Title
Fracture resistance of endodontically treated simulated immature teeth obturated with mineral trioxide aggregate

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Publication Date
2006

Peer reviewed|Thesis/dissertation

FRACTURE RESISTANCE OF

ENDODONTICALLY TREATED SIMULATED IMMATURE TEETH

OBTURATED WITH MINERAL TRIOXIDE AGGREGATE

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ORAL AND CRANIOFACIAL SCIENCES

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

[redacted]

DEDICATION

To

my beautiful wife *Betty*

and

my lovely daughter *Michelle*

for

their love and support

ACKNOWLEDGMENTS

I would like to express my gratitude to:

The American Association of Endodontists Foundation for the Endodontic Research Grant in support of this research.

Dentsply Tulsa Dental for donating the ProRoot MTA and Lexicon products used in this research.

Dr. Sally Marshall for permission to use the Biomaterials Research Laboratory.

Dr. John Featherstone for permission to use the Biomaterials, Biophysical Sciences and Engineering Laboratory.

Dr. Harold Goodis, my mentor, Dr. Sally Marshall, Dr. Adam Lloyd and Dr. Diana Gekelman for serving in my research committee. Their guidance and support has made my research experience a memorable one.

Dr. Stuart Gansky for providing his statistical expertise. His patience with me was much appreciated.

Mr. Larry Watanabe, Ms. Grace Nonomura and Ms. Marcia Rapozo-Hilo for their help and training in gamma irradiation sterilization, fracture resistance testing, wet SEM and microhardness testing. They made me feel welcome in their laboratory and made those countless hours in the laboratory enjoyable.

My fellow endodontic residents for their camaraderie.

My dental colleagues in the San Francisco Bay area who had helped with the specimen collection.

Last but not least, my parents, siblings and in-laws for their love and encouragement.

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INTRODUCTION

Traumatized immature permanent teeth pose a challenge to endodontic and restorative treatment. (Bishop and Woollard, 2002) When the root and the apex are not completely formed, it is not possible to obturate the root canal system in a predictable manner. The thin, underdeveloped root is prone to fracture. (Cvek, 1992) With a vital pulp, apexogenesis may be possible and, if successful, the root will develop normally. (Webber, 1984) If the pulp is necrotic, apexification is traditionally performed, using calcium hydroxide. This requires long-term follow up of the patient until a calcific bridge is formed at the apex and obturation with gutta-percha can be achieved. (Rafter, 2005) This poses a problem as patients may not return for follow up appointments. (Steinig *et al.*, 2003) Mineral Trioxide Aggregate (MTA) enables obturation of the root canal system before the apex is formed. (Torabinejad and Chivian, 1999)

Studies have shown that long-term calcium hydroxide treatment weakens root dentin. (Grigoratos *et al.*, 2001; White *et al.*, 2002; Andreasen *et al.*, 2002) Various techniques to strengthen immature endodontically treated teeth using bonding, composite, and resin glass ionomer have been reported. (Rabie *et al.*, 1985; Rabie *et al.*, 1986; Katebzadeh *et al.*, 1998; Goldberg *et al.*, 2002) The physical and chemical properties of MTA have been studied. (Torabinejad *et al.*, 1995a) However, to date no study has been done on the strength of endodontically treated immature teeth with roots that have been obturated entirely with MTA. No study has been done on the effect of MTA on the microhardness of human dentin. MTA has good sealing ability (Torabinejad *et al.*, 1993; de Leimburg *et al.*, 2004; Al-Kahtani *et al.*, 2005) and is biocompatible. (Torabinejad *et al.*, 1995b;

Sousa *et al.*, 2004) If MTA proves able to strengthen immature teeth, then it will be a treatment of choice to avoid lengthy follow up in apexification cases and will improve the overall prognosis of such cases by minimizing root fractures.

REVIEW OF THE LITERATURE

Dental trauma and the consequential pulpal necrosis are common in children. In a clinical study on traumatic dental injuries in 1,298 patients in Copenhagen, 48% of the patients were 10 years of age or younger. (Andreasen, 1970) 24% were between the age of 6 and 10. The maxillary central incisor was most frequently involved in both the primary and permanent dentitions. Tooth fractures were more common in the permanent dentition while luxations were more frequent in the primary dentition. Repeated dental injuries were found in 24% of the cases.

In a survey done in Ireland, 7,171 children aged 8, 12 and 15 years were examined as part of a national survey of children's dental health. (Holland *et al.*, 1988) The prevalence of traumatic injury to teeth was found to increase with age, reaching a peak at 12 years.

The prevalence of traumatic injuries to permanent incisors in a group of 2,798 patients in Italy was studied. (Zerman and Cavalleri, 1993) The patients, aged 6 to 21 years, were examined over a 5-year period. The prevalence of traumatic injuries to permanent incisors was found to be 7.3%. A very large number of dental injuries occurred in children aged 6 to 13 years. About 80% of the teeth injured were maxillary central incisors. In the follow-up study of the crown fractured permanent incisors with incomplete root formation in this group of patients, there were 55 patients with 84 injured incisors. (Cavalleri and Zerman, 1995) The most common type of trauma (80%) was fracture of enamel and dentin without pulpal exposure. At the 5-year-control, 4 of 67 teeth (6%) with fracture of the enamel and dentin without pulpal involvement showed pulpal necrosis. 8 of 14 teeth (57%) with fractures of the enamel and dentin with pulpal involvement showed pulpal necrosis. 34 restored teeth (40%) had to be retreated because of a new trauma. The authors concluded that while crown fractures without pulpal involvement in permanent incisors with incomplete root formation have a low percentage of pulpal complications, 60% of the teeth with crown fractures with pulpal involvement had pulpal complications.

A study on dental injuries in 1,275 Norwegian children aged 7 to 18 years found nearly 50% of the children who had suffered dental trauma were between 8 to 10 years of age at the time of the injury. (Skaare and Jacobsen, 2005) The most accident-prone were 8-year-olds. 97% of the accidents involved one of the incisors with the maxillary central incisors being most often affected.

One study followed the pulpal healing patterns of 637 luxated permanent incisors in 400 patients for up to 11 years. (Andreasen, 1989) The results showed that the risk of pulpal necrosis depended on the stage of root development and the type of luxation injury suffered. Risk of pulpal necrosis was greatest in extrusion, lateral luxation and intrusion, in that order. Teeth with completed root development had greater risk of pulpal necrosis. In teeth with open apices, the risk of pulpal necrosis was 9% after extrusion, 9% after lateral luxation, and 63% after intrusion.

Thus it is common that endodontic treatment is necessary in traumatized immature or young permanent teeth. The objective of root canal obturation procedures is the complete three-dimensional filling of the root canal systems and all portals of exit. (Schilder, 1967) When the root and the apex are not completely formed, it is not possible to obturate the system in a predictable manner. (Goldman, 1974) The thin, underdeveloped root is prone to fracture. In a study on 885 luxated nonvital incisors with respect to periodontal tissue healing, occurrence of ankylosis and cervical root fractures, a significantly higher frequency of cervical root fractures was found in immature teeth when compared to mature teeth. (Cvek, 1992) The frequency of fractures was dependent on the stage of root development, ranging from 77% in teeth with the least to 28% in teeth with the most developed roots. In the 168 teeth that suffered cervical root fractures, 103 teeth fractured during calcium hydroxide treatment and 65 fractured after the obturation of the root canal with gutta-percha. Fractures occurred 3 months to 6 years after the start of treatment with the majority (63%) within the first 3 years.

When endodontic treatment is necessary in an immature tooth with an open and divergent apex, apexification is traditionally performed using calcium hydroxide prior to obturation. (Webber, 1984) Three clinical cases were reported where apexification was performed using a thick paste of calcium hydroxide and camphorated *p*-chlorophenol. (Frank, 1966) The patients were recalled at 3- to 6-month intervals until closure of the apex was evident on the radiograph and a definite apical stop was verified clinically. Apexification requires long-term follow up of the patient until a calcific bridge is formed at the apex and obturation with gutta-percha can be achieved. (Rafter, 2005) The effect of apexification using calcium hydroxide was studied with the time taken and the type of apical barrier formed evaluated. (Ghose *et al.*, 1987) In the 51 pulpless immature permanent incisors studied, 96% developed an apical barrier within 3 to 10 months. The initial apical diameter did not affect the time taken and the type of apical barrier formed. This long-term follow up poses a problem as patients may not return for follow-up appointments. (Steinig *et al.*, 2003) The coronal seal may fail during the process leading to coronal leakage and failure. (Saunders and Saunders, 1994) The thin, immature root may fracture before the treatment is completed. (Cvek, 1992) A case was reported where a 10-year-old patient did not return for 9 months after initiation of calcium hydroxide apexification. (Heling *et al.*, 1999) The temporary filling was lost and the patient developed pain, swelling and a sinus tract.

Furthermore, studies have shown that long-term calcium hydroxide treatment weakens root dentin. The effect of saturated calcium hydroxide solution and sodium hypochlorite solutions (3% and 5%) on the flexural strength and modulus of elasticity of standardized human dentin bars was studied. (Grigoratos *et al.*, 2001) Standardized plano-parallel dentin bars cut from human teeth were placed into vessels containing 3% or 5% sodium hypochlorite for 2 hours or calcium hydroxide solution for 1 week. The dentin bars were then subjected to three-point bend tests. The results showed a significant decrease in the flexural strength of the dentin bars after exposure to calcium hydroxide though the modulus of elasticity was not affected significantly. In both the sodium hypochlorite groups, there was a significant decrease in the flexural strength and the modulus of elasticity.

The effect of calcium hydroxide, MTA and sodium hypochlorite on the strength of root dentin was also examined. (White *et al.*, 2002) In this study, bovine dentin blocks with standardized dimension were placed in Petri dishes containing calcium hydroxide, MTA, or sodium hypochlorite for 5 weeks. The dentin specimens were subsequently shear tested. There was a 32% mean decrease in strength in the calcium hydroxide group, a 33% decrease in strength in the MTA group and a 59% decrease in the sodium hypochlorite group. The authors concluded that root dentin was weakened after 5 weeks of exposure to the various materials and suggested that the weakening was caused by a breakdown of the protein structure caused by the alkalinity of the materials used.

The effect of long-term calcium hydroxide used as a root canal dressing on the risk of root fracture was investigated. (Andreasen *et al.*, 2002) The root canals of sheep mandibular incisors with immature root formation were filled with a calcium hydroxide paste. The teeth were tested for fracture strength after 0.5, 1, 2, 3, 6, 9, and 12 months. The results showed a significant decrease in fracture strength with increasing storage time after 2 months. The fracture strength was not significantly affected when the storage time was 1 month or less. The results indicated that the fracture strength would be halved in about 12 months. The authors suggested that the alkaline nature of calcium hydroxide might have neutralized, dissolved or denatured the acid proteins and proteoglycans containing phosphate and carboxylate groups in the organic matrix of dentin, thus weakening the dentin.

The effect of two calcium hydroxide combinations on root dentin microhardness was determined. (Yoldas *et al.*, 2004) Dentin samples from the middle third of the root were treated with either a calcium hydroxide-glycerin combination or calcium hydroxide-water combination for 1, 3 and 7 days. Knoop hardness was tested 1 mm from the root canal walls. Results showed a significant decrease in dentin microhardness after 3 and 7 days of treatment compared to the pretreatment controls.

The fracture resistance of human root dentin after exposure to intracanal calcium hydroxide was studied. (Doyon *et al.*, 2005) The roots were sectioned horizontally into 1-mm thick disks that were subsequently loaded to fracture. The authors found no difference in the peak load at fracture after 30 days of exposure compared to the control. However, roots treated with calcium hydroxide for 180 days showed a significant decrease in peak load at fracture compared to the control group and the 30-day groups.

Dentin microhardness can also be affected by the irrigants used in root canal treatment. The effect of endodontic irrigation solutions on the microhardness of root canal dentin in 18 extracted human incisors was evaluated. (Saleh and Ettman, 1999) Knoop hardness was measured at 500 μm and 1 mm from the pulpo-dentinal interface of the cervical, middle and apical transverse section of each root. Measurements were made before and after irrigation with 3% hydrogen peroxide and 5% sodium hypochlorite used alternatively, or 17% ethylenediaminetetraacetic acid (EDTA). 1 ml of each solution per segment was applied for 60 seconds of exposure time. The results showed that both irrigants decreased the microhardness of root dentin with EDTA producing more reduction.

The effect of 0.2% chlorhexidine gluconate on the microhardness and roughness of root canal dentin was compared to 5.25%, 2.5% sodium hypochlorite, 3% hydrogen peroxide, 17% EDTA, and distilled water (control). (Ari *et al.*, 2004) Each irrigant was used for 15 minutes. A total of 180 specimens were tested for Vickers hardness. The results showed that all the irrigation solutions except chlorhexidine and the control significantly decreased the microhardness of root canal dentin.

The effects of irrigation with 2.5% and 6% sodium hypochlorite for 5, 10 or 20 minutes were evaluated using 42 bovine roots. (Slutzky-Goldberg *et al.*, 2004) The irrigants were replenished every minute and saline served as control. The roots were cut into two 10-mm segments and Vickers hardness measured at 500 µm, 1000 µm, and 1500 µm from the root canal lumen. A decrease in microhardness was found at 500 µm between the control and sodium hypochlorite groups at all irrigation periods. There was also a significant difference in groups irrigated for 10 or 20 minutes. At all distances, the decrease in microhardness was more marked after irrigation with 6% than 2.5% sodium hypochlorite.

The effect of citric acid and EDTA on the microhardness and roughness of root dentin in 45 longitudinally sectioned human teeth was studied. (Eldeniz *et al.*, 2005) The specimens were randomly divided and treated with either 19% citric acid for 150 seconds followed by 5.25% sodium hypochlorite, or 17% EDTA for 150 seconds and rinsed with 5.25% sodium hypochlorite, or rinsed with distilled water as control. Vickers hardness testing demonstrated significant difference between the groups in the following order of hardness, control > EDTA > citric acid.

The effect of 10% citric acid, 17% EDTA and 17% EDTA plus Cetavlon (EDTAC) solutions on the microhardness of human root canal dentin was determined. (De-Deus *et al.*, 2006) Vickers microhardness values were obtained in transverse sections of 16 maxillary human canines before and after the specimens were exposed to 50 μL of the chelator solutions for 1, 3 and 5 minutes. There was no significant decrease in microhardness after 1 minute of exposure. After 3 minutes, EDTA produced a significantly greater decrease in microhardness. After 5 minutes, both EDTA and EDTAC caused significantly more reduction in microhardness than citric acid.

To overcome the problem of the weakened immature teeth, various strengthening techniques have been proposed. 3 cases were presented where immature incisors with horizontal fractures in the cervical region were restored with an acid-etch resin technique. (Rabie *et al.*, 1985) The gutta-percha was removed from the root canals to a level 2 to 3 mm apical to the osseous crest after endodontic treatment. The root canal dentin was acid-etched and a post to retain a crown was cemented into the canal system with a composite resin. The authors suggested that the acid-etch technique could strengthen the root considerably.

A later study reported a case where acid-etched composite resin was used to strengthen a nonvital immature maxillary central incisor. (Rabie *et al.*, 1986) A 13-year-old patient presented with a traumatized right maxillary central incisor with an incompletely formed root, thin root canal walls and an open apex. After initiation of root canal treatment, the root canal was filled with a paste of calcium hydroxide and saline up to a level 3 mm apical to the osseous crest. A thin layer of Intermediate Restorative Material (IRM) cement was placed over the paste. The enamel and dentin of the access cavity was acid-etched and a composite resin was injected into the cavity. A plastic post with a diameter of 1.5 mm coated with Vaseline was placed centrally into the resin until contact was made with the IRM cement. The post was removed after polymerization of the composite resin to allow subsequent access to the root canal. The apexification and root canal treatment was completed after 12 months. At the 12 months post-treatment follow-up examination, there was no evidence of vertical or horizontal fractures of the tooth. The authors concluded that the described method could strengthen severely weakened nonvital immature teeth with thin dentin walls during long-term endodontic treatment.

A more recent study evaluated the reinforcing effect of a resin modified glass ionomer in simulated immature teeth. (Goldberg *et al.*, 2002) The root canals of maxillary human incisors were enlarged to a size of 1.65 mm to simulate immature teeth with incomplete root formation. The crowns were removed to obtain a standardized root length. After obturation of the apical 2 mm of the root canal with gutta-percha, the root canal was filled with resin modified glass ionomer. A translucent curing post was inserted into the glass ionomer to facilitate light curing. The post was then removed. Using a stainless steel cone, the compressive force necessary to result in root fracture was determined. The reinforced teeth showed a significant increase in resistance to fracture when compared to the control teeth.

The strengthening of immature teeth during and after apexification was investigated. (Katebzadeh *et al.*, 1998) 100 extracted mature human maxillary central incisors were used in their study. The cervical areas were thinned to simulate the thin dentinal walls of immature teeth. The root canals were prepared and obturated with gutta-percha. In one test group, composite and a clear curing post was placed to the level of the gutta-percha, 3 mm apical to the cemento-enamel junction (CEJ). The post was removed after curing, leaving a patent channel. In another test group, an opaque post was used instead. In the third test group, a metal post was cemented in the channel left by the curing post. Cervically unprepared teeth were used as a positive control. In the negative control, the access was restored with composite to the level of the CEJ. The teeth were tested for resistance to cervical fracture. All the test groups were significantly stronger than the negative control group and none of the test groups were significantly different from the positive control group.

The use of a fiber-composite laminate in reinforcing immature maxillary central incisors has been suggested. (Pene *et al.*, 2001) In that study, 26 extracted human maxillary central incisors were machined to simulate immature teeth. Dentin-bonded composite was used to fill the canal in one test group while a piece of Reinforcement Ribbon was used together with the dentin-bonded composite in another test group. Unrestored teeth served as control. The fracture strength test showed that dentin-bonded composite with or without the reinforcement ribbon significantly strengthened the simulated immature teeth, with the composite only group being stronger.

An in vitro study examined the effect of root reinforcements in immature teeth. (Carvalho *et al.*, 2005) 56 bovine incisors were prepared to simulate immature teeth and divided into 4 groups. In group 1, the cervical and middle third of the root was reinforced by composite resin light-cured with the aid of a translucent curing post. In group 2, the root was reinforced by a zirconium fiber post cemented with a dual-cure resinous cement. The remaining 2 groups were the positive and negative controls. The teeth were subjected to a compressive force and the authors concluded that the use of root reinforcements with zirconium fiber posts or composite resins significantly increased the structural resistance of the weakened teeth.

The reinforcement and strengthening effect of Resilon was compared to gutta-percha and a self-cured flowable composite resin in endodontically treated roots of simulated immature teeth. (Stuart *et al.*, 2006) 60 single rooted teeth were prepared with a #5 Peeso reamer through the apex to simulate immature roots with an open apex of 1.5 mm. The root ends were filled with a 4 mm barrier of MTA and backfilled with the test materials. Positive and negative controls were used. Fracture strength testing showed no significant difference between the groups. The authors concluded that canal wall reinforcement of teeth with a canal diameter of 1.5 mm or less might not be necessary.

An alternative to using calcium hydroxide for apexification over multiple visits is to perform a one-visit apexification by creating an apical stop. (Morse *et al.*, 1990) Various materials have been used in one-visit apexification, including resorbable tricalcium phosphate ceramic (Koenigs *et al.*, 1975), dental amalgam (Dimashkieh, 1975), oxidized regenerated cellulose (Dimashkieh, 1977), dentin chips (Tronstad, 1978), tricalcium phosphate (Coviello and Brilliant, 1979), calcium hydroxide (Coviello and Brilliant, 1979), freeze-dried bone (Rossmeisl *et al.*, 1982a), and freeze-dried dentin (Rossmeisl *et al.*, 1982b). Recently MTA has been suggested for apexification, enabling obturation of the root canal system before the apex is formed. (Torabinejad and Chivian, 1999)

In a review of the use of MTA, the technique using MTA for apexification was described and radiographs of a successful case were presented. (Schmitt *et al.*, 2001) The authors used MTA to create a 3 to 4 mm apical plug and suggested obturating the rest of the root canal system with thermoplastic gutta-percha and sealer or with composite in thin walled teeth.

3 cases were reported of central incisors where trauma had caused pulpal necrosis and interruption of root development. (Giuliani *et al.*, 2002) After initiation of root canal treatment, calcium hydroxide was placed in the canal for 1 week. Subsequently, the apical 4 mm of the root canals were filled with MTA and the remaining portion of the system was obturated with thermoplastic gutta-percha. The teeth showed resolution of the periapical lesions clinically and radiographically at 6-month and 1-year follow-up.

A case where MTA was used to treat a nonvital immature incisor was reported. (Maroto *et al.*, 2003) A 9-year-old boy had suffered an intrusion and a complicated enamel-dentin crown fracture in both of his upper central incisors with immature roots and open apices. The teeth became nonvital 3 months after the initial treatment that included pulpotomy and pulp capping. Apexification using calcium hydroxide was then started. An apical stop formed in one of the incisors after 2 years of treatment and the tooth was obturated with gutta-percha. In the other incisor, an apical stop failed to form after 3 years of treatment. The canal was then obturated with a 2-mm apical plug of MTA with the remainder of the system filled with gutta-percha. At the 1-year follow up, the tooth was asymptomatic and there was radiographic evidence of healing of the radiolucent lesion.

In a case where MTA was used for obturation of two mandibular central incisors with open apices, a 58-year-old patient presented with swelling and a sinus tract at the apices of teeth #24 and #25. (Hayashi *et al.*, 2004) The teeth had been endodontically treated conventionally 13 years previously. Non-surgical retreatment was instituted. After rinsing with 17% EDTA and 5% sodium hypochlorite and dried, the canals were obturated with a thick mix of MTA using a small plugger and the large end of sterilized paper points. At the 2-year follow-up radiographic examination, the teeth were asymptomatic and regeneration of periradicular tissue was evident.

2 cases were reported where MTA was used for one-step apexification. (Kratchman, 2004) One case involved a maxillary central incisor with a blunderbuss apex and thin root canal walls. The second case involved a maxillary central incisor with an open apex

where calcium hydroxide apexification had failed. In both cases, calcium sulphate was used as an apical barrier before MTA was condensed to form the apical plug.

A technique of intraradicular rehabilitation of weakened anterior roots was described. (Tait *et al.*, 2005) The authors suggested one-visit apexification using MTA to form an immediate apical seal. The root was then reinforced with composite resin cured via a clear plastic light-transmitting post. Where insufficient coronal tooth structure remained, a quartz-fiber post was cemented with adhesive resin cements to retain a composite core.

MTA is available commercially as gray ProRoot MTA or white ProRoot MTA (Dentsply Tulsa Dental, Tulsa, Oklahoma). White MTA consists of a hydrophilic powder mixture of 75% Portland cement clinker, 20% bismuth oxide, and 5% gypsum by weight. (Dentsply Tulsa Dental, 2002) The major compounds are tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, and calcium sulfate dihydrate (Gypsum). Small amounts of crystalline silica, calcium oxide, magnesium oxide, potassium sulphate and sodium sulphate are also present. The composition of gray MTA is similar to that of white MTA, containing 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight. (Dentsply Tulsa Dental, 1998) In addition, gray MTA contains tetracalcium aluminoferrite. The powder is mixed with sterile water at a 3 to 1 ratio to provide a thick, creamy consistency. After mixing, the pH of MTA is 10.2 and rises to 12.5 at 3 hours and remains constant thereafter. (Torabinejad *et al.*, 1995a) The compressive strength of MTA is 40.0 ± 4.4 MPa at 24 hours and increases to 67.3 ± 6.6 MPa at 21 days. The mean setting time of MTA was 2 hours 45 minutes ± 5 minutes. MTA is more radiopaque than conventional gutta-percha and dentin. Suggested clinical applications of MTA include apexification, capping of pulps with reversible pulpitis, repair of root perforations surgically or non-surgically, and as a root-end filling material. (Torabinejad and Chivian, 1999)

Though MTA is similar to Portland cement, they are not the same. The chemical and physical properties of white ProRoot MTA in the bulk form and at the surface was analyzed and compared to two commercially available Portland cements. (Dammaschke *et al.*, 2005) The authors found that the ProRoot MTA consisted of less Copper, Manganese, Strontium, chromophores (Fe^{3+}) and Al-species than the Portland cements. The ProRoot MTA contained bismuth while the Portland cements did not. The Portland cements had a wide range of particle size while ProRoot MTA had a uniform and smaller particle size.

The constitution of white and gray ProRoot MTA was compared to ordinary Portland cement using energy dispersive analysis by X-ray in a scanning electron microscope and X-ray diffraction. (Camilleri *et al.*, 2005) Results showed that the constitution of ProRoot MTA to be similar to ordinary Portland cement in general except for the addition of bismuth compounds. The energy dispersive analysis showed the presence of calcium, silicon, bismuth and oxygen in white MTA and calcium, silicon, aluminum, iron, bismuth and oxygen in gray MTA with calcium and silicon being the predominant elements in both. White MTA was composed primarily of tricalcium silicate and bismuth oxide while gray MTA was composed primarily of tricalcium silicate, dicalcium silicate and bismuth oxide.

The major constituents in gray ProRoot MTA, white ProRoot MTA, ordinary Portland cement and white Portland cement was compared using powder X-ray diffraction. (Islam *et al.*, 2006) The main constituents were found to be tricalcium silicate, tricalcium aluminate, calcium silicate, and tetracalcium aluminoferrite in all four cements with the additional presence of Bi_2O_3 in the gray and white ProRoot MTA.

The effects of physiological environments on the hydration behavior of MTA were studied. (Lee *et al.*, 2004) The authors found that an acidic environment of pH 5 adversely affects both the microhardness and the hydration behavior of MTA.

The solubility and porosity of MTA was found to increase as the water-to-powder ratio increased. (Fridland and Rosado, 2003) At a 0.33 water-to-powder ratio, as recommended by the manufacturer, the pH level of the solution ranged from 11.94 to 11.99 with calcium as the main element. The authors concluded that the calcium was in the form of calcium hydroxide. In a subsequent study, the same investigators projected that MTA could solubilize 31% at the manufacturer's recommended water-to-powder ratio. (Fridland and Rosado, 2005)

The solubility, microhardness and radiopacity of white ProRoot MTA were compared to two commercially available Portland cements. (Danesh *et al.*, 2006) The results showed that the solubility of white MTA in double-distilled water was significantly lower than the Portland cements. The microhardness and radiopacity of white MTA was significantly higher than the Portland cements.

The effect of setting conditions on the flexural strength of MTA has been determined. (Walker *et al.*, 2006) MTA beam specimens were allowed to set for either 24 or 72 hours with the specimens exposed to moisture on either one or two surfaces. The specimens that were allowed to set for 24 hours with moisture on 2 sides had significantly higher flexural strength from the three-point bend test. The authors concluded that a moistened cotton pellet should be placed on the intracanal MTA surface under a temporary restoration and the pellet should only remain in place for 24 hours to optimize flexural strength.

The fracture resistance of immature sheep teeth after various treatment modalities using calcium hydroxide or MTA as a root filling were compared. (Andreasen *et al.*, 2006) Sheep incisors with 80% root completion were divided into 4 groups. One group was root filled with calcium hydroxide and sealed apically with IRM. Another group was root filled with MTA. A third group was filled with calcium hydroxide and sealed with IRM for 30 days after which the calcium hydroxide was replaced with MTA. The negative control group was stored in saline. Fracture resistance was tested after 100 days of storage. The group filled with calcium hydroxide for 100 days showed a decrease in fracture resistance compared to the other groups. The authors concluded that there was no significant decrease in strength of the root when calcium hydroxide was present in the canals of immature sheep teeth for only 30 days followed by root filling with MTA.

The sealing ability of MTA had been evaluated as a root end filling material and as an orthograde apical obturation material. Rhodamine B fluorescent dye and a confocal microscope were used to evaluate the sealing ability of amalgam, super EBA and MTA as root end filling materials. (Torabinejad *et al.*, 1993) Results showed that MTA leaked significantly less than amalgam and super EBA. Bacterial leakage of MTA as a root end filling material was also evaluated. (Torabinejad *et al.*, 1995c) The time taken for Staphylococcus epidermidis to penetrate a 3-mm thickness of amalgam, super EBA, IRM or MTA was determined. MTA did not show any leakage throughout the experimental period of 90 days, whereas the other materials started leaking at 6 to 57 days.

Bacterial leakage of pulpless teeth with open apices that were obturated with MTA was studied. (de Leimburg *et al.*, 2004) After cleaning and shaping the root canals of 34 extracted human single-rooted teeth, the apical foramina were enlarged to ISO size 80. The roots were divided into three groups of 10 with the remaining 4 roots used as controls. The apical portions of the roots in the test groups were obturated with MTA of 1-mm, 2-mm or 3-mm thickness using a MTA Endo Gun and compacted with a plugger. The sterilized specimens were inoculated with Enterococcus faecalis and incubated in sterile medium. Contamination was checked at 10, 20, 30, 40, and 50 days. No specimens were contaminated at day 10. 17% of the specimens were contaminated at day 50 with no statistical difference between the groups. The authors concluded that MTA provided an adequate seal when used as an orthograde apical obturation of pulpless teeth with open apices.

The in vitro microleakage of an orthograde apical plug of MTA in permanent teeth with simulated divergent open apices was evaluated. (Al-Kahtani *et al.*, 2005) The groups compared were 2 mm MTA plug, 5 mm MTA plug, 2 mm MTA plug with a second 2 mm increment 24 hours later, 2 mm MTA plug with gutta-percha back fill 24 hours later, and a positive control. Actinomyces viscosus was used in a bacterial leakage model. The 5 mm apical plug completely prevented bacterial leakage up to 70 days. The other groups were not significantly different from the positive control.

The interactions of MTA with a synthetic tissue fluid composed of a neutral phosphate buffer saline solution and root canal dentin in extracted human teeth were studied. (Sarkar *et al.*, 2005) Two extracted single-rooted human teeth were endodontically prepared and the root canal filled with MTA after smear layer removal. After 2 months of storage in synthetic tissue fluid at 37° C, an adherent interfacial layer that resembled hydroxyapatite in composition was formed at the dentin wall. The authors concluded that calcium, the dominant ion released from MTA, reacted with phosphates in the synthetic tissue fluid, yielding the hydroxyapatite like interfacial layer. From this limited study, they attributed MTA's sealing ability, biocompatibility and dentinogenic activity to these physicochemical reactions.

Several authors have evaluated the placement technique for MTA. The adaptation of MTA using two different placement and condensation methods was studied. (Aminoshariae *et al.*, 2003) MTA was placed into 80 polyethylene tubes in 3-, 5-, 7- or 10-mm lengths using either a hand condensation method or an ultrasonic condensation method. The samples were evaluated by light microscopy and radiography. The hand method resulted in better adaptation to the tube wall and fewer voids than the ultrasonic method. The authors suggested hand condensation for placement of MTA.

A later study compared the sealing ability and set hardness of white and gray MTA using a one- or two-step apical barrier technique. (Matt *et al.*, 2004) 44 root segments were prepared to an ISO size #90 to simulate open apices. Apical barriers of white or gray MTA were placed to a thickness of 2 or 5 mm using a Messing gun and pluggers. The root canals were then obturated with thermoplasticized gutta-percha and sealer either immediately (one-step) or after the MTA had set for 24 hours (two-steps). Dye leakage analysis showed that the gray MTA leaked significantly less than the white MTA and the two-step technique leaked less than the one-step technique. Vickers microhardness testing showed that the 5-mm thick barrier was significantly harder than the 2-mm barrier regardless of the type of MTA or number of steps.

Another study evaluated the bacterial leakage of MTA as an apical barrier when placed ultrasonically with or without an intracanal composite resin. (Lawley *et al.*, 2004) The investigators also looked at the resistance to root fracture when either flowable self-cured composite resin or gutta-percha and sealer was placed against the apical barrier of MTA which had been condensed using ultrasonic vibration. A divergent open apex 1.24 mm in size was simulated in extracted human single-rooted mandibular premolars and maxillary central incisors. MTA was condensed with or without ultrasonic vibration. Enterobacter aerogenes, Enterococcus faecalis and Staphylococcus epidermidis were used for the leakage study. The ultrasonics group with or without composite leaked significantly less at 45 days. At 90 days, only the ultrasonics with composite group leaked significantly less than the others. A 4-mm thickness of MTA followed with an intracanal composite resin was significantly more resistant to root fracture than MTA with gutta-percha.

While MTA has been studied extensively in the last decade, to date no study has evaluated the strength of endodontically treated immature human teeth with roots that were obturated entirely with MTA. No study has determined the effect of MTA on the microhardness of human dentin. If MTA proves able to strengthen immature teeth, then it will be a treatment of choice to avoid lengthy follow-up and the resulting complications in apexification cases and will improve the overall prognosis of such cases by minimizing root fractures.

SIGNIFICANCE OF THIS STUDY

This study investigates the possibility of strengthening immature roots to resist fracture by obturating the root canal system with MTA. To date, no study has been done on the fracture strength of endodontically treated immature human teeth with roots that have been obturated entirely with MTA. No study has been done on the effect of MTA on the microhardness of human dentin.

HYPOTHESES AND SPECIFIC AIMS

The purpose of this study is to determine the effect on the fracture strength and microhardness of endodontically treated, simulated immature teeth when MTA is used as the root canal filling material.

Null Hypotheses

1. There is no difference in the fracture strength of endodontically treated, simulated immature human roots obturated entirely with MTA or gutta-percha or unobturated.
2. There is no difference in the microhardness of the dentin of endodontically treated, simulated immature human roots obturated entirely with MTA or gutta-percha or unobturated.

Specific aims

1. To measure and compare the fracture strength of simulated immature human roots obturated entirely with MTA or gutta-percha or unobturated.
2. To measure and compare the microhardness of the dentin of simulated immature human roots obturated entirely with MTA or gutta-percha or unobturated.

MATERIAL AND METHODS

Specimen collection

Human incisors, cuspids and bicuspids from patients up to 30 years old were collected through local dental offices. The Human Subjects Protection Program of the Committee on Human Research (CHR) of the University of California, San Francisco (UCSF) has approved an exempt status to collect teeth that were scheduled for extraction. The teeth were collected in such a manner that the patients were not identifiable. UCSF standard operating procedures for infectious agents and biosafety were followed. The teeth were placed in deionized water in plastic bottles immediately following extraction.

A power analysis was done to determine the number of specimens needed. 10 teeth were used per group for a total of 60 teeth, based on the sample size calculation using the following parameters: family error rate (α) of 0.05, power of 0.8, minimum detectable difference between any groups of 25%, standard deviation of 15% of the mean, and 3 groups. Additional teeth were collected to allow for unexpected loss during processing.

The specimens were stored in deionized water until use and were sterilized using γ-irradiation (White *et al.*, 1994). The specimens were examined with a SurgiTel 3.5X telescope (General Scientific Corporation, Ann Arbor, Michigan) for the presence of craze lines, fractures, caries, and existing restorations. Such specimens were excluded from the study. The specimens were digitally radiographed mesio-distally and bucco-lingually to determine the number of root canal systems. The Trophy Mac 1.5 digital imaging system and the Trophy ETX Model AD215 X-ray unit (Trophy Radiologie, Vincennes, France) were used at 70 kVp, 8 mA, 0.08 msec and a focus to film distance (FFD) of 8 inches with the size 1 sensor. Specimens with multiple roots or multiple root canal systems were excluded from part I of the study. Some specimens with multiple roots or multiple root canal systems were selected for part II of the study. Only the facial root canal systems were used in these specimens. The collected teeth were assigned a number, grouped according to the tooth type and randomly assigned to the control and treatment groups by the throw of a die. Only one tooth from each patient was used.

Root canal preparation

One operator using a standardized technique prepared all specimens as described below. Because immature teeth with open apices were extremely difficult to obtain, the root canals of young human teeth were simulated to be similar to immature teeth with open apices. The apical size of the final preparation was standardized at 1.5 mm. In part II of the study, only the facial root canal systems were prepared when specimens with multiple roots or multiple root canal systems were used.

A Sharpie extra-fine point permanent marker (Sanford, Bellwood, Illinois) was used to mark 3 lines, one at the facial CEJ, one at 3 mm coronal to the facial CEJ, and one at 3 mm (part I) or 4 mm (part II) gingival to the facial CEJ. The crown was removed at 3 mm coronal to the facial CEJ using a water-cooled Isomet low speed saw (Buehler, Lake Bluff, Illinois) prior to canal system preparation. Access was made with Diamond Access Burs (Dentsply Tulsa Dental, Tulsa, Oklahoma) and refined with Pulp Shaper

Burs (Dentsply Tulsa Dental, Tulsa, Oklahoma). Pulpal tissues were removed with stainless steel barbed broaches (Union Broach, New York, New York). The root canal system was cleaned and shaped to the apical foramen with Flex-O-files (Dentsply Maillefer, Tulsa, Oklahoma). The root canal system was enlarged sequentially by number 1 to 6 Lexicon Gates Glidden drills (Dentsply Tulsa Dental, Tulsa, Oklahoma) to a final apical size of 1.5 mm, the size of the number 6 Gates Glidden drill. 1 ml of 3% sodium hypochlorite (1:1 dilution with deionized water, Clorox Regular-bleach, Clorox Professional Products Co., Oakland, California) was used for irrigation between each instrument. The concentration of the sodium hypochlorite was confirmed with a Hach Test Kit for Hypochlorite (C12 Model CN-HRDT, Hach Company, Loveland, Colorado). Smear layer was removed by a final irrigation with 5 ml of 17% EDTA (Henry Schein Inc., Melville, New York) over a period of 1 minute and then 5 ml of 3% sodium hypochlorite. (Baumgartner and Mader, 1987) A Casio 1310 AW-43 stopwatch (Casio Computer Co., Ltd., Tokyo, Japan) was used to ensure that the total exposure time to sodium hypochlorite was 30 minutes per tooth. The canal was dried with Lexicon absorbent paper points (Dentsply Tulsa Dental, Tulsa, Oklahoma).

Root canal obturation

One operator using a standardized technique obturated all specimens as described below. In part II of the study, only the facial root canal systems were obturated when specimens with multiple roots or multiple root canal systems were used.

Group 1 (Positive Control)

The root canal system was not obturated with any root canal filling material.

Group 2 (Gutta-percha)

The root canal system was obturated entirely with gutta-percha and sealer from the apical foramen to the facial CEJ using standard endodontic procedures. The apical foramen was occluded with a gloved finger to serve as a matrix against which the gutta-percha was compacted. A Lexicon ISO size 120 .02 taper gutta-percha cone (Dentsply Tulsa Dental, Tulsa, Oklahoma) was trimmed to fit to 0.5 mm from the apical foramen. The root canal system was obturated with the gutta-percha cone and Pulp Canal Sealer EWT (SybronEndo, Orange, California) using the warm vertical compaction technique. (Schilder, 1967) The System B heat source (SybronEndo, Orange, California) and Schilder pluggers (Dentsply Maillefer, Tulsa, Oklahoma) were used. The canal system was back filled to the facial CEJ using the Obtura III system with a 23-gauge needle and Obtura dental gutta percha (Obtura-Spartan, Fenton, Missouri). Figure 1 is the digital radiograph of an obturated specimen in Group 2.

Group 3 (MTA)

The root canal system was obturated entirely with tooth colored (white) ProRoot MTA root canal repair material (Dentsply Tulsa Dental, Tulsa, Oklahoma) from the apical foramen to the facial CEJ. The apical foramen was occluded with a gloved finger to serve as a matrix against which the MTA was compacted. The MTA was carried into the canal using the MAP system with the curved needles (Roydent Dental Products, Johnson City, Tennessee) and compacted with Schilder pluggers (Dentsply Maillefer, Tulsa, Oklahoma) in small increments. A SP-1 ultrasonic tip (SybronEndo, Orange, California) in a Satelec P5 ultrasonic booster (Acteon Inc., Mount Laurel, New Jersey) at the lowest power setting was used to condense the MTA. A moist cotton pellet was placed in the pulp chamber for 24 hours to facilitate setting of the MTA. The cotton pellet was removed 24 hours after obturation. Figure 2 is the digital radiograph of an obturated specimen in Group 3.

All the dental materials used were mixed and handled according to the manufacturer's recommendation. Digital radiographs using the afore-mentioned parameters were taken to confirm the quality of the obturation. The obturated specimens were wrapped in cotton gauze and stored in Hank's balanced salt solution (HBSS) in a sealed container for 24 hours to ensure that the sealer and the MTA were set. A DG16 endodontic explorer (Hu-Friedy Mfg. Co., Inc., Chicago, Illinois) was used to confirm the set of the obturation materials. The cotton pellet was then removed and the coronal access and the apical foramen were sealed with Kerr sticky wax (KerrLab Corp., Orange, California). The specimens were stored in HBSS for 5 weeks before testing to allow time for the MTA to attain maximum strength. (Torabinejad *et al.*, 1995a)

Figure 1. Digital radiograph of a specimen obturated with gutta-percha

Figure 2. Digital radiograph of a specimen obturated with MTA

Testing of physical properties

Part I: Fracture strength testing

The teeth were mounted in Rockite (Hartline Products Co. Inc., Cleveland Ohio) to 3 mm below the CEJ. ¾" PVC coupling sockets (Lasco Fittings, Inc., Brownsville, Tennessee) were used as molds for the mounting as shown in Figure 3. Wooden tongue blades and glue were used to secure the specimens to the PVC molds during mounting. The Rockite was mixed at a ratio of 32 grams of powder to 8 grams of deionized water. A Tooth Master investment vibrator (Whaledent Inc., Cuyahoga Falls, Ohio) was used during pouring to avoid entrapment of bubbles in the mounting material. Part of the coupling was removed to facilitate the fracture strength testing.

A jig was fabricated using Coe tray plastic (GC America Inc., Alsip, Illinois) so that the long axis of the mounted specimen could be placed at 30° to the horizon as shown in Figure 4.

Figure 3. Specimen mounted in Rockite and PVC coupling

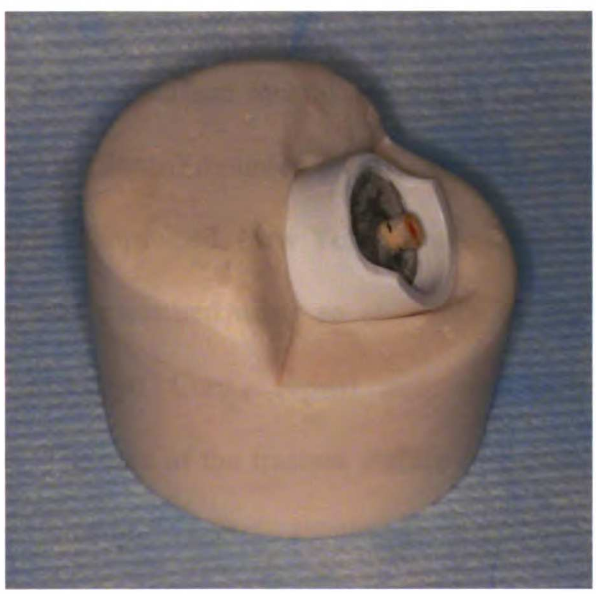

Figure 4. Mounted specimen in the acrylic jig

Load was applied to the specimen root from the facial direction at the facial CEJ at a 120° angle to the long axis of the root until failure, using the Instron materials testing machine Model 1122 with a custom fabricated force application apparatus (Instron, Norwood, Massachusetts) as shown in Figure 5. A flat area of approximately 1 mm wide was made just coronal to the facial CEJ to facilitate the load application. The load at fracture in Kilogram-force (Kgf) was recorded.

The fractured specimen and a metal wire of a known length were mounted on a Fisherbrand 1"x3" microscope glass slide (Fisher Scientific, Pittsburgh, Pennsylvania) using Bostik Blu-Tack (Emhart Australia Pty. Ltd., Thomastown, Victoria, Australia) so that the fracture surface and the wire were on the same focal plane as shown in Figures 6 and 7.

A digital image of the fracture surface was taken using a Canon EOS 10D digital SLR camera (Canon Inc., Tokyo, Japan) mounted on a Zeiss OPMI pico surgical microscope (Carl Zeiss Surgical, Inc., Thornwood, New York) as shown in Figure 8. The length of the metal wire in mm was measured using an electronic caliper (Model #CD-S6 CT, Absolute Digimatic, Mitutoyo Corp., Japan) and was used for calibrating the measurement software. The area of the fracture surface was measured in mm^2 using the Image J software version 1.34s (National Institutes of Health USA, http://rsb.info.nih.gov/ij/).

Figure 5. Fracture strength testing using the mechanical testing machine

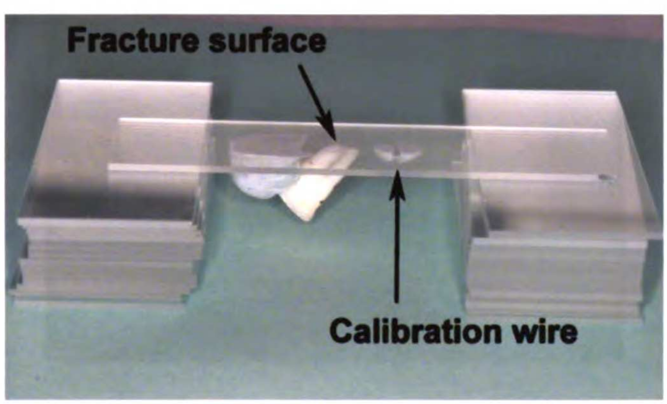

Figure 6. Fractured specimen mounted with the calibration wire

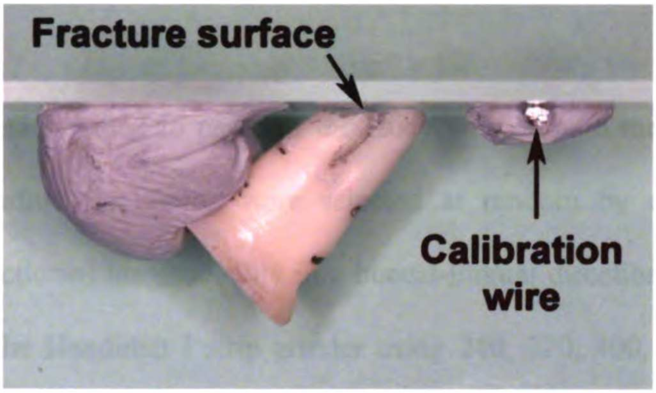

Figure 7. Fractured surface mounted on the same focal plane as the calibration wire

Figure 8. Apparatus for taking digital image of the fracture surface

Wet SEM

3 specimens from each group in part I of the study with enough mesio-distal width for subsequent longitudinal sectioning were selected at random by drawing lots. The specimens were sectioned longitudinally in a buccal-lingual direction and hand polished sequentially with the Handimet I strip grinder using 240, 320, 400, 600, 800 and 1200 grit silicon carbide paper and 6, 3 and 1 micrometer Metadi diamond suspension (Buehler, Lake Bluff, Illinois). The fracture surfaces were examined at 20X magnification for differences in the fracture pattern between the test groups and the control group using the Topcon ISI SX-40-A scanning electron microscope (Topcon Instruments, Pleasanton, California) at low vacuum (wet). Micrographs of the representative specimens were taken.

Part II: Microhardness testing

A 4 mm transverse section of the root was obtained by sectioning the tooth at the CEJ and at 4 mm gingival to the CEJ using a water-cooled Isomet low speed saw (Buehler, Lake Bluff, Illinois). The apical sectioned surface was tested. A groove was placed with a #34 inverted cone bur (Brasseler USA, Savannah, Georgia) on the mid-lingual root surface for orientation. The sectioned specimens were hand polished sequentially with the Handimet I strip grinder using 240, 320, 400, 600, 800 and 1200 grit silicon carbide paper and 6, 3 and 1 micrometer Metadi diamond suspension (Buehler, Lake Bluff, Illinois). The polished specimens were mounted on Fisherbrand 1”x3” microscope glass slides (Fisher Scientific, Pittsburgh, Pennsylvania) with Super Glue epoxy adhesive

(Pacer Technology, Rancho Cucamonga, California) such that the polished surface was planar parallel with the glass slide as shown in Figure 9.

Knoop hardness testing of the dentin in the mid-facial direction was performed using the Micromet 2100 microhardness tester (Buehler, Lake Bluff, Illinois). Indents were made with a load of 25 gram-force starting at 25 μm from the root canal wall. The indents were made at 25 μm intervals for the first 500 μm from the root canal wall, after which the indents were made at 100 μm intervals until the cemento-dentinal junction (CDJ) was reached. Since the size of the specimens varied, an additional indent was made 25 μm inside of the CDJ to standardize the location of the last indent in relation to an anatomical landmark. The indents were stained with rhodamine dye and digitally photographed using the Olympus BX50 light microscope at 50X magnification (Olympus Corp., Tokyo, Japan). The image was captured with XCAP-Lite for Windows version 2.2.030227 (EPIX, Inc., Buffalo Grove, Illinois) and Image-Pro Plus for Windows version 4.5.1.29 (Media Cybernetics, Inc., www.mediacy.com). The length of the indent was measured using the Image J software version 1.34s (National Institutes of Health USA, http://rsb.info.nih.gov/ij/). The Knoop hardness (KH) in GPa was calculated.

Figure 9. Specimen mounted for microhardness testing

Data Collection and Analysis

Data collected

The load at fracture of the roots in Kgf was recorded. The area of the fracture surface in mm^2 was measured from digital photographs. The fracture strength of the root in MPa was calculated by dividing the load at fracture in Newtons (N) by the area of the fracture surface in mm^2.

The length of the long diagonal of the indentation created by the microhardness test of the dentin was measured and the Knoop hardness was calculated using the formula $KH = P/CL^2$, where KH is the Knoop hardness in GPa, P is the load applied to the indenter in N, L is the measured length of the long diagonal of the indentation in mm and C is 0.07028, which is the constant of the indenter relating the projected area of the indentation to the square of the length of the long diagonal.

Statistical Analysis

The data were analyzed using the SPSS Graduate Student Version 14.0 for Windows statistical software (SPSS Inc., Chicago, Illinois).

Part I: Fracture resistance testing

The means and standard deviations of the fracture strength were compared. One-way analysis of variance (ANOVA) was used to determine any significant difference among the groups. Bonferroni-Holm t-tests for pairwise multiple comparisons were used to identify the group(s) with significant differences with a family error rate of 0.05.

Part II: Microhardness testing

A linear mixed effects model statistical analysis was used to analyze the microhardness results equally spaced from 25 µm to 200 µm from the root canal wall. The microhardness was the dependent variable. Distance from the root canal wall was the repeated effect (i.e. sample was the random effect). Orthogonal polynomial contrasts were used to test for linear, quadratic and cubic trends. The type of obturation material, the intercept and the contrast coefficients were used as the fixed effects. A compound symmetric (exchangeable) correlation structure was used for the random effect. A first-order autoregressive correlation structure with homogenous variances was used for the repeated measures.

RESULTS

Part I: Fracture resistance testing

The mean +/- standard deviation fracture strengths for the control, gutta-percha and MTA groups were 73.3 ± 13.9 MPa, 71.3 ± 17.3 MPa and 74.6 ± 17.9 MPa respectively, as shown in Figure 10. The tests for normality did not show the fracture strength data to depart from normal distribution. The one-way ANOVA showed no significant difference in fracture strength between the test and control groups. ($F = 0.098$; $p = 0.907$) Thus post hoc tests were not warranted.

All specimens fractured similarly. The fracture lines went obliquely in an apical direction from the lingual to the facial side of the specimens. The fractures occurred in the part of the roots that were embedded in the Rockite mounting as shown in Figures 11 to 13.

Wet SEM

A total of 9 specimens, 3 from each group, were examined under wet SEM. No difference in the fracture pattern was observed between the test and control groups. At 20X magnification, cracks could be seen originating from the fracture surface and ran along the long axis of the root as shown in Figures 14 to 22. Cracks were not found in other parts of the specimens.

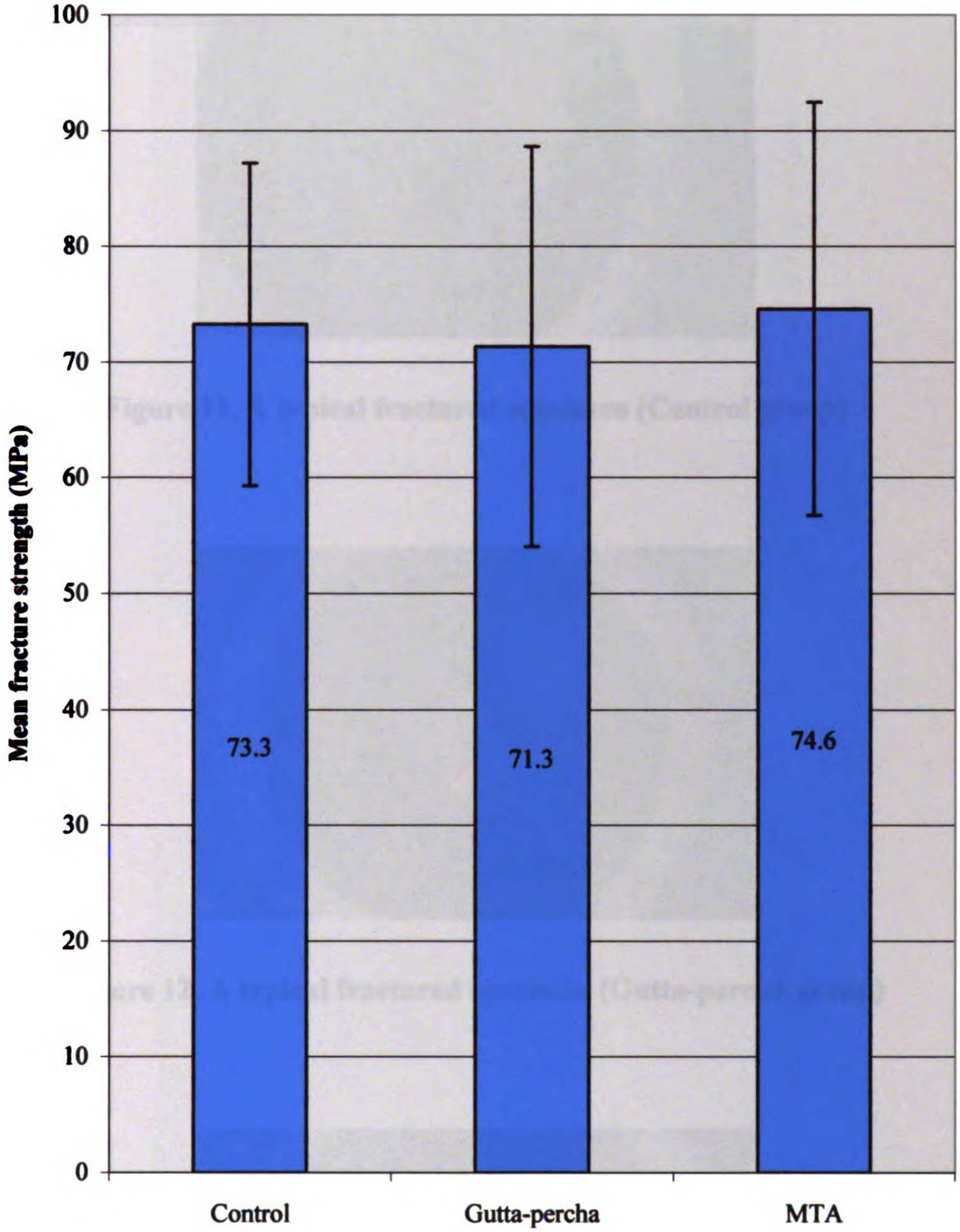

Figure 10. Fracture strength in MPa (Vertical bars = standard deviation)

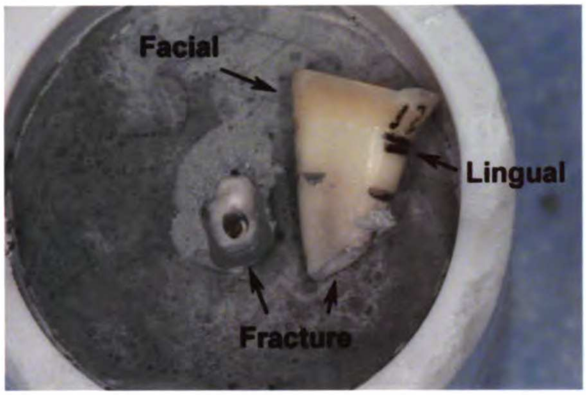

Figure 11. A typical fractured specimen (Control group)

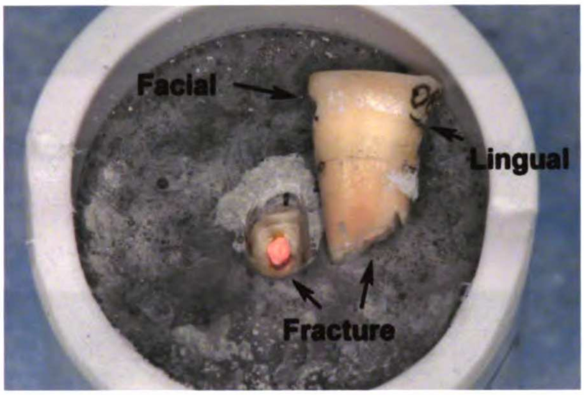

Figure 12. A typical fractured specimen (Gutta-percha group)

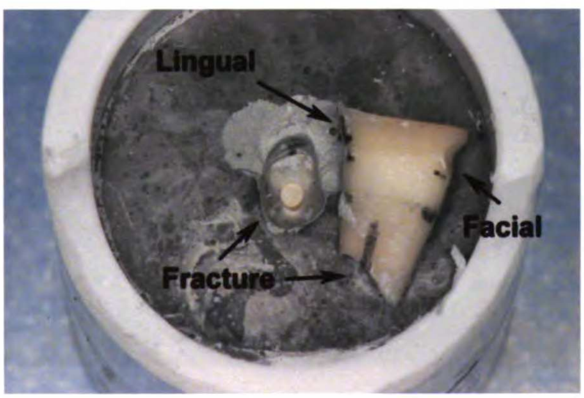

Figure 13. A typical fractured specimen (MTA group)

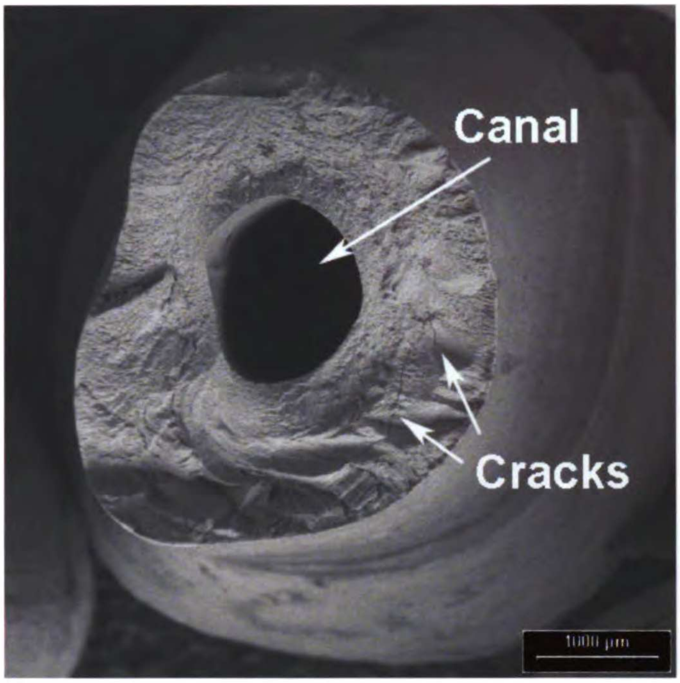

Figure 14. Fracture surface of a typical specimen (Control group)

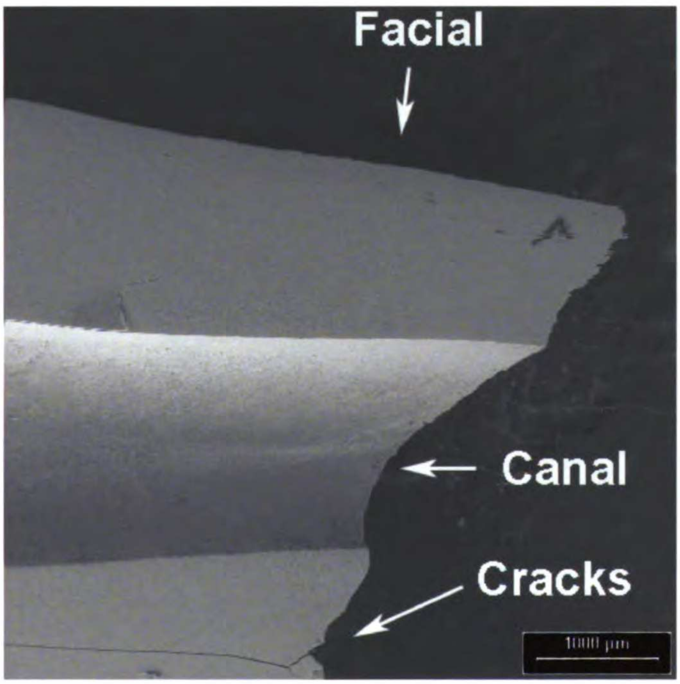

Figure 15. Longitudinal section of the fracture end (Control group)

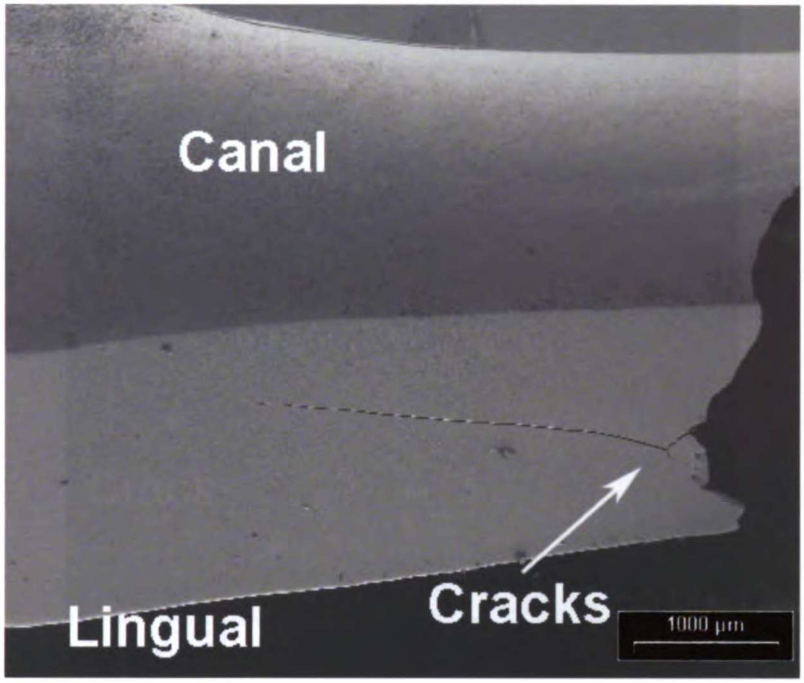

Figure 16. Longitudinal section of the fracture end (Control group)

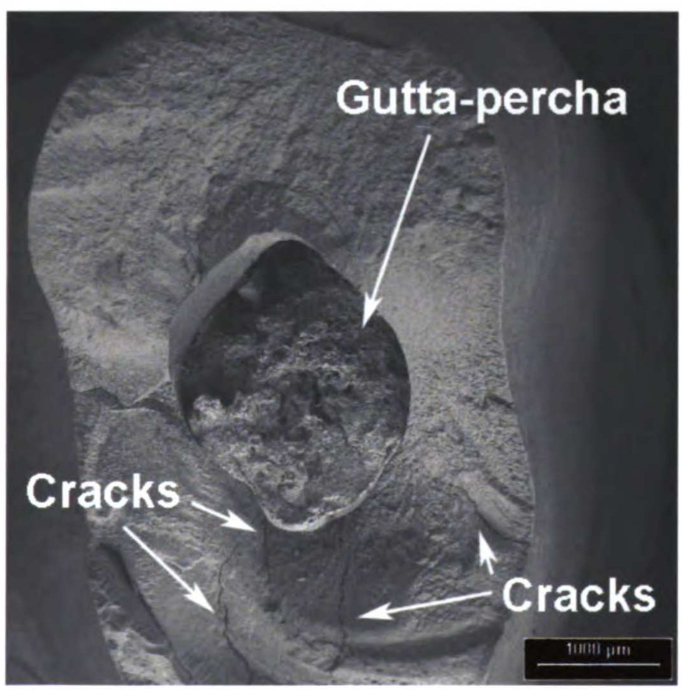

Figure 17. Fracture surface of a typical specimen (Gutta-percha group)

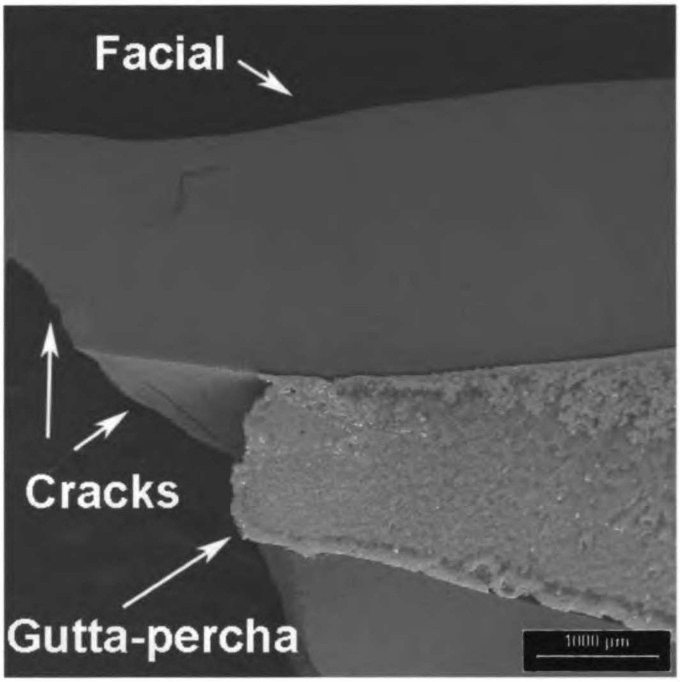

Figure 18. Longitudinal section of the fracture end (Gutta-percha group)

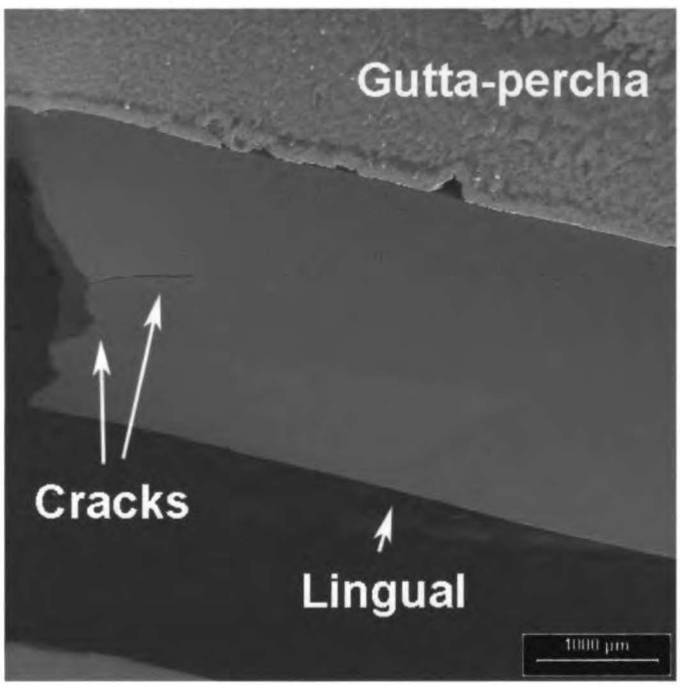

Figure 19. Longitudinal section of the fracture end (Gutta-percha group)

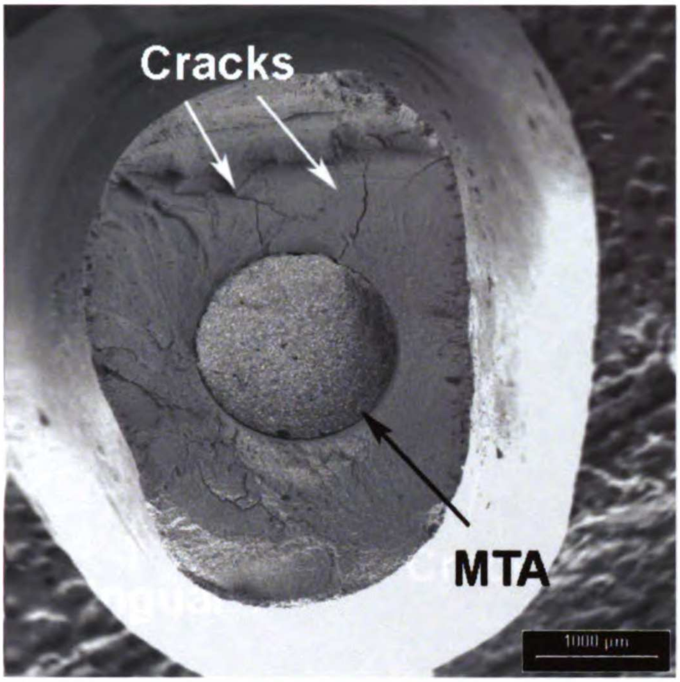

Figure 20. Fracture surface of a typical specimen (MTA group)

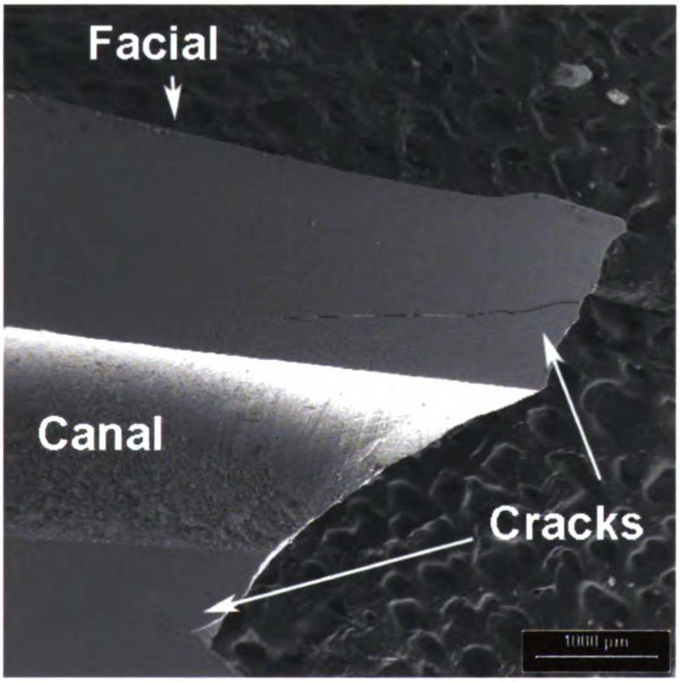

Figure 21. Longitudinal section of the fracture end (MTA group)

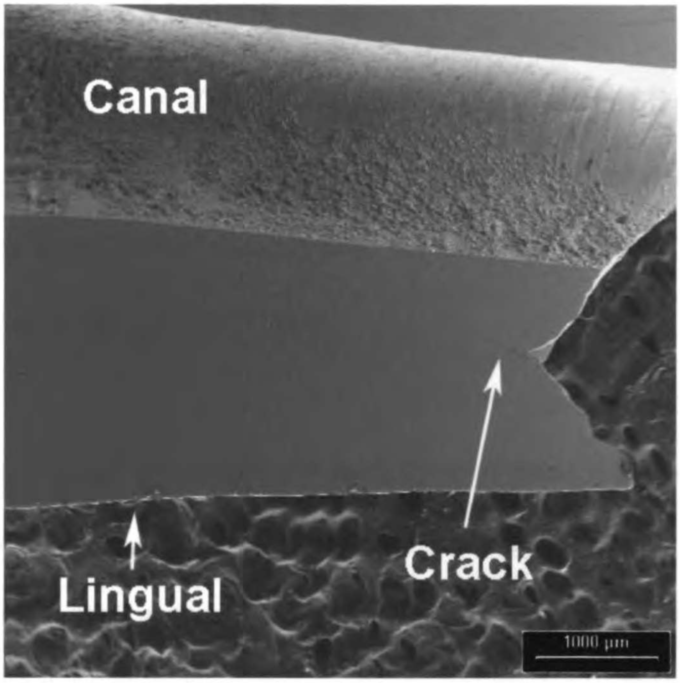

Figure 22. Longitudinal section of the fracture end (MTA group)

Part II: Microhardness testing

The following graphs are plots of the Microhardness (GPa) against the distance from the root canal wall (μm) (Figures 23 to 25) and plots of the Microhardness (GPa) against the percentage distance from the root canal wall to the CDJ (%) (Figures 26 to 28). A cubic polynomial was used to plot the trend lines.

The microhardness from 25 μm to 200 μm from the root canal wall was analyzed using a linear mixed effects model. There was no significant difference in the microhardness between the test and control groups. ($F = 1.552$; $p = 0.230$)

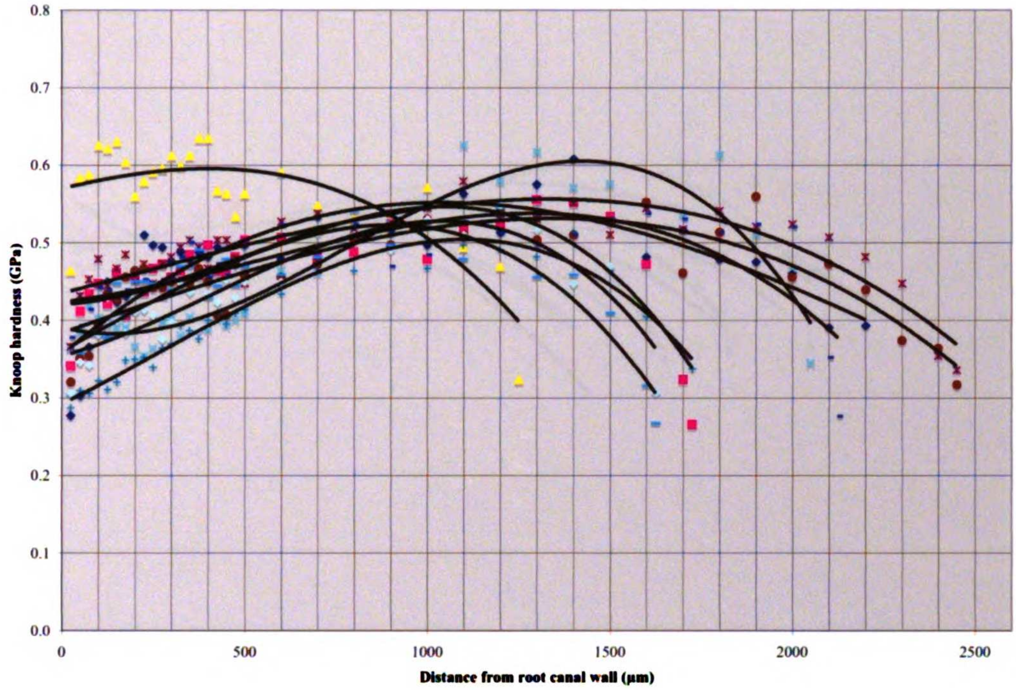

Figure 23. Microhardness vs. distance from canal wall (Control group)

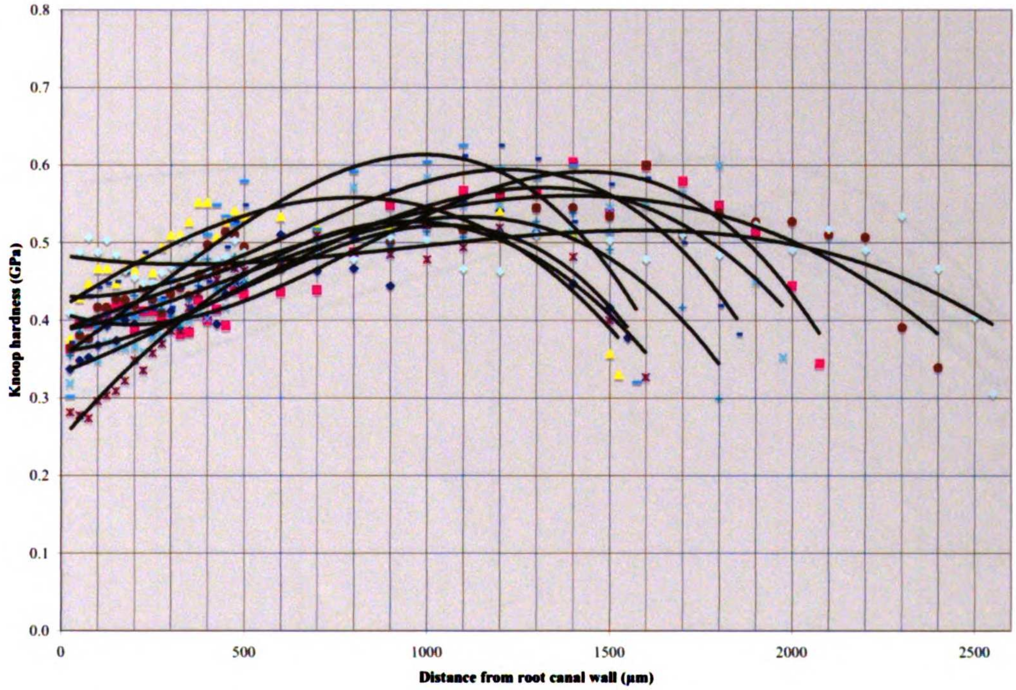

Figure 24. Microhardness vs. distance from canal wall (Gutta-percha group)

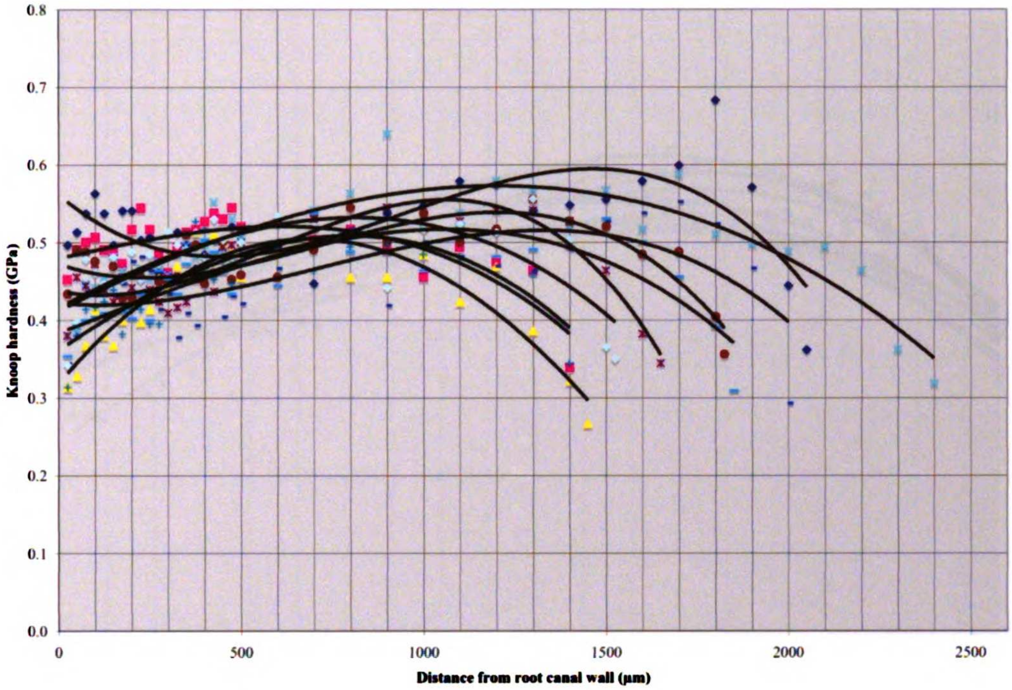

Figure 25. Microhardness vs. distance from canal wall (MTA group)

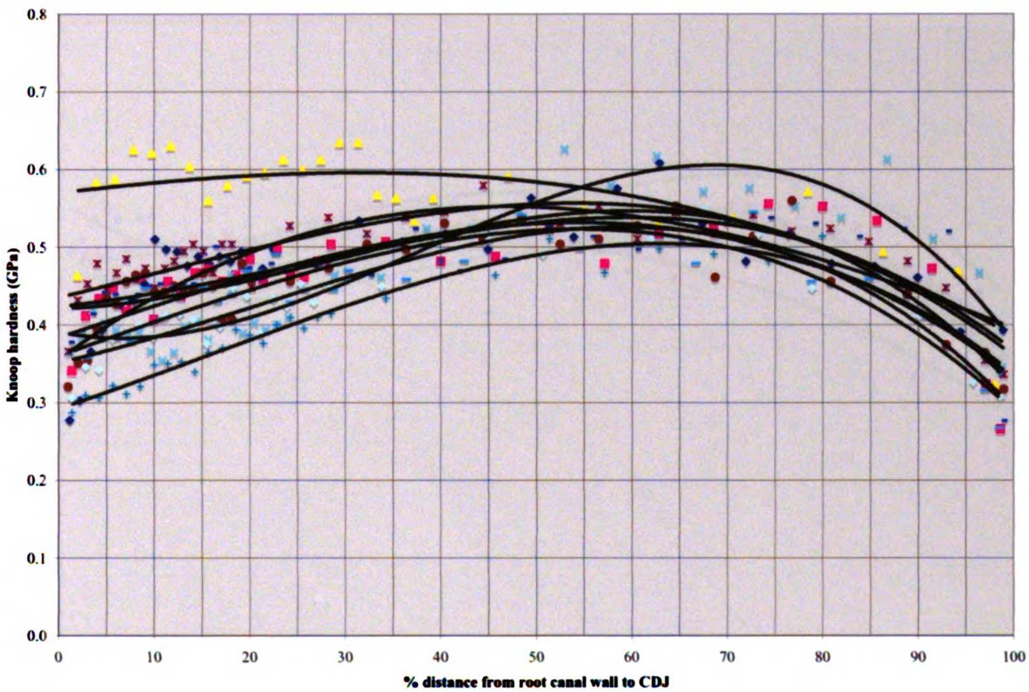

Figure 26. Microhardness vs. % distance from canal wall to CDJ (Control group)

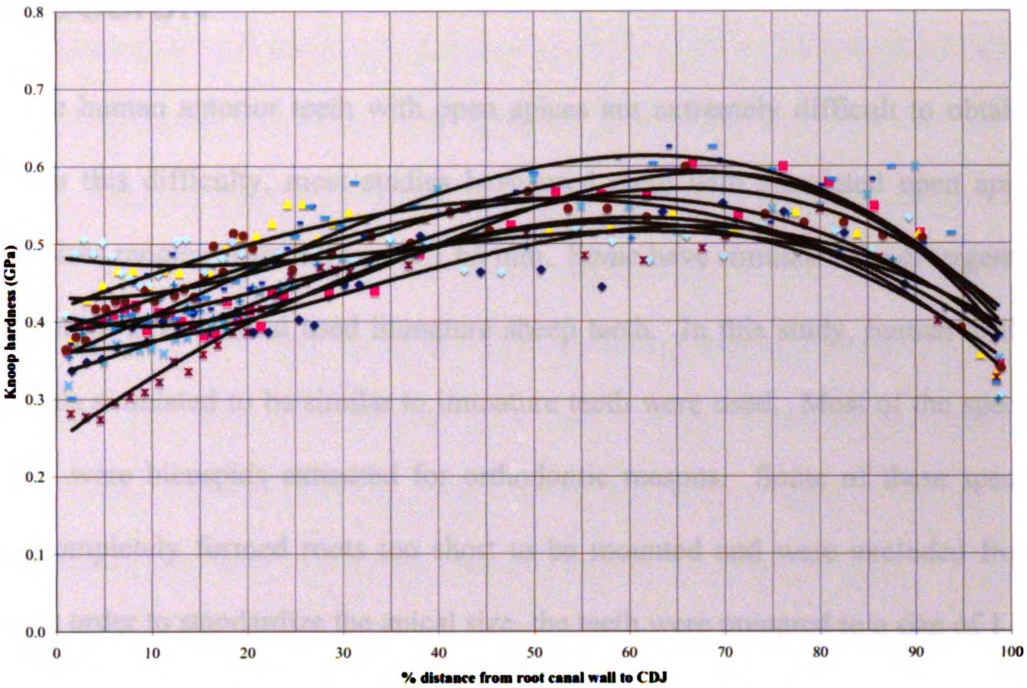

Figure 27. Microhardness vs. % distance from canal wall to CDJ (Gutta-percha group)

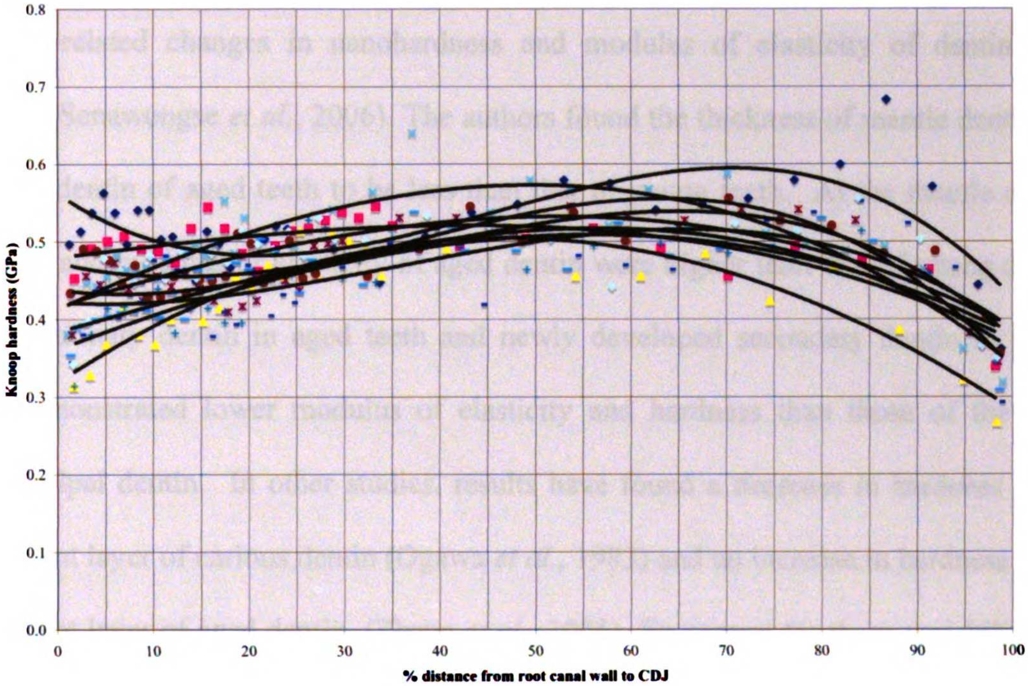

Figure 28. Microhardness vs. % distance from canal wall to CDJ (MTA group)

DISCUSSION

Immature human anterior teeth with open apices are extremely difficult to obtain. To overcome this difficulty, most studies have used teeth with simulated open apices of various sizes ranging from 0.8 mm to 1.65 mm. Some have simulated the divergent apex. Other studies have instead used immature sheep teeth. In this study, human teeth with root canals simulated to be similar to immature teeth were used. Most of the specimens collected were bicuspids extracted for orthodontic reasons. Some of these specimens have incompletely formed roots too short to be mounted and were excluded from the study. In order to standardize the apical size, the teeth were prepared to a size of 1.5 mm. This is the minimum size of the root canal where root reinforcement may be necessary. (Stuart *et al.*, 2006)

The age-related changes in nanohardness and modulus of elasticity of dentin were studied. (Senawongse *et al.*, 2006) The authors found the thickness of mantle dentin and globular dentin of aged teeth to be less than that of young teeth. At the mantle dentin, hardness and modulus of elasticity of aged dentin were higher than that of young dentin. The reactionary dentin in aged teeth and newly developed secondary dentin in young teeth demonstrated lower modulus of elasticity and hardness than those of the other circumpulpal dentin. In other studies, results have found a decrease in hardness in the transparent layer of carious dentin (Ogawa *et al.*, 1983) and an increase in hardness in the transparent layer of aged dentin. (Zheng *et al.*, 2005) To control for these variabilities of age-related and caries-related changes in dentin, only caries-free teeth from patients 30 years or younger were used in this study.

In the fracture resistance testing, the load was applied at 120° to the long axis of the tooth. A pilot study found this angle to be optimum in creating consistent fracture of the specimen. Due to the conical shape of the roots, some of the specimens pulled out of the mounting when the load was applied at a lower angle to the long axis of the teeth. When too great an angle was used, the blade of the mechanical testing machine slid down the root without fracturing the specimen. In a clinical trauma situation, the angle of the traumatic force cannot be predicted. There are no studies in this area and the angulations of the fracture load used in previous fracture resistance studies were not consistent. A study on the effect of load angulation on the fracture resistance of post-restored teeth showed that the mean failure loads increased as load angle approached parallelism to the long axis of the tooth. (Loney *et al.*, 1995) Since the load angulation used in this study was standardized at 120°, the comparison between the test groups was valid.

In our pilot study, attempt was made to simulate the clinical situation by applying a coating of Plasti Dip rubber (Plasti Dip International, Blaine, Minnesota) on the root surface to simulate the periodontal ligament. Most of the specimens pulled out of the mounting when a fracture load was applied at a 120° angle. Therefore, no attempt to simulate the effect of the periodontal ligament was made in this study.

Most traumatic dental injuries involve maxillary incisors (Andreasen, 1970). When gray MTA is used in upper anterior teeth, the gray color may show through the crown and may

compromise esthetics. Therefore white MTA was used in this study to simulate the choice of material in a clinical situation.

According to the manufacturer's directions for use, the ProRoot MTA root repair material will set over a period of four hours. (Dentsply Tulsa Dental, 2002) In this study, the MTA was allowed to set for 24 hours with a moistened cotton pellet in the pulp chamber. This optimized the flexural strength of the MTA. (Walker *et al.*, 2006) After the MTA is mixed, moisture may evaporate from the mixture, making it unusable because of the thicker consistency. Some clinicians may add water to the mixture to restore the working consistency. The water-to-powder ratio affects the solubility and porosity of MTA. (Fridland and Rosado, 2003) In turn this may affect the strength. Therefore the water-to-powder ratio recommended by the manufacturer was followed strictly in this study. The specimens were stored for 5 weeks before fracture testing since a previous study had shown that it took at least 21 days for MTA to attain it's maximum strength. (Torabinejad *et al.*, 1995a) Since MTA can solubilize 31% at the manufacturer's recommended water-to-powder ratio over the long term (Fridland and Rosado, 2005), the storage time of the obturated specimens before fracture testing may affect the results. The storage time in our study was standardized. Therefore the comparison between the test groups was valid.

A hybrid placement method was used in this study where the MTA was compacted by hand using pluggers and also condensed using low energy ultrasonics. While a previous study had suggested hand condensation for placement of MTA over the ultrasonic method for better adaptation and less voids (Aminoshariae *et al.*, 2003), another study

had found significantly less bacterial leakage when MTA was condensed with ultrasonics (Lawley *et al.*, 2004). No voids were evident in the MTA after obturation in our study using digital radiography. Further investigation to evaluate this hybrid placement method is needed.

The wet SEM showed that the specimens fractured in a similar manner. Preexisting flaws or cracks may cause premature fracture of the specimens. The flat area created to facilitate the fracture loading may also create a weak point where a fracture may originate. All specimens fractured in a similar manner in this study and no crack or fracture had originated from the flat area. The wet SEM did not show any unusual fracture pattern.

The results showed that MTA did not increase the fracture resistance of the roots. A previous study had reported a hydroxyapatite like interfacial layer between the MTA and the dentin wall when MTA was exposed to a synthetic tissue fluid. (Sarkar *et al.*, 2005) But to date, no study has reported bonding between MTA and dentin. In a study on the effect of sodium hypochlorite and calcium hydroxide on dentin, the mean flexural strength of the dentin beam specimens in the control group was found to be 87 MPa. (Grigoratos *et al.*, 2001) Another study had found the mean flexural strength of MTA beam specimens to be 14 MPa at 24 hours (Walker *et al.*, 2006), using similar setting conditions as our study. Therefore it is not surprising that the MTA did not strengthen the teeth. However, the results might be different if a different apical size was selected.

A bigger apical size will further weaken the specimen root and thus make any effect of the obturation materials more apparent. Further study in this area is warranted.

The results from this study did not agree with a previous study where MTA decreased the strength in root dentin by 33%. (White *et al.*, 2002) The latter study used bovine dentin blocks rather than whole human roots. Their specimens were placed in Petri dishes containing MTA and were kept moist by adding water every 3 to 5 days. By contrast, the present study allowed the MTA to set inside the roots. Therefore this study appears to be a better simulation of the clinical situation and the results seem more clinically relevant.

Based on the results shown in Figures 23 to 25, there appeared to be a linear increase in microhardness in the root dentin from the root canal wall towards the central dentin. Therefore only the first 200 μm from the root canal wall was included in the statistical analysis. It is assumed that any changes in microhardness caused by the obturation materials would be noted within the first 200 μm. As mentioned previously, the sodium hypochlorite used to irrigate the canal might have affected the microhardness. (Saleh and Ettman, 1999; Ari *et al.*, 2004; Slutzky-Goldberg *et al.*, 2004) The literature is contradictory in the effect of 17% EDTA used for 1 minute on dentin microhardness. An earlier study had found a weakening effect (Saleh and Ettman, 1999) while a more recent study had found no effect (De-Deus *et al.*, 2006). But since the exposure time and the strength of the sodium hypochlorite and EDTA used were standardized, the comparison between the control and the tests groups was valid. Furthermore, the current study attempted to simulate a typical clinical procedure and sodium hypochlorite is a

commonly used root canal irrigant while 17% EDTA is a commonly used chelating agent.

Figure 26 showed a trend in the microhardness of root dentin from the canal wall across to the CDJ in the control group, with an increase in microhardness from the canal wall towards the CDJ. The microhardness then decreased as the CDJ was approached. There was a similar trend in the MTA and the gutta-percha groups as shown in figures 27 and 28. The correlation of the differences in structure and the microhardness of human dentin were studied using transverse sections of recently extracted human teeth. (Craig *et al.*, 1959) The authors found that the Knoop hardness of dentin increased as the distance from the pulp chamber increased and a consistent value was obtained about 0.5 mm from the pulp chamber. They attributed the softer dentin adjacent to the root canal to the presence of less calcified predentin. The relationship between dentin microhardness and tubule density in normal human permanent teeth was investigated. (Pashley *et al.*, 1985) A highly statistically significant inverse correlation was found between dentin microhardness and tubular density. As tubular density increased from the dentino-enamel junction (DEJ) towards the pulp chamber, there was an associated decrease in dentin microhardness. The authors suggested that this might be due to a decrease in the amount of intertubular dentin and an increase in individual tubular diameter. In a study of human root dentin, a systematic change in microhardness from the cementum towards the pulp cavity was found. (Wang and Weiner, 1998) The microhardness of root dentin increased from a very low value near the CDJ to a maximum, and then decreased towards the pulp. A study of nanohardness of human peritubular and intertubular dentin using atomic-force

microscopy found that the hardness of fully hydrated peritubular dentin was independent of location while that of intertubular dentin depended upon location and was significantly greater near the DEJ than near the pulp. (Kinney *et al.*, 1996) The authors suggested that the observed decreases in dentin microhardness in previous studies were mostly due to the decreased hardness of the intertubular dentin rather than the increased tubular density. The results from the present study were in agreement with these studies.

Based on the results of this study and previous studies, a regimen for treating necrotic immature permanent teeth can be proposed. After initiation of root canal treatment, the root canal system can be dressed with calcium hydroxide for no more than 1 month to avoid any weakening effect from calcium hydroxide. (Andreasen *et al.*, 2002; Andreasen *et al.*, 2006) The tooth can then be treated with one visit apexification using a 5-mm MTA plug to provide a hard barrier (Matt *et al.*, 2004) and an adequate seal (Al-Kahtani *et al.*, 2005). If the apical size is 1.5 mm or more (Stuart *et al.*, 2006) and if sufficient coronal tooth structure remains, the root can be reinforced by restoring with a flowable self-cured composite resin (Lawley *et al.*, 2004) If insufficient coronal tooth structure remains, a post can be cemented with adhesive resin cements to retain a composite core. (Katebzadeh *et al.*, 1998) A long-term outcome study is needed to evaluate the effectiveness of this approach.

CONCLUSIONS

Within the parameters of this study, the following conclusions can be drawn:

1. There is no difference in the fracture strength of endodontically treated, simulated immature human roots obturated entirely with MTA or gutta-percha compared to the unobturated control.
2. There is no difference in the microhardness of the dentin of endodontically treated, simulated immature human roots obturated entirely with MTA or gutta-percha compared to the unobturated control.

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