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Sealing ability of ProRoot MTA when placed as an apical barrier using three different techniques

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List of contents

List of Figures:

List of Tables:

1 Introduction

Endodontic treatment of immature teeth remains a challenge due to open apices and flaring root canals accompanied by thin immature dentinal walls. The major challenges are achieving complete debridement, canal disinfection, and optimal sealing of the root canal system.

In case of pulp necrosis in immature teeth, apexification is the treatment of choice inducing a calcified barrier at the apex allowing a more favorable condition for conventional root canal filling with gutta-percha and a root canal sealer.

The aim of the apexification procedure is to limit bacterial infection and create an environment conducive to the production of mineralized tissue in the apical region. Most endodontic failures occur as a result of leakage of irritants into the periapical tissues (Hoen and Pink 2002; Ng et al., 2008; Siqueira and Rocas 2008). An ideal orthograde filling material should seal the pathways of communication between the root canal system and its surrounding tissues. It should also be nontoxic, nongenotoxic, noncarcinogenic, biocompatible with the host tissues, insoluble in tissue fluids, and dimensionally stable (Torabinejad and Pitt Ford 1996; Ribeiro 2008).

Furthermore, the presence of moisture should not affect its sealing ability; it should be easy to use and be radiopaque for recognition on radiographs (Torabinejad and Pitt Ford 1996).

There are many reports that disclose successful treatment of teeth with necrotic pulps and open apexes by using Mineral Trioxide Aggregate (MTA) as an apical barrier (Giuliani et al., 2002; Maroto et al., 2003; Hayashi et al., 2004; Villa and Fernández, 2005; Ghaziani et al., 2007; Gaitonde and Bishop, 2007).

However, it was not clearly known how well MTA adapts to root canal walls when placed using an orthograde approach. To date, there have been no studies comparing the full density of MTA when placed from an orthograde approach using three different placement methods.

Therefore, the purpose of this study was to comparatively evaluate the root canal sealing efficiency of ProRoot MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) produced by three different placement techniques:

a) hand condensation with pluggers.
b) hand condensation with paper points.
c) condensation with ultrasonic sound apparatus.

2 Literature Review

Several materials have been used as an apical plug to prevent extrusion of filling materials during obturation of the teeth with open apexes (Shabahang et al., 1999).

Calcium hydroxide pastes have become the material of choice to induce apexification (Leonardo et al., 1993; Estrela et al., 2001; Felippe et al., 2005; Rafter 2005).

Despite its efficacy, this chemical has several disadvantages, such as financial concerns, psychological status, or aesthetic demands of the patient (Schumacher and Rutledge, 1993), difficulty in patient follow-up, delayed treatment (Shabahang et al., 1999), variability of treatment time with an average 12.19 months, and number of appointments and radiographs (Dominguez Reyes et al., 2005).

The calcium hydroxide is renewed periodically until an apical barrier is formed (Leonardo et al., 1993). The time needed to form an apical barrier is unpredictable and depends on the size of the apical foramen, the presence of infection and the host (Leonardo et al., 1993; Felippe et al., 2005). Mostly, a long-term treatment is required and success is depending on the cooperation of the young patient (Sheehy and Roberts, 1997).

It has been pointed out by Cvek (1992) that endodontically treated immature teeth have a relative high incidence of cervical root fracture either spontaneously or due to minor impacts. According to White et al. (2002) a 5-week exposure to calcium hydroxide results in a 32% decrease in strength to bovine dentin.

Andreasen et al. (2002, 2006) in an in vitro experiment with sheep mandibular incisors with open apices demonstrated that long-term calcium hydroxide dressings had a significant negative effect on the strength of the root, but up to 4 weeks of calcium hydroxide did not adversely affect the fracture resistance. Another in vitro experiment demonstrated that a previous traumatic event reduced the resistance to fracture of a tooth by up to 85% (Schatz et al., 2001).

Furthermore, root filling with calcium hydroxide reduced the micro tensile fracture resistance of teeth by 43.9% between 7 and 84 days (Rosenberg et al., 2006), and when immersed in a saturated solution of calcium hydroxide for 1 week, a reduction in the flexural strength of human dentin was demonstrated (Grigoratos et al., 2001).

Alternatives to calcium hydroxide have been proposed, the most promising being Mineral Trioxide Aggregate (MTA) (Shabahang et al., 1999; Shabahang and Torabinejad 2000; Witherspoon and Ham, 2001; Steinig et al., 2003; Andreasen et al., 2006).

MTA is a powder consisting of fine hydrophilic particles that bind in the presence of moisture (Torabinejad et al., 1995a). MTA has shown to be biocompatible, promoting tissue regeneration in the apical and periapical zone. So it has been recommended for apexification (Torabinejad et al., 1997; Torabinejad and Chivian, 1999).

An in vitro study using bovine incisors has shown that teeth with MTA root filling showed higher fracture resistance in comparison to teeth that had calcium hydroxide placed as an intra-canal medicament (Bortoluzzi et al., 2007).

An investigation on immature sheep roots used various time intervals from 2 weeks to 1 year for evaluating fracture resistance; results showed that after 1 year, the teeth filled with MTA had significantly more resistance to fracture compared with those filled with calcium hydroxide or the controls (Hatibović-Kofman et al., 2008).

The effect of MTA on the apexification of teeth with incomplete root information and the necessity of employing calcium hydroxide paste before using MTA were evaluated in premolars teeth of dogs. The apical calcified tissue barrier was present in all specimens but the percentage of complete barriers was 53.8% for the teeth treated with MTA appose to 16.7% for specimens treated first with calcium hydroxide. The barrier was formed in the interior of the canal in 69.2% of roots from MTA group only, while because of MTA extrusion in specimens treated first with calcium hydroxide, 75% of the barriers occurred beyond the limits of the root canal walls (Felippe et al., 2006).

In an investigation on dog's teeth with immature apexes, Shabahang et al. (1999) reported that the teeth treated with MTA showed a higher incidence of apical closure and fewer inflammatory cells in comparison to calcium hydroxide. In an experiment on monkey's teeth with infected root canals and open apexes, Ham et al. (2005) reported that root canals filled with MTA had the highest amount of hard tissue formation and the lowest level of inflammation after 90 days when compared to calcium hydroxide.

Two separated studies compared the apexification and the apexogenesis with MTA and calcium hydroxide for 12 months (El-Meligy and Avery, 2006a; 2006b). None of the MTA-treated teeth

showed any clinical or radiographic pathology while 2 teeth treated with calcium hydroxide in each study revealed failure; therefore the authors concluded that MTA was a suitable alternative to calcium hydroxide.

Simon et al. (2007) reported a decrease in the size of the pre-existing periapical lesion in 81% of the cases with at least 12 months follow-up when MTA was used in one-visit apexification treatment. Another study evaluated the clinical efficacy of MTA as an apexification material when used in non-vital immature teeth. The results showed clinical success in 94.1% of the cases with mean follow-up 12.53 months (Sarris et al., 2008).

Mente et al. (2009) reported in a retrospective study with a mean follow-up 30.9 months that 84% of the treated open apices teeth were healed. It was also showed that teeth without or with preoperative periapical radiolucency had a healed rate of 100% and 78% respectively.

Set MTA provides a good seal and excellent marginal adaptation (Fernández-Yáñez Sánchez et al., 2008).

2.1 Structure and chemical composition of MTA

MTA was developed at Loma Linda University, California, USA and the first literature about the material appeared in 1993 (Lee et al., 1993). It has been approved by the U.S. Food and Drug Administration in the year 1998 (Schwartz et al., 1999). Some of the commercially available MTA are ProRoot MTA (GMTA), White ProRoot MTA (WMTA), MTA-Angelus (Solucoes Odontologicas) MTA-Angelus Blanco (Solucoes Odontologicas), and MTA Bio (Solucoes Odontologicas) (Srinivasan et al., 2009). Several investigations have reported that the main elemental components of MTA are calcium and silica, as well as bismuth oxide (Asgary et al., 2005; Camilleri et al., 2005a; Asgary et al., 2006; Belío-Reyes et al., 2009). MTA is a mixture of a refined Portland Cement and bismuth oxide (Bi_2O_3), and contains trace amounts of SiO_2, CaO, MgO, K_2SO_4, and Na_2SO_4. Portland Cement is a mixture of dicalcium silicate ($2CaO.SiO_2$), tricalcium silicate ($3CaO.SiO_2$), tricalcium aluminate ($3CaO.Al_2O_3$), gypsum ($CaSO_4.4H_2O$), and tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$) (Dammaschke et al., 2005; Sarkar et al., 2005). The powder of MTA was composed mainly of tricalcium and dicalcium silicate with bismuth oxide also present for radiopacity (Camilleri et al., 2005a). Another study performed by Islam et al., (2006a) reported that the main constituents present in ProRoot MTA and Portland Cement are

tricalcium silicate, tricalcium aluminate, calcium silicate, and tetracalcium aluminoferrite. The actual composition of GMTA is 75% Portland Cement, 5% dehydrated calcium sulfate, and 20% bismuth oxide, while MTA-Angelus is composed of 80% Portland Cement and 20% bismuth oxide (Duarte et al., 2003; Oliveira et al., 2007). By using energy dispersive spectroscopy to compare GMTA and MTA-Angelus, it was showed that the amount of calcium in MTA-Angelus is higher than in GMTA, whereas the amounts of carbon, oxygen, bismuth, and silica are higher in GMTA (Oliveira et al., 2007). The authors reported also the presence of aluminum and the absence of iron in MTA-Angelus; conversely, GMTA exhibited the presence of iron and an absence of aluminum. No difference was found in the presence of 14 elements between MTA and Portland Cement except for the bismuth which was present in MTA (Funteas et al., 2003). Song et al. (2006) reported that the primary differences among GMTA, WMTA, and Portland Cement are the lack of potassium and the presence of bismuth oxide. Bismuth oxide is present in both hydrated and non-hydrated MTA and is also a part of calcium silicate hydrate (Camilleri, 2007). The amount of gypsum in ProRoot MTA is approximately half of the amount found in Portland Cement (Dammaschke et al., 2005). Torabinejad et al. (1995a) reported that the main molecules present in MTA are calcium and phosphorous ion. However, Asgary et al. (2005) using energy dispersive analysis with X-ray (EDAX) could not detect the presence of phosphorus. Another study showed also that MTA did not contain phosphorous (Camilleri et al. 2005a). Modifying the gypsum content in MTA, result a significant reduction of setting time, thereby reducing the number of treatment visits (Camilleri et al., 2005b). The differences between Portland Cement and ProRoot MTA, by comparing the same chemical, physical surface, and bulk material characteristics were described by Dammaschke et al. (2005). They concluded that ProRoot MTA contains lower level of potentially toxic heavy metals (Cu, Mn, Sr), chromophores (Fe3+), aluminum, iron, magnesium, and sulfur, but contains about 2 atom% bismuth, also ProRoot MTA showed an equal and smaller particle size, whereas Portland Cement is composed of particles with a wide range of sizes. Two studies reported more homogenous chemical composition as well as particle sizes and shapes for GMTA in comparison to MTA-Angelus (Song et al., 2006; Komabayashi and Spangberg, 2008). MTA is prepared as a mixture of powder and water and is used in a slurry form, which gradually hardens in the oral environment (Sarkar et al., 2005). When MTA powder is mixed with water, calcium hydroxide and calcium silicate hydrate are initially formed and eventually transform into a poorly crystallized and porous solid gel (Camilleri, 2007). Dammaschke et al. (2005) reported that calcium hydroxide is a product of tricalcium aluminate hydrogenation, while Camilleri (2008)

believed that calcium hydroxide is formed from dicalcium and tricalcium silicate after mixing MTA powder with water. Bismuth affects calcium hydroxide precipitation after MTA hydration, (Camilleri, 2007). The differences between GMTA and WMTA are the concentrations of Al_2O_3, MgO, and FeO (Dammaschke et al., 2005; Islam et al., 2006b). Another study has reported that GMTA contained a significant amount of iron when compared with WMTA, and gray MTA-Angelus had a lower content of bismuth oxide than ProRoot MTA (Song et al., 2006). WMTA has 54.9% less Al_2O_3, 56.5% less MgO, 90.8% less FeO which leads to the conclusion that the FeO reduction is most likely the cause for the color change (Asgary et al., 2005). The WMTA lacks the aluminoferrite phase that imparts the grey color to GMTA (Camilleri et al., 2005a).While it was also suggested that the reduction in iron and manganese could also contribute to the lighter color of WMTA (Dammaschke et al., 2005). A study comparing WMTA to white Portland Cement (WPC) showed the cements to have similar constituent elements except for the bismuth oxide in the MTA (Asgary et al., 2004). Comparing the particle size and shape of WMTA, GMTA, it was reported that WMTA has finer particle size than GMTA (Camilleri et al., 2005a; Asgary et al., 2006; Komabayashi and Spangberg, 2008a). Another study showed that the particle size of GMTA powder ranging from 1-10 μm (Lee et al., 2004), whereas Camilleri (2007) reported that the WMTA powder has particles less than 1 mm to 30 μm before hydration. ProRoot MTA contains fewer large particles than MTA-Angelus; in addition, MTA-Angelus contains a higher number of small particles than ProRoot MTA (Komabayashi and Spangberg, 2008a). Portland Cement has a cumulative percentage of particles diameter between 0.5 and 3 μm, which may be able to penetrate dentine tubuli (Komabayashi and Spangberg, 2008b). Since bismuth oxide dissolves in an acidic environment, it has been suggested that placing MTA in an acidic environment such as inflammatory tissues might result in the release of bismuth oxide (Camilleri, 2007). This might decrease the biocompatibility of MTA because bismuth oxide does not encourage cell proliferation in cell culture (Camilleri et al., 2004).

2.2 pH of MTA

Hydrated MTA products have an initial pH of 10.2 which rises to 12.5 after 3 hours of setting which is almost similar to calcium hydroxide (Torabinejad et al., 1995a; Camilleri et al., 2005a; Dammaschke et al., 2005). Another study reported that the pH level of the solution of MTA was highly alkaline, ranging between 11.94 and 11.99 (Fridland and Rosado 2003). Comparing pH values at different periods of time, both GMTA and WMTA exhibit significantly higher pH values

than 2 types of Portland Cement immediately after mixing, while at 60 minutes, GMTA has a significantly lower pH value than WMTA and both types of Portland Cement (Islam et al., 2006b). MTA has kept its high pH value for an extended period of time (Fridland and Rosado, 2005). Because of the high pH level of MTA, many studies reported that the biologic activity is due to the formation of calcium hydroxide (Duarte et al., 2003; Fridland and Rosado 2003). An investigation assessed pH value and calcium release for ProRoot MTA and MTA-Angelus; results showed that MTA-Angelus produced a slightly higher pH value and calcium release than ProRoot MTA (Duarte et al., 2003). Santos et al. (2005) reported that calcium and hydroxyl ions may be released from MTA-Angelus during storage in moist condition for periods up to 360 hours. It was also reported that the presence of $CaCl_2$ increased significantly the pH of MTA immediately after mixing, and increased the release of calcium ions in the 24-hour period (Antunes Bortoluzzi et al., 2006).

2.3 Setting time and compressive strength of MTA

MTA is prepared by mixing its powder with sterile water in a 3:1 powder-to-liquid ratio (Torabinejad et al., 1993a). The mixing time of MTA is crucial. If the mixing of MTA is prolonged, it results in dehydration of the mix. Sluyk et al. (1998) reported in their study that the mixing time should be less than 4 minutes. Although moisture is needed for MTA to set, excess moisture can result in a mix that is soupy and difficult to use (Schwartz et al., 1999; Naik and Hegde 2005). The setting process is described as a hydration reaction of tricalcium silicate ($3CaO.SiO_2$) and dicalcium silicate ($2CaO.SiO_2$) which is responsible for the development of material strength (Dammaschke et al., 2005). The mean setting time of GMTA was reported by Torabinejad et al. (1995a) as 2 hours and 45 minutes, which is longer than amalgam, Intermediate Restorative Material (IRM), and Super-EBA (reinforced zinc oxide-eugenol cement). GMTA exhibits significantly higher initial and final setting times than WMTA (Chng et al., 2005; Islam et al., 2006b). The manufacturers' data sheets for MTA-Angelus indicate that the absence of dehydrated calcium sulfate lowers the material setting time to 10 minutes (Oliveira et al., 2007). Another study showed that the setting time for MTA-Angelus is 14.28 ± 0.49 minutes (Santos et al., 2008), which is lower than GMTA and WMTA on the basis on previous studies (Torabinejad et al., 1995a; Chng et al., 2005). The longer setting time of WMTA in comparison with Portland Cement is attributed to the lower levels of sulfur and tricalcium aluminate in WMTA (Dammaschke et al., 2005). The effect of mixing MTA powder with different liquids and additives

has shown that the choice of preparation liquid can have an effect on setting time and compressive strength. Saline and 2% lidocaine anesthetic solution increased setting time and compressive strength as compared to the sterile water. Preparation with five percent calcium chloride ($CaCl_2$) solutions, a water based lubricant, and sodium hypochlorite gels decreased setting time and the final compressive strength was significantly lower than that obtained prepared with sterile water. It was also reported that when MTA powder was mixed with chlorhexidine gel (CHX gel), this mixture had not set 7 days after mixing, and hence a compressive strength measurement was not obtainable for this material (Kogan et al., 2006). In addition, it has been shown that the setting time of MTA reduced when it is mixed with Na_2HPo_4 buffer solution (Ding et al., 2008). Another study showed that MTA sets one third faster when it is mixed with 1% methylcellulose and 2% calcium chloride (Ber et al., 2007). It was reported that the placement of Glass-Ionomer Cement (GIC) used as permanent filling material does not affect the setting of MTA when placed over it (Nandini et al., 2007). It was also concluded that the setting of both GIC and MTA was not affected when GIC was layered over partially set MTA after 45 minutes (Ballal et al., 2008). At 24 hours MTA has the lowest compressive strength (40 MPa) in comparisons to amalgam, Super-EBA, and IRM, but it increased after 21 days to 67 MPa which is comparable to that of IRM and Super-EBA but significantly less than that of amalgam (Torabinejad et al., 1995a). GMTA has greater compressive strength in comparison to WMTA and Portland Cement in a similar in vitro study (Islam et al., 2006b). In contrast, two other investigations reported more compressive strength for WMTA (Watts et al., 2007; Holt et al., 2007). The setting reaction of MTA products is a hydration reaction; therefore sufficient water in potential preparation liquids must be present for reaction (Gancedo and Garcia, 2006). However, Walker et al. (2006) reported that the flexural strength of WMTA was higher when a moistened pellet placed on the intra-canal surface for 24 hours under a temporary restoration. The authors suggested that cotton pellets should be removed after 24 hours because the flexural strength decreases 72 hours after WMTA receives moisture from both sides. Nekoofar et al. (2007) examined the effect of condensation pressure of WMTA on the compressive strength and surface hardness. They reported no statistically significant effect of condensation pressure on the compressive strength of WMTA, but there was a significant reduction in surface hardness. A recent investigation reported significantly lower compressive strength for WMTA when the material was etched by phosphoric acid 37%, therefore, it was suggested that the restoration with acid-etch composite after MTA placement should be postponed for at least 96 hours (Kayahan et al., 2009). Tunc et al. (2008) evaluated the bond strength of a composite and a

compomer to WMTA using two different bonding systems. The authors concluded that the total-etch one-bottle adhesive system exhibited a stronger bond to WMTA than the self-etch one-step system. The retentive strength of GIC, zinc-phosphate cement and MTA was compared. The results showed that the prefabricated posts luted with GMTA provided significantly less retentive strength than glass-ionomer and zinc-phosphate luting cements (Vargas et al., 2004).

2.4 Solubility and radiopacity of MTA

MTA solubility levels have been reported to be stable over time (Fridland and Rosado, 2005). Earlier study proved no signs of solubility of ProRoot MTA in water when tested under modified International Organization for Standardization (ISO) and American Dental Association specification (Torabinejad et al., 1995a). The solubility and porosity of MTA showed significantly increasing trend that follows the amount of water used when preparing the mix under ISO specifications (Fridland and Rosado, 2003). Budig and Eleazer (2008) studied the potential of dry ProRoot MTA powder to set solely by moisture absorbed through the root, they found a statistical significant difference of roots in which dry MTA powder set at 72 hours versus 12 hours. When comparing the physical properties of WMTA with those of GMTA, the former material demonstrates significantly more solubility (Islam et al., 2006b). WMTA solubility, micro hardness, and radiopacity have been compared to two Portland cements, reported that WMTA was significantly less soluble, exhibited higher micro hardness, and was more radiopaque (Danesh et al., 2006), which can be attributed to the different chemical and physical properties of the tested materials (Dammaschke et al., 2005; Song et al., 2006; Komabayashi and Spangberg, 2008). In contrast, another study reported that both Ordinary Portland Cement (OPC) and WPC exhibit significantly less solubility than WMTA (Islam et al., 2006b). An investigation on the effect of an alkaline environment on the micro hardness of WMTA has shown significantly lower micro hardness in normal 7.4 and high 10.4 pH values in comparison to 8.4 and 9.4 pH values (Saghiri et al., 2009). A recent in vitro study showed that 2% chlorhexidine gluconate has significantly reduced the surface hardness within 24 hours of WMTA placement, while Ethylenediaminetetraacetic acid (EDTA) has no effect on surface hardness of WMTA (Nandini et al., 2010). It was reported that MTA is more radiopaque than IRM, and Super EBA. Also the mean radiopacity of MTA is 17.7 mm of equivalent thickness of aluminum, which is sufficient to make it easy to visualize radiographically (Torabinejad et al., 1995a). Shah et al., (1996) recommended that the root-end filling materials should have a

radiopacity greater than that for root canal sealers. The authors also found that the radiopacity of MTA is higher than that reported for Super-EBA or IRM. In contrast, another study compared the same materials and reported more radiopacity for Super-EBA (9.9 mm Al) and IRM (9.3 mm Al) than MTA (6.4 mm Al), but in the same range as zinc oxide-eugenol based root canal sealers (Laghios et al., 2000). A positive correlation between bismuth oxide concentration and radiopacity was observed (Coutinho-Filho et al., 2008). Comparing the radiopacity of WMTA with that of GMTA has reported more radiopacity for WMTA (Chng et al., 2005; Islam et al., 2006b). It was also reported that the radiopacity of Portland Cement is lower than of ProRoot MTA (Islam et al., 2006b; Coutinho-Filho et al., 2008). The push-out strength of MTA was similar to Super-EBA and IRM when exposed to sodium hypochlorite or saline when used as a perforation repair material, but MTA was more susceptible to oxidizing agent (Loxley et al., 2003). Another report proved that a hydrogen peroxide-based canal preparatory agent has significantly reduced the push-out strength of MTA to dentin, whereas 5.25% sodium hypochlorite and 2% chlorhexidine did not (Yan et al., 2006). It was also reported that the humidity has significantly increased the push-out strength of MTA obturations (Gancedo and Garcia, 2006). In contrast, Sluyk et al. (1998) reported no significant difference in MTA retention in the presence of dry or moist cotton pellets placed over the material after its insertion in perforation sites. The perforation retention strength was not affected when MTA mixed with either saline, sterile water, or lidocaine but the bond strength to blood contaminated root dentin was significantly less in comparison to uncontaminated dentin (Vandeweele et al., 2006).

2.5 Antibacterial activity and bacterial leakage of MTA

ProRoot MTA cements and IRM showed generally greater antibacterial activity than other tested root end filling materials, completely inhibited Pseudomonas aeruginosa, and either delayed or limited the growth of Enterococcus faecalis (Eldeniz et al., 2006). Substituting 0.12% chlorhexidine gluconate has provided more antibacterial activity against Actinomyces odontolyticus, Fusobacterium nucleatum, Streptococcus sanguis, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans, than WMTA prepared with sterile water alone (Stowe et al., 2004). In another study 2% chlorhexidine mixed with MTA powders has reported a significant increase in the antibacterial effect of WMTA and GMTA against Enterococcus faecalis (Holt et al., 2007). GMTA and WMTA in concentrations of 50 and 25 mg/ml respectively were equally inhibitive against Candida albicans

for up to 7 days, but at lower concentrations only GMTA was effective (Al-Hezaimi et al., 2006a). In another in vitro study the antibacterial effects of GMTA and WMTA against Enterococcus faecalis and Streptococcus sanguis was assessed and the result to be reached was that lower concentrations of GMTA were required than the WMTA to exert the same antibacterial effect (Al-Hezaimi et al., 2006b). However, a recent investigation reported similar antibacterial properties for both types of GMTA and WMTA (Asgary and Kamrani, 2008). Torabinejad et al. (1995b) reported that MTA shows no antimicrobial activity against any of the anaerobes but did have some effect on five (Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, Lactobacillus and Staphylococcus epidermidis) of the nine facultative bacteria. In an antimicrobial study on MTA and Portland Cement against Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, a wild fungus, and a mixture of these bacterial and fungal species, both materials exhibited diffusion in agar without inhibition of microbial growth (Estrela et al., 2000). A similar study showed that MTA and Portland Cement have no antimicrobial activity against Candida albicans, Enterococcus faecalis, Escherichia coli and Staphylococcus aureus (Miyagak et al., 2006). While Sipert et al. (2005) reported that both MTA and Portland Cement showed antimicrobial activity for six of the seven strains tested except Escherichia coli. One experiment showed that, both the freshly mixed and the 24-hour set GMTA have an antifungal effect on Candida albicans (Al-Nazhan and Al-Judai, 2003). A study comparing the effect of MTA and Portland Cement on Candida albicans, Staphylococcus aureus, and Escherichia coli showed no antimicrobial effect for either of the tested materials (Al-Hezaimi et al., 2005a). Another investigation reported antimicrobial activity of GMTA, WPC, and OPC on Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, and Enterococcus faecalis (Tanomaru-Filho et al., 2007). However, recent investigation has reported similar suitable antibacterial activity in WMTA and MTA-Angelus against the standard strains Streptococcus mutans, Streptococcus sanguis and Streptococcus salivarius (Luczaj-Cepowicz et al., 2008). The microleakage of MTA materials has also been evaluated using bacterial penetration methods. GMTA was found to have significantly more resistance to Staphylococcus epidermis penetration than amalgam and ZOE preparations, and no leakage evident for the next 90 days, while the other materials exhibited bacterial penetration within the next 6 to 57 days (Torabinejad et al., 1995c). Another study found that GMTA resisted Serratia marcescens penetration for up to 49 days after inoculation, while zinc-free amalgam and ZOE materials showed trends for more bacterial penetration (Fischer et al., 1998). GMTA was found to have the same bacterial penetration

resistance as a ZOE preparation, amalgam, a bonded resin composite, and bonded amalgam during a 12-week evaluation using streptococcus salivarius (Adamo et al., 1999). ProRoot MTA and a bonded polymer based material were found to exhibit similar root-end bacterial leakage resistance using Streptococcus salivarius model, with both materials having significantly less bacterial leakage than a ZOE preparation (Maltezos et al., 2006). In an endotoxin leakage study, MTA was more resistant to leakage than amalgam, Super EBA, and IRM in most time intervals tested (Tang et al., 2002). By using prevotella nigrescens, GMTA was found to have the same bacterial penetration resistance as polyacid-modified resin composite and a ZOE preparation after 47 days (Scheerer et al., 2001). It was shown that WMTA root-end fillings contaminated with either blood, saline, or saliva during placement has displayed varying resistance to staphylococcus epidermidis. The study showed that saliva contamination has caused significantly more bacterial leakage than the uncontaminated WMTA (Montellano et al., 2006). GMTA did not demonstrate any bacterial leakage during a 45-day evaluation when used as perforation repair materials, while approximately half of the amalgam-repaired furcations allowed penetration and transmission of Fusobacterium nucleatum (Adamo et al., 1999). Nakata et al. (1998) compared the effectiveness of MTA and amalgam in repairing furcal perforations using a dual-chambered anaerobic bacterial leakage model. The results of this study showed that MTA has superior seal compared with amalgam in preventing leakage of Fusobacterium nucleatum. Furthermore, no significant difference was found between GMTA and WMTA in the resistance to Fusobacterium nucleatum penetration after 60 days when they were used as furcation repair materials (Ferris and Baumgartner, 2004). When GMTA is used in the treatment of immature apices, it has been reported to provide resistance to bacterial penetration by Enterococcus faecalis and Staphylococcus epidermis but not Enterobacter aerogenes (Hachmeister et al., 2002). A similar study reinforced GMTA resistance to Enterococcus faecalis penetration with no leakage after 10 days (de Leimburg et al., 2004). GMTA was also evaluated against Actinomyces viscosus microleakage in simulated immature apices which had received 2 or 5 mm apical GMTA restoration. The reached result was that only the 5 mm thick restoration resisted the microleakage for the entire evaluation and exhibited significantly less leakage compared to the positive control and other GMTA groups (Al-Kahtani et al., 2005). When evaluated as a coronal barrier, no difference against human saliva bacterial penetration was found among GMTA, WMTA, or resin-modified glass-ionomer restorative material (Tselnik et al., 2004).

2.6 Sealing ability and marginal adaptation of MTA

The success of an endodontic material may largely depend on its sealing ability, because most of the post-treatment endodontic disease is thought to occur due to tissue and other materials in uncleaned and/or unobturated areas of the root canal system that egress into the surrounding tissues (Torabinejad et al., 1995c). GMTA has been reported to have less microleakage than amalgam (Lee et al., 1993; Torabinejad et al., 1993; Torabinejad et al., 1994; Bates et al., 1996; Yatsushiro et al., 1998; Wu et al., 1998; Aqrabawi 2000; Fogel and Peikoff, 2001; Pereira et al., 2004) zinc-oxide-eugenol preparations (Lee et al., 1993; Torabinejad et al., 1993; Torabinejad et al., 1994; Wu et al., 1998; Aqrabawi 2000; Martell and Chandler, 2002; Pereira et al., 2004; Asgary et al., 2008) a conventional glass ionomer material (De Bruyne et al., 2005) and a resin modified glass ionomer cement (Daoudi and Saunders, 2002). While other studies reported no difference in leakage among MTA materials, zinc-oxide-eugenol preparations (Bates et al., 1996; Roy et al., 2001; Davis et al., 2003; De Bruyne et al., 2005) and conventional glass ionomer materials (Wu et al., 1998). According to Torabinejad et al. (1995d) MTA seals very superiorly and no gaps were found in any of the experimental specimen, while amalgam, Super-EBA and IRM exhibited gaps ranging from 3.8 to 14.9 microns. Presence or absence of blood had no significant effect on the amount of dye leakage (Torabinejad et al., 1994), while the addition of calcium chloride enhances the sealing ability of both GMTA and WMTA (Bortoluzzi et al., 2006). Roy et al. (2001) reported that an acid environment did not hinder the sealing ability of MTA. In one study, MTA samples were placed in normal saline, distilled water, or EDTA. The samples that were placed in EDTA had poor cell adhesion; which had led the authors to conclude that poor cell adhesion to the surface of MTA might be due to poor hydration of the material, which leads to a higher concentration of toxic ions on the surface of EDTA-stored MTA (Lee et al., 2007). Total GMTA-dentin bond strength is heightened by increased surface area, as one report states that 4 mm of GMTA has been reported to afford more resistance to displacement than 1 mm thick applications, and was not affected by previous calcium hydroxide placement (Hachmeister et al., 2002). A study performed by Valois and Costa (2004) by using protein leakage showed that 4 mm thick MTA was significantly more effective than others (1, 2, 3 mm) in preventing apical leakage. While another fluid filtration investigation showed that the minimal thickness for MTA to effectively seal the apical area was at least 3 mm (Lamb et al., 2003). However, Matt et al. (2004) reported that the thickness of 5 mm of MTA as apical barrier allowed less leakage than 2 mm, and the obturation with gutta-percha after 24 hours showed less microleakage than the immediately obturation. In contrast, Rahimi et al. (2008) reported that the

dye penetration was not significantly different between 1 mm, 2 mm, or 3 mm thickness of MTA when used as a root-end filling material. De Leimburg et al. (2004) used a polymerase chain reaction for bacterial leakage detection. They showed no significant difference between 1 mm, 2 mm, and 3 mm thickness of MTA as an apical barrier in teeth with open apexes. It was found by using visual topography evaluations of MTA and ZOE as root-end filling materials that the marginal adaptation of MTA was good with or without finishing procedures (Gondim et al., 2003), and the finishing technique did not significantly affect the incidence of microleakage (Gondim et al., 2005). Shipper et al., (2004) in vitro study compared MTA with amalgam as a root-end filling material and the results showed that MTA demonstrated better marginal adaptation to the root end cavity wall than amalgam. The authors suggested that the expansion of the material during the hydration setting reaction contributed to the superior adaptation to dentine. A scanning electron microscopic (SEM) study showed that GMTA root-end marginal adaptation might be slightly affected after occlusal loading (Peters and Peters, 2002). Camilleri and Pitt Ford (2008) showed that MTA has better marginal adaptation than GIC. A comparative SEM study of the marginal adaptation of WMTA, GMTA and Portland Cement showed no significant difference among the tested materials, although GMTA showed better marginal adaptation (Bidar et al., 2007). The microleakage and sealing ability of MTA and Portland Cement has been evaluated by using bacterial penetration showing no statistically significant difference between both materials (De-Deus et al., 2006). An SEM investigation has reported similar marginal adaptation when WPC was compared with white MTA-Angelus as root-end filling materials (Costa et al., 2009). Another investigation showed no significant difference between GIC and MTA-Angelus as root-end filling materials in a dye leakage model (de Martins et al., 2009). Another investigation compared WMTA and White MTA-Angelus as apical barrier for open apex teeth. Results showed no significant difference in dye penetration (Lolayekar et al., 2009). For repair of furcation preparation, it was reported that Super EBA provided a better seal than GMTA only at the 24-hour observation period, after which no difference in leakage was observed (Weldon et al., 2002). Another investigation compared sealing furcation perforations by using MTA, One-Up Bond or Super EBA. After 24 hours, MTA demonstrated significantly more leakage than the other groups. However, after 1 month there was no significant difference between the test materials (Hardy et al., 2004). Another study showed that when MTA is used to repair furcation perforations, less strain penetration has been exhibited in comparison to the other tested materials (Tsatsas et al., 2005). Hamad et al. (2006) found no difference in leakage between GMTA and WMTA when used to repair the furcation perforation, and also found that the

microleakage from the orthograde direction was significantly more than the retrograde direction. Another study compared MTA-Angelus with IRM and GMTA as perforation repair materials. GMTA samples showed the least amount of dye leakage (Hashem and Hassanien, 2008). The microleakage of MTA materials used for root canal obturation has been reported by Vizgirda et al. (2004). They suggested that MTA displayed more apical microleakage than laterally-condensed and thermoplasticized gutta-percha. However in another report, both GMTA and WMTA allowed less saliva microleakage than vertically condensed gutta-percha (Al-Hezaimi et al., 2005). It was also reported that root resection of canals obturated with MTA did not affect its sealing ability (Andelin et al., 2002). Calcium hydroxide intra-canal medication has been shown to affect the sealing ability of WMTA, and it was also shown that residual calcium hydroxide intra-canal medication could interfere with the adaptation of MTA to the root canal walls by being a mechanical obstacle, and by chemically reacting with MTA, thus influencing its surface characteristics (Stefopoulos et al., 2008). Zou et al. (2008) investigated the use of the internal matrix concept for repair furcal perforations to limit the flow of the MTA material and improve its sealing ability. They found that calcium sulphate provided a successful barrier against over extension of MTA, but decreased significantly its sealing ability, and also found that collagen plug did not prevent over extension or improve its sealing ability.

2.7 Biocompatibility of MTA

The biocompatibility of MTA has been reported widely in vitro and in vivo studies.

2.7.1 in Vitro

Kettering and Torabinejad (1995) have evaluated the mutagenicity of GMTA, ZOE-based, root-end filling materials as well as positive and negative controls by using Salmonella typhimurium LT-2 strains. They concluded that none of the root end filling materials including GMTA would be considered mutagens as measured by Ames Test. Cytomorphology of in vitro osteosarcoma cells and quantification for cytokine by ELISA showed very similar results for MTA and Portland Cement, as well as Portland Cement accelerated by calcium chloride (CaCl2) (Abdullah et al., 2002). The biological compatibility of MTA was examined by testing the adherence, viability, proliferation and secretion of collagen of osteoblasts-like cells. The cells on MTA were viable, grew, and released some collagen even at 72 hours (Pelliccioni et al., 2004). Nakayama et al. (2005) evaluated the behavior of rat bone marrow cells on MTA by using Scanning (SEM) and

Transmission Electron Microscopy (TEM). It was concluded that MTA has a low toxicity and the material does not inhibit cell growth but suppresses differentiation of osteoblast-like cells. Other evaluations have reported no genotoxic effects of MTA or WPC by single cell gel assay on human peripheral lymphocytes (Braz et al., 2006), mouse lymphoma cells (Riberio et al., 2005), cell proliferation (Camilleri et al., 2005b) and Chinese hamster ovary cells (Riberio et al., 2006). No genotoxic effects with ProRoot MTA, MTA-Angelus and Portland Cement have been reported when using human ECV 304 endothelial cells (De Deus et al., 2005). By using a cell viability assay for mitochondrial dehydrogenase activity in human periodontal ligament fibroblasts after 24 hours exposures to extracts of varying concentrations of MTA, Super-EBA, amalgam, in both freshly mixed and 24-hours set states. The cytotoxicity was measured and it was found that in the freshly mixed state, the sequence of toxicity was amalgam>Super-EBA>MTA. In the 24-hr set state, the sequence of toxicity at a low extract concentrations was Super-EBA>MTA>amalgam, while at a higher extract concentrations was Super-EBA>amalgam>MTA (Keiser et al., 2000). The reactions of periodontal ligament fibroblasts and gingival fibroblasts to MTA were evaluated by using Fluorescence method (Camp et al., 2003) and enzyme assay method (Pistorius et al., 2003). Both methods showed that MTA was biocompatible. Another report reinforced that MTA showed little effect on mitochondrial dehydrogenase activity of human periodontal ligament fibroblast, while GIC and amalgam showed mild cytotoxicity (Lin et al., 2004). Balto (2004) found by using Scanning Electron Microscope (SEM) analysis that periodontal ligament fibroblast showed normal morphology and exhibited growth and attachment to 24 set MTA surfaces. He reported also that set MTA appears to be less cytotoxic than fresh MTA if the quality and quantity of cell attachment to the root-end filling materials considered as a criterion to evaluate materials toxicity. In a comparable study involving resected root surfaces, periodontal ligament cell attachment was observed on MTA but was absent on gutta-percha (Fayad et al., 2004). Another study indicated that MTA induced a general osteogenic phenotype in periodontal ligament fibroblasts, with induction of alkaline phosphatase activity, as well as production of osteonidogen, osteonectin, and osteopontin (Bonson et al., 2004). In contrast, Haglund et al. (2003) showed that MTA was cytotoxic to both macrophages and fibroblasts and no cytokine production was observed. The cytotoxicity of amalgam, ZOE preparations, and GMTA was reported by two methods. The first method was via agar overlay which showed that freshly mixed and set amalgam were significantly less toxic compared to the other materials, while fresh and set GMTA was significantly less toxic than the ZOE preparations. The radiochromium method suggested that both fresh and set GMTA was significantly least toxic

followed by amalgam, ZOE preparations (Torabinejad et al., 1995e). Osorio et al. (1998) assessed the cytotoxic effects of MTA on human gingival fibroblasts and L929 cells by using enzyme assay. They concluded that MTA was not cytotoxic, but was biocompatible. GMTA was shown not to affect the cell viability or the prostaglandin E2 synthesis of macrophages and fibroblasts (Melegari et al., 2006). The percentage of toxicity was compared when MTA mixed with chlorhexidine or sterile water. It was suggested that the potentially beneficial antimicrobial effect of chlorhexidine may increase the cytotoxicity of MTA based-materials (Hernandez et al., 2005). It was concluded that GMTA causes osteoblast adhesion with release of cytokines from the attached osteoblasts, and Osteocalcin production has been increased when cells were grown on MTA (Koh et al., 1997). Osteocalcin levels were also increased in the presence of MTA (Thomson et al., 2003). By using Scanning Electron Microscope, osteoblasts were found to demonstrate good adhesion and spreading on GMTA surface, but did not demonstrate the same ultrastructural characteristics when exposed to a ZOE preparation and amalgam (Zhu et al., 2000). To assess the osteoblast biocompatibility of MTA by observing the expression of Interleukin (IL)-1alpha, IL-6, IL-8, IL-11 and macrophage colony stimulating factor (M-CSF), ELISA assays have been used. The growth of osteoblast cell was reported, production of (IL)-1alpha and IL-11 were not detected from the cells exposed to the GMTA, osteoblastic IL-6 and IL-8 were detected as well as M-CSF (Mitchell et al., 1999). Conversely, Koh et al. (1997, 1998) showed a rise of both IL-1alpha and IL-1 beta together with IL-6 after the cells were in contact with the material for 6 days. Another investigation on mouse preosteoblasts exhibited production of IL-6 in the presence of both types of GMTA and WMTA (Deller-Quinn and Perinpanayagam, 2009). Another study showed that in the presence of MTA, cells grow faster and produce more mineralized matrix gene expression in osteoblasts (Tani-Ishii et al., 2007). The antiproliferative activity of MTA was the lowest one in comparison to GIC and ZOE when the materials were used against three fibroblastic cell lines (Koulaouzidou et al., 2005). It was reported that MTA induced proliferation and not apoptosis of pulp cells in vitro (Moghaddame-Jafari et al., 2005). The biocombatibilty of MTA, 4META/MMA-TBB resin (Super-bond), IRM on osteoblast adhesion, proliferation, and matrix formation was compared. The results showed that MTA and Super-bond have good biocompatibility and allow hard- tissue forming cells to create a matrix layer which might enhance apical tissue regeneration, while the adhered cells to IRM decreased with time (Yoshimine et al., 2007). By using X-ray diffraction to compare the biocompatibility of calcium and silicate cement (CS) and MTA in bone cells (MG63), it was found that both showed the evident type I collagen, osteocalcin, alkaline phosphatase, bone sialoprotein, and osteopontin

expression (Chen et al., 2009). Another investigation has confirmed cementoconductivity, cementoinductivity, and osteoconductivity of WMTA (Hakki et al., 2009). Both GMTA and WMTA displayed biocompatibility and exhibited reduced cell growth when exposed to a Saos-2 osteosarcoma cell line (Camilleri et al., 2004). In contrast, Perez et al. (2003) using a different type of cell showed that WMTA was not as biocompatible as GMTA and postulated that the difference might be due to surface morphology of the materials. Duarte et al. (2005) compared the amount of arsenic ion release for ProRoot MTA, MTA-Angelus, and Portland Cement. They concluded that the materials release similar amounts of arsenic at values that were not harmful to the human body. In contrast, another study compared the amount of arsenic in GMTA, OPC, and WPC showed that OPC contains more than 6 times the amount of arsenic compared with GMTA (Monteiro Bramante et al., 2008). Another investigation showed that GMTA and gray MTA-Angelus did not show any trace of arsenic; while WMTA showed the minimum amount of arsenic (De-Deus et al., 2009). However, a recent review concluded that both MTA and Portland Cement exhibit no genotoxicity (Ribeiro, 2008). Huang et al. (2003) reported that a good cell growth was demonstrated on material extracts when tested using methyltetrazolium (MTT) assay. However, another study investigated the effects of calcium hydroxide-based, ZOE-based (Super-EBA), and MTA-based, on a human osteosarcoma cell line (U2OS). The results showed both cell attachment and IL-4 and IL-10 cytokine production for the MTA group (Huang et al., 2005). Freshly mixed or set GMTA has been reported to display little to no neurotoxicity of fetal mice, while freshly mixed or set amalgam, ZOE preparation (Super-EBA), and a resin endodontic sealer exhibited significantly neuronal death (Asrari and Lobner, 2003). WMTA was found to be more biocompatible than GMTA in supporting human cementoblast and keratinocyte growth, and both cell types showed significantly higher proliferation when grown on 12-day set MTA compared to 24-hour set MTA (Oviir et al., 2006). It was also suggested that MTA had more of a stimulating effect on human dental pulp cells than a commercial calcium hydroxide preparation (Dycal), and the number of calcium ions released from MTA was significantly higher than that released from Dycal (Takita et al., 2006). The amount of calcium release in WMTA is reported to be much more than that released by WPC, and the mechanism of hydration is different in these materials (Camilleri, 2008). An in vitro study compared the toxicity of primary teeth pulpotomy medicaments. MTA showed the lower toxicity in comparison to calcium hydroxide, ferric sulfate and formocresol (de Menezes et al., 2009). An experiment comparing the cytotoxicity of GMTA and calcium hydroxide demonstrated that calcium hydroxide produces more cytotoxicity and decreases cell metabolic activity approximately 3 times more than GMTA

(de Souza Costa et al., 2008). A recent investigation examined the effect of MTA and calcium hydroxide on 3T3 fibroblast cells and showed that MTA has a significantly shorter duration of cytotoxicity in comparison to calcium hydroxide (Sepet et al., 2009). Yasuda et al. (2008) compared MTA and Dycal by using rat dental pulp cell cultures. They concluded that MTA has no cytotoxicity after 72 hours, and it not only significantly increases mineralization by simulating dental pulp cells but also increases the amount of bone morphogenetic protein-2 (BMP-2) production. In contrast, Dycal decreases BMP-2 production and increases cell death.

2.7.2 in Vivo

The tissue reaction of Super-EBA and GMTA was examined when implanted in the mandibles of guinea pigs. It was recorded that the tissue reaction to MTA implantation was slightly milder than that observed in Super-EBA (Torabinejad et al., 1995f). Another study reported a more favorable tissue reaction to GMTA in comparison to amalgam and two ZOE preparations (Super-EBA, IRM) that were implanted in guinea pigs tibias and mandibles with direct bone apposition observed on some MTA samples (Torabinejad et al., 1998). Sousa et al. (2004) compared the osseous reaction of guinea pigs to MTA and ZOE. Four weeks after implantation, MTA response was rated as none to slight, ZOE showed bone resorption, necrosis, and infiltration of mononuclear inflammatory and foreign body giant cells. After 12 weeks, MTA and ZOE exhibited biocompatible characteristics. However, a different study found no difference in rat bone tissue response between GMTA and a calcium hydroxide root canal sealer (Cintra et al., 2006). Implantation of MTA and Portland Cement in rat connective tissue showed that both materials were biocompatible (Holland et al., 2001 a). Also it has been reported that both GMTA and Portland Cement demonstrated similar tissue reactions with bone healing and minimal inflammatory response on the materials surfaces (Saidon et al., 2003). Another study found no inflammatory response difference in rat connective tissue when exposed to both GMTA and Portland Cement with iodoform (de Morais et al., 2006). MTA, pure Portland Cement and a mixture of Portland Cement and bismuth oxide have been compared in subcutaneous connective tissue reactions. Histological evaluation suggested that all materials were biocompatible (Coutinho-Filho et al., 2008). On direct contact MTA produce minimal or no inflammatory reaction in soft tissues and are capable of inducing tissue regeneration (Sumer et al., 2006). Histological evaluation of tissue reaction to MTA has been evaluated by subcutaneous implantation of the materials in test animals. Subcutaneous implantation in rats

showed that MTA initially elicited severe reactions with coagulation necrosis and dystrophic calcification (Moretton et al., 2000). Another study showed that GMTA induced calcification in a rat connective tissue (Yaltirik et al., 2004). It was reported that WMTA, GMTA, and amalgam have similar inflammatory cell after 3 weeks, although GMTA was more biocompatible than WMTA and amalgam after 1 week (Shahi et al., 2006). Another study showed that there were no significantly differences on inflammatory cells after 15 days between WMTA and GMTA when implanted into subcutaneous connective tissue of rats (Vosoughhosseini et al., 2008). A recent investigation showed similar mild to moderate tissue reaction when Portland Cement and MTA were implanted in the subcutaneous tissue of rats (Hwang et al., 2009). An improved rabbit ear chamber was used to evaluate the biocompatibility of MTA and calcium hydroxide-containing root canal sealer (Sealapex). Both of them showed revascularization of connective tissue with complete recovery of microcirculation (Masuda et al., 2005). Another study used a rat aortic ring model to simulate the pulpal vessels smooth muscle contraction. Results showed that MTA induces vessel contraction in a dose-dependent manner (Tunca et al., 2007). GMTA was reported to be associated with significantly less periapical inflammation and more fibrous capsules than amalgam when used as root-end filling materials in a canine model. In addition, almost all of the GMTA specimens exhibited new cementum tissue growth on the GMTA surface (Torabinejad et al., 1995g). Regan et al. (2002) reported that both of GMTA and an epoxy-based root canal cement exhibited excellent canine periradicular tissue response at 60 days with no statistically significant difference between the materials for new cementum, bone, or periodontal ligament formation. While another study found a presence of periodontal ligament formation and a hard tissue growth on the GMTA surface compared to a ZOE preparation (Economides et al., 2003). The periapical tissue response of monkeys to GMTA and amalgam as root-end filling materials was evaluated histologically after 5 months. Periradicular tissues adjacent to the amalgam restorations displayed inflammation, while only one tissue specimen adjacent to the GMTA displayed inflammation. However, cementoblast activity associated with thick cementum was observed on the GMTA surface with five of six specimens, but no cementum was observed on the amalgam surface (Torabinejad et al., 1997). A Microscopic analysis in monkeys showed that both of MTA and calcium hydroxide were good root canal filling materials for immediately reimplanted teeth, providing organized periodontal ligament with no inflammation (Panzarini et al., 2007). MTA showed after 5 months the most favorable periapical tissue response; with formation of cemental coverage over MTA compared to Super-EBA and amalgam as a root-end filling materials on endodontically treated dog premolars and molars (Baek et al., 2005). Neither

freshly prepared nor fully-set GMTA was found to make a difference in periapical healing, with new cementum deposition and bone healing observed in both groups (Apaydin et al., 2004). Another study evaluated the effect of calcium sulfate (CS) and GMTA placement on the healing of periapical tissue following periradicular surgery. After 4 months it was found that the use of calcium sulfate and GMTA does not significantly affect periradicular healing (Apaydin and Torabinejad, 2004). Use of MTA in combination with calcium hydroxide in one study has shown that the periodontium may regenerate more quickly than either material used on its own in apexification procedures (Ham et al., 2005). The pulpal reactions were reported when MTA was used for pulp capping or pulpotomy. MTA-capped pulps showed complete bridge formation with no signs of inflammation (Faraco and Holland 2001; Tziafas et al., 2002; Andelin et al., 2003). The same results were obtained when MTA was placed over pulp stumps following pulpotomy (Holland et al., 2001b). MTA has been evaluated in rats as a pulpotomy agent. In comparison to bioactive glass, formocresol, and ferric sulfate, it was reported that MTA performed ideally as a pulpotomy medicament causing dentine bridge formation and simultaneously maintaining normal pulpal histology (Salako et al., 2003). For furcation repair, Ford et al. (1995) examined the histologic response of intentional perforations in the furcations of mandibular premolars of dogs repaired with MTA or amalgam. Their results showed the presence of cementum formation and very little inflammation in the immediately repaired samples with MTA, while the samples repaired with amalgam showed an absence of cementum and the presence of many inflammatory cells in the repaired areas. GMTA was compared to a resin-based calcium hydroxide root canal sealer (Sealapex) repairing lateral root perforation. At 30 days, GMTA–treated samples displayed no inflammation and deposition of cementum, while the root canal sealer exhibited chronic inflammation. At 180 days, GMTA- repaired specimens showed no ankylosis with most specimens exhibiting cementum formation with periodontal ligament. In contrast, sealer-repaired specimens exhibited some cementum formation but were associated with chronic inflammation (Holland et al., 2001c). The histologic response of periapical tissues was reported comparing GMTA and a glass-ionomer material (Ketac-Endo) as a sealer. All root canals obturated with GMTA exhibited no inflammation with apical closure, while a minority of glass-ionomer sealer showed partial closure with different degrees of chronic inflammatory reaction (Holland et al., 1999). It was found that the uncontaminated lateral root perforations which sealed immediately showed better reparation than the contaminated, and the addition of bacterial agent such as calcium hydroxide-based paste did not improve the repair of contaminated perorations (Holland et al., 2007). The physicochemical basis for the biological

properties of MTA was attributed to the production precipitates with a composition and structure similar to that of hydroxyapatite when the calcium ions released by the MTA came into contact with tissue fluid (Sarkar et al., 2005). This report was reinforced by Bozeman et al. (2006) who reported that the crystal precipitates on GMTA and WMTA materials were chemically and structurally similar to hydroxyapatite. It was also found that GMTA produced twice as much hydroxyapatite crystals as WMTA which leads to speculation that GMTA and WMTA may not possess the same level of bioactivity (Bozeman et al., 2006). Min et al. (2008) capped human third molar with MTA or Dycal and examined dentin bridge formation. Results indicated the presence of significantly more positive immunostaining in the MTA group than in the Dycal group for dentin sialoprotien (DSP) and heme oxygenase-1.

2.8 Clinical application of MTA

MTA has many clinical applications such as pulp capping, pulpotomy, root-end filling material, repair of root perforations, and apexification (Torabinejad, 2004).

2.8.1 Pulp-capping

GMTA has been compared with calcium hydroxide as a pulp-capping medicament using cynomolgus monkeys. After 5 months all of the pulps capped with MTA showed thick and continuous dentin bridge formation and all but one were free of inflammation. In contrast, only two samples capped with the calcium hydroxide preparation had dentin bridges, and all samples had severe pulpal inflammation (Ford et al., 1996). Abedi et al. (1996) found a significantly higher frequency of calcific bridge formation and less inflammation with MTA compared with calcium hydroxide in an animal study. Faraco and Holland (2001) emphasized the advantages of MTA over calcium hydroxide for pulp capping. Thirty teeth of three dogs were capped with either calcium hydroxide or MTA. More inflammation and less frequent dentinal bridging were observed in the calcium hydroxide group in addition to material resorption and microorganisms. Another study reported that GMTA when used as pulp-capping material induced deposition of hard tissue of osteotypic form at 3 weeks that typically observed with reparative dentin (Tziafas et al., 2002). Both GMTA and WMTA showed a calcified bridge formation at 2 weeks when used as a pulp-capping medicament in dog's teeth (Parirokh et al., 2005). It was also reported that GMTA induced complete bridge formation at 2 weeks that stained positive for DSP (Andelin et al.,

2003). The properties of GMTA were compared with calcium hydroxide in human tooth pulp-capping treatment. Samples capped with calcium hydroxide showed necrosis, chronic inflammation with absence of odontoblastic layer, and 0.15 mm thickness dentinal bridge at 6 months. In Contrast, GMTA samples displayed no necrosis, no pulp tissue inflammation with a near-regular odontoblastic layer, and 0.43 mm thickness dentinal bridge at the same period (Aeinehchi et al., 2003). Another study evaluated the pulpal response to direct pulp capping with MTA or calcium hydroxide cement in human third molars. The mean thickness of the dentin bridges observed in the MTA group was statistically greater than that of calcium hydroxide group (Min et al., 2008). A similar study compared WMTA and a calcium hydroxide preparation as direct pulp capping material in 48 third molars at 30 days and 136 days. At both evaluation periods, no significant difference was found between the groups in regards to the clinical presentation as well as the histological status (Iwamoto et al., 2006). Nair et al. (2008) showed that MTA resulted in less pulpal inflammation and more predictable hard tissue barrier formation in permanent teeth in comparison to hard setting calcium hydroxide. It was also reported that MTA healed the pulp tissue at a faster rate than calcium hydroxide, although after 2 months both materials were successful for pulp capping in human teeth (Accorinte et al., 2008). Bogen et al. (2008) reported an observational study where MTA was placed over carious exposures in permanent teeth with reversible pulpitis over 9 years. The authors concluded that 97.6% of the samples showed favorable outcomes based on radiographic assessment, clinical examination, and cold testing. In addition, all immature teeth in younger patients showed subsequent complete root formation. Another study showed that the clinical and radiographic success rate for using MTA as a pulp capping material in young permanent teeth was 93% over 2 years, with evidence of continued root growth (Farsi et al., 2006). A prospective study comparing MTA to calcium hydroxide when used as a pulp capping material in primary teeth was showed that MTA was as successful as calcium hydroxide after two years follow up (Tuna and Olmez, 2008). Another study showed no statistically significant differences were found between MTA and calcium hydroxide when used as a pulp capping agent in rats' teeth (Dammaschke et al., 2010).

2.8.2 Pulpotomy

A histological study showed that the pulps capped with MTA showed a homogenous dentin bridge formation and less inflammation when compared to the pulps capped with calcium hydroxide (Chacko and Kurikose, 2006). Histological evaluation of pulpotomies in dogs using ProRoot MTA, MTA-Angelus, and Portland Cement showed hard tissue bridge over the pulp and

all the materials were equally effective as pulp protection materials (Menezes et al., 2004). A randomized controlled trial study showed no significant difference between MTA and Portland Cement when they are used as a pulp capping agent in primary molar pulpotomies, and all the pulpotomised teeth were clinically and radiographically successful over 2 years follow up (Sakai et al., 2009). MTA and formocresol were compared as pulpotomy dressings in primary molars with carious pulp exposures over 6-30 months. Only one failure with internal resorption was reported in the teeth treated with formocresol, while none of the MTA-treated teeth showed radiographic or clinical pathology. Pulp canal obliteration was noted at a higher frequency in MTA-treated samples (Eidelaman et al., 2001). A similar study with a mean follow-up 38 months showed 97% success rate for MTA and 83% for formocresol, with higher pulp canal obliteration in MTA group, although both groups did not induce undesirable responses (Holan et al., 2005). Another study compared GMTA, WMTA and formocresol as pulpotomy dressings in primary teeth. Histological evaluation showed that both types of MTA induced thick dentin bridge formation, while formocresol induced thin, poorly calcified dentin. The authors concluded that GMTA was found to provide significantly better clinical and radiographical outcomes than WMTA and formocresol (Agamy et al., 2004). A randomized prospective study showed that none of the MTA treated teeth showed any clinical or radiographic pathology over 2 years when used in pulpotomized primary molars, while formocresol-treated teeth demonstrated approximately 13% radiographic and 2% clinical failure (Farsi et al., 2005). Percinoto et al. (2006) compared MTA to calcium hydroxide as a pulpotomy dressing for one year follow-up, and reported both materials to be equally effective in primary teeth. Two studies have compared formocresol to GMTA and WMTA, and reported MTA to be an acceptable alternative to formocresol as a wound dressing in the pulpotomy of primary teeth (Aeinehchi et al., 2007; Noorollahian 2008). Another study showed that the success rate of formocresol as a pulp capping agent for primary tooth pulpotomy over 2 years follow up was 85%, whereas MTA showed 95% success (Subramaniam et al., 2009). Maroto et al. (2005, 2006, and 2007) have reported longitudinal and observational clinical studies on GMTA and WMTA. In these studies no statistical difference was found in the rate of pulp canal obliteration between GMTA and WMTA whereas GMTA was found to produce significantly more dentin bridges. MTA has also been compared to formocresol and calcium hydroxide as a pulpotomy dressing in primary molars over 2 years with 100% clinical and radiographic success, whereas internal resorption was a common radiographic finding in teeth treated with calcium hydroxide. Although MTA and formocresol were equally effective, it was suggested that MTA is a suitable replacement for formocresol concerns about cytotoxicity and

potential mutagenicity of formocresol (Moretti et al., 2008). An evidence based assessment was used to compare MTA, formocresol, ferric sulfate and calcium hydroxide as a pulpotomy agent in primary molars. The clinical and radiographic data showed that MTA was significantly more successful than formocresol, ferric sulfate and calcium hydroxide (Ng and Messer, 2008). Another study showed that outcomes for MTA pulpotomy were superior to ferric sulfate after a median follow-up of 2 years (Doyle et al., 2010). A pulpotomy study evaluated and compared WMTA, WPC, beta-tricalcium phosphate (Beta-TCP), ferric sulfate and formocresol when used in primary pig teeth. It showed equally good results in WMTA, WPC and Beta-TCP with normal pulp tissue preservation, while formocresol and ferric sulfate showed irritated pulp tissue and more inflammatory pulp response (Shayegan et al., 2008). Discoloration of teeth was observed in 60% of the deciduous molars treated with MTA, but this was not of significance since the tooth was later restored with a stainless steel crown (Naik and Hegde, 2005). The results of a meta-analysis comparing success rate of pulpotomies using formocresol or MTA showed that MTA has significantly fewer failures compared with formocresol (Peng et al., 2006). The success of using MTA for partial pulpotomy in cariously exposed young permanent molars was evaluated by two observational studies. More than 75% of the samples in both studies showed favorable clinical outcomes (Barrieshi-Nusair and Qudeimat, 2006; Witherspoon et al., 2006). The histological success of MTA when used as a pulpotomy agent in human permanent molars with irreversible pulpitis was evaluated. All the samples showed complete dentin bridge formation and the pulps were vital and free of inflammation (Eghbal et al., 2009). Dentinogenesis of MTA can be due to its sealing ability, biocompatibility, alkalinity or induction of osteogenic transcripts, such as alkaline phosphates, osteonidogen, osteonectin and osteopontin (Bonson et al., 2004).

2.8.3 Root-end filling material and repair of root perforations

A prospective clinical study compared GMTA to IRM as root-end filling materials in 122 adult patients. At the 12-month review the results for both materials were good but the healing dynamics were more rapid with MTA compared with IRM. At the 24-month review the success rate for MTA was higher (92%) compared with that of IRM (87%). However, statistical analysis showed no significant difference in success rate between both materials (Chong et al., 2003). The same results were found by Lindeboom et al. (2005). Kim and Kratchman (2006) reported that MTA is the most biocompatible root-end filling material and can be used with predictable outcomes in endodontic surgery. Saunders (2008) reported 88.8% clinical and radiographic

success after 4-72 months when MTA was used as a root-end filling material. A meta-analysis showed that MTA has a high clinical success rate, provides the best seal, shows superior biocompatibility, and is the only root-end filling material that promotes tissue regeneration when compared with amalgam, IRM, and Super-EBA (Fernández-Yáñez Sánchez et al., 2008). A long-term study concerning GMTA used for root perforation repair in 16 cases has been reported. All treatments showed clinical and radiographic signs of healing with return of normal radiographic architecture to the repair sites (Main et al., 2004). Many clinical case reports in which MTA has been used to repair perforation showed clinical and radiographic healing signs (Hembrough et al., 2003; Bargholz 2005; Menezes et al., 2005; Yildirim and Dalci, 2006; Pace et al., 2008). Ghoddusi et al. (2007) repaired mechanical or strip perforations with MTA and followed them clinically and radiographically for 6-12 months. They reported that more than 82% of treated teeth displayed radiographic success, whereas all cases were symptom-free.

3 Materials and Methods

3.1 Selection of teeth

Sixty freshly extracted, human single-rooted teeth, stored in 0.2% thymol, were used in this study (Fig.1). They were cleaned of calculus, soft tissue tags, attached bone or other debris by hand-scaling instruments (Fig.2).

Preoperative radiographs showed an absence of multiple canals, calcifications, or severe apical curvatures (Fig.3).

Teeth were inspected for root surface cracks using the surgical operating microscope at X5 and X20 magnification (Fig.4 & 5, SZH-10, Olympus Optical Co. GmbH, Hamburg, Germany).

Standardized radiographs were taken prior to instrumentation with the initial root canal instrument of size 10 inserted into the canal. The inclusion criteria selected:

1- Sound teeth.
2- Teeth without any resorption due to periodontal reasons.
3- Teeth without root fractures.
4- Teeth with complete root formation.

Fig.1: Sixty single-rooted human teeth selected for this study

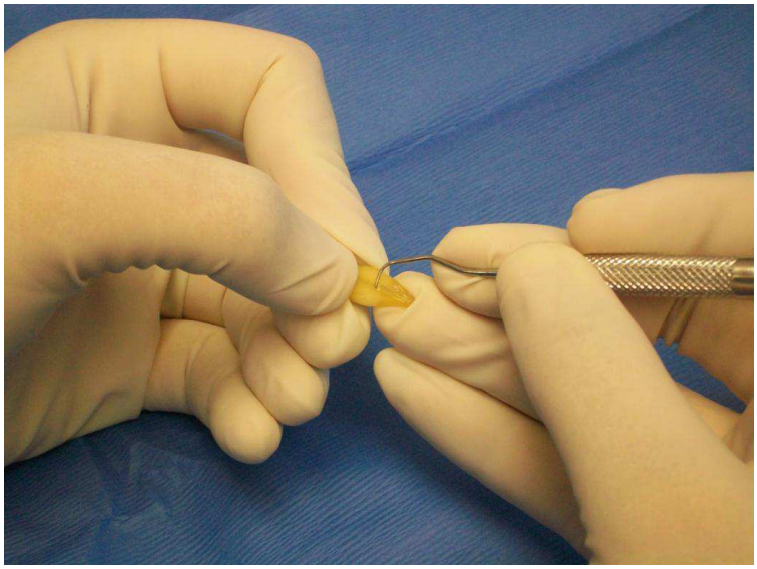

Fig.2: Scaling of debris from external root surface

Fig.3: Digital radiograph of tooth in proximal view to ensure one-root canal system

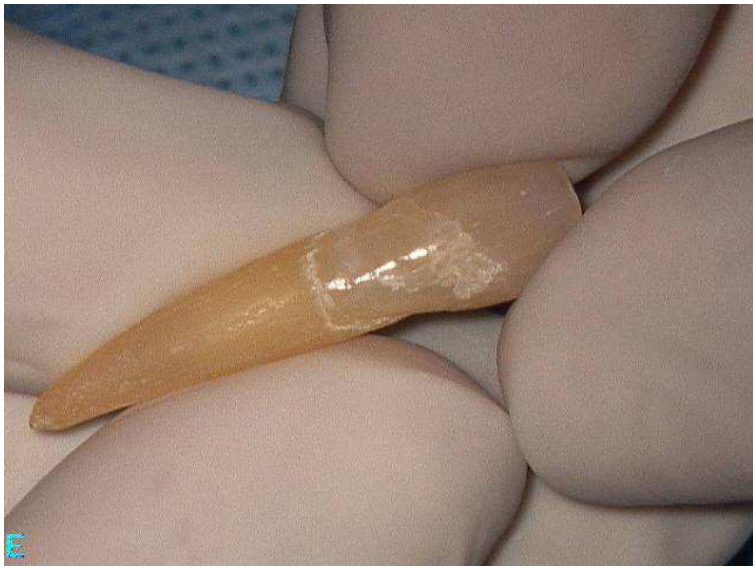

Fig.4: Inspection of external root surface to screen for cracks at X5 magnification

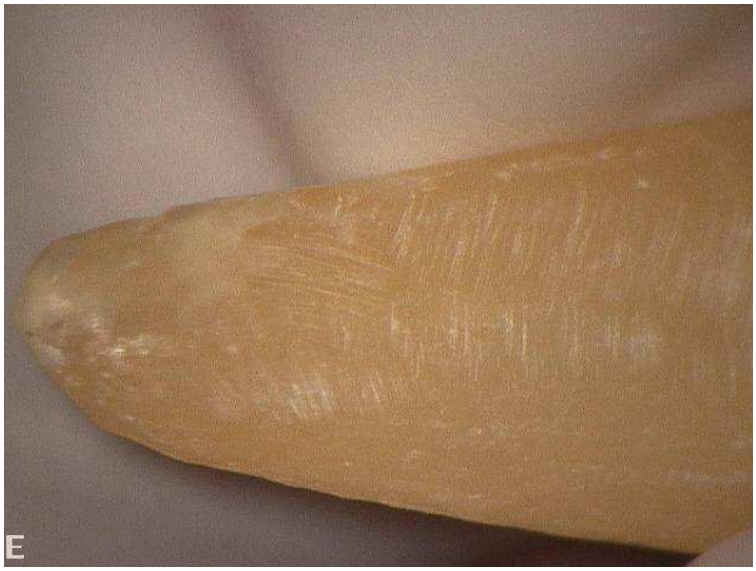

Fig.5: Inspection of external root surface to screen for cracks at X20 magnification

3.2 Canal instrumentation

The teeth were decoronated to standardized 15 mm root lengths with micromotor handpiece (NSK, Japan) and diamond discs (Dentaurum, Germany).

The root segments were then prepared to simulate the clinical situation of an open apex with Gates Glidden Drills # 1-6 (Mani Inc, Japan).

The drills were used in a crown-down manner until a # 6 drill could pass through the apical foramen. Once the Gates Glidden Drill # 6 had proceeded to the end of the canal and had rotated freely, it was removed.

Root canal irrigation was performed using 1 ml of 3% sodium hypochlorite between each file. A No.-10 K-type file was used to maintain apical patency.

Upon completion of instrumentation, the smear layer was removed by rinsing with 2 ml of a 17% EDTA solution for 3 minutes with sonic activation from the EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) (Fig.6).

The teeth were then irrigated with a final rinse of 5 ml of 2 % chlorhexidine gluconate.

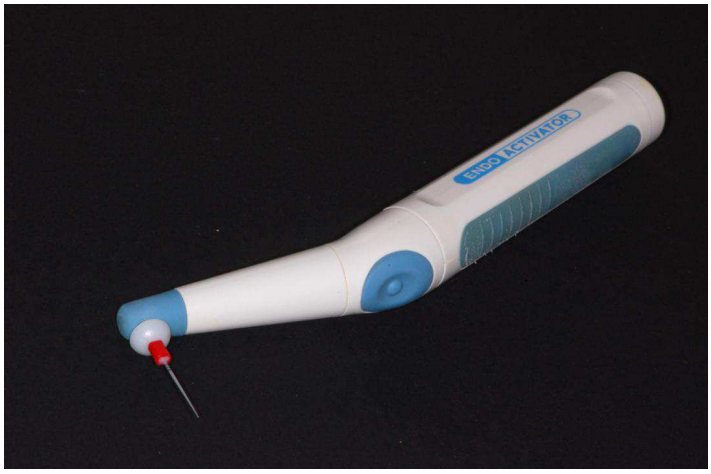

Fig.6: Handpiece for sonic activation (EndoActivator, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA)

The open apexes of 60 root segments were sealed with a small cotton pellet (Roeko, Coltène/Whaledent, Germany) moistened with modeling wax (Cavex Set Up Wax, Cavex Holland BV, The Netherlands). Polysiloxane precision impression material (Optosil®, Comfort®

Putty, Heraeus Kulzer, Germany) was mixed with its Activator paste (Universal Plus, Heraeus Kulzer, Germany).The mixtures were putted in Disposable Plastic Dappen Dishes (Diadent Europe B.V., The Netherlands), then before its setting, the 60 root segments were immersed vertically in the mixture.

3.3 Assignment of teeth and root canal filling

The 60 root segments were then randomly divided into three groups of 20 roots each.

Group 1: ProRoot® MTA was placed as 5mm apical barriers and condensed into the preparations using hand pluggers (Fig.7).

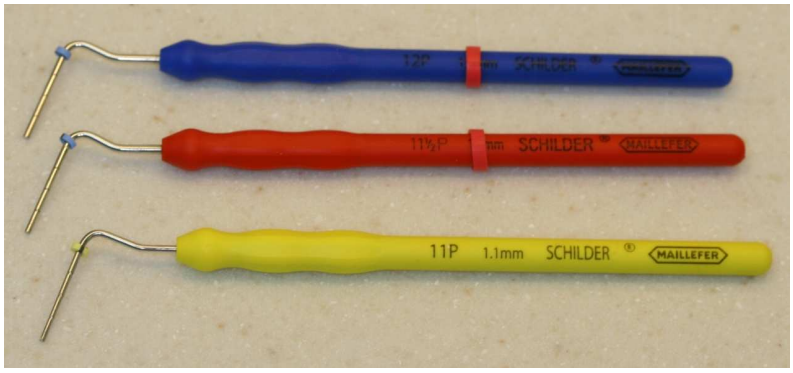

Fig.7: Hand pluggers for condensing the MTA into the apical region (Dentsply, Maillefer, Switzerland)

Group 2: ProRoot® MTA was placed as 5mm apical barriers and condensed into the preparations using paper points (Fig.8).

Fig.8: Paper points for condensing the MTA into the apical region (Roeko, Coltène/Whaledent, Germany)

Group 3: ProRoot® MTA was placed as 5mm apical barriers and condensed into the preparations using ultrasonic endodontic tips (Fig.9).

Fig.9: Endodontic ultrasonic tips for condensing the MTA into the apical region (Start-X™ # 4 & 5, Dentsply, Maillefer, Switzerland)

The MTA used in all 3 groups was mixed on a paper pad with distilled water in 3:1 powder ratio.When the mixture exhibited putty like consistency after about 30 seconds of mixing, it was immediately placed as apical barrier using the MTA carrier (Dentsply, Maillefer, Switzerland) (Fig.10).

Fig.10: MTA carrier for dispencing the MTA into the apical region (Dentsply, Maillefer, Switzerland)

Group1: Using the MTA carrier, ProRoot® MTA was dispensed into the apical region as 5mm apical barrier by the help of endodontic microscope (Carl Zeiss OPMI, PROergo, Zeiss, Germany).

Hand pluggers (Dentsply, Maillefer, Switzerland) with sizes 11, 11.5, 12 were used to condense the MTA into the appropriate length in the apical third.

Group 2: Using the MTA carrier, ProRoot® MTA was dispensed into the apical region as 5mm apical barrier by the help of endodontic microscope.

Paper Points (Roeko, Coltène/Whaledent, Germany) with sizes 120, 130, 140 were used to condense the MTA into the appropriate length in the apical third.

Group 3: Using the MTA carrier, ProRoot® MTA was dispensed into the apical region as 5mm apical barrier by the help of endodontic microscope.

The ultrasonic endodontic tips (Start-X™ # 4 & 5, Dentsply, Maillefer, Switzerland) connected to endodontic scaler (EMS Piezon® Master 400, EMS SA, Switzerland) was used to condense MTA to the apical third (Fig.11).

Fig.11: Endodontic ultrasonic apparatus for condensing the MTA in the apical region (EMS Piezon® Master 400, EMS SA, Switzerland)

The MTA was packed into the apical portion of the roots by activating the ultrasonic endodontic tip and slowly moving the MTA material apically using a 1- to 2-mm vertical packing motion (Fig.12).

The packing procedure was accomplished in 45 seconds for each root.

Fig.12: An apical plug of MTA deposited in the apical 5 mm of the root canal

Radiographs were then taken for all root segments in all 3 groups to verify the placement of the apical barriers. Apical barriers with visible voids, with less than 5 mm of material, or with more than 5 mm of material were excluded (Fig.13).

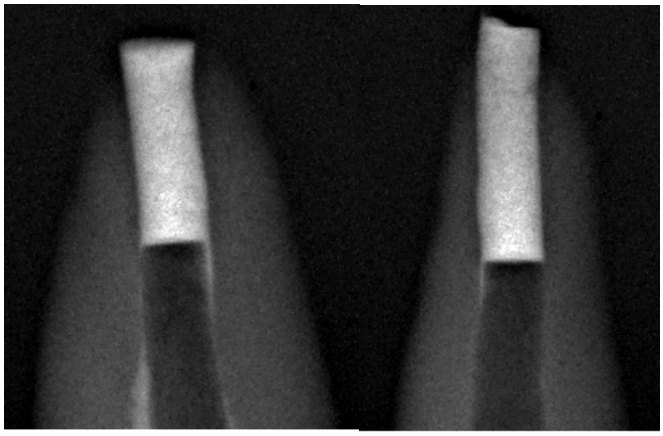

Fig.13: Radiograph showing placement of apical barriers

A cotton pellet moistened with 1 ml of saline was placed in the root canals till the material set and the coronal portion sealed with IRM (Caulk, Dentsply, USA). After 24 hours, the IRM and cotton pellet were removed and the canals dried and obturated using the endodontic microscope with vertically condensed Heated Gutta-Percha System (Obtura Spartan Endodontics, Fenton, USA) and AH 26® (Dentsply DeTrey GmbH, Germany) as root canal sealer (Fig.14 &15).

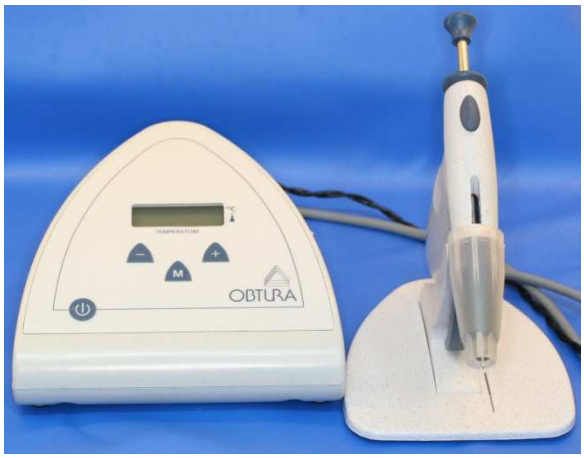

Fig.14: Obtura System for obturation the canals with vertically condensed heated gutta-percha (Obtura Spartan Endodontics, Fenton, USA)

Fig.15: Root canal filling by warm compaction of gutta-percha.

Coronal portions of all samples were then sealed with Cavit™ G (Temporary Filling Material 3M ESPE, Seefeld, Germany) (Fig.16).

Fig.16: Root segments after sealing with Cavit™ G

All root segments in all 3 groups were stored at 37°C and 100% humidity for 4 weeks.

The root segments from all groups were double coated with nail varnish except for the apical 1mm (Fig.17).

Fig.17: Teeth after coating with nail varnish

Roots were then totally immersed in methylene blue dye (Merck KGaA, Darmstadt, Germany) for 48 hours at room temperature.

After removal from the dye, the teeth were washed with tap water and then embedded in cold-curing resin for metallographic testing (Technovit 4071, Heraeus Kulzer GmbH, Hanau, Germany) (Fig.18 & 19).

Fig.18: Teeth after removing from the methylene blue dye (Merck KGaA, Darmstadt, Germany)

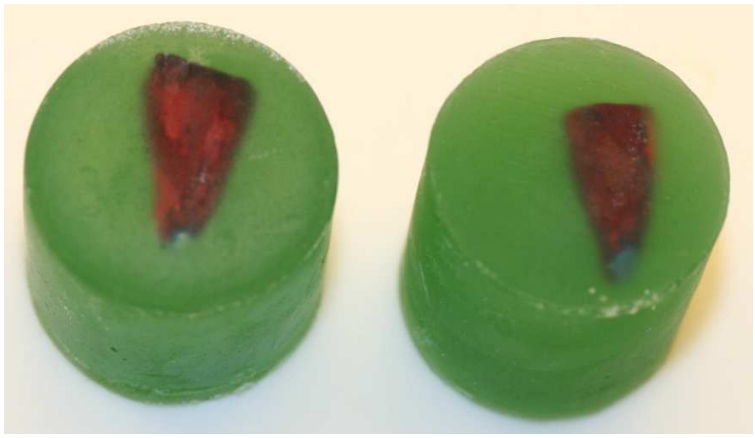

Fig.19: Teeth after embedding in the cold-curing resin testing (Technovit 4071, Heraeus Kulzer GmbH, Hanau, Germany)

The teeth were then longitudinally sectioned with saw microtome (Leica SP1600, Leica Microsystem, Wetzlar, Germany) along the long axis of the teeth with the sections passing through the obturated root canals and apical barriers in 0.4 mm thick slices (Fig.20 & 21).

Fig.20: Leica SP1600 saw microtome for sectioning the teeth in 0.4mm thick slices (Leica SP1600, Leica Microsystem, Wetzlar, Germany)

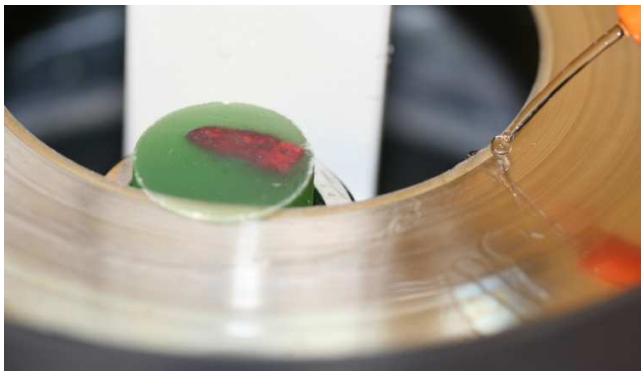

Fig.21: Tooth during sectioning by Leica SP1600

3.4 Measurement of dye leakage

Before the measurement of dye leakage, samples were randomly numbered, so the examiner was blind to which group belong each sample. Linear dye penetration was measured from apical root surface to the most coronal extent of dye penetration using the stereomicroscopic (SZH-10, Olympus Optical Co. GmbH, Hamburg, Germany) at 20X magnification, connected to the color video camera (Sony Power HAD, Model 950 P, Tokyo, Japan) (Fig.22).

Fig.22: Stereomicroscope for measuring the dye penetration (SZH-10, Olympus Optical Co. GmbH, Hamburg, Germany)

The dye penetration was then measured with an image analysis program (analysis 3.0, Soft Imaging System, Münster, Germany). All procedures were completed by one operator, whilst the scanning electron microscope evaluations were carried out by a second examiner who was blind in respect of all experimental groups and who underwent a training process with reference to the scoring system of the scanning electron microscope evaluations.

3.5 Statistical analysis

The leakage in all roots was examined. The linear extent of dye penetration from the apical end of the canal preparation to the coronal direction was measured by means of a light stereomicroscope at (20 ×) magnification.

The mean values and the standard deviations were calculated by using Microsoft Excel 2010.

Quantitative data were tabulated and analyzed using SPSS 16 (SPSS, Inc., Chicago, IL, USA) software. Level of significance was set at P less than or equal to 0.05.

4. Results

The highest length of methylene blue dye penetration along the wall of root end preparation for hand pluggers group is shown in Table 1.

Tooth Number	Value (µm)
35	2135.11
2	2246.93
57	1783.22
4	1353.99
46	727.81
6	713.60
49	825.92
19	1178.90
32	849.64
10	666.14

Tooth Number	Value (µm)
50	935.84
12	1538.72
43	1121.06
53	1355.45
24	2310.24
16	709.75
21	1826.77
18	1184.13
29	1721.19
52	1638.51

Table 1: The highest score of dye leakage (µm) along the wall of root end preparation in the hand pluggers group.

A microscopic image showing dye penetration for tooth 53 of hand pluggers group is shown in Fig.23.

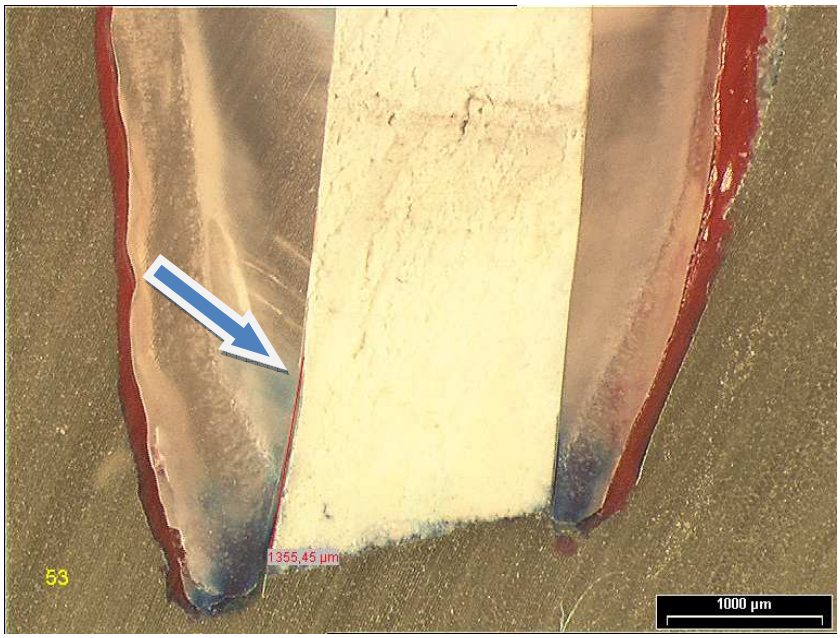

Fig.23: Microscopic image from hand pluggers group showing dye leakage (tooth 53)

The highest length of methylene blue dye penetration along the wall of root end preparation for paper points group is shown in Table 2.

Tooth Number	Value (µm)
41	1692.44
26	1361.84
13	801.30
34	1057.33
45	1355.80
5	1040.27
33	1183.89
48	911.27
7	1231.27
56	1924.61

Tooth Number	Value (µm)
60	1007.38
20	1366.95
23	1090.91
54	1091.11
27	1182.50
11	923.18
3	1889.68
58	2175.40
25	568.54
38	1166.91

Table 2: The highest score of dye leakage (µm) along the wall of root end preparation in the paper points.

A microscopic image showing dye penetration for tooth 26 of paper points group is shown in Fig.24.

Fig.24: Microscopic image from paper points group showing dye leakage (tooth 26)

The highest length of methylene blue dye penetration along the wall of root end preparation for ultrasonic tips group is shown in Table 3.

Tooth Number	Value (µm)	Tooth Number	Value (µm)
17	1637.65	31	545.63
22	1275.91	9	1223.80
47	1330.90	36	585.49
14	1300.87	44	1469.60
59	1110.55	1	832.29
42	979.02	51	1472.79
55	729.99	37	986.63
28	1356.66	15	2029.18
8	713.15	39	783.50
30	720.59	40	1618.35

Table 3: The highest score of dye leakage (µm) along the wall of root end preparation in the ultrasonic tips group.

A microscopic image showing dye penetration for tooth 1 of ultrasonic tips group is shown in Fig.25.

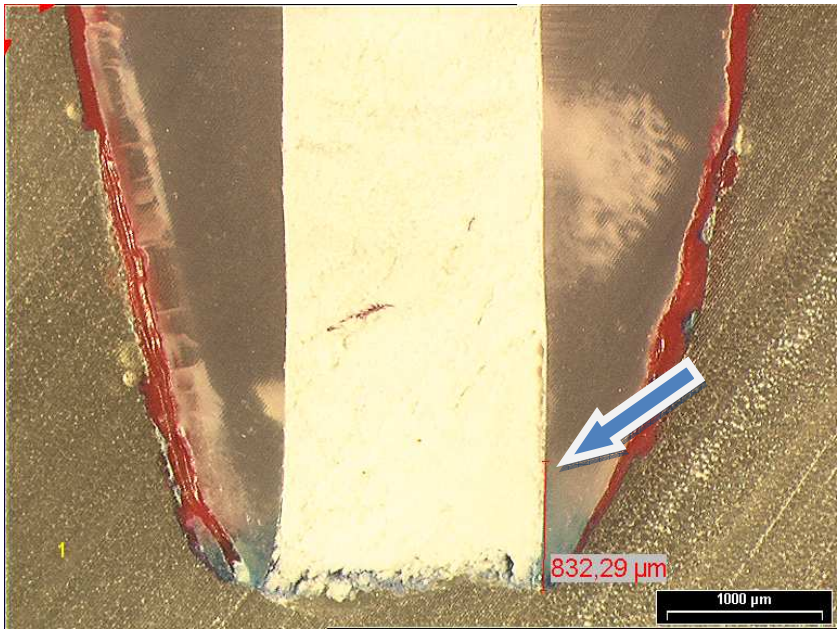

Fig.25: Microscopic image from ultrasonic tips group showing dye leakage (tooth 1)

The summary of mean values and the standard deviations, and maximum and minimum values of apical dye penetration for three groups is listed in Table 4.

This table shows that group 1 with an MTA application via hand pluggers had the highest mean value of apical dye penetration with 1.34mm ±0.5mm followed by group 2 using paper points with 1.25mm ±0.4mm. Group 3 with the ultrasonic endodontic tips showed the lowest mean value of apical dye penetration of 1.14mm ±0.4mm.

Groups	N	Minimum	Maximum	Mean (µm)	Standard deviation
1 hand pluggers	20	666.14	2246.93	1341.14	537.46
2 paper points	20	568.54	2175.40	1251.12	400.50
3 ultrasonic endodntic tips	20	545.63	2029.18	1135.12	402.61

Table 4: Descriptive statistical analysis of apical dye penetration in µm for the three experimental groups of MTA application with hand pluggers, paper points, and ultrasonic endodontic tips

Still, all three techniques exhibited considerable variation ranging from about 0.5 to well over 2mm dye penetration and leakage (Table 4, Fig.26). Using paper points for the application of MTA resulted in the most consistent results around a mean value of about 1.25mm.

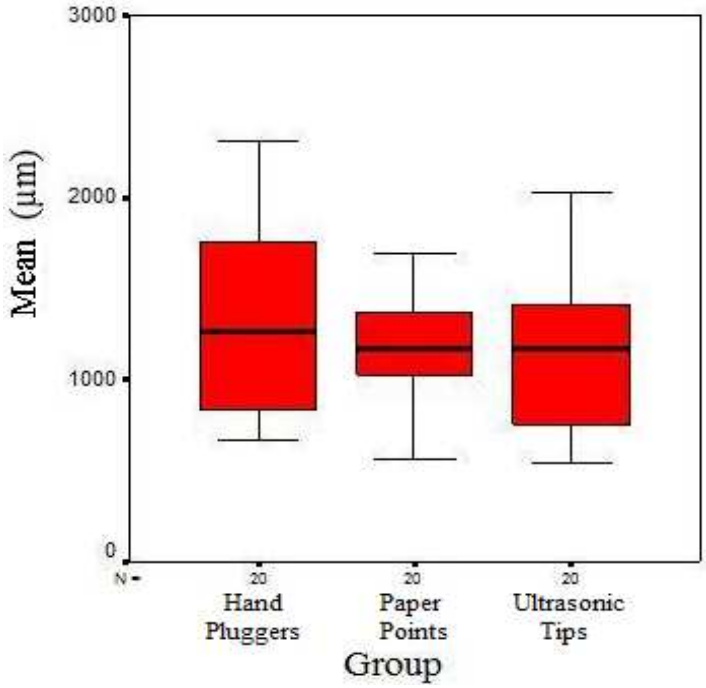

Fig.26: Distribution of apical dye penetration in µm for the three experimental groups for MTA application with hand pluggers, paper points, and ultrasonic endodontic tips.

According to the results obtained, it was analyzed statistically using the Student's *t*-tests for comparison (Table 5, 6 and 7).

Group 1-2		Levene test for equality of variance		t-test for Equality of Means						
		F	Significance	t	df	P-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Variable	Equal variances assumed	3.150	0.084	0.601	38	0.552	90.017	149.87	-213.39	393.43
	Not equal variances assumed			0.601	35.12	0.552	90.017	149.87	-214.21	394.24

Table 5: Statistical analysis and independent samples test between group 1 (hand pluggers) and group 2 (paper points).

Group 1-3		Levene test for equality of variance		t-test for Equality of Means						
		F	Significance	t	df	P-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Variable	Equal variances assumed	2.166	0.149	1.372	38	0.178	206.01	150.16	-97.96	510
	Not equal variances assumed			1.372	35.21	0.179	206.01	150.16	-98.75	510.79

Table 6: Statistical analysis and independent samples test between group 1 (hand pluggers) and group 3 (ultrasonic tips).

Group 2-3		Levene test for quality of variance		t-test for Equality of Means						
		F	Significance	t	df	P-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Variable	Equal variances assumed	0.234	0.631	0.914	38	0.367	116	126.98	-141.06	373.06
	Not equal variances assumed			0.914	37.99	0.367	116	126.98	-141.06	373.06

Table 7: Statistical analysis and independent samples test between group 2 (paper points) and group 3 (ultrasonic tips).

The statistical analysis showed that there were no significant differences among the 3 experimental groups ($0.178<p<0.552$).

5 Discussion

5.1 Single visit apexification with MTA

In children, dental caries and trauma are the most common challenges to the integrity of a tooth as it matures. If the dental pulp is damaged before development of the root length and closure of the apical foramen, normal root development is altered or halted completely (Ham et al., 2005).

In such teeth, due to lack of apical constriction, hermetic seal of the root canal system during obturation is not possible. For such cases, the best management technique is apexification.

Apexification is defined as a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp (Rafter 2005).

The advantage of a material that promotes the immediate formation of an artificial apical plug and that maintains the capability to induce apexification with time means that the definitive root filling can be placed immediately after the material sets (Felippe et al., 2006).

Single visit techniques using apical barriers have been proposed as alternatives to the long term calcium hydroxide apexification procedure which enables immediate obturation of the root canal (Witherspoon and Ham 2001; Linsuwanont 2003; Andreasen et al., 2006; Simon et al., 2007).

The idea of single visit apexification is not new and has been discussed and tested for many years. Several case reports have been published. Biological apical closure appears later, i.e. after the filling of the root canal, contrary to the technique with calcium hydroxide, where obtaining the apical barrier is necessary to complete the root canal treatment.

Pradhan et al. (2006) showed that the time taken to complete the treatment of non-vital teeth with unformed apices and for obtaining an apical biological barrier formation by using MTA was significantly less than that for calcium hydroxide.

It was found that medication with calcium hydroxide had no significant effect on grey MTA leakage (Hachmeister et al., 2002; Stefopoulos et al., 2008).

Other studies dealing with the use of MTA as apical barrier (de Leimburg et al., 2004; Al-Kahtani et al., 2005) have not addressed that problem since they did not consider a calcium hydroxide medication.

Another study revealed that white MTA sealed better when used without previous intracanal medication with calcium hydroxide (Stefopoulos et al., 2008).

The in vivo findings of Felippe et al (2006) suggest that the calcium hydroxide premedication is not a prerequisite, as the mere presence of MTA as an apical plug led to favorable results in terms of periapical inflammation and hard tissue formations in dogs' teeth.

MTA is osteoconductive, which may help the periapical tissue to adapt and heal. Its effectiveness has been shown in many case reports (Torabinejad and Pitt Ford, 1996).

Felippe et al. (2006) showed that the application of MTA immediately after root canal preparation favored the establishment of a normal periodontal ligament and formation of new bone and cementum. The histological responses observed in this study indicate that MTA is a reliable material and should be considered effective in teeth with incomplete root formation.

Its application results in predictable apical closure and reduction of the treatment time, number of appointments and radiographs, particularly in young patients.

Another study suggested MTA for apexification because it provides an adequate seal in the root canal, and it appears to offer a biological active substrate that stimulates periodontal cell production (Villa and Fernández, 2005).

Because of potential discoloration effect of grey MTA, white MTA has been introduced into endodontic treatment for the same purpose (Parirokh et al., 2005).

5.2 Methodological considerations

Bozeman et al (2006) showed that white MTA had a significantly higher compressive strength than grey MTA at 24 hours and that grey MTA had a significantly longer time to final setting time compared to white MTA. Based on this study, the results suggest that white MTA is an effective substitute for grey MTA. Hence white MTA was used for this study.

Natural teeth were used in this experiment because it would certainly better simulate the actual clinical situation than the plastic tubes.

Matt et al. (2004) and Al-Kahtani et al. (2005) reported that 5-mm MTA apical plug provided better microleakage resistance than 2-mm MTA apical plug. Considering these results, 5-mm apical plug was used in this study to provide sufficient volume of MTA for reduction of dye leakage.

The open apexes of the segments were sealed with a small cotton pellet moistened with modeling wax to simulate a periapical environment. MTA was condensed into root-end preparation against a cotton pellet absorbed with wax for support.

The root segments were immersed vertically in a mixture of polysiloxane precision impression material putted in disposable plastic dappen dishes to enable the application of MTA for all groups in the same position and to avoid additional variables.

Orthograde delivery is a sensitive technique. Placement must be verified with radiographs and condensation is limited because of minimal resistance of the open apex. In addition to the difficulty in delivering the material to the apex, the irregularities and divergent nature of the anatomy may limit adaptation to the dentinal walls, creating marginal gaps at the dentinal interface (Al-Kahtani et al. 2005).

Therefore after placement of MTA, radiographs were taken to verify the thickness of the barrier and its placement at the apex.

The goal of a 5 mm thickness barrier was not easily achieved because of the difficulties of handling the material of the apex. The use of an operating microscope may facilitate placement and handling at the apex. Hence ProRoot® MTA was dispensed into the apical region as 5mm apical barrier by the help of endodontic microscope.

Due to the prolonged setting time of ProRoot MTA, these segments were obturated with gutta-percha in 24 hours. Matt et al. (2004) reported also that the obturation with gutta-percha after 24 hours showed less apical dye leakage than the immediately obturation.

These findings concur with the manufacturers' recommendation of placing a moist cotton pellet in the canal, and allowing the material to set for a minimum of 4 hours.

The long hardening time of ProRoot MTA is said to reduce internal tensions and the incidence of marginal infiltrations, but it forces to definitively fill the tooth in the following sitting (Casella and Ferlito, 2006).

After obturation, the coronal portions of all samples were sealed with Cavit to prevent any chances of dye penetration from the coronal aspect. They were stored at 37˚C and 100% humidity for 4 weeks to simulate a periapical environment.

Many techniques have been advised to test the sealing properties of restorative materials in vitro and in vivo. These techniques include use of dyes, chemical tracers, radioactive isotopes, neutron activation analysis, electrical conductivity, and bacterial penetration.

In the present study, methylene blue dye penetration method was used for assessing the degree of microleakage; because it is easy and economic to use. It has a high degree of staining and has a molecular weight even lower than that of bacterial toxins (Ahlberg et al., 1995; Tamse et al., 1998; Kontakiotis et al., 2001). Dye studies, however, usually employed to screen new restorative filling materials. When a filling material does not allow penetration of small molecules, it has the potential to prevent leakage of larger substances such as bacteria and their by-products (Aqrabawi 2000; Oliver and Abbott 2001).

The limitation of dye leakage studies is that they measure the degree of leakage in only one plane, making it impossible to evaluate the total amount of leakage (Wu and Wesselink 1993; Ahlberg et al., 1995; Tamse et al., 1998).

Before placing the prepared samples in methylene blue dye, all the root segments except the apical 1mm were double coated with nail varnish to prevent dye penetration into the lateral and accessory canals.

5.3 Discussion of results

Maximum linear extent of dye penetration was defined as the most coronal level of dye visible on the root canal walls or gutta-percha, regardless of whether it was continuous with the apical area.

It has generally been considered that a potential root end filling material should set as it is placed in the root end cavity without significant shrinkage. This condition would allow dimensional stability of the material after placement and less time for an unset material to be in contact with vital tissues.

In general terms, the quicker a material sets the more it shrinks. This phenomenon may explain why ProRoot MTA in previous studies had significantly less dye and bacterial leakage than other materials used as root end filling materials (Torabinejad et al., 1995a).

The benefit to use ultrasonic activation for placement of MTA apical barriers is still in question.

When orthograde MTA is placed in teeth with open apices, it has been shown that a good seal and adequate retention is difficult to achieve (Hachmeister et al., 2002).

Another study has shown that ultrasonic energy applied while placing the barrier may improve the seal of the set cement (Lawley et al., 2004). Our findings do not support this hypothesis as handpluggers and paper points achieved the same results as ultrasonic application of MTA.

6 Conclusion & recommendations

This study compared the fill density of white ProRoot MTA in extracted single-rooted teeth, using three placement methods: hand condensation with pluggers (group 1), hand condensation with paper points (group 2), and direct ultrasonic endodontic tips (group 3).

The results showed considerable variation, but mean values and variability were very similar for all three application methods. Paper points achieved the most consistent results with few minimal values for dye penetration, but also few high values.

Although ultrasonic vibration showed less dye leakage compared with Group 1 and 2, but the statistical analysis showed that there were no significant differences among the 3 experimental groups ($0.178<p<0.552$).

In a one-visit apexification technique, it must keep in mind that an apical barrier is used mainly to produce an apical stop against which gutta-percha will be adequately condensed.

The obturation of the canal with gutta-percha and sealer will play the key role for a bacteria tight apical seal as in every non-surgical endodontic treatment.

7 Summary

A one-visit apexification protocol with Mineral Trioxide Aggregate (MTA) can be seen as an alternative to the traditional treatment practices with calcium hydroxide. The aim of this study was to investigate the sealing ability of ProRoot MTA when placed as an apical barrier using three different techniques.

Sixty freshly extracted single-rooted human teeth were decoronated and standardized to a root length of 15mm. The root segments were prepared with Gates Glidden burs (size 1-6) to simulate the clinical situation of an open apex and randomly assigned into 3 experimental groups of 20 samples each. 5mm MTA was placed by pluggers (Group 1) paper points (Group 2) or ultrasonic tips (Group 3). Radiographs were taken to verify the placement of the apical barriers. After obturation of all samples with warm gutta-percha (Obtura) and AH26 sealer, the coronal portion of all samples was sealed with Cavit. The root segments were then double coated with nail varnish except for the open apex and were exposed to methylene blue dye for 48 hours at room temperature. Afterwards, the samples were sectioned longitudinally and the extent of dye penetration was measured with a stereomicroscope.

The mean depth of dye leakage for Group 1 was 1.34mm (±0.5 SD) Group 2 1.25mm (±0.4 SD) and Group 3 1.14mm (± 0.4 SD). Statistical analysis showed that there were no significant differences among the 3 experimental groups ($0.178<p<0.552$).

In conclusion, ProRoot MTA has a similar sealing ability when placed as an apical barrier with pluggers, paper points or ultrasonic tips.

8 Zusammenfassung

Die einzeitige Apexifikationstechnik mit Mineral Trioxid Aggregat (MTA) kann als Alternative zu den herkömmlichen, langwierigen Behandlungsmethoden mit Calciumhydroxid gesehen werden. Ziel der Studie war es, die Dichtigkeit von ProRoot MTA als apikale Barriere bei drei verschiedenen Applikationstechniken zu untersuchen.

Bei sechzig, frisch extrahierten, einwurzligen, humane Zähnen wurden die klinischen Kronen abgetrennt, um eine standardisierte Wurzellänge von 15 mm zu erhalten. Die Wurzeln wurden mit Gates-Bohrern (Größe 1-6) erweitert, um die klinische Situation eines offenen Apex zu simulieren. Anschließend wurden jeweils 20 Proben nach dem Zufallsprinzip den drei Versuchsgruppen zugeordnet. 5 mm MTA wurde mit einem Plugger (Gruppe 1), Papierspitze (Gruppe 2) oder Ultraschallspitzen (Gruppe 3) gestellt. Die Röntgenbilder wurde die korrekte Platzierung der apikalen Barrieren überprüft. Nach Verschluss aller Proben mit warmer Guttapercha (Obtura, Sealer AH26) wurde der koronale Anteil aller Proben mit Cavit abgedichtet. Mit Ausnahme des Apex (ca. 1 mm) wurden die Proben dann doppelt mit Nagellack beschichtet und für 48 Stunden in eine Methylenblaufärbung bei Raumtemperatur getaucht. Abschließend wurden in den Längsschnitten der Proben (Schichtstärke 0.4 mm) das Ausmaß der Farbpenetration mit einem Stereomikroskop (Vergrößerung 20X) gemessen.

Die mittlere Farbpenetrationstiefe betrug 1,34 mm (± 0,5 SD) für Gruppe 1, 1,25 mm (± 0,4 SD) für Gruppe 2, und 1,14 mm (± 0,4 SD) für Gruppe 3. Die statistische Analyse zeigte, dass es keine signifikanten Unterschiede zwischen den drei experimentellen Gruppen ($0{,}178 < p < 0{,}552$).

Schlussfolgernd ergibt sich, dass ProRoot MTA eine fast identische Farbpenetration und damit Abdichtungsfähigkeit bei der Applikation als apikale Barriere mit Plugger, Papier- oder Ultraschallspitzen hat.

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10 Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät, keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Datum Unterschrift

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