healed	Healed
21	Not-Healed	Not-Healed	Not-Healed
22	Healed	Healed	Healed
23	Not-Healed	Healed	Not-Healed
24	Healed	Healed	Healed
25	Healed	Not-Healed	Not-Healed
26	Healed	Healed	Healed
27	Healed	Healed	Healed

Figure 2. Example of a healed maxillary molar.

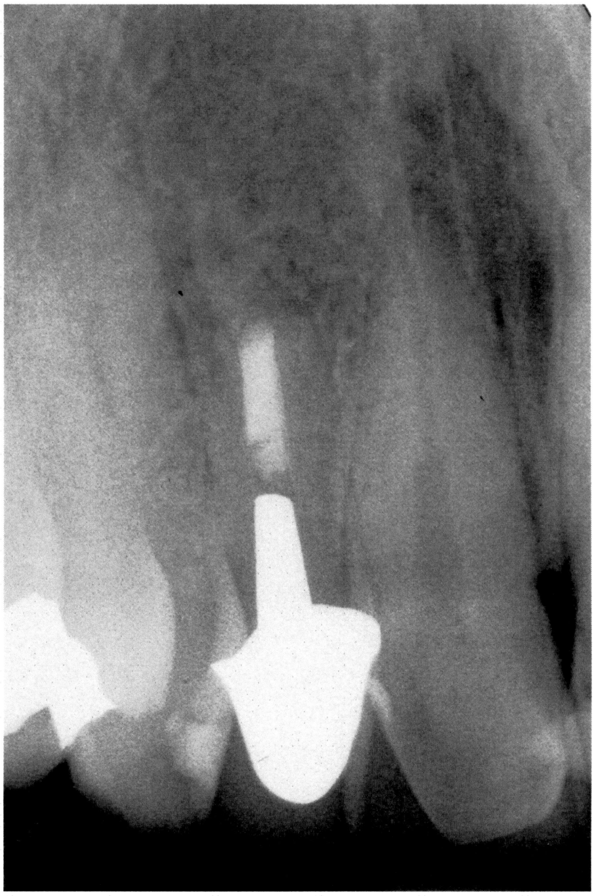

Figure 3. Example of a healed maxillary incisor.

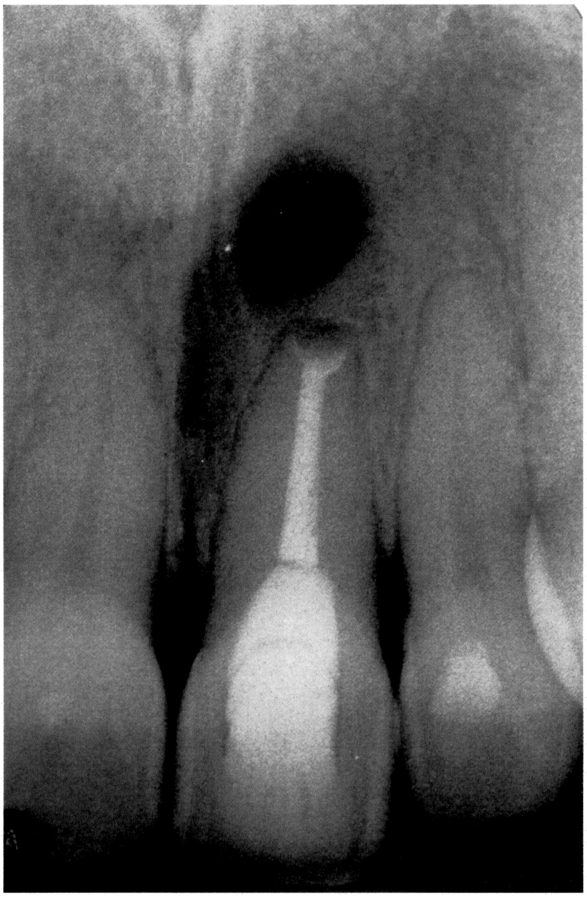
Figure 4. Example of a healed maxillary incisor with a residual scar.


Figure 5. Example of a healed mandibular molar

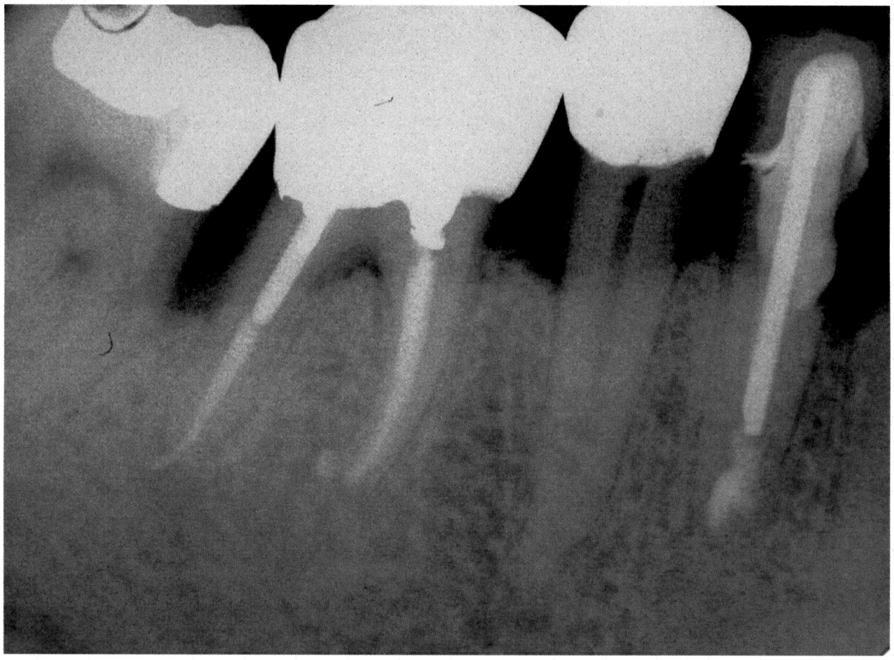

Figure 6. Example of a healed mandibular premolar.

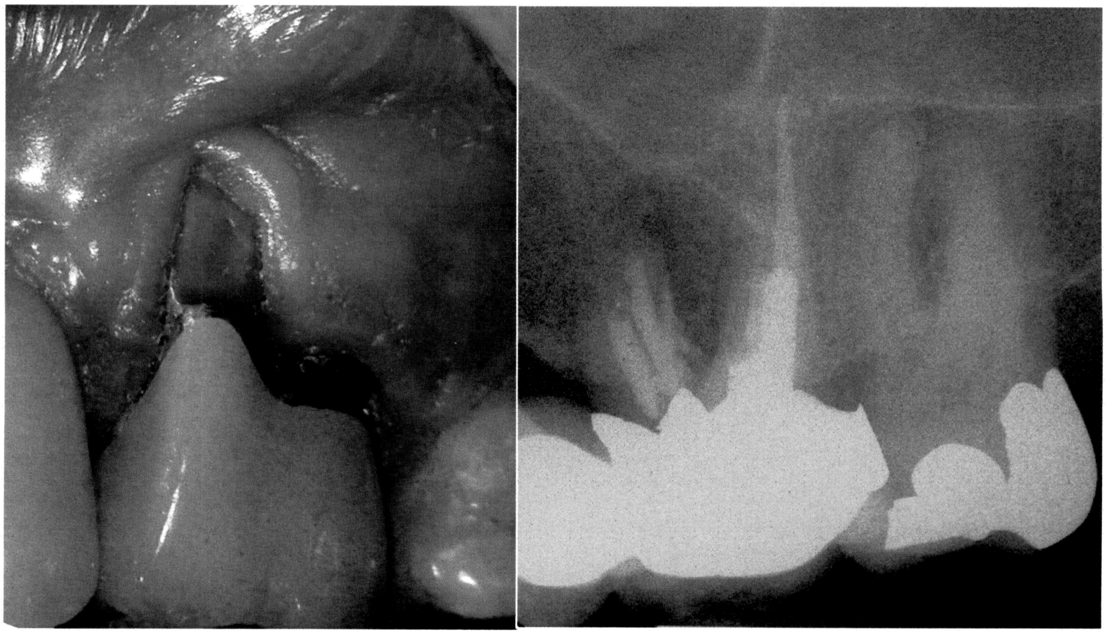

Figure 7. Example of a not-healed maxillary molar with a MB root dehiscence.

Figure 8. Example of a not-healed maxillary incisor.

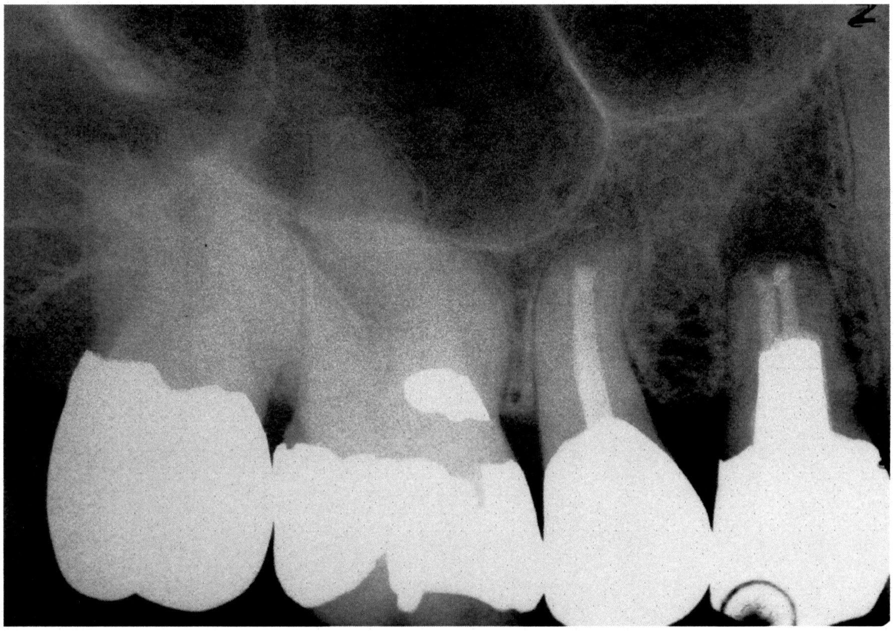

Figure 9. Example of a not-healed maxillary premolar.

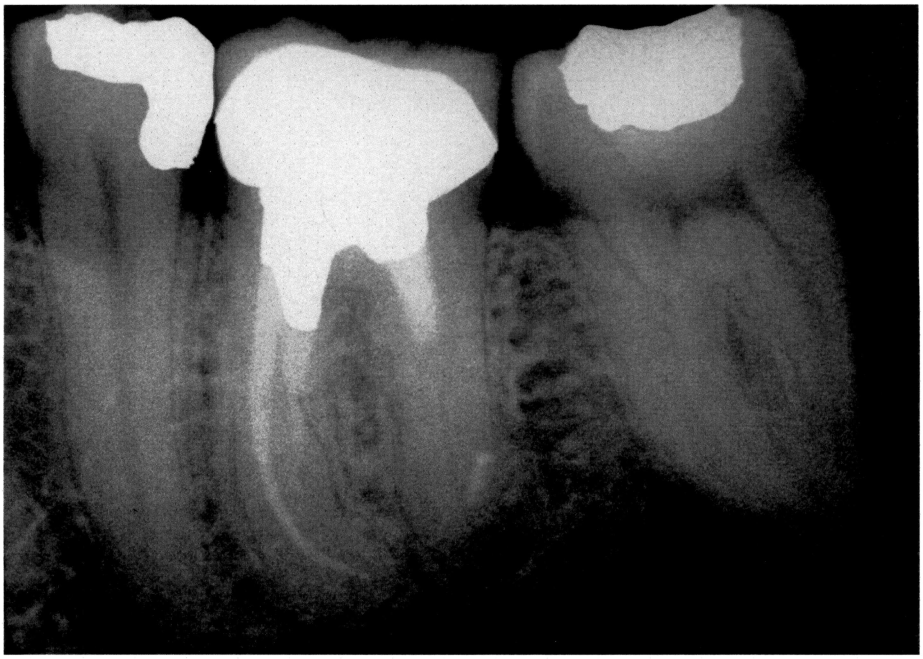

Figure 10. Example of uncertain healing in a mandibular molar

DISCUSSION

The recalled patients in this study had their surgical procedures completed by second year endodontic residents at Loma Linda University. These residents likely exhibited various degrees of experience and surgical skills. No strict controls were in place during the surgical procedures, but a certain extent of similarity should be expected due to the residency training protocols for endodontic surgery and MTA retrofill procedures.

Our recall rate of 33% was in line with recent publications reporting on the clinical outcome of endodontic procedures (Farzaneh et al. 2004; Friedman et al. 2003). We attribute the low recall rate to the lack of a research protocol at the time of surgery and the high migration rate in Southern California. Recent publications with a controlled trial study design have reported much higher recall rates (Gorni et al. 2004; Wang et al. 2004).

All of the patients recalled in this study presented with adequate coronal restorations in the form of amalgam or composite resin cores, full gold crowns, or porcelain fused to metal crowns. Even though no patients were turned away for the lack of a coronal restoration, the presence of these permanent restorations was required for inclusion in this study. This position is based on the notion that the quality of the coronal restoration is likely as important as the quality of the root canal filling (Ray et al. 1995; Tronstad et al. 2000). Even though MTA has been shown to resist bacterial leakage in vitro (Torabinejad et al. 1995b; Fischer et al. 1998), the complexity of endodontic infections in situ cannot be underestimated. Therefore, we expect that a true assessment of the endodontic surgery success could not be made without good coronal restorations.

The only two clinical findings that were considered for a *not-healed* outcome were trans-mucosal palpation sensitivity over an involved root end and/or a sinus tract traceable to a retrofilled root. We expect that these two clinical conditions are specific for a failing endodontic surgery and should not be mistaken for other symptomatic conditions relative to endodontically treated teeth. For example, percussion sensitivity or bite pain may be due to traumatic occlusion, parafunctional habits, sinus infections or cracked teeth. The additional clinical parameters that were measured are listed on the recall exam form (Appendix), but these conditions were generally within normal limits for the patients recalled in this study.

In order to streamline the radiographic evaluation process, we elected to digitize traditional radiographs with a high quality digital camera. Digitizing the conventional radiographs with a digital camera has been shown to be superior to utilizing a flat bed scanner (Goga et al. 2004). The diagnostic quality of digitized images is as effective for the assessment of periradicular lesions as traditional films (Barbat et al. 1998). Our independent examiners found the image quality to be excellent, and the clarity and detail of the radiographs on a 19" flat-panel monitor was acceptable.

The outcome of surgical endodontic cases has largely been assessed by radiographic interpretation alone (Allen et al. 1989; August 1996; Dorn et al. 1990; Malmstrom et al. 1982; Testori et al. 1999). We included clinical parameters, but the radiographic assessment had a more profound effect on the overall outcome of our recalled cases (Table 2-4). In fact, in this study, only once did the clinical assessment determine the overall outcome of a particular case (Table 2, Case #25). This demonstrates the accuracy of the radiographic interpretation in determining the outcome of periapical surgery. The reliability of radiographic interpretation for determining success and failure in endodontics has been questioned for many years (Bender et al. 1966a, 1966b; Goldman et al. 1972). In an attempt to address this problem, Orstavik and associates (1986) proposed a periapical index (PAI) scoring system to evaluate the radiographic healing following non-surgical root canal therapy. Unfortunately, the PAI system does not correlate with various healing conditions encountered in endodontic surgery. Therefore, we proposed a Surgical Healing Index (SHI) to account for other possible healing processes including residual scars, residual cysts, ankylotic processes, or fibrous encapsulation. These conditions are sometimes difficult or impossible to detect radiographically, but the diagrammatic representation of each category presented to the examiners (Figure 1) should have helped account for the existence of these conditions at the time of observation. Overall, the SHI system was used as a tool to help the independent examiners determine if the case should fall within the three main categories of healed, not-healed, or uncertain. No histologic evidence exists supporting or refuting the proposed SHI outcome categories, but theoretically the radiographic appearance may correlate with the definitions.

The Inter Correlation Coefficient which evaluates the inter-examiner reliability was low, but in line with *kappa* values reported by Delano and associates (2001) for the PAI system. A possible explanation might be the projection of two periapical radiographs simultaneously for each case. It was originally thought that the presentation of different angulations would provide the examiners with greater information about the outcome of each case, but perhaps various examiners focused their evaluation procedure on a specific radiograph as compared to their cohorts.

The present study design only provides a level of evidence (LOE) 4 case series, which falls short of recommendations made by Mead and associates in 2005. They found that the majority of reports on the outcome of endodontic surgery are a LOE 4 and that future studies should strive for better study design and stronger evidence. High quality randomized control trials are difficult to accomplish, expensive, and require many years to complete. The scope of this study was to acquire baseline long term data that may be useful to clinicians as they present prognostic information to their patients. This information may also be useful for researchers who are planning long term randomized control trials or multi-center longitudinal studies at a higher level of evidence.

The success rates within our study are not as high as those reported by Chong and PittFord in 2003. In their randomized control trial, MTA retrofilled cases were 92% successful after 2 years. Our result of 81% falls short; however, our recall term was a minimum of 3 years and up to 8 years. One can only extrapolate that perhaps the decrease in success may be due to the early success and late failure concept that has been reported in the endodontic literature (Rud et al. 1972; Kvist et al. 1999; Rubenstein et al. 1999, 2002). In this study, all of the failures occurred at least 4 years following surgery. Another possible reason for the discrepancy may include the lack of an immediate post-operative radiograph for comparison against the recall radiograph. Our examiners were asked to evaluate the current condition without reference radiographs, which deviates from the protocol of Chong and PittFord. Additionally, variance in surgical skills and experience may have affected the outcome of the MTA retrofill procedures.

It is difficult to compare the results of our study with those obtained by Wang et al. in 2004. Their report in phase one and two trials of the Toronto study fails to separate the outcome data of MTA as a root end filling material against other materials. Their healed outcome at 4-8 years following surgery was 74%, which is lower than that reported by Chong et al. in 2003 and lower than our findings.

Based on the results of this study and others (Chong et al. 2003; Wang et al. 2004), it appears that the use of MTA as a root end filling material produces a clinical and radiographic outcome similar to other materials such as Super-EBA or IRM (Dorn et al. 1990; Testori et al. 1999; Rubenstein et al. 1999, 2002; Chong et al. 2003; Gagliani et al. 2005). The benefit of MTA over other materials appears to be its regenerative properties. MTA has consistently demonstrated cementoconductive and osteoinductive properties that are unique to the material (Torabinejad et al. 1995e, 1997; Shabahang et al. 1999; Holand et al. 1999; Regan et al. 2002; Economides et al. 2003; Thomson et al. 2003; Baek et al. 2005). The presence of cementum deposition over the resected root ends and over the MTA root end filling material itself has been reported and may provide a "double seal" against penetrating bacteria and bacterial byproducts (Torabinejad et al. 1995e, 1997; Regan et al. 2002; Economides et al. 2003; Baek et al. 2005). No reports have been identified to declare that cementum itself is impervious to penetrating bacteria, but calcifications or pathologic granules have been shown within the cementum of heavily infected teeth (Simon et al. 1981; Armitage et al. 1983). Nonetheless, cementum is probably impervious to bacterial penetration based on the radiographic relationship between periradicular lesions and portals of exit of the root canal system.

Many researchers have attempted to determine or propose a mechanism for the inductive nature of MTA. This process began in 1995 with the investigation of the physical properties of MTA (Torabinejad et al. 1995f), but more recent publications have evaluated the chemical reaction, physical changes, and biologic response in more detail.

In 1995, Torabinejad and associates determined that a sharp pH rise occurs with MTA about three hours after mixing. This pH rise is likely due to the release of calcium hydroxide from the material, which occurs when calcium oxide contacts tissue fluids (Duarte et al. 2003; Fridland et al. 2003). This finding is supported by Holland and associates (1999) who concluded that the mechanism of action for hard tissue formation may be similar for both calcium hydroxide and MTA. Even if the mechanism of action is similar, however, there may be differences relative to the inductive capacity of MTA and calcium hydroxide. Shabahang and associates in 1999 determined that the degree of hard tissue formation adjacent to MTA was about twice that of calcium hydroxide. Additionally, the ability of MTA to promote hard tissue formation is probably related to the application state (fresh vs set) of the material. Freshly placed MTA appears to result in a significantly higher incidence of cementum formation than set MTA (Apaydin et al. 2004).

Numerous investigators have begun to evaluate local physiologic responses to MTA. It appears that MTA upregulates the production of IL-1α, IL-1β, and IL-6 from osteoblasts (Koh et al. 1998). MTA permits cementoblast attachment and allows the continued local production of mineralized matrix genes and protein expression (Thomson et al. 2003). MTA seems to elevate local levels of cellular PGE_2 synthesis from gingival fibroblasts and these cells appear to demonstrate a normal rate of protein synthesis in the presence of MTA. Bonson and associates (2004) have determined that PDL fibroblasts display enhanced proliferation on MTA as compared to gingival fibroblasts. They report that MTA preferentially induces alkaline phosphatase expression in both PDL and gingival fibroblasts. In addition, extracellular calcium ion released from MTA has been shown to upregulate intracellular calcium ion levels in osteogenic and odontogenic cells, which in turn activates intracellular phosphorylation pathways to induce cell proliferation (Shin 2004 Thesis).

Ongoing and future research should focus on high level of evidence studies evaluating the outcome of MTA as a root end filling material. Additionally, more studies are needed to determine the exact mechanisms of action for MTA's inductive properties and the benefit of a "double seal" at the root end.

Conclusion

Based on the results of this study, it appears that the use of MTA as a root end filling material leads to a >80% long term healed rate and a 93% long term survival rate.

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Appendix

Clinical Recall Examination Form

Patient Name: ____ Tooth #: __________

Original Diagnosis: ____

Comments: ____

Today's Date:				Initial Tx Date:			
Med Hx Review:				Vital Signs:			
VAS Pain Report: (Subjective)	(No Pain) 1 < 2 < 3 < 4 < 5 < 6 < 7 < 8 < 9 < 10 (Severe Pain)						
Symptom History:	None	Report:					
Oral cancer screening:	WNL	Abnormal:					
Percussion:	WNL	Slightly Sensitive		Painful			
Biting:	WNL	Slightly Sensitive		Painful			
*Palpation:	WNL	Tender	Describe Location				
Swelling:	None:	Intra-Oral:		Extra-Oral:			
Periodontal status including probing depths:	MB	B	DB	ML	L	DL	Furcal
Mobility:	Grade 0	Grade I	Grade II	Grade III			
Occlusion	WNL	Hyperocclusion		Fremitis			
*Sinus Tract:	Absent	Present	Traced To:				
Final restoration:	Present	Absent	When Placed				

*Clinical Data for Retrofill Healing Outcome