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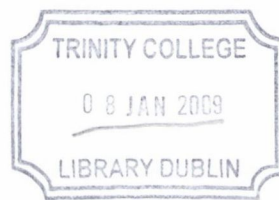
One Step Apexification using two types of Mineral Trioxide Aggregate

A thesis submitted in partial fulfilment of
D.Ch.Dent

2008

Paediatric Dentistry

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*Thesis
8666*

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Summary

Study Aim

This research investigated the clinical and radiographic success of one step apexification of non-vital immature permanent incisors in children using 2 types of white MTA; white MTA Angelus and white MTA ProRoot®. The objective was to establish the effectiveness of white MTA as an apexification material for immature permanent incisors and propose one or both brands as a suitable and time efficient replacement to calcium hydroxide.

Materials and methods

Ethical approval for this study was obtained from the Faculty of Health Sciences Research Ethics Committee, Trinity College, Dublin. A total of 21 children with 22 non-vital traumatised immature permanent incisors were recruited from the Trauma Clinic of Dublin Dental School & Hospital. Participants were alternatively assigned to Group 1 (white MTA ProRoot®) or Group 2 (white MTA Angelus). One step apexification was carried out following a standardised protocol. Clinical and radiographic review took place at 3, 6, 12 and 18 months. Two calibrated, blinded examiners evaluated all baseline and follow up radiographs using the periapical index (PAI) and change in the periapical radiolucency (PARL) where applicable. The outcome was recorded as absolute success, relative success, absolute failure or relative failure according to set criteria. The stage of root development, apical anatomy, position of the MTA plug and timing of apical barrier formation were also recorded by the examiners. Inter-examiner and intra-examiner agreement was tested using Kappa-Cohen tests. Fisher's Exact tests were used to assess relationships between variables and the study groups and also the healing of PARL over time. A Repeated Measures ANOVA was used to examine the change in PAI over time.

Results

Results were reported for the 6 months time point, with all 22 cases included for evaluation. The clinical success rate was 90.9% in Group 1, 100% in Group 2 and 95.5% in the group as a whole. No

statistically significant differences were calculated between the radiographic outcomes of Group 1 and Group 2. A statistically significant reduction in pathology was seen over time in both PAI and PARL outcomes. A relative radiographic success rate of 86.4% was recorded overall, when both the PAI change and PARL healing were considered. A statistical relationship was established between non-divergent apical anatomy and ideal positioning of the MTA plug. Cervical discolouration was observed in 18.2% of teeth after MTA placement, all of these teeth were in Group 2.

Conclusions

Both commercial brands of white MTA showed similar clinical and radiographic outcomes. These outcomes compare favourably to existing literature on both grey MTA and calcium hydroxide. It was interesting to note that tooth discolouration was associated with the use of MTA Angelus in a small number of teeth. This is the only clinical study reporting the success of using white MTA for apexification in non-vital, traumatised immature permanent incisors in children. One step apexification using both white MTA Angelus and ProRoot® can be proposed as a suitable, time efficient, acceptable alternative to traditional calcium hydroxide apexification. Larger, multi-centre clinical trials would be required to recruit sufficient numbers in order to establish robust statistical results.

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1 Introduction

Root development and apical closure are normally completed within 3-4 years of tooth eruption (Moorrees *et al.* 1963). If pulp death occurs before apical closure, further root development may be arrested. Most commonly pulp death is caused by traumatic injuries but may also be due to caries, a structural defect or an anomaly of the tooth.

When a non-vital tooth is immature, endodontic therapy will be challenging due to the lack of apical stop, divergent apical anatomy, thin dentinal walls and young age of the patient. It may not be possible to achieve an adequate three-dimensional apical seal using conventional root canal preparation and obturation. An apical barrier is needed to provide a stable platform against which gutta percha can be compacted. A procedure known as apexification has been developed to assist completion of the root canal treatment by forming an apical barrier. Many medicaments have been proposed in times past to achieve an apical barrier. Apexification using calcium hydroxide was introduced by Frank in 1966. Calcium hydroxide has been the most widely used and clinically accepted for over 40 years. This technique is easy and clinically acceptable, with high clinical success rates reported (Sheehy and Roberts 1997). The treatment time frame is however unpredictable and often prolonged, risking loss of patient compliance. The presence of a temporary coronal restoration for increased lengths of time leaves these teeth at risk of bacterial contamination. There have also been reports of an increased risk of root fracture in teeth with long-term calcium hydroxide dressings (Andreasen *et al.* 2002, Andreasen *et al.* 2006). The disadvantages of the calcium hydroxide apexification technique may be overcome by using a one step apexification technique. As the name suggests, this entails placement of an artificial apical stop in one visit, without the need for awaiting biological apical closure. This allows prompt obturation and completion of the endodontic treatment.

Mineral Trioxide Aggregate (MTA) has been introduced as the material of choice in the one step apexification technique (Torabinejad *et al.* 1995a). There have been many *in vitro* studies investigating the properties of this material, but very few clinical trials using the cement for apexification (Camilleri and Pitt Ford 2006). Existing clinical trials have shown favourable results, comparable or superior to apexification with calcium hydroxide. Since the introduction of grey MTA in 1993, a more aesthetic white version has been developed. White MTA ProRoot® (Dentsply Tulsa Dental, Tulsa, Okla, USA) has gained popularity following reports of coronal discolouration with grey MTA (Sarris *et al.* 2001). The composition of white MTA ProRoot® has been shown to differ from grey MTA ProRoot® only in the metal oxides content (Asgary *et al.* 2005). Even more recently, another commercial brand of MTA cement, MTA Angelus (Angelus Dental Solutions, Industria de Produtos Odontologicos Ltda, Londrina, Parana, Brazil) has entered the market. MTA Angelus has a faster setting time and it is also claimed to possess improved handling characteristics. It is available as a grey or white cement. MTA Angelus has been shown *in vitro* to be very similar in chemical composition and biocompatibility to MTA ProRoot® cement (Song *et al.* 2006).

There is limited current research using white MTA ProRoot® as an apexification material. To date, there are no clinical trials or case reports involving the use of MTA Angelus for apexification. There is therefore, an immense gap in the literature of *in vivo* research involving white MTA ProRoot® and white MTA Angelus. It is important that more clinical trials using these newer materials are carried out to assess the clinical success and efficacy of one-step apexification. Research should aim to compare the clinical outcomes of apexification using MTA with outcomes using the traditional calcium hydroxide apexification technique. This will enable clinicians to make an evidence based choice regarding apexification material and technique.

The purpose of this prospective research is to investigate the clinical and radiographic success of one-step apexification, using white MTA

ProRoot® and white MTA Angelus in children with non-vital, immature permanent incisors. The findings will be compared to the extensive literature available on the success and efficacy of the traditional calcium hydroxide apexification technique.

2 Literature Review

2.1 Causes of pulpal necrosis

Pulpal necrosis may be a consequence of dental trauma, dental caries or a defect in tooth development.

2.1.1 Trauma

Trauma is the most frequent cause of pulp necrosis in immature anterior permanent teeth. Worldwide, 20-30% of 12 year-old children have experienced dental trauma. Boys suffer approximately one third more injuries than girls. The peak age for anterior trauma is 9-10 years old, which coincides with root development of permanent incisors. The most common aetiologies of trauma are falls, sports injuries, road traffic accidents and violence (Andreasen *et al.* 2003).

Loss of vitality following trauma is dependent upon:

- i) The stage of root development
- ii) The type and severity of traumatic injury
- iii) The presence of bacteria (Barnett 2002).

Loss of vitality is also related to the degree of disruption of the neurovascular bundle. In-growth of new vessels begins 4 days after trauma at a rate of 0.5mm/day in immature teeth (Andreasen *et al.* 2003). Revascularisation is less likely in teeth with closed apices (<1mm) as the area of pulpo-periodontal interface is paramount (Andreasen *et al.* 1985). Concussion and subluxation injuries have a 3-6% risk of vitality loss while extrusive and lateral luxation injuries hold more risk of pulp obliteration for immature incisors and pulp necrosis in only 9% of teeth (Andreasen *et al.* 1985). In avulsion injuries, revascularisation is seen in 41-93% of teeth with open apices (>1mm) replanted within <5 minutes (Andreasen *et al.* 1995). Intrusive luxation may cause pulp necrosis in 100% teeth with closed apices and 63% of immature teeth with a higher incidence seen as the extent of intrusion increased (Andreasen *et al.* 1985, Andreasen *et al.* 2006). Crown

fractures carry a minimal risk of vitality loss but this increases with additional luxation injury and pulp exposure (Barnett 2002).

2.1.2 Dental Caries

Caries may lead to loss of vitality in anterior permanent teeth. Caries in newly erupted anterior teeth in children is not commonly seen, the disease tends to be seen in an older population, when root development is complete. Due to the visible position of anterior teeth, treatment may be sought before pulp necrosis occurs. Some factors may predispose children to aggressive anterior carious lesions such as special medical or intellectual needs, sugary medications or an excessively high frequency of consumption of cariogenic substances. Reduced salivary flow may predispose teeth to decay, in children this may be due to medications, radiation and chemotherapy or salivary gland surgery.

2.1.3 Defects of tooth development

Dens invaginatus has been defined by The American Association of Endodontists (2003) as “a defect in tooth development, characterised by invagination of the enamel organ before the calcification phase.”

Oehlers in 1957 classified dens invaginatus into three categories (Table 2.1.1).

Table 2.1.1 **Oehlers Classification of Dens Invaginatus**

TYPE 1	• enamel lined minor invagination within crown • does not extend past the CEJ
Type 2	• enamel lined, invades past the CEJ into the root • remains as a blind sac • may or may not connect with the pulp
Type 3	• enamel or cementum lined and penetrates the root • perforates apical area and forms a second foramen • no immediate connection with the pulp

Dens invaginatus occurs in 1-5% permanent teeth, with males affected twice as commonly as females. Maxillary lateral incisors are most

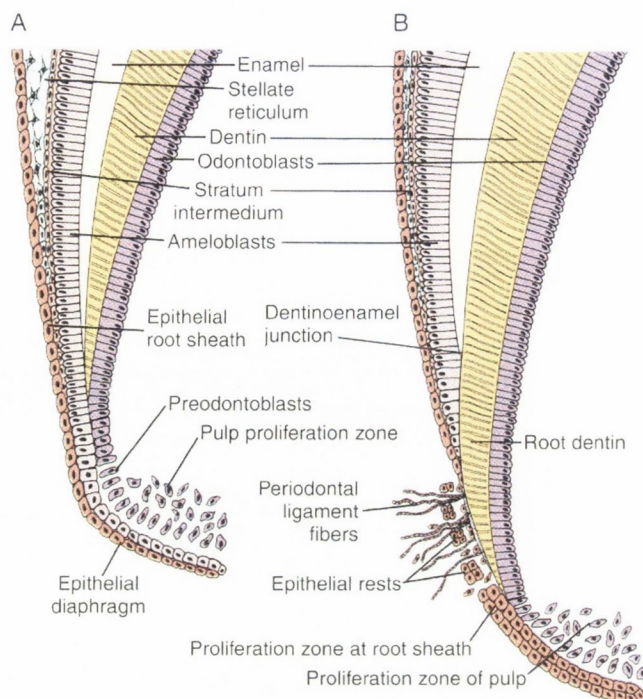
commonly affected with a reported prevalence of 9% (Hamasha and Alomari 2004). There is a risk of bacterial invasion, and subsequent necrosis in cases where the enamel lining of the invagination is thin or incomplete or the channels extend into the pulp. Early necrosis before root development completion may leave the tooth with an open apex and further complicate its management (Subay and Kayatas 2006).

Various enamel and dentine defects may predispose immature teeth to weakness and infection risk. Amelogenesis imperfecta, enamel hypoplasia or hypomineralisation, dentinogenesis imperfecta, hereditary opalescent dentine, dentine dysplasia and regional odontodysplasia may all lead to pulpal disturbances (Witkop 1988, Weerheijm *et al.* 2001, Hamdam *et al.* 2004). These defects may allow more rapid bacterial invasion of the tooth, either due to attrition of weakened structure or increased porosity. Penetration of the pulp leads to increased risk of vitality loss (Witkop 1988).

2.2 Root development

The root sheath is formed where the inner and outer enamel epithelium cells merge in the cervical region of the enamel organ at the future cemento-enamel junction. Following crown completion cells in the cervical region proliferate into 2 layers to form Hertwig's root sheath (Figure 2.2.1). The inner layer originates from the inner enamel epithelium. In the crown this layer produces enamel from ameloblasts but in the root portion the dental papilla is stimulated to differentiate into odontoblasts and produce dentine. These cells dictate the length, curvature, thickness and number of roots. The outer cell layer deposits intermediate cementum on the newly formed root dentine. This thin layer of acellular cementum seals the dentine tubules. The outer cell layer then immediately breaks up and moves away from the root surface in clusters as epithelial rests, which persist in the dental follicle as epithelial rest cells of Malassez.

Figure 2.2.1 **Diagram of root formation. A, early stage. B, later stage.**



(Reproduced from Avery and Cheigo 2006)

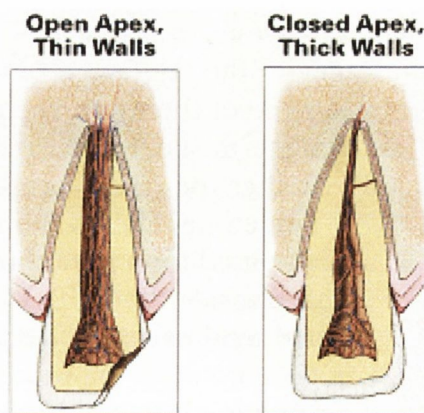
Mesenchymal cells from the follicle then move between the rest cells and differentiate into cementoblasts secreting cementoid on the root surface. This cementoid eventually calcifies into mature cementum. At the apical end the root sheath bends at an almost 45° angle to form the epithelial diaphragm which encircles the apical opening of the dental pulp during root development. These cells proliferate and cause root growth. Dentinogenesis continues from the crown into the root. The pulp adjacent to the dentine becomes the pulp proliferation zone, which is thought to provide new cells needed for root growth. Once root length is complete the dentine walls thicken and the apical opening closes to 1-3mm. This is normally complete 3-4 years after tooth eruption (Avery and Chiego 2006).

Hertwig's root sheath may be damaged by trauma. In some cases the vascularity and cellularity of the periapical area may preserve the root sheath, allowing root formation to continue despite the presence of puplal pathology (Frank 1966, Cvek 1972). Every effort should be made to maintain the root sheath vitality, as it is a source of undifferentiated cells which function to produce hard tissue and prevent ingrowth of periodontal ligament cells into the canal space (Andreasen

and Andreasen1992, Rafter 2005). Some authors have noted continued apical root development in non-vital, immature teeth following dressing with various antiseptic and antibiotic pastes (Cooke and Robotham 1960, Ball 1964, Frank 1966, Sato *et al.* 1996, Thibodeau and Trope 2007). Cooke and Robotham (1960) speculated that even in necrotic cases the root sheath can continue to organise mesodermal tissue into root components, once apical trauma is minimised. This apical mesodermal tissue is now considered as the apical papilla. Stem cells from this apical papilla (SCAP) may survive infection and allow root maturation (Sonoyama *et al.* 2008).

Complete destruction of the root sheath and apical papilla will halt normal development of the root. In these cases there may still be hard tissue formation by fibroblasts and cementoblasts of the dental follicle and periodontal ligament (Torneck 1982). Cessation of root development results in an immature root. An incomplete root was defined by Moorrees *et al.* (1963) as, a quarter to full root length with a fully or half open apex. Immature teeth have blunt ends and wide open apices. Incomplete dentinogenesis may render the dentinal walls thin (Figure 2.2.2), predisposing them to fracture (Andreasen *et al.* 2002).

Figure 2.2.2 Diagram showing thin walls of an immature tooth compared with thick walls of a mature tooth



The immature apical anatomy may be divergent, making an optimal apical seal difficult to achieve. Sealing the root canal using lateral condensation techniques is challenging due to the absence of an apical

stop (Walton and Torabinejad 2002). Consequently, the combination of these factors, pose a difficult situation for the clinician.

2.3 Diagnosing pulp status in immature teeth

The diagnosis of pulpal necrosis in immature teeth is challenging. A full and comprehensive history must be taken prior to clinical and radiographic assessment. Careful note must be made of any history of trauma or pain in the tooth. Prolonged or spontaneous pain may be indicative of irreversible pulpitis as it arises from stimulation of unmyelinated C fibres. Clinical observations of tenderness to percussion, mobility and presence of a swelling or sinus may also indicate necrosis. Discolouration is not always associated with necrosis so should be considered in conjunction with other factors (Jacobsen 1980). Sensibility testing using thermal and electrical tests is unreliable and erratic in immature teeth as the subodontoblastic nerve plexus is undeveloped. Trauma itself may cause pressure or tension on nerve fibres resulting in inconclusive pulp testing for four-six weeks following trauma (Skieller 1960, Andreasen *et al.* 2003). Laser Doppler Flowmetry, which measures blood flow seems to be the most reliable recent method of testing pulp status but is still not widely available (Strobl *et al.* 2004).

Radiographic examination may also be difficult to decipher. Healthy developing teeth often have radiolucencies around the apex, which may resemble pathology. Transient apical breakdown may resemble pathological changes radiographically (Andreasen *et al.* 2003). Regular radiographic review is recommended to monitor for occurrence of periapical pathology, continued root development and to enable comparison with the contralateral tooth (Rafter 2005). A combination of the history with frequent clinical and radiographic investigations will allow an accurate diagnosis of pulp status in these difficult cases.

2.4 Apexification

Apexification has been defined by Walton and Torabinejad (2002) as:

preparation of the thin walls and optimise access for proper condensation of a root end filling (Dawood and Pitt Ford 1989). The disadvantage is a reduction in the crown root ratio, which may further compromise the longevity of the tooth (Rafter 2005). Compliance with invasive surgery is also an issue in this young patient group (Frank 1966, Witherspoon and Ham 2001). Periapical clot induction was explored in monkey teeth by Ham *et al.* (1972) as a means of inducing apical closure and interestingly, a case report where no treatment was provided yet apical closure was achieved was described by Lieberman and Trowbridge in 1983. Endodontic therapy and control of infection has been found adequate to promote apical barrier formation (Das 1980). Minimal or no root canal instrumentation has been advised to avoid interfering with root development in necrotic teeth (Das 1980, Das and Das 1997).

Many techniques have described first stage endodontics followed by the placement of various dressings to induce an apical hard tissue barrier (Table 2.4.2).

Table 2.4.2 **Materials to induce apical closure**

Material	Author	Year
Antiseptic paste	Cooke and Robotham	1960
Antibiotic paste	Ball	1964
Calcium & CMCP	Kaiser	1964
Calcium hydroxide	Frank	1966
Calcium & CMCP	Dylewski	1971
Tricalcium phosphate	Roberts and Brilliant	1975
Collagen-calcium phosphate gel	Nevins <i>et al.</i>	1978

Early medicaments included antiseptic and antibiotic dressings. Ball (1964) demonstrated apical closure following the use of an antibiotic paste, as did Cooke and Robotham (1960) with an antiseptic material of zinc oxide paste with cresol and iodoform. Other medicaments included calcium hydroxide combined with various medicaments and calcium phosphate derivatives.

2.5 Calcium Hydroxide as an Apexification Material

Despite early success of other materials, it is calcium hydroxide (CH) that has been the most commonly used medicament for over 40 years. In 1964, Kaiser was the first to propose the use of calcium hydroxide combined with camphorated monochlorophenol (CMCP), to induce apical barrier formation. In 1966 Frank described an apexification technique using calcium hydroxide, which still forms the basis of apexification methods to the present day. The clinical success of calcium hydroxide and CMCP for barrier induction was confirmed by Dylewski (1971). The cytotoxicity of CMCP has led to further investigations using plain calcium hydroxide mixed with saline, sterile water or distilled water and showed similar clinical results with reduced cytotoxicity (Rafter 2005).

2.5.1 Antibacterial Properties

The antibacterial properties of calcium hydroxide have been well documented (Foreman and Barnes 1990). Bacteria and their subproducts stimulate organic reactions, which are the main cause of pathological alterations in root canals and the periapical area (Sjogren *et al.* 1991). If bacteria persist in the root canal and dentinal tubules, following trauma, inflammatory root resorption may occur which can destroy significant amounts of tooth structure in relatively short time periods (Sjogren *et al.* 1991, Trope 2002). The primary cause of failure of root canal therapy is the persistence of bacteria (Sundqvist *et al.* 1998). The complex anatomy of root canals provides an excellent opportunity for the growth and proliferation of microorganisms. Brystrom and Sundqvist (1981) demonstrated that not all bacteria are removed by instrumentation alone, therefore an adjunct such as calcium hydroxide is necessary to complement mechanical cleaning. Apical barrier formation is much more likely once bacterial levels have decreased (Ham *et al.* 1972).

The antibacterial properties of calcium hydroxide have been mainly attributed to both ionic dissociation and also to its alkaline pH of >12. The alkaline pH is thought to act as a local buffer against acidic products of inflammation and also may neutralise lactic acid produced

by osteoclasts, preventing further destruction of mineralised tissues (Heithersay 1975, Foreman and Barnes 1990). The alkaline environment created is also unfavourable to the majority of aciduric endopathogens (Sjogren *et al.* 1991). It has also been postulated that calcium ions can stimulate the enzyme pyrophosphatase, which denatures pyrophosphates and leads to increased repair mechanisms (Heithersay 1975).

An *in vitro* study by Estrela in 1998, examined the direct antimicrobial effect of calcium hydroxide, and demonstrated that the ionic dissociation of calcium hydroxide into Ca^{2+} and OH^- imparted antimicrobial and mineralising properties. The ions were shown to inhibit cytoplasmic membrane enzymes of bacteria, denature bacterial proteins, damage bacterial DNA and activate alkaline phosphatases within 3 days. Further research by Estrela (1995) led to the hypothesis that the high pH of calcium hydroxide causes reversible and irreversible bacterial enzymatic inactivation. Sjogren *et al.* (1991) investigated the effect of calcium hydroxide on bacterial samples from 30 root canals with pulp necrosis and apical periodontitis. The results showed 100% of bacteria that survived biomechanical preparation were eliminated by calcium hydroxide in 7 days. Sjogren *et al.* proposed that 7 days was adequate to allow movement of the free hydroxyl ions into the dentine tubules by diffusion gradient.

Tronstad *et al.* (1981) investigated pH changes in periradicular dental tissues in green vervet monkey teeth, following root canal dressing with calcium hydroxide. He suggested that an increase in the pH at the root surface due to free hydroxyl ions, may decrease dissolution of mineral tissues by neutralising acid produced and activating alkaline phosphatases. The study demonstrated a favourable influence of the pH in the dentinal tubules and at the root surface. Kontakiotis *et al.* (1995) performed an *in vitro* study to look at the antimicrobial action of calcium hydroxide, concluding that the ability of calcium hydroxide to absorb carbon dioxide from root canals may contribute to antimicrobial properties. The antibacterial action of calcium hydroxide has been found useful for preventing external inflammatory root resorption in

traumatised teeth that lose vitality. Inflammatory root resorption is defined as “*an unprotected or altered tooth surface attracting resorbing cells and an inflammatory response maintained by infection*” (Trope 2000). The initial damage to the protective root cementoid can be further propagated by bacteria from a necrotic pulp (Trope 2002). The denuded root is exposed to resorption by clastic cells and products of inflammation as long as stimulation exists from the pulp bacteria (Trope *et al.* 1995, Trope 2002).

A dressing of calcium hydroxide placed one week following injury has been shown to be effective at removing bacteria and halting inflammatory root resorption (Trope *et al.* 1992). It is not recommended to place calcium hydroxide earlier than 1 week due to potential cytotoxicity and damage of cementum and PDL cells (Foreman and Barnes 1990, AAE Guidelines 2004). Cvek (1992) looked retrospectively at 885 luxated mature and immature maxillary incisors. It was found that 97% of inflammatory root resorption was arrested after dressing with calcium hydroxide and filling with gutta percha.

Another property, which enhances the ability of calcium hydroxide to cleanse a root canal system is a proteolytic action. This is thought to help canal debridement by dissolving necrotic tissue remnants (Andersen *et al.* 1992). On the negative side this proteolytic effect may weaken circumpulpal dentine and result in increased weakening of an immature tooth (Cvek 1992).

2.5.2 Apical Barrier Formation

The mechanism of apical closure using calcium hydroxide is still unclear. In 1958 Mitchell and Shankwalker implanted calcium hydroxide into connective tissue of rats and found tissues not normally prone to calcification became calcified. This suggests an osteogenic potential of calcium hydroxide. It is thought that calcium hydroxide produces a response in periapical tissues similar to its effect on pulp tissue, namely a multilayered necrosis with adjacent mineralisation (Holland *et al.* 1977). Schroeder and Granath (1971) proposed that the necrotic tissue layer formed adjacent to calcium hydroxide causes a

low grade inflammatory reaction which stimulates calcification. Some dispute exists as to where the calcium ions incorporated into the hard tissue barrier originate. Pisanti and Sciaky (1964) demonstrated that the calcium ions in apical barriers of dog teeth originated from the calcium reserves of the animal. This would suggest the role of calcium hydroxide as an initiator but not a substrate (Foreman and Barnes 1990).

The calcific barrier shape has been described as a cap, bridge or ingrown wedge, a cap being the most frequent finding (Ghose *et al.* 1987). The apical shape has been described as either physiological, rounded or a straight bridge. In a recent prospective study by Dominguez Reyes *et al.* (2005), physiological apical shape was most frequently observed (73.11%), followed by a rounded form (19.2%) and a straight form or bridge (7.7%). This differs from Ghose *et al.* (1987) who reported rounded apical closure in 65% and straight bridges in 24% cases.

The composition of the calcific barrier has been described as osteocementum (Chosack *et al.* 1997), mineralised scar tissue (Foreman and Barnes 1990), and cementum like tissue with loose connective tissue inclusions (Heithersay 1975). Baldassari-Crus *et al.* (1998) examined the barrier under a scanning electron microscope (SEM). The findings were a 2-layered porous and irregular cap-like structure. The dense outer layer was acellular-like cementum and the dense inner layer consisted of irregular fibrous collagen connective tissue with granular inclusions of foreign material and irregular calcified fragments. Steiner and Van Hassel (1971) histologically examined sections of apical barriers in primate teeth. They described the barrier composition as cementum-like, and formation proceeding from the periphery of the apex towards the centre in concentric circles.

Establishing the presence and quality of a calcified barrier can be challenging. A barrier may be palpated clinically using a file but not be evident radiographically (Yates 1988, Sheehy and Roberts 1997,

Abbott 1998). Abbott (1998) does not agree that radiographs can reliably assess the quality of calcium hydroxide dressing or barrier formation and recommends regular dressing change at two or three month intervals to allow clinical barrier assessment. Walia *et al.* (2000) reported a porous structure despite radiographic and clinical evidence of a complete barrier.

The position of apical stop seems to be variable and may be related to presence of calcium hydroxide in the apical area. Mitchell and Skankwalker (1958) emphasised the necessity of direct calcium hydroxide contact with the periapical tissues for apical barrier formation. Finucane and Kinirons (1999) found most barriers formed at the radiographic apex but some more coronally. A relationship was found between more apically positioned barriers and increased frequency of calcium hydroxide dressing changes (<2 monthly), suggesting that dissolution of calcium hydroxide may allow ingrowth of apical tissue and result in a more coronal barrier. Conversely, Kinirons *et al.* (2001) also recorded the majority of barriers were formed at or near the radiographic apex, but were unable to confirm a relationship with frequency of calcium hydroxide dressing, with a variety of dressing protocols resulting in an acceptable barrier position. Dissolution (wash out) may be affected by pH of the periapical tissues, fluid exudates and the repeated pumping effects of mastication (Metzger *et al.* 2001). Wash out is more likely in wide open apices immediately following placement (Yates 1988).

Many clinical studies have investigated the success rates and time for barrier formation using calcium hydroxide (Table 2.5.1). In a summary of 10 studies success rates for barrier formation were reported of 74-100% and the mean length of time for barrier formation of 5-20 months (Sheehy and Roberts 1997).

Table 2.5.1 Studies of Calcium Hydroxide Apexification Outcome

Reference	Number	CH used	Barrier (months)	Success Rate
Heithersay 1970	21	CH & Methylcellulose	15-75	90%
Cvek et al. 1972	55	CH powder & saline	18.2 mean	90%
Winter et al. 1977	34	Reogan Rapid* =27 CH powder & sterile water=7	Not stated	74%
Chawla et al. 1986	26	Reogan Rapid*	6 (65%) 12 (35%)	100%
Ghose et al. 1987	51	Calasept	3-10	96
Thater et al. 1988	34	Pulpdent	Not stated	74%
Mackie et al. 1988	112	Reogan Rapid	10.3 mean	96%
Yates et al. 1988	22 study protocol 26 control (no protocol)	CH powder & saline Hypocal	9 (study) 20.2 (control)	100%
Kleier et al.1991	48	CH paste & CMCP Pulpdent	12 +/- 7	100%
Mackie et al. 1994	19 19	Reogan Rapid Hypocal	6.8 mean 5.1 mean	100% 100%
Finucane & Kinirons 1999	44	Not stated	7.8 mean (parallel) 11 mean (divergent)	100% 100%
Kinirons et al.2001	107	Not stated	10.8 mean	100%
Dominguez Reyes et al.2005	26	CH & distilled water	12.19 mean	100%

* *Reogan Rapid*: see Table 2.5.2

2.5.3 Factors influencing barrier formation

There are many factors thought to influence the rate of apical barrier formation during apexification including frequency of dressing change, type of trauma, size of apical foramen, apical anatomy, age of patient, presence of infection and inter-appointment symptoms.

Much controversy exists amongst the literature as to the desirable frequency of calcium hydroxide dressing change. Some authors advocate a single placement while some recommend once monthly or three monthly changes, and others believe change should only occur when radiographs indicate resorption of the apical third of dressing.

A single application is favoured by Felipe *et al.* (2005) who recently investigated the influence of renewing calcium hydroxide paste on apexification and periapical healing in 40 dog teeth. The conclusion was that replacement of calcium hydroxide paste is not necessary for apexification to occur but did decrease periapical inflammation. Similarly, Chosack *et al.* (1997) examined the development of apical osteodentine in monkey teeth and concluded that nothing was to be gained by repeated dressing with calcium hydroxide either monthly or 3 monthly for at least 6 months. Gupta *et al.* (1999) reported on a case of completed root development 7 months following a single application of calcium hydroxide. It was proposed that root development continued because necrosis had not involved the root sheath or odontoblasts. Morse *et al.* (1990) postulated that CH is necessary to promote initial healing and canal disinfection, but multiple visits re-accessing a canal with repeated cleansing may disturb the process of apexification.

Repeated applications comprising of a dressing change at 1 month and then 3 monthly until a barrier is palpated is recommended by the RCSE Guidelines (Mackie 1998). Kleier and Barr (1991) performed apexification on 48 teeth changed the dressing 3-6 monthly routinely, achieving 100% success in an average of 1 year +/- 7 months. In a recent prospective study by Dominguez Reyes *et al.* (2005) CH apexification was carried out on 26 necrotic, immature incisors. A success rate of 100% barrier formation was recorded in an average time of 12.19 months. The calcium hydroxide dressing was changed 3 monthly, an average of 3.23 sessions were required for apexification. The main disadvantage of this study was that multiple operators (undergraduates) performed the treatment so reproducibility is questionable. Finucane and Kinirons (1999) looked retrospectively at

44 teeth that had undergone apexification and found increased frequency of dressing change had a positive relationship with speed of apical barrier formation. A multicenter study of 107 teeth by Kinirons *et al.* (2001) supports this finding that barrier detection was significantly earlier in more frequently dressed teeth.

Replacement of the calcium hydroxide dressing only when a radiograph indicates resorption of medicament in the apical third or if symptoms develop is also an option (Walia *et al.* 2000, Yates 1988, Ghose *et al.* 1987). Yates (1988) examined the success rates of apexification in 22 teeth following a strict protocol, compared to 26 teeth that had been treated without any protocol. Radiographs were used to assess calcium hydroxide dressing quality at 3 and 6 monthly intervals and calcium hydroxide dressing change was carried out if found inadequate. Interestingly it was noted that more calcium hydroxide was resorbed after 1 month in teeth with wide open apices and often on changing a dressing a barrier was palpated clinically although not evident radiographically.

The initial size and anatomy of the apical foramen has been shown to affect the speed of barrier formation. Apical closure was a significant factor in relation to speed of barrier formation, with divergent apices requiring significantly more time in some reports (Yates 1988, Finucane and Kinirons 1999, Domingues Reyes *et al.* 2005). However other authors did not find apical opening significant (Ghose *et al.* 1987, Kleier and Barr 1991).

Age may be inversely related to barrier formation time, with children older than eleven needing significantly less treatment time (Mackie *et al.* 1988). This is likely to be a consequence of more mature root development.

Periapical infection has been proposed to influence barrier formation. Both Cvek (1972) and Chawla (1986) found the presence of infection increased apical barrier formation time. This emphasises the

advantage of diagnosing pulp necrosis early to avoid the development of infection and ensure a good coronal seal between appointments, preventing bacterial ingress from compromising barrier formation. Other studies found infection to be a weak predictor of the speed of barrier formation (Finucane and Kinirons 1999, Mackie *et al.* 1988, Yates 1988). Kleier and Barr (1991) found that teeth with a sizeable periapical radiolucency (>5mm) were at significantly more risk of inter-appointment symptoms. Inter-appointment symptoms were found to delay apexification by 5 months while periapical infection was found to delay or reverse barrier formation and healing.

The type of trauma is also thought to influence treatment time, as some injuries will inflict more damage on the tooth and periodontium than others. Finucane and Kinirons (1999) found that degree of displacement by trauma increased the time taken for apexification and Mackie *et al.* (1988) observed more failed of apexification in teeth that had suffered displacement injuries.

Many types of calcium hydroxide preparations have been developed over the years, some commercially available premixed (Table 2.5.2) and others mixed at chairside. Proprietary brands of calcium hydroxide paste have been developed which are usually composed of calcium hydroxide in an aqueous solutions with variable additional components that affect consistency or radiopacity (Zmener *et al.* 2007). The commercially available pastes have an advantage over chair side preparations as they often have a more creamy consistency and come with a user friendly syringe delivery system (Zmener *et al.* 2007). No robust evidence exists to demonstrate the superiority of any one commercial brand (Morse *et al.* 1990, Steinig *et al.* 2003). An *in vitro* study by Zmener *et al.* (2007) investigated the pH of three calcium hydroxide dressing materials and found Calasept (Nordiska Dental, Angelholm, Sweden) and Ultracal XS (Ultradent Products Inc., South Jordan, UT, USA) to have significantly higher alkalinizing properties than calcium hydroxide/distilled water paste. More studies are required

on the available proprietary brands to help clinicians make an evidence based decision on material choice.

Table 2.5.2 Comercial brands of calcium hydroxide preparations

Product	Composition
Pulpdent (Pulpdent Corporation, Watertown, MA, USA)	CH Methycellulose
Reogan Rapid (Vivadent, Liechtenstein)	CH Casein paste
Hypocal (Ellman, New York, USA)	98% CH paste
Calasept (Nordiska Dental, Angelholm, Sweden)	Pure CH powder Isotonic saline
Ultracal XS (Ultradent Products Inc., South Jordan, UT, USA)	CH Hydroxyapatitie Thickeners + water

Many methods of calcium hydroxide paste delivery exist, including injection, use of lentulospiral fillers, McSpadden compactors or compaction with paper points or hand pluggers (Metzger *et al.* 2001). The best method of calcium hydroxide placement is unclear from the literature, however most authors advocate filling of the canal to its full extent with a minimum amount of voids and replacement of the dressing if unsatisfactory (Yates 1988, Metzger *et al.* 2001). As previously discussed, hydroxyl ions need to be able to diffuse from the calcium hydroxide dressing via dentinal tubules or the apical foramen to reach periapical tissues and induce healing and hard tissue formation. Some authors state (Mitchell and Shankwalker 1958) that calcium hydroxide should be in contact with vital periapical tissues but this has not been definitively proven (Morse *et al.* 1990). Metzger *et al.* (2001) compared 3 methods of calcium hydroxide placement *in vitro*, with results strongly supporting condensation of thick calcium hydroxide paste using paper points over delivery with a lentulo-spiral filler or injection of a commercial paste (Calasept). It was also proposed that barium sulphate should not be included as a radiopacifier due to residual opacity effects. Peters *et al.* (2005) performed an *in vitro* study on canal preparation and modes of calcium hydroxide delivery, finding

canals prepared with a wider taper and apical size showed fewer voids. They also favoured paste delivery using lentulo-spiral fillers over paste injection to reduce voids. This study did not examine hand condensation.

2.5.4 Disadvantages of Calcium Hydroxide

Despite reports of good clinical success, there are some inherent disadvantages of using calcium hydroxide as an apexification material (Table 2.5.3).

Table 2.5.3 Disadvantages of calcium hydroxide

Diadvantages of CH
• Variable time barrier formation • Multiple visits • Compliance • Unpredictable barrier formation • Variable barrier quality and quantity • Difficult barrier detection • Prolonged temporary coronal seal • Cytotoxicity • Weakened root dentine

Although rates of barrier formation are good, the time taken to achieve this ranges from 5-20.2 months (Sheehy and Roberts 1997). This variation prevents the clinician from being able to give patients a definitive time scale of treatment completion (Steinig *et al.* 2005). It may be difficult to maintain positive morale when patients must attend indefinitely. Multiple visits and extended treatment times may be problematic and it is crucial that these teeth are not lost to follow up. The development of a hard tissue barrier is unpredictable. In some cases, despite prolonged treatment with calcium hydroxide, failure to achieve an apical stop is noted (Maroto *et al.* 2003, Karp *et al.*2006). Diagnosing the presence of an apical stop can be challenging. Often radiographic examination does not reveal a barrier palpated clinically (Yates 1988, Sheehy and Roberts 1997, Abbott 1998). The quality of barrier has also been questioned, Walia *et al.* (2000) reported a porous structure despite radiographic and clinical evidence of a complete

barrier and examination under SEM has also described a porous structure (Baldassari-Crus *et al.* 1998).

The importance of a coronal seal in endodontically treated teeth has been well documented (Ray and Trope 1995, Tronstad *et al.* 2000). Often between visits a temporary restoration is used to seal the access cavity, with a limited life span (Abbott 1998). Invasion of a root canal with bacteria is the most frequent cause of periapical pathology and root canal failure (Sjogren *et al.* 1991). Ray and Trope (1995) examined 1010 endodontically treated teeth. Good coronal restorations resulted in significantly less periapical inflammation than good endodontic obturation. A combination of good coronal restoration and good endodontic obturation showed 91.4% absence of periapical inflammation compared to 18.1% of teeth with both poor coronal and apical restorations.

Patients with immature teeth are usually young children, often 8-9 years of age and may require behaviour shaping to accept treatment. Teeth undergoing apexification may need treatment extending well into adulthood, so management must aim at producing a relaxed and compliant patient. It has been shown that over multiple visits children may become sensitised to stressful procedures (Venham and Quatrocelli 1977). If not properly managed, an anxious patient may become increasingly less able to cope with treatment over time. If cooperation of a child is lost, completion of treatment may be inhibited which will greatly decrease the prognosis for these fragile teeth and if the tooth is lost replacement will be extremely challenging with a noncompliant recipient. In cases with wide open apices in very immature roots, the treatment time will be even more prolonged, further challenging compliance issues (Witherspoon and Ham 2001). Following removal of the necrotic pulp, any symptoms will usually be resolved and patients experience little further discomfort. An asymptomatic tooth is not a good motivator for encouraging patients to return for multiple follow up visits, particularly as the benefits of

treatment provided may not be perceived by the patient (Witherspoon and Ham 2001).

The cytotoxicity of calcium has been questioned when placed immediately in a traumatised tooth. PDL cell death, cementum damage and acceleration of replacement root resorption have been reported (Foreman and Barnes 1990, Lengheden *et al.* 1991). It has been recommended to delay the placement of calcium hydroxide dressing for 7-10 days following trauma when pulp extirpation is required to avoid further inflammation of the traumatised periodontal tissues (AAE Traumatic Dental Injuries Guidelines 2004).

There have been reports in the literature that prolonged application of calcium hydroxide may weaken teeth (Cvek 1992, Andreasen *et al.* 2002, Andreasen *et al.* 2006). Dentine strength is determined by links between hydroxyapatite and collagenous fibrils and it is thought that the alkalinity and proteolytic ability of calcium hydroxide may denature carboxylate and phosphate groups causing reduced organic support and weakening of the root dentine (Andreasen *et al.* 2002). Andersen *et al.* (1992) has previously described the proteolytic capacity of calcium hydroxide. Cvek (1992) looked retrospectively at 885 luxated, non-vital maxillary incisors treated with calcium hydroxide. Calcium hydroxide was placed for an average of 24 months in the immature teeth, compared to 11 months in mature teeth prior to filling with gutta percha. Periapical healing occurred in 95% teeth following calcium hydroxide treatment, at 4-year review, with no difference between mature and immature teeth. 19% teeth experienced cervical root fracture, 63% of these within 3 years of treatment commencing. Fracture occurred after further trauma in 8% of cases, during chewing or biting in 41% and 48% of patients did not notice the fracture occurring. The frequency of fracture in immature teeth was significantly higher and relative to stage of root development with 77% of fractures occurring in the least developed roots and 28% in the most developed roots. Fracture was also significantly related to healed areas of inflammatory resorption in the cervical area. Cvek (1992)

demonstrates the high risk of root fractures in immature teeth following calcium hydroxide therapy, therefore it would be judicious to warn patients and parents of the risk of this complication. The retrospective nature, multiple operators of varying skill levels and inconsistent treatment protocol weakens this study, however it is strong on numbers allowing for meaningful statistical analysis and has an excellent follow up time.

Mackie *et al.* (1993) followed up 93 non-vital immature incisor treated with calcium hydroxide apexification and gutta percha root filling. Life table analysis suggested that 86% of teeth treated by this modality would be present after 5 years. 7% (6) of the teeth had been lost at minimum 6 month follow up, half the losses (3) were due to fracture following further trauma, 1 from an attempt at post and core placement and 2 from progressive resorption. The authors concluded that immature teeth may be weak and brittle and prone to loss from even minor trauma or operative procedures.

Andreasen *et al.* (2002) following the results of Cvek (1992) tested the hypothesis that immature teeth are weakened by calcium hydroxide dressing prior to gutta percha root filling. The study used 90 immature mandibular incisors from sheep. The teeth were divided into groups of 10 with calcium hydroxide being placed for varying time intervals from 0.5-12 months plus a control group then the fracture strength was tested. The authors concluded that calcium hydroxide dressing had a significantly negative effect on root strength, with marked decrease in fracture strength with increasing time of calcium hydroxide placement over 30 days. The fracture strength of immature sheep teeth filled with calcium hydroxide for about 1 year was halved. It was postulated that this finding may account for clinical observations of frequent fractures in immature teeth treated by prolonged calcium hydroxide therapy. Andreasen *et al.* (2006) following their *in vitro* research in 2002, examined at 30 immature mandibular incisors from sheep. The teeth were dressed with saline, calcium hydroxide, MTA or calcium hydroxide (30 days) followed by MTA. Fracture testing was carried out after 100

days (3 months). Teeth in the prolonged calcium hydroxide group showed a significant reduction (22%) in fracture strength resistance compared to the other groups. This study confirms the weakening effect of long-term calcium hydroxide on root dentine. The authors promoted short-term calcium hydroxide to dissolve pulp remnants, disinfect the canal and remove apical infection before MTA barrier placement with less detrimental effect of root strength. This *in vitro* study is well explained and executed, but is weak in numbers, with less than 10 teeth in each group. Clinical trials in this area would be beneficial in order to make evidence based treatment decisions, however this would pose ethical issues.

An *in vitro* study by Rosenberg *et al.* (2007) measured the effect of calcium hydroxide root filling on the microtensile fracture strength (MTFS) of 40 extracted human teeth. Teeth matched for size and dentine thickness, were filled with calcium hydroxide for 7, 28 or 84 days or with gutta percha (control group) and sealed. Study teeth were placed in a humidor to mimic the oral environment for the specified time period then removed for fracture testing. The results and statistical analysis showed intracanal placement of calcium hydroxide significantly weakened the teeth by 0.157 MPa/day. There was a significant difference between the MTFS of the control group and 84-day calcium hydroxide group. Overall the root dentine in the calcium hydroxide groups was weakened by 23-43.9%, leading the authors to suggest re-evaluation of the use of calcium hydroxide in clinical practice.

Overall, the evidence is leading us to reduce the time calcium hydroxide is placed in teeth to avoid detrimental consequences. The variable time frame and unpredictable nature of apical barrier formation and risk of patient compliance loss, questions the standard use of calcium hydroxide for apexification.

2.6 One visit apexification

One visit apexification has been defined by Morse *et al.* (1990) as “*non-surgical condensation of a biocompatible material into the apical portion of a root canal*”. The rationale is that an immediate apical stop is created allowing prompt canal obturation and definitive coronal restoration. It is unnecessary to wait for a biological response before obturation can proceed, the biological response occurs after the artificial barrier has been placed. The barrier formation would be of predictable thickness and quality, facilitating adequate condensation of gutta percha, and ensuring 3-dimensional obturation of the canal. This technique would allow predictable completion of treatment in a shorter time frame requiring fewer visits, decreasing the risk of attendance failure and compliance loss. Temporary coronal restorations are *in situ* for a short period, reducing the risk of coronal restoration leakage and canal re-infection.

Numerous materials have been proposed to achieve an end seal (Table 2.6.1).

Table 2.6.1 Materials for an apical end seal

Material	Author	Year
Tricalcium phosphate ceramic	Koenings et al.	1975
Dentine chips	Tronstad	1978
Freeze dried cortical bone	Rossmeil et al.	1982
Mineral Trioxide Aggregate	Torabinejad et al.	1995a
Tricalcium phosphate	Harbert	1996

Favourable clinical results have been reported with all these materials, however it is mineral trioxide aggregate (MTA) that has generated huge interest and gained significant popularity over recent years.

2.7 Mineral Trioxide Aggregate

Lee, Monsef and Torabinejad first described mineral trioxide aggregate, or MTA in 1993 at Loma Linda University, California as a new material for perforation repair. MTA received Food and Drug Administration (FDA) approval in 1998. MTA is marketed as MTA ProRoot®, (Dentsply Tulsa Dental, Tulsa, Okla, USA). Most early research has been carried out using grey MTA ProRoot®. White MTA ProRoot® was

introduced in 2002, which is more aesthetic and designed to cause less tooth discolouration (Witherspoon and Ham 2001). A new Brazilian product has been introduced onto the market, MTA Angelus (Angelus Dental Solutions, Industria de Produtos Odontologicos Ltda, Londrina, Parana, Brazil). This product is available in white or grey colour, with a very similar in composition to MTA ProRoot®, but a shorter initial setting time and improved handling characteristics (Duarte *et al.* 2003).

2.7.1 Composition and physical properties

MTA is a powder of fine hydrophilic particles that sets in the presence of moisture to form a colloidal silicate hydrate gel, which further sets to form a hard structure. Camilleri and Pitt Ford (2006) carried out a literature review on 13 papers addressing the constituents of MTA. MTA is composed of 75% Portland cement, 20% bismuth oxide and 5% calcium (Duarte *et al.* 2003). The main compounds are dicalcium silicate, tricalcium silicate, tricalcium aluminate, tricalcium oxide and tetracalcium aluminoferrite (ProRoot® MTA Product Literature, Dentsply, Tulsa Dental, Tulsa, OK 74136). MTA also contains small amounts of other mineral oxides, which modify its chemical and physical properties. A radiopacifier, bismuth oxide powder is added to enhance radiographic diagnosis (Torabinejad *et al.* 1995a). Interestingly, Asgary *et al.* (2005) found phosphorous levels close to the limit of detection, which contradicts Torabinejad *et al.* (1995a), who recognised phosphorous as an important constituent of MTA. The authors concluded that a change in MTA composition has occurred since described by Torabinejad *et al.* (1995a). Many papers have compared the constituent elements of MTA to Portland cement. Estrela *et al.* (2000) chemically compared MTA with Portland cement and concluded the chemical elements were the same except for additional bismuth in the MTA.

A comparison in the composition of grey and white MTA ProRoot® was performed by Asgary *et al.* (2005). The results showed calcium oxide, silica and bismuth oxide were the main components of both MTA

mixtures, with the main difference being increased aluminium trioxide, iron oxide and magnesium oxide in the grey MTA. Another study using Energy Dispersive Analysis by x-ray also showed white MTA and grey MTA to be very similar in composition apart from notable peaks for iron and aluminium in the grey material (Camilleri *et al.* 2005). The reduction of iron oxide in white MTA is thought to be responsible for the colour change as FeO is black. Dammaschke *et al.* (2005) examined the chemical and physical properties of white MTA ProRoot® and two common Portland cements using x-ray photoelectron spectroscopy and Energy Dispersive Analysis. The MTA contained 50% less gypsum in addition to less heavy metals, chromophores and aluminium species. The particle size of the MTA was shown to be smaller and uniform unlike the wide range of particle size in the Portland cements. Therefore MTA cannot not be substituted by ordinary Portland cement (Damaschke *et al.* 2005, Danesh *et al.* 2006).

The properties of MTA Angelus have been compared to MTA ProRoot® and found to be similar. MTA Angelus contains less bismuth oxide (Song *et al.* 2006, Duarte *et al.* 2003), and consists of 80% Portland Cement as compared with 75% in MTA ProRoot® (Duarte *et al.* 2003). Arsenic release from MTA ProRoot® and Angelus were found to be well below levels considered harmful (Duarte *et al.* 2005).

The setting reaction of MTA involves dissolution of the anhydrous cement followed by crystallisation of hydrates in an interlocking mass of crystals, with needle like projections form delineated bundles that fill the gaps between cubic crystals (Lee *et al.* 2004). The recommended mixing liquid for MTA is sterile water. An *in vitro* study has investigated the effects of mixing with alternative liquids and additives including saline, 2% lidocaine, 3.0% NaOCl gel, chlorhexidine gluconate gel, K-Y Jelly and 3 or 5% CaCl₂. Setting time was altered by the various additives, with setting time increased by lidocaine and saline, decreased by CaCl₂ and K-Y Jelly and notably an MTA product mixed with chlorhexidine gel failed to set. Compressive strengths were

significantly lower in the set materials than MTA mixed with water (Kogan *et al.* 2006).

MTA has been shown to have low solubility and porosity in aqueous environments (Torbinejad *et al.* 1995a). The soluble fraction of set MTA is calcium hydroxide, in an aqueous environment fresh water is absorbed and calcium hydroxide is released. This is a continuous osmotic process and may be responsible for some advantageous properties (Fridland and Rosado 2003). More calcium hydroxide is released with a high water to powder ratio but the consistency becomes unworkable, the water to powder ratio of 0.33 as recommended by the manufacturer is found to be ideal, allowing both calcium hydroxide release and workability (Fridland and Rosado 2003).

The radiopacity of MTA is slightly greater than dentine and gutta percha so should be easily distinguishable on radiographs (Torbinejad *et al.* 1995a). MTA has a pH of 10.2 when freshly mixed which increases to 12.5 after 3 hours (Torbinejad *et al.* 1995a).

The setting time of MTA is 3-4 hours, this may account for minimum shrink on setting and excellent sealing potential. The setting time of MTA Angelus is 15 minutes (Duarte *et al.* 2003). The faster setting time may be an advantageous quality of MTA Angelus in some applications, but little information is available as to how the change in setting time affects the workability of the material.

MTA has a low compressive strength of 40 MPa after 24 hours and 67.3 MPa after 3 days, which is comparable to intermediate restorative material (IRM®, Dentsply Caulk, Milford, DE). It is therefore not ideal for load bearing functional areas (Torbinejad *et al.* 1995a). Plugs placed in thicker sections (5mm vs. 2mm) have shown significantly higher microhardness, regardless of whether white or grey MTA was placed using a 1 or 2 step technique (Matt *et al.* 2004).

2.7.2 The physiochemical basis of MTA

MTA ProRoot® has been shown to release calcium ions and promote a high pH on immersion in solution (Duarte *et al.* 2003, Sarkar *et al.* 2005). MTA Angelus has also been shown to produce calcium ions and promote a high pH on storage in solution (Santos *et al.* 2005). Duarte *et al.* (2003) showed that pH and calcium ion release were slightly higher for MTA Angelus than ProRoot® on immersion.

The biological response to MTA is thought to be similar to calcium hydroxide (Holland *et al.* 1999), with similar mechanisms of action (Holland *et al.* 2001). This could be explained by the findings of Camilleri *et al.* (2005) that on hydration MTA produces silicate hydrate gel and calcium hydroxide. Torabinejad *et al.* (1995a) also postulated that MTA divides into discrete calcium oxide crystals and amorphous calcium phosphate. The calcium oxide crystals are thought to react with tissue fluid, forming calcium hydroxide. Sarkar *et al.* (2005) examined the physicochemical basis of the biological properties of MTA. The study investigated the interaction of MTA with a synthetic tissue fluid and root canal dentine in extracted human teeth. In phosphate rich fluids MTA undergoes sparing dissolution and releases calcium ions, which trigger precipitation of hydroxyapatite (HA) on the MTA surface (Sarkar *et al.* 2005). Amorphous calcium phosphates are produced initially, which eventually hydrolyse to crystalline apatites (Martin *et al.* 2007). The gradual dissolution of MTA leads to HA crystals growing and filling the microscopic space between the root dentine and MTA, enhancing the sealability of MTA. This seal is mechanical initially, but becomes a chemical bond due to a diffusion-controlled reaction between the root dentine and HA layer. The hard tissue layer that forms on MTA in a biological environment has previously been suggested to be calcite, a compound of calcium and carbonate due to its birefringent characteristics under polarized light (Holland *et al.* 1999). Sarkar *et al.* (2005) suggest this layer is in fact HA, which would contribute to the excellent biocompatibility of MTA. Bozeman *et al.* (2006) used SEM and X-ray diffraction (XRD) to analyse the crystal

precipitate from GMTA and WMTA and also found chemical and structural similarities to HA.

It may be concluded that MTA is not an inert, but a bioactive material in a simulated oral environment (Sarkar *et al.*2005).

2.7.3 Antimicrobial and antifungal properties

A pH of 12.5 after setting may impart some antimicrobial properties (Torabinejad 1995b). Estrela and Pesce (1996) postulated that antimicrobial activity is related to hydroxide ion release, which raises pH and creates an unfavourable bacterial environment.

Torabinejad *et al.* (1995b) used an agar diffusion test to measure the effectiveness of amalgam, ZOE, SuperEBA and MTA against nine facultative bacteria and seven strict anaerobes. MTA was found to have an antibacterial effect on five of the facultative bacteria but no effect on the anaerobes, similar to the other materials. Another study used the direct contact test to evaluate the antibacterial activity of leachable components of various root fillings. Set samples of IRM and MTA were generally more potent inhibitors of bacterial growth and activity (Eldinez *et al.*2006). Kettering and Torabinejad (1995) found MTA to be nonmutagenic against strains of *Salmonella* using the Ames mutagenicity assay.

Fungal colonization has been associated with root canal treatment failure (Al-Hezaimi *et al.*2005). Effective antifungal properties of freshly mixed and set MTA have been demonstrated *in vitro* using tube dilution tests (Al-Nazhan and Al-Judai 2003, Mohammadi *et al.*2006). WMTA was shown to be effective against *C. albicans* for up to 3 days at strong concentration (Al-Hezaimi *et al.*2005).

2.7.4 Sealability and Marginal adaptation

Microleakage is considered an important factor in the success of root end fillings. The sealability of MTA is enhanced by the formation of

hydroxyapatite crystals at the interface between root dentine and MTA (Martin *et al.* 2007). It has also been reported that both white and grey MTA expand slightly on setting, further contributing to the excellent sealing characteristics (Storm *et al.* 2008).

Various *in vitro* techniques are available to investigate microleakage including dye penetration, bacterial penetration and fluid filtration models. Torabinejad *et al.* (1993) stated that an ideal root filling should adhere and adapt to the dentinal walls of the root and prevent microleakage of bacteria and their by-products into the periradicular area. Torabinejad *et al.* (1993) in an *in vitro* study used dye penetration to evaluate the sealability of 3mm root fillings of amalgam, SuperEBE and MTA. Statistical analysis revealed that MTA leaked significantly less than the other materials. Torabinejad *et al.* (1995c) investigated marginal gaps in root end fillings and found gaps ranging from 3.8-14.9 microns with amalgam, SuperEBA and IRM, but no gaps were identified with MTA use. Tang *et al.* (2002) used a fluid filtration technique to confirm *in vitro* superior sealing ability against endotoxin compared to Super-EBA, IRM and amalgam as a root end filling. *In vitro* results for sealing and marginal adaptation of MTA Angelus were also superior to both Super-EBA and Vitremer™ as root end fillings (Xavier *et al.* 2005). Wu *et al.* (1998) assessed the seal of various root end fillings using a fluid transport model and found that the leakage of MTA decreased over time while the leakage of amalgam and SuperEBA increased. The improved seal of MTA was maintained for the full length of the experiment, 12 months. Another *in vitro* study demonstrated no significant difference against bacterial penetration of MTA compared to other root end fillings (Scheerer *et al.* 2001). Sealing ability remained unaffected by the presence of blood when compared with IRM® and SuperEBA as a root end filling, an important quality in the periapical environment (Torabinejad *et al.* 1994).

MTA has been shown by many studies to produce an excellent seal in retrograde root canal procedures but there is less evidence to support the efficacy of seal in apexification procedures (Martin *et al.* 2007).

Orthograde delivery of MTA is difficult and may allow more leakage than surgical placement as a root end filling. This has been attributed to irregularities and divergence of the apical anatomy, limited operator visibility and the necessity for lighter compaction forces to avoid extrusion through open apices (Hachmeister *et al.* 2002). Orthograde placement has been demonstrated *in vitro* to provide adequate seal against bacteria (de Leimburg *et al.* 2004), however when compared to a root filling control orthograde placement showed significantly more bacterial penetration at 60 days (Hachmeister *et al.* 2002). One *in vitro* study showed GMTA ProRoot® demonstrated significantly less dye penetration than white (Matt *et al.* 2004) when placed using a 1 or 2 step technique.

2.7.5 MTA plug thickness

Determining the optimum thickness of apical barrier is important, as it is imperative to prevent microleakage, while at the same time preserving maximum root canal length for reinforcing restorative techniques.

A 5mm orthograde apical plug of MTA has been shown *in vitro* to completely prevent bacterial leakage (Al-Kahtani *et al.* 2005). The manufacturers of MTA ProRoot® (Tulsa Dental) recommend a 3-5mm apical plug, which has been shown to demonstrate a reasonable seal and improve over 4 weeks in the presence of phosphate containing fluids (Martin *et al.* 2007). Other studies have shown no impact of plug thickness on microleakage (Hachmeister *et al.* 2002, Aminosharaie *et al.* 2003, de Leimburg *et al.* 2004). Complete obturation of the root canal space with MTA has shown no seal advantage over a 3-5mm apical plug (Martin *et al.* 2007). Plug thickness may affect resistance to displacement forces, which is important in preventing extrusion of material during condensation of gutta percha. A 4mm plug has been shown to be adequate (Hachmeister *et al.* 2002). Plugs of increased thickness (5mm vs. 2mm) have demonstrated significantly higher microhardness (Matt *et al.* 2004).

2.7.6 Method of placement and seal

Matt *et al.* (2004) carried out an *in vitro* study to investigate the effect of 1 step (immediate obturation) or 2 step apexification (24 set time). The authors hypothesised that if MTA sets in the presence of periodontal moisture during root perforation repair, then by extrapolation MTA as a root end plug can set using periapical moisture without the stage of intracanal moisture also. The authors concluded that a 2 step procedure allowed significantly less dye penetration than the 1 step technique. Another *in vitro* study demonstrated no difference in microleakage using 1 or 2 stages (Al-Kahtani *et al.* 2005).

The effect of MTA delivery method on microleakage is unclear. MTA can be placed by hand, hand assisted ultrasonic, or ultrasonic delivery. An *in vitro* study compared hand and ultrasonic condensation of MTA. Light microscopy and radiographic assessment revealed better adaptation and fewer voids within the hand condensed MTA group (Aminoshariae *et al.* 2003). Hand condensation assisted by indirect ultrasonic activation has been shown to produce heavier and more dense MTA plugs compared to hand condensation alone (Yeung *et al.* 2006). Lawley *et al.* (2004) looked *in vitro* at the effect of MTA placement techniques on bacterial leakage. A 4mm MTA plug was placed with and without ultrasonic vibration. After 90 days the ultrasonic group provided a significantly better bacterial seal. The ideal method of MTA condensation merits further investigation.

2.7.7 Use with calcium hydroxide

Some controversy exists as to whether or not it is advantageous to dress teeth with calcium hydroxide prior to MTA placement. The manufacturers of both MTA ProRoot® and MTA Angelus recommend dressing with calcium hydroxide paste for 1 week prior to apexification. Ham *et al.* (2005) evaluated apexification in monkey teeth and showed that MTA combined with calcium hydroxide may promote more rapid regeneration of the periodontium than either material in isolation. Felipe *et al.* (2006), using immature dog premolars, found an initial

application of calcium hydroxide unnecessary for apexification to occur and it was shown to be strongly associated (75%) with MTA extrusion and barrier formation beyond root canal walls. Hachmeister *et al.* (2002) found premedication with calcium hydroxide had no significant effect on MTA leakage or displacement resistance. It has been suggested that dressing with calcium hydroxide prior to obturation with gutta percha may lead to improved microleakage properties (Poraew *et al.* 1990, Holland *et al.* 1995) but this has been disputed and attributed to the model of dye penetration used (Kontakiotis *et al.* 1997).

2.7.8 Cytotoxicity

Torabinejad *et al.* (1995d) reported less cytotoxicity of fresh and set MTA than of amalgam, SuperEBA or IRM on mouse fibroblasts. Keiser *et al.* (2000) also reported low cytotoxicity of both freshly mixed and set MTA in contact with human periodontal ligament fibroblasts when compared to Super EBA and amalgam. Mixing MTA ProRoot® with chlorhexidine in place of sterile water has been shown *in vitro* to increase cytotoxicity to fibroblasts and macrophages (Hernandez *et al.* 2005). A cytotoxic assay of human endothelial cells showed similar cytotoxicity of Portland cement, ProRoot and MTA Angelus (De-Deus *et al.* 2005). Ribeiro *et al.* (2005) found no difference in the cytotoxicity of MTA Angelus compared with 2 Portland cements. MTA is therefore minimally cytotoxic and suitable for use within the periradicular tissues.

2.7.9 Biocompatibility

MTA has been reported as biocompatible in many *in vitro* and *in vivo* studies. MTA is an excellent material for use in the periapical area, as it consistently allows bone formation (Koh *et al.* 1997), cementum overgrowth (Torabinejad *et al.* 1995e, Thomson *et al.* 2003) and may facilitate repair of the periodontal ligament (Balto 2004). MTA has been shown to be biocompatible, but is not just inert and may also actively promote hard tissue formation (Sakar *et al.* 2005). Biocompatibility has been investigated using various techniques, including cell expression

and proliferation, subcutaneous or intra-osseous implants and direct tissue contact (Camilleri and Pitt Ford 2006).

Biocompatibility of MTA has been assessed *in vitro* by cell culture techniques of periodontal cells including osteoblasts, cementoblasts and fibroblasts (Chong *et al.* 2005). MTA seems to offer a biologically active substrate for osteoblasts, allowing adherence of healthy cells and stimulation of cytokine (interleukin and macrophage colony-stimulating factor) production (Koh *et al.* 1997, Koh *et al.* 1998). Haglund *et al.* (2003) did not detect stimulation of cytokine production in mouse fibroblasts and macrophages in contact with MTA. MTA Angelus and ProRoot® both showed excellent results regarding cell viability and non-interference of cytokine production in mouse macrophages (Rezende *et al.* 2005). Zhu *et al.* (2000) observed the adhesion of human osteoblasts to root end fillings by scanning electron microscopy. Attachment and spreading of an osteoblast monolayer was observed on MTA. Human alveolar cells, derived from oral surgery procedures, have been shown to attach and proliferate on set grey and white MTA. Cells have been shown to form an interfacing matrix layer in 7 days. This clinically applicable model demonstrates the ability for MTA to promote periradicular alveolar repair (Al-Rabeah *et al.* 2006). Perez *et al.* (2003) compared the reaction of rat osteoblasts and MG-63 osteosarcoma cells on GMTA ProRoot® and WMTA. The cells initially attached to both MTA types but osteoblasts did not survive on the WMTA past day 13. Primary osteoblasts were felt to be more appropriate for testing endodontic materials *in vitro*. In contrast, reports of good biocompatibility with human osteosarcoma cell line (Saos-2) with 1 day set GMTA and WMTA. 28 day set samples were less biocompatible (Camilleri *et al.* 2004).

Thomson *et al.* (2003) investigated the effect of cementoblast growth and osteocalcin production in tissue culture. Results showed MTA permitted attachment and growth of cementoblasts so can therefore be considered cementoconductive. A histological study using MTA for perforation repair in dogs, demonstrated the growth of cementum over

MTA with minimum inflammatory reaction, even when the material was extruded into the tissues (Ford *et al.* 1995). Oviir *et al.* (2006) investigated the effects of grey and white MTA on the proliferation of oral keratinocytes and cementoblasts. Cells were grown for 72 hours on either 24 hour or 12 day set MTA. Both cell types grew significantly better on the WMTA, and showed significantly higher proliferation on 12 day set GMTA than 24 hour set.

The attachment and morphology of human periodontal ligament cells in direct contact with MTA has been evaluated using a scanning electron microscope. Fibroblast cells appeared tightly attached to set MTA and proliferation occurred as the incubation period increased (Balto 2004). MTA has been shown to preferentially induce fibroblasts to express genes associated with cementum formation and osseous repair, specifically alkaline phosphatase which is a phosphate releasing protein, indicative of bone formation activity (Bonson *et al.* 2004).

An *in vivo* investigation placed intraosseous implants of MTA and Portland cement into the mandibles of guinea pigs and showed bone healing with minimal inflammatory response (Saidon *et al.* 2003). Holland *et al.* (1999b) placed dentine tubes filled with MTA or calcium hydroxide subcutaneously in rats. Results were similar for both materials, with the formation of an irregular calcific bridge at the tube openings. An initially severe reaction of coagulation necrosis was followed by induction of dystrophic hard tissue formation in a rat study by Yalirik *et al.* (2004). A similar investigation in rats also found a coagulation necrosis reaction followed by dystrophic calcification (Moretton *et al.* 2000). These studies confirm the ability of MTA to induce hard tissue formation in connective tissue cells.

2.7.10 Tissue response and barrier formation

Favourable long-term periapical tissue response has been shown following the placement of MTA. Economides *et al.* (2003) examined the use of MTA as a root end filling in dog teeth. The formation of connective tissue with occasional inflammation 1 week postoperatively

was a characteristic early tissue response. Early tissue healing was associated with hard tissue inclusions, activated by the peripheral root walls. MTA has been shown to produce a favourable periapical inflammatory tissue response and stimulation of cementum when compared with other root end fillings (Torabinejad *et al.* 1995e, Torabinejad *et al.* 1997, Baek *et al.* 2005). It is thought that MTA may produce initial coagulation necrosis, which subsides over time and is followed by calcification (Moretton *et al.* 2000). Felipe *et al.* (2006) observed a layer of necrotic tissue between the MTA plug and hard tissue barrier. When considering a biological response, initial effects are not as important as late harmful effects.

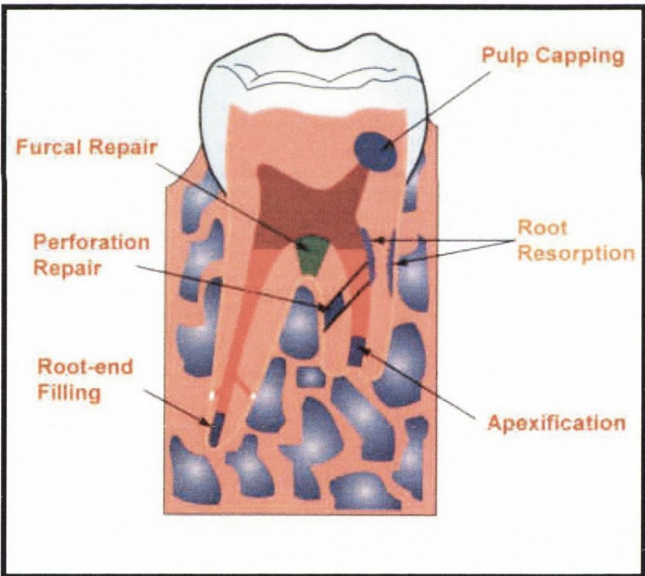
The mechanism of barrier deposition on MTA is not fully understood, however the biological cell responses have a role to play. MTA is cementoinductive as it has shown the capacity to attract cementoblastic cells and promote a favourable environment for cementum formation and osteocalcin production (Ford *et al.* 1995, Thomson *et al.* 2003). MTA has also shown stimulation of cell adhesion, proliferation and cytokine production by osteoblasts (Koh *et al.* 1997, Zhu *et al.* 2000), and stimulation of alkaline phosphatase expression by fibroblasts (Bonson *et al.* 2004). Shabahang *et al.* (1999) investigated the formation of periapical hard tissue barriers in experimentally infected, immature dog teeth using bone morphogenic protein, osteogenic protein-1, MTA and calcium hydroxide. Calcium hydroxide was placed in all teeth for 1 week to disinfect the canal, the teeth were then unfilled (control) or filled to the root apex with a dressing. After 12 weeks, complete apical closure was found in 93% of roots filled with MTA, 38.5% of calcium hydroxide and osteogenic protein-1 and none of the controls. The MTA plug that did not demonstrate barrier formation at low magnification had been extruded 2-3mm. A thin band of barrier was observed at higher magnification. The authors concluded that MTA produced hard tissue with significantly greater consistency but not quantity. Although the differences between the groups may be partly due to the short time frame it does demonstrate the rapid and reliable barrier formation with MTA. No significant difference has been shown

in the quantity of cementum formed or osseous healing associated between freshly placed or set MTA in dog teeth (Apaydin *et al.* 2003). A regenerative response is thought to occur in pulp when MTA is used as a pulpotomy medicament (Ng and Messer 2008). Nair *et al.* (2007) compared pulp healing in extracted wisdom teeth following pulpotomy with either MTA or calcium hydroxide. A necrotic layer was seen in contact with the pulp with less inflammation in the MTA group. The hard tissue barrier formed in the MTA group was thicker and less porous than in the calcium hydroxide group.

2.7.11 Clinical applications of MTA

The characteristics of MTA enable diverse endodontic applications (Figure 2.7.1).

Figure 2.7.1 Clinical applications of MTA



Possible applications include root end filling (Torabinejad and Chivian 1999, Chong *et al.* 2003), direct pulp capping (Faraco and Holland 2001), root perforation repair (Lee *et al.* 1993, Main *et al.* 2004), furcation repair (Ford *et al.* 1995), pulpotomies (Maroto *et al.* 2007) and in the treatment of internal resorption defects (Schmitt *et al.* 2003). MTA is also suitable for apexification and due to its characteristics this can be achieved in a one step procedure (Witherspoon and Ham 2001).

MTA has been described as fulfilling all the requirements of a root end filling (Torabinejad *et al.* 1995a). Chong and Pitt Ford (2005) reviewed the ideal requirements of a root end filling material (Table 2.7.1).

Table 2.7.1 Requirements of an ideal root end filling

Requirements
• Biocompatible • Adaptable to root end • Seal root end 3-dimensionally • Antibacterial • Dimensionally stable in moisture • Non-toxic • Stimulate periodontal regeneration • Not stain tooth or tissues • Radio-opaque • Long shelf life • Easy to handle • Inexpensive

Chong *et al.* (2003) carried out the first randomised prospective clinical study on the use of MTA as a root end filling. 108 patients were divided randomly into IRM or MTA groups for treatment. After 24 months the highest number of teeth with complete healing was seen in the MTA group, however this was not statistically significant. Lindebloom *et al.* (2005) reported similar findings. Guidelines for an ideal apexification material may be extrapolated from root end filling requirements.

2.7.12 MTA as an apexification material

MTA has some clear advantages over calcium hydroxide when used as an apexification material (Table 2.7.2).

Table 2.7.2 Advantages of MTA as an apexification material

Advantages of MTA
• One step technique • Predictable barrier formation • Predictable treatment time • Reduced time of temporary coronal seal • Reduced time of calcium hydroxide dressing • No decrease in fracture strength resistance • No increased risk of replacement resorption • Fulfils all criteria of an ideal root end filling • Definitive restoration not delayed

The main advantages are that a single stage technique shortens treatment time, produces a predictable barrier and encourages patient compliance (Witherspoon and Ham 2001). A reduction in calcium hydroxide dressing time is possible. This may have a positive effect on root fracture resistance (Andreasen *et al.* 2006) and avoid the increased risk of replacement resorption (Lengheden *et al.* 1991). The tooth is available for a definitive restoration almost immediately allowing reinforcement and improved aesthetics. Interruption in root development of immature teeth leaves thin, fragile dentine walls, which are liable to fracture. Teeth restored with intracanal composite have shown significantly greater fracture resistance than gutta percha filled teeth (Lawley *et al.* 2004). As MTA apexification can be performed in 1 step, there is little delay in placing a reinforcing restoration, which may further improve the long-term prognosis of MTA sealed teeth.

2.7.13 Disadvantages of MTA

MTA also has some disadvantages (Table 2.7.3). It has been reported that MTA when mixed with water has a sandy consistency, which can be difficult to manipulate. (El Meligy *et al.* 2006). Sarris *et al.* (2008) reported difficulty with rapid dehydration and retention of MTA on root canal walls. Retention of the walls may inhibit vision and reduce canal diameter during gutta percha obturation.

Table 2.7.3 Disadvantages of MTA as an apexification material

Disadvantages of MTA
• Expensive • Difficult storage • Handling properties • Microscopy required • Compliance • Technique dependant • Discolouration • Recent material-lack of familiarity • Limited evidence base

Hachmeister *et al.* (2002) identified the difficulties of orthograde apexification as vision is limited. It is thought that correct use of microscopy can significantly increase operator visibility (Sarris *et al.*

2008). The additional expense and skills required in using a microscope may discourage some operators from MTA use. The provision of MTA apexification requires fewer visits, which is favourable to encourage patient compliance. The visits are however long, may require local anaesthesia and necessitate a compliant, still patient. Not all children have the ability to cope with these demands and calcium hydroxide apexification may be a more realistic option.

The handling characteristics of MTA and microscope use, combined with the anatomical characteristics of an open apex may render the predictable placement of an MTA stop technique sensitive and difficult to achieve (Steinig *et al.* 2003). Calcium hydroxide apexification has been the standard of care for many years and is a technique with which clinicians are familiar with comfortable providing. Introducing a new, more technique sensitive and more expensive material requiring specialist equipment may be a barrier to the wider use of MTA. A recent survey on the teaching and use of MTA in 13 UK dental schools revealed only 2 schools teaching MTA for apexification (Pitt Ford *et al.* 2007). Mooney and North (2008) investigated the current opinions on MTA apexification among UK paediatric dental consultants using a questionnaire. It was reported that most consultants felt MTA was an appropriate material for apexification, however only 68.2% had been involved in its use. The biggest advantage cited was reduced visits, while only a third of applicants felt that tooth weakening was reduced. Perceived disadvantages were cost and limited evidence base, inhibiting standard use (Mooney and North 2008).

2.7.14 Role for calcium hydroxide apexification

In some situations calcium hydroxide is still, however an invaluable tool for the clinician (Table 2.7.4).

Table 2.7.4 Role for calcium hydroxide apexification

Role for calcium hydroxide
• Persistent infection • Inflammatory root resorption • Compliance • Operator skill • Lack of equipment • Limited finances

In cases where a large periapical radiolucency exists with excessive suppuration or a persistent sinus tract, calcium hydroxide may need to be replaced several times to eliminate infection and thoroughly disinfect the canal.

Long-term calcium hydroxide is indicated in the control of established external inflammatory root resorption (AAE Traumatic Injuries Guidelines 2004). It is thought that a longer dressing time is required to allow full penetration of the infected dentine tubules (Trope *et al.* 1995). Until root resorption is stable it would be unwise to proceed with obturation, tooth structure can be lost at an aggressive rate while inflammation persists (Andreasen and Andreasen 1992). Once the pulp canal is disinfected, the stimulus for resorption is removed and healing may occur (Trope *et al.* 1995).

Due to the age group involved with most apexification cases, there will be occasions when young patients will not have the ability to cope with treatment. One step apexification requires several long appointments and due to the use of microscopy patients must be able to remain motionless. Local anaesthesia is also recommended for MTA placement, as it is necessary to pack the cement directly on the periapical tissues. With a calcium hydroxide dressing, the formation of a barrier removes the necessity for anaesthesia and visits can be much swifter and less demanding of operator skill and patient co-operation. If compliance is an issue, placement of calcium hydroxide on a long-term basis may be a more acceptable option for both the child and clinician.

2.8 Existing literature

There is an abundance of *in vitro* evidence supporting the characteristics of MTA, which make it suitable as an apexification material. *In vivo* evidence is less available and severely lacking in well designed, prospective clinical trials in humans. Most of the literature investigates grey MTA ProRoot, some white MTA ProRoot, but only a handful of studies involve MTA Angelus.

2.8.1 Animal studies

Much of the original *in vivo* work by Torabinejad *et al.* investigated the favourable biological periapical response to MTA as a root end filling material in animals (Torabinejad *et al.* 1995e, Torabinejad *et al.* 1997). More recent work by Baek *et al.* (2005) also investigated tissue response caused by various root end fillings in dog teeth. MTA demonstrated the most advantageous periapical response and cementum deposition.

Other animal studies have investigated the biological reactions of MTA when placed using an orthograde technique for apexification purposes. 100% barrier formation was reported in dog teeth by Felipe *et al.* (2006). Initial placement of calcium hydroxide was strongly associated with MTA extrusion and an unnecessary adjunct for apexification. Dog teeth were also employed to compare root-end induction using MTA, calcium hydroxide and osteogenic protein, with MTA producing an apical stop with greater consistency (Shabahang *et al.* 1999). Monkey teeth enabled Ham *et al.* (2005) to demonstrate BMP-2 expression in periapical tissues of teeth with an MTA barrier, indicating bone healing. Studies investigating the effect of MTA implantation subcutaneously and intraosseously have been carried out using animal models (Holland *et al.* 1999, Saidon *et al.* 2003, Yaltirik *et al.* 2004). These studies have demonstrated the biocompatible tissue response following MTA placement.

2.8.2 Human Studies

The vast majority of literature reporting the clinical success of MTA as an apexification material is in the form of case reports. (Giuliani *et al.* 2002, Linsuwanot 2003, Maroto *et al.* 2003, Karp *et al.* 2006, D’Arcangelo *et al.* 2007). All of these papers reported successful, predictable apexification using MTA in non-vital, immature teeth that had experienced trauma. Some cases reported success of MTA following failed calcium hydroxide apexification (Maroto *et al.* 2003, D’Arcangelo *et al.* 2007) and conventional root treatment (Giuliani *et al.* 2002, Linsuwanot 2003, D’Arcangelo *et al.* 2007). Pinar Erdem and Sepet (2008) reported on 5 cases of MTA apexification in immature teeth with necrotic pulps. 2 year follow up showed clinical and radiographic success in 4 of the 5 teeth treated. Karp *et al.* (2206) and D’Arcangelo *et al.* (2007) used WMTA ProRoot, the other operators either did not specify MTA colour or used grey MTA ProRoot®.

There have been 5 more extensive clinical studies to date using MTA as an apexification material (Table 2.8.1).

Table 2.8.1 Clinical studies on MTA apexification

Reference	Study	No.	MTA	Age	F/U	Success
Pace <i>et al.</i> 2007	Case series	11 MTA	NS	11-32 years	24/12	91% complete 9% incomplete
El Meligy <i>et al.</i> 2006	Prospective	15 MTA 15 CH	NS	6-12 years	12/12	100% MTA 87% CH
Pradhan <i>et al.</i> 2006	Prospective	10 MTA 10 CH	NS	8-15 years	NS	100%
Simon <i>et al.</i> 2007	Prospective	43 MTA	GMTA + WMTA	7-53 years	12/12	81%
Sarris <i>et al.</i> 2008	Prospective	17 MTA	GMTA	7-17 years	12/12 mean	94.1% clinical 76.5% radiographic

CH= calcium hydroxide, NS = not specified

Pace *et al.* (2007) reported on a case series of 11 immature, non-vital incisors with a history of trauma, treated by MTA apexification. After 2 years, 10 teeth showed complete healing, 1 tooth showed incomplete healing however, this was thought to be related to the severity of the initial trauma.

El Meligy and Avery (2006) prospectively carried out apexification on 30 necrotic permanent incisors, in 15 children aged 6-12 years old. There was a history of trauma in 24, while 6 were non-vital due to caries. The teeth were randomly assigned to the MTA ProRoot or calcium hydroxide groups. Clinical and radiographic success at 12-month follow up was 87% and 100% for the calcium hydroxide and MTA groups respectively. The authors suggested MTA as a suitable replacement for calcium hydroxide for the apexification procedure. Although this study is carried out on a relatively small patient numbers and over a relatively short time (for calcium hydroxide apexification) it is a well designed, prospective, controlled, one operator study. It is unclear as to whether grey or white MTA ProRoot® was used and how examiners were blinded, despite different radiographic appearances between MTA and calcium hydroxide.

Another evaluation of MTA apexification was undertaken by Pradhan *et al.* (2006) in 20 teeth. The non-vital, traumatised immature incisors were alternatively allocated to either an MTA or calcium hydroxide apexification group. The age range of the 16 patients was 8-15 years. The teeth were stratified according to size of periapical radiolucency and stage of root development. The treatment was 100% successful. 70% of MTA barriers showed radiographic evidence of a biological hard tissue barrier. The 3 teeth failing to show a barrier had extruded MTA plugs. 100% of teeth in the calcium hydroxide group showed radiographic evidence of an apical barrier. The mean time for barrier formation was significantly less in the MTA group of 3.0 +/- 2.9 months versus 7.0 months +/- 2.5 months in the calcium hydroxide group. All radiolucencies were found to eventually heal, with larger lesions taking more time. There was comparable healing between the 2 groups. The

total treatment time from start to gutta percha obturation was significantly shorter in the MTA group. One operator carried out all treatments. The operator was also an examiner, and the other examiners were unable to be blinded to the material used due to possible radiographic distinction. It was unclear as to whether white or grey MTA ProRoot® was used. The teeth were reviewed radiographically every 4 weeks for at least 11 months, which seems excessive under current guidelines for traumatised teeth (AAE Traumatic Injuries Guidelines, 2004).

Simon *et al.* (2007) carried out a prospective study on one-visit apexification using MTA. 43 teeth non-vital teeth with open apices received an apexification procedure performed by one operator with minimum of 12 months follow up. Two blinded examiners assessed pre-treatment, post-treatment and review radiographs for each tooth. The average age of patients was 18 years with a range of 7-53 years. All teeth with open apices were included, incisors and molars. The reason for loss of vitality of the selected teeth is not specified. Both white and grey MTA ProRoot® were used due to suspension of marketing of the grey material. Healing was observed in 81% of cases when periapical index (PAI) and decrease of periapical radiolucency were taken into account. An apical barrier was distinguishable in only 26% cases, inferring that 55% of cases were successful but barrier detection was not possible. The authors hypothesised that the healing rate may have been lower compared to other studies due to the inclusion of posterior teeth and older adults. In addition barrier detection was low due to limited thickness of the dentine bridge being indistinguishable by radiographic assessment. This was a very well designed study with the largest sample numbers recorded using MTA. However, the lack of a control group and the large age range and tooth type are notable.

Very recently, a pilot study on the clinical evaluation of MTA for root end closure of non-vital, traumatised, immature permanent incisors in children has been published (Sarris *et al.* 2008). A total of 17 teeth

were treated in 15 children with an average age of 11.7 years. One operator carried out all treatments. GMTA ProRoot was used. Mean follow up time was 12.53 months (6-16 months range). Clinical success was seen in 94.1% of cases, 76.5% were radiographically successful and 17.6% showed uncertain radiographic outcome. Cervical discolouration was evident in 2 cases. The quality of MTA was adequate in 76.5% of cases. The final obturation was inadequate in 11.8%. It is unclear if microscopy was used for the MTA placement. This study is the first to concentrate on non-vital traumatised incisors in children. Despite the small study numbers it is well designed and addresses a gap in the literature. The authors conclude that larger, multi centre clinical trials are needed. They recommend further research on white MTA for clinical use and postulate that the expense of MTA and difficulty of handling may restrict its application.

All the existing literature mentions a need for further *in vivo* studies with larger sample sizes and longer follow up. A recent survey of UK paediatric dentistry consultants revealed that 50% felt that a lack of available robust evidence was a disadvantage for the use of MTA, despite 86.3% citing MTA as a suitable material for apexification. This further highlights the need for a multi-centre randomised controlled trial (Mooney and North 2008).

Only the most recent clinical pilot study by Sarris *et al.* (2008) investigates apexification specifically in a young paediatric population. It is often the under 11 year old age group that require apexification due to incomplete root development; therefore it seems logical that investigation of the success rates in this age group is most relevant.

Overall, MTA ProRoot® as an apexification material has experienced excellent and radiographic success of 76-100%. However, only one clinical study specified the use of white and grey MTA ProRoot® and did not compare the outcome of each material (Simon *et al.* 2007). No clinical trials have reported the use of WMTA Angelus.

In summary, there is a large gap in the literature for well designed *in vivo* studies using white MTA Pro-Root and white MTA Angelus as apexification materials in young children with non-vital, immature incisors.

2.9 Aims and objectives

The purpose of this study was to evaluate the clinical and radiographic success of white MTA ProRoot® and white MTA Angelus in one step apexification in children with non-vital, immature permanent incisors.

2.9.1 The study aims

- To investigate the clinical and radiographic success of one step apexification in traumatised, non-vital, immature permanent incisors in children using white MTA.
- To compare the clinical and radiographic success of one step apexification procedures using white MTA Angelus with a white MTA ProRoot® control.
- To compare the clinical and radiographic success of apexification using white MTA with a calcium hydroxide control group as reported in the literature.

2.9.2 The study objectives

- To establish the effectiveness of white MTA as an apexification material in immature, non-vital teeth.
- To recommend either white MTA Pro-Root or white MTA Angelus as a superior one step apexification material in teeth with immature apices requiring root canal treatment based.
- To propose white MTA as an adequate or superior replacement apexification material for calcium hydroxide in the correct clinical circumstances.

2.9.3 The null hypothesis

- There will be no difference in the clinical and radiographic success of one step apexification between the white MTA Angelus and the white MTA ProRoot® groups.
- There will be no difference in the clinical and radiographic success of apexification between the white MTA groups and the calcium hydroxide literature control group.

3 Materials and Methods

3.1 Study population

The study population was drawn from patients referred to the Trauma Clinic of the Department of Paediatric Dentistry of Dublin Dental School and Hospital. The study period was from January 2007 to June 2008. Some difficulty was anticipated in achieving adequate numbers so the study was advertised via the A&E service in Dublin Dental Hospital and the HSE Public Dental Service (Appendix 1).

3.2 Inclusion and exclusion criteria

All patients who met the inclusion criteria were eligible for participation (Table 3.2.1).

Table 3.2.1 Inclusion and Exclusion Criteria

Inclusion Criteria*	
1.	< 16 years old
2.	Immature permanent incisor root
3.	Non-vital tooth requiring endodontic therapy
4.	Restorable tooth
5.	No horizontal or vertical root fractures
6.	No active root resorption (inflammatory or replacement)
7.	≥½ root length developed
8.	Fit and healthy patient (ASA I/II)
9.	Informed consent achieved
Exclusion Criteria	
1.	Un-cooperative patient
2.	ASA>II
3.	Medical condition contraindicating endodontic therapy
4.	Root fracture
5.	Active root resorption
6.	Unrestorable tooth
7.	< ½ root length developed (<stage 2)
8.	Informed consent not achieved

(*Based on apexification criteria by Walton and Torabinejad 2002.)

3.3 Study design

3.3.1 Permission and Consent

Ethical approval was attained from the Faculty of Health Sciences Research Ethics Committee, Trinity College Dublin (Appendix 2).

A gatekeeper was appointed from the nursing staff of the Trauma Clinic. Each potential participant was approached by the gatekeeper. The study was explained and initial request for participation was sought. Information leaflets were given to all children and parents or guardians at the initial visit (Appendix 3). At a subsequent visit, prior to treatment commencement, informed consent was obtained from all participants using the consent form (Appendix 4). Letters were written to referring dentists, where applicable, explaining the nature of the intended treatment.

3.3.2 Confidentiality

Children were allocated a participant number for identification purposes. All consent forms and data capture forms were stored in a locked cupboard and computerised records were stored on a password-protected computer. Information regarding the treatment was available only to members of the study group.

3.3.3 Group allocation

Participants were numbered as they were recruited for the study. The subjects were alternately assigned to either Group 1 or Group 2 (Table 3.3.1). 1 tooth was considered 1 unit of study.

Table 3.3.1 Study Group Allocation

Group	Material
1	White MTA ProRoot®
2	White MTA Angelus

3.3.4 Pre-operative screening

A standard full assessment was carried out on each patient to assess the suitability of each candidate based on the inclusion and exclusion criteria.

A comprehensive history was taken for the following categories, presenting complaint, trauma, pain, medical, social, and dental.

Clinical examination included extra and intra oral examination, full dental charting and sensibility testing of involved teeth.

Radiographic examination was performed if none was available or if the existing radiograph was not of diagnostic quality. A periapical radiograph was taken using a size 2 film (Kodak, Carestream Health Inc., Rochester, New York 14608) and an anterior film holder (Densply Rinn Corp) at exposure 70 Kv, 0.16 seconds. This was used as a baseline radiograph for those included in the study to avoid excessive exposures.

The co-operation of the patients was assessed and recorded using the Frankl Behaviour Rating Scale (Table 3.3.2).

Table 3.3.2 Frankl Behaviour Scale

Rating	Symbol	Description	Explanation
1	- -	Definitely Negative	Refusal of treatment, crying forcefully, fearful
2	-	Negative	Reluctant, uncooperative, sullen, withdrawn
3	+	Positive	Acceptance of treatment, at times cautious
4	++	Definitely Positive	Good rapport, interested

(Frankl *et al.* 1962)

All treatment options were explained and discussed with the children and parents/guardians. Children fulfilling inclusion criteria at this point were given information sheets, consent forms and an explanation of the

study by the gatekeeper and a subsequent appointment was scheduled.

3.4 Baseline Data Capture

Baseline data was recorded for all children eligible and consented for the study using the designed data capture forms (Appendix 5).

3.4.1 Trauma history

A full and detailed trauma history was recorded, including source of referral, type of trauma, teeth involved, patient age at time of trauma, emergency treatment received e.g. splinting and subsequent management. Any pain history and need for medication was recorded in relation to the trauma. If root canal therapy had already been commenced the date of pulp extirpation and where possible, the medicament used was recorded.

3.4.2 Clinical data

Clinical information regarding the traumatised and adjacent teeth, was recorded (Table 3.4.1). Information included pain, tenderness to percussion, mobility, percussion tone, tooth colour, presence of related a sinus tract or abscess, periodontal probing depths and response to thermal (EndoFrost, -50°C, Coltene Whaledent, Langenau, Germany) and electric pulp sensibility testing (SybronEndo vitality scanner Model 2006, Sybron Dental Specialities, Glendora, CA 91740, USA).

Table 3.4.1 Clinical data recording

Tooth	TTP	Mobility	Percussion Tone	Colour	Sinus tract/abscess	PPD	EF	EPT

3.4.3 Radiographic data

A standardised baseline periapical radiograph was taken for all eligible participants, if not taken previously at the assessment stage. A size 2 film (Kodak, Carestream Health Inc., Rochester, New York 14608) with an anterior film holder using a paralleling technique (Densply Rinn Corp) at exposure setting 70 Kv, 0.16 seconds, was employed.

Radiographic data from these radiographs was recorded by 2 blinded examiners at the evaluation stage (3.6.2).

3.5 Apexification Procedure

All treatment was carried out using the same standardised protocol. The same 2 operators (MFH and AM) carried out all treatments during the Trauma Clinic sessions. Treatment was completed over three visits comprising of; root canal access, placement of the MTA plug (apexification) and finally canal obturation.

3.5.1 Visit 1: Root canal access & debridement

Local anaesthesia was administered if clinically indicated. Benzocaine gel (20%) topical anaesthetic (Topicale™, Premier Dental Products Co., Plymouth Meeting, PA 19462, USA) was applied for 5 minutes followed with infiltration with 2% xylocaine 1:80000 adrenaline (Lignospan Special, Septodont, Deproco UK LTD., Kent, England). Rubber dam isolation was achieved using either a standard Dental Dam (Coltene Whaledent Inc., Langenau, Germany) or a Dry Dam (SDI, Vasby, Sweden).

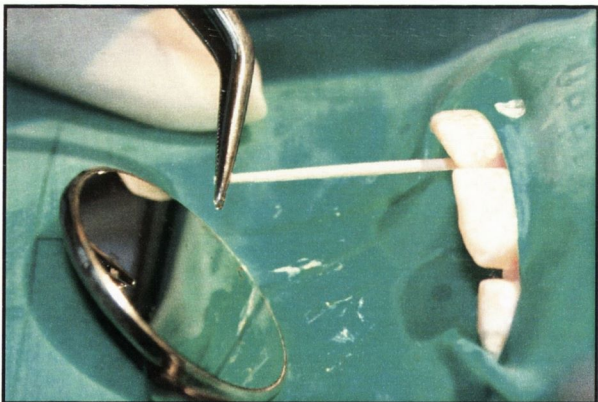
Access to the pulp chamber was gained with a diamond burr (541 Hi-Di Once, Dentsply, Ash Instruments, UK). Gates Glidden numbers 2, 3, and 4 (Dentsply, Maillefer, Switzerland) were used coronally, to gain straight line access. Mechanical debridement was achieved using K File endodontic files (Dentsply, Maillefer, Switzerland). Chemical debridement was achieved using copious irrigation with 0.2% chlorhexidine gluconate (Corsodyl, GlaxoSmithKline, Dublin 16, Ireland) (Figure 3.5.1).

Figure 3.5.1 **Irrigation of the root canal**



The working length was established using an apex locator (Root ZX, J Morita MFG Corp. Kyoto 612 Japan) and a working length radiograph. An open apex was diagnosed from radiographic appearance and confirmed clinically, by lack of apical resistance detected using a size 80 K File at the working length. Canals were dried with large sterile, paper points (Uniflex, 3M) (Figure 3.5.2).

Figure 3.5.2 **Drying of the root canal**



UltraCal XS, a calcium hydroxide paste (Ultradent Products Inc, South Jordan, Utah, USA) was placed for at least 1 week to disinfect the canal, as recommended by the manufacturers of MTA ProRoot (Dentsply Tulsa Dental) and MTA Angelus (Angelus Dental Solutions). A cotton wool pellet was placed over the calcium hydroxide, a temporary material was placed in the access cavity (Cavit, 3M ESPE, AG, Seefeld, Germany), with a glass ionomer cement (Fuji IX Extra, GC Corporation, Tokyo, Japan) in the coronal 2mm, and occlusal adjustment made.

3.5.2 Visit 2: Placement of the MTA plug

Review of signs and symptoms and a clinical examination were carried out prior to treatment commencement.

Local anaesthesia was administered where required and rubber dam isolation was achieved. The canal was re-accessed, calcium hydroxide paste was removed by chemomechanical means with copious 0.2% chlorhexidine irrigation and dried with sterile paper points. The canal was viewed under x6 magnification using a microscope (Optomic Microlux 150, Optomic, Espana). The allocated MTA was mixed according to the manufacturer instructions (see sections 3.5.3 and 3.5.4). An initial increment was delivered into the canal using a size 25 Messing Gun (Produits Dentaires, S.A, Vevey, Switzerland) (Figures 3.5.3 & 3.5.4). The MTA plug was tapped to the desired working length using premeasured, large, sterile paper points (Figure 3.5.4). A periapical radiograph was taken to confirm the initial MTA increment position (Figure 3.5.5). Following confirmation of the apical position 3-5mm MTA (as recommended by the manufacturers) were placed in the canal in increments and gently compacted using paper points. A further periapical radiograph was taken to check the correct position, thickness and condensation of the MTA plug.

A paper point, moistened with sterile water, was placed in the canal in contact with the MTA plug, followed by a cotton pellet. A temporary material was placed in the access cavity (Cavit, 3M ESPE, AG, Seefeld, Germany), with a glass ionomer cement (Fuji IX Extra, GC Corporation, Tokyo, Japan) in the coronal 2mm, and occlusal adjustment made.

Figure 3.5.3 Messing Gun

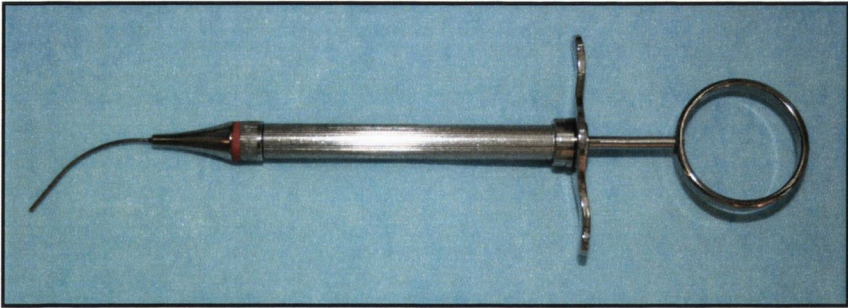


Figure 3.5.4 MTA delivery and positioning using a measured paper point

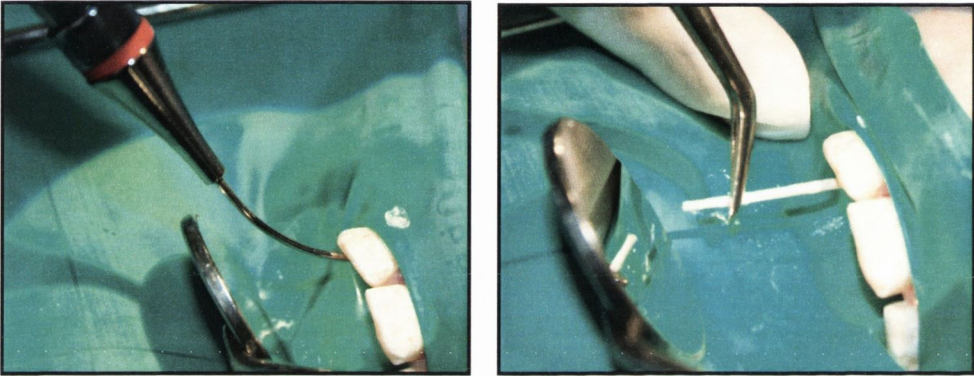
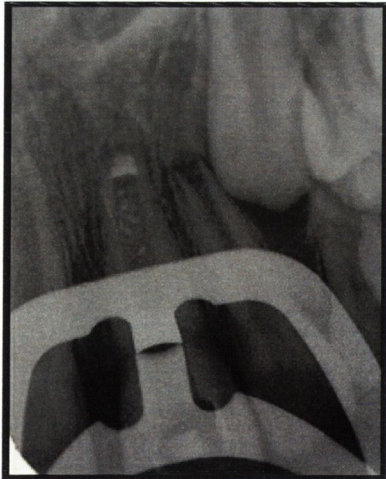


Figure 3.5.5 Radiograph to confirm position of initial MTA increment



3.5.3 White MTA ProRoot® preparation

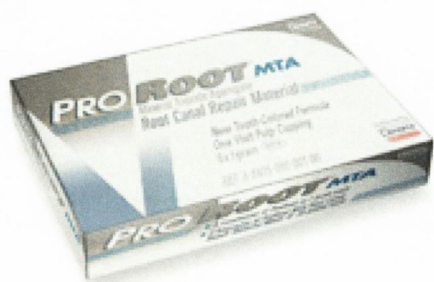
White ProRoot® MTA (Dentsply Tulsa Dental, Tulsa, Okla, USA) is marketed as two, 1 gram sachets of powder with micro ampoules of sterile water and a mixing stick at a cost of €135. The setting time is 4 hours (Figure 3.5.6).

The same batch was used for all study participants:

Batch Number: 05004913

Expiry date: 2008/12

Figure 3.5.6 White MTA ProRoot



The instructions for mixing are found on the package insert and are as follows:

The powder should be dispensed onto a mixing pad. The contents of a micro-dose ampoule of sterile water should be squeezed next to the MTA powder. The liquid should be gradually incorporated into the cement using the ProRoot[®] mixing stick and mixed for 1 minute until all particles are hydrated. More liquid may be added if necessary to make a thick creamy paste. Careful mixing is advised to make the material easier to handle. The working time is 5 minutes, but this can be extended by covering the material with wet gauze to prevent evaporation. The manufacturers recommend that each 1-gram packet is for a single use, however if smaller quantities are required the remaining MTA can be stored in sterile empty film canisters to keep from hydration. Once an ampoule of sterile water is opened it should be discarded if not finished. The MTA cement should be stored in a cool, dry place.

It is recommended that 3-5mm of MTA should be placed at the apex for apexification and a minimum of 4 hours should be allowed before obturation.

3.5.4 White MTA Angelus preparation

White MTA Angelus (Angelus Dental Solutions, Industria de Produtos Odontologicos Ltda, Londrina, Parana, Brazil) is supplied in a 2-gram container of powder, 3ml sterilized water and a scoop at a cost of €135.

The setting times are 10 minutes initial set, 15 minutes final set.

The same batch was used for all study participants:

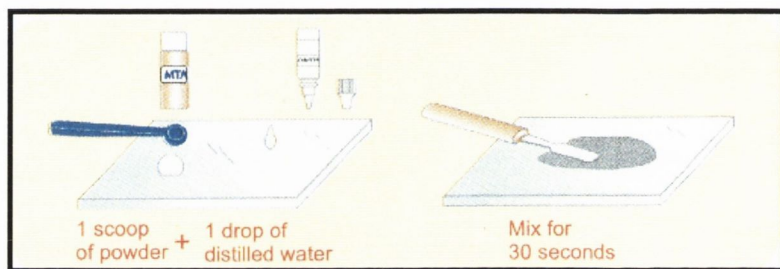
Batch number 5495

Expiry date: 07/09

The instructions for mixing are found on the package insert and are as follows:

The directions recommend dispensing 1 scoop MTA-Angelus powder and 1 drop of distilled water onto a sterilized glass slab.

Figure 3.5.7 Mixing of MTA Angelus



The powder and water should be mixed for 30 seconds with a spatula, until homogenous and with a consistency similar to wet sand (Figure 3.5.7). The scoop should be sterilized before use. The cap of the powder must be replaced immediately and the MTA should be stored in a dry, well ventilated place protected from direct sunlight.

It is recommended that 3-4mm of MTA should be placed at the apex for apexification and a minimum of 24 hours should be allowed before obturation.

3.5.5 Visit 3: Obturation

Review of signs and symptoms and a clinical examination were carried out. Rubber dam isolation was achieved and the canal was viewed under x6 microscopy. The presence of a firm barrier of MTA cement was assessed using a K File. The canal was backfilled with thermoplasticised gutta percha (Obtura II, Obtura Corp. Fenton, MO, USA), according to manufacturers' instructions and condensed using an endodontic plugger (Figure 3.5.8).

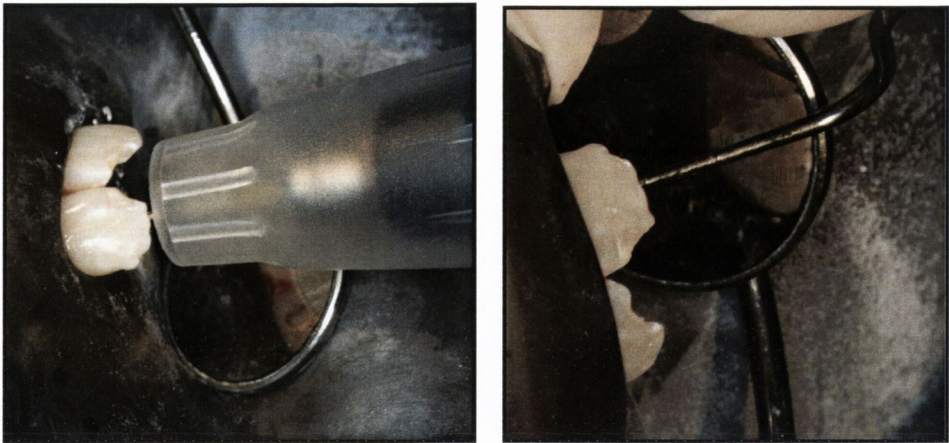


Figure 3.5.8 Injection and condensation of gutta percha

At this stage a composite resin (Esthet-X, Dentsply, Caulk, Millford, USA) coronal restoration was placed if no further restorative procedures were deemed necessary. A very bright shade (W-0) of composite was used to enable visualisation for ease of removal if necessary in the future. If further treatment was planned, a glass ionomer cement (Fuji IX Extra, GC Corporation, Tokyo, Japan) was placed on an intermediate basis. Occlusal adjustment was carried out. All restorative procedures were completed by the principal researcher.

3.6 Evaluation

Participants were scheduled for clinical and radiographic evaluation at 3, 6, 12 and 18 months.

3.6.1 Clinical evaluation

A blinded examiner carried out the periodic clinical examinations. The tooth/teeth involved in treatment and adjacent teeth were assessed for pain, tenderness to percussion, mobility, percussion tone, tooth colour, presence of a related sinus tract or abscess and periodontal probing depths and thermal and electrical sensibility testing (Table 3.6.1).

Table 3.6.1 Clinical Evaluation

Tooth	TTP	Mobility	Percussion Tone	Colour	Sinus tract/abscess	PPD	EF	EPT

3.6.2 Radiographic evaluation

A standardised periapical radiograph was taken at each review visit by the chief investigator.

Two examiners were asked to evaluate all baseline and review radiographs. The examiners were blinded to the type of MTA cement used, and also to the stage of treatment follow up. The participants were identified using their allocated study number only.

The evaluation was carried out in a darkened room with light boxes and a magnifier (x 2.5, Lic Dental, Sweden).

3.6.3 Root Development Index

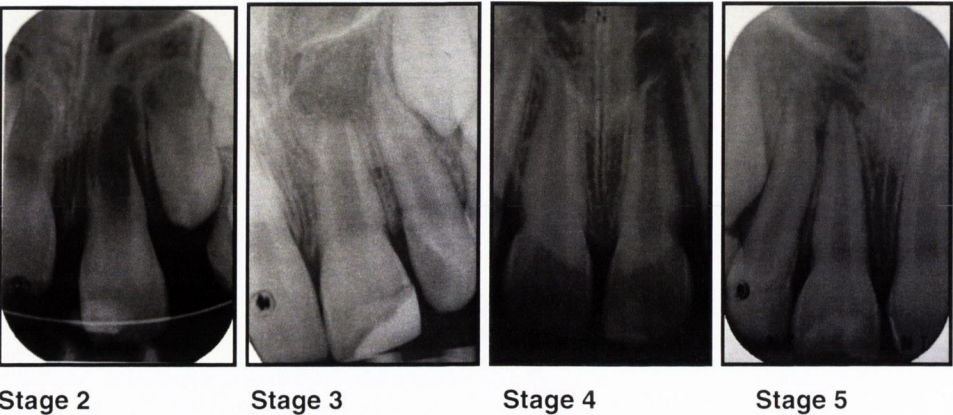
An index was used to categorise the degree of root development of each tooth involved at baseline (Table 3.6.2, Figure 3.6.1).

Table 3.6.2 Stage of root development

Stage of root development*	Description
1	¼ root length
2	½ root length
3	¾ root length
4	Full root length, open apex
5	Full root length, ½ open apex
6	Full root length, closed apex

(* developed by Moorrees et al. 1963, modified by Andreasen and Pedersen 1985)

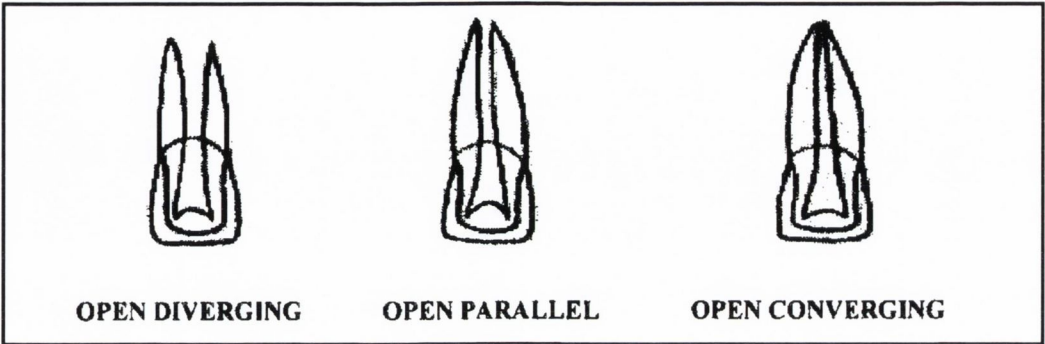
Figure 3.6.1 Stages of root development



3.6.4 Apical Anatomy

The anatomy of the apical portion of the root was recorded as parallel, diverging or converging at baseline (Figure 3.6.2).

Figure 3.6.2 Diagram of Apical Anatomy



3.6.5 PARL

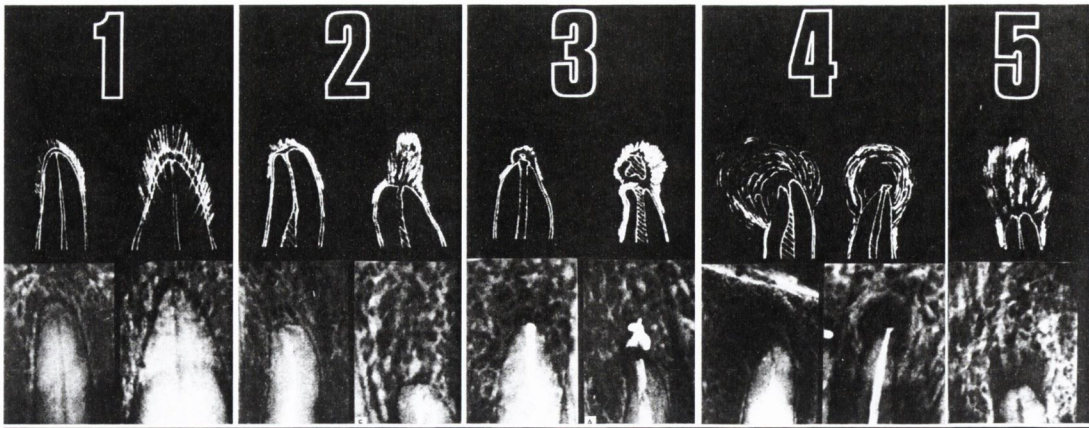
The presence or absence of a periapical radiolucency was recorded at baseline and follow up. If a PARL was present the maximum horizontal diameter was measured where possible. The presence of a PARL at baseline and the longest review radiograph were compared.

3.6.6 Periapical Index (PAI)

The periapical status of each tooth was categorised at baseline and follow up using the PAI (Orstavik *et al.* 1986). This index is recognised as a valid, accurate and reproducible way of quantifying periapical status (Orstavik *et al.* 1986). Each stage represents part of a biological continuum.

Observers were given reference radiographs, corresponding line drawings (Figure 3.6.3) and an explanation of categories, numbered 1-5 (Table 3.6.3).

Figure 3.6.3 Reference radiographs, corresponding line drawings and associated PAI score.



(Courtesy of Dag Orstavik, 2008)

Table 3.6.3: Description of PAI categories

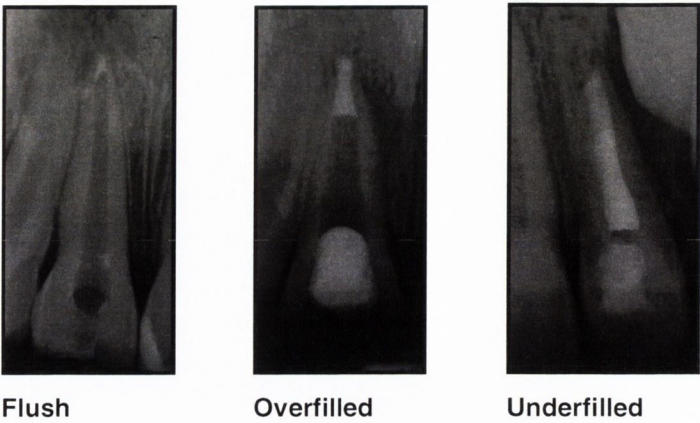
Category	Description
1	normal periapical structure
2	small changes in bone structure
3	changes in bone structure & some mineral loss
4	periodontitis & well defined periapical radiolucency
5	severe periodontitis with exacerbating features

* when in doubt, assign a higher score

3.6.7 Position of MTA plug

The MTA plug was recorded as flush with the tooth apex (F), overfilled (O) or underfilled (U); this is shown in Figure 3.6.4.

Figure 3.6.4 Radiographs showing position of the MTA plug



3.6.8 Apical Barrier

The presence of a radiographic barrier over the MTA plug was assessed on follow up radiographs. The barrier was classified as present, absent (A) or partial (P) or complete (C). This was assessed on each follow up radiograph in order to put a time scale on barrier development.

3.6.9 Calibration

The examiners were comprehensively instructed in use of the radiographic measurements by the chief investigator, using sample radiographs and diagrams.

A calibration exercise of 8-10 radiographs was carried out at the beginning of each radiographic evaluation session to ensure inter-examiner calibration. Once the examiners were calibrated, evaluation of all baseline and follow up radiographs was undertaken. Baseline radiographs were scored by both examiners, on two separate occasions, time 1 and time 2. This allowed for statistical analysis of inter-examiner reliability and intra-examiner reliability for baseline data. The follow up radiographs were scored by both examiners at 1 time period and the results were recorded on examiner recording sheets (Appendix 6).

Data from calibration, baseline and follow up radiograph scoring was recorded on formulated tables and subsequently entered into an Excel 2003[®] (Microsoft Inc., Redmond, WA, USA) spreadsheet for future statistical analysis.

3.6.10 Clinical and Radiographic criteria of success

The criteria for clinical and radiographic are outlined in Table 3.6.4. radiographic success was divided into absolute or relative success and absolute or relative failure.

Table 3.6.4 Criteria of Clinical and Radiographic success

Clinical Success
• Absence of signs and symptoms of periapical disease • Absence of pain, tenderness to percussion, periodontal probing defects or presence of a swelling/sinus tract.
Radiographic Criteria
Absolute success • Normal periodontal ligament space, contour width and structure • PAI = 1 • Absence PARL
Absolute Failure • Increase in PAI • Increase or development of PARL
Relative Success* • Decrease PAI >1 • Decrease PARL but not complete resolution
Relative Failure** • Stable PAI >1 • Stable PARL

* relative success rate = absolute success + relative success

** relative failure rate = absolute failure + relative failure

3.6.11 Statistical Analysis

All data were recorder on Microsoft Excel 2003[®] (Microsoft Inc., Redmond, WA, USA). Graphical representation of the descriptive data was generated by Microsoft Excel 2003[®].

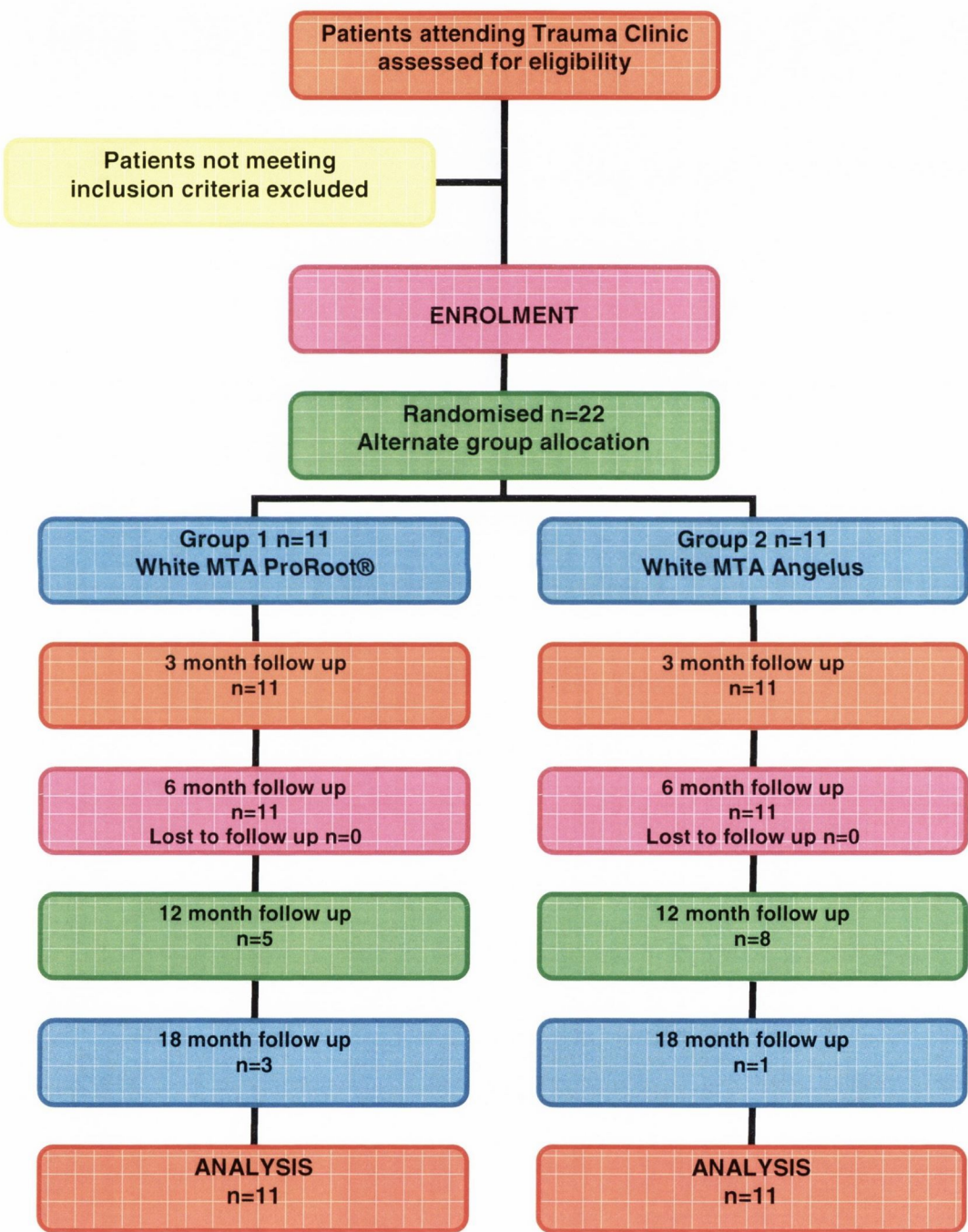
Statistical analysis was carried out using InStat[®] (GraphPad Software inc., CA, USA) and JMP[®] (SAS Institute, Cary, NC, USA) statistical softwear. Inter-examiner and intra-examiner calibration was analysed using the Kappa-Cohen test. Fisher Exact test were used to compare groups of data and a Repeated Measures ANOVA was used to assess the PAI score change over time.

4 Results

4.1 CONSORT diagram

A diagram to show the flow of participants through each stage of the study is shown (Figure 4.1.1)

Figure 4.1.1 Flow of participants through the study



4.2 Epidemiological Data

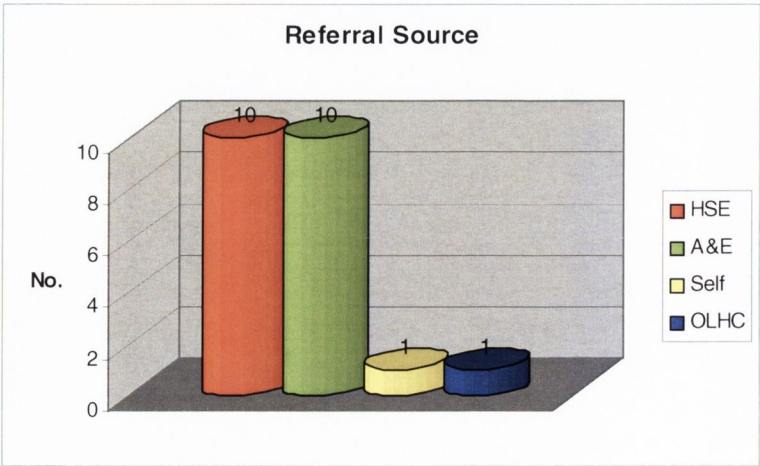
The study population was sourced from children who attended the Trauma Clinic of the Department of Child and Dental Health, Dublin Dental School and Hospital between January 2007 and January 2008. 21 children, with 22 teeth were found suitable for inclusion. Table 4.2.1 shows the distribution of sex among the study participants. There were more boys than girls in both groups and overall 72.7% males compared to 27.3% females.

Table 4.2.1 Distribution of sex in the study groups

	Male		Female	
	n	%	n	%
Group 1	10	90.9	1	9.1
Group 2	6	54.5	5	45.5
Total	16	72.7	6	27.3

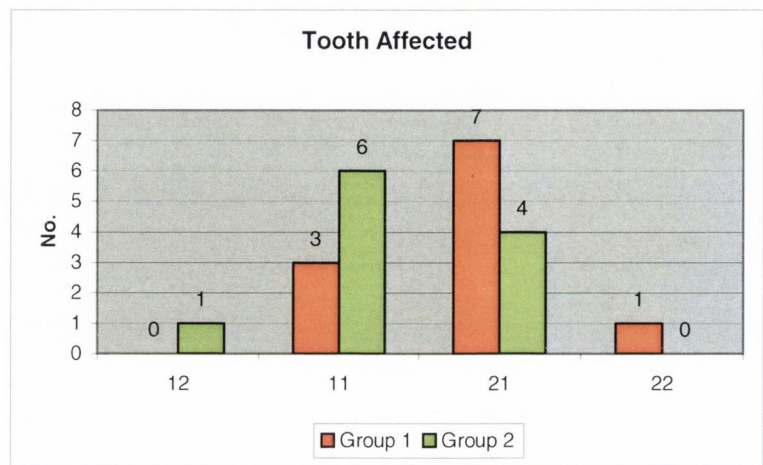
The mean age was 10 years (±2 years) with a range of 8-14 years. When considering the medical history, 22.7% (5) children had asthma and 1 child had mild von Willebrands Disease. Figure 4.2.1 illustrates the distribution of referral source. The community dental service, the HSE (Health Service Executive) was the source of 45.5%, A&E in Dublin Dental Hospital the source of 45.5% while 4.5% came from Our Ladies Hospital for Children (OLHC) and 4.5% were self referred.

Figure 4.2.1 Distribution of referral source



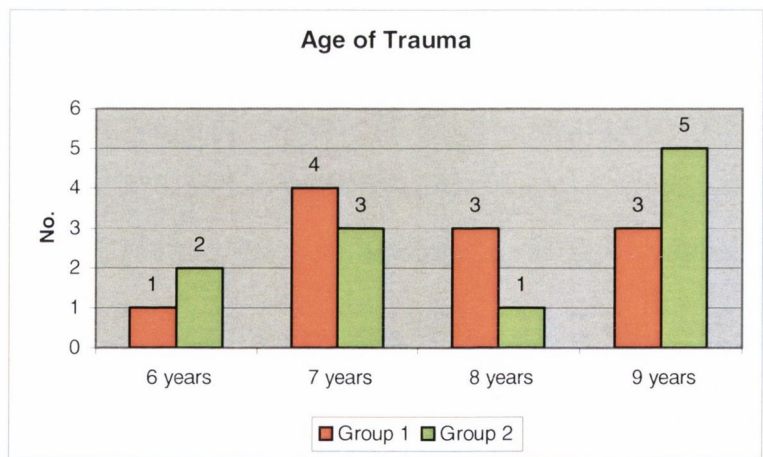
Overall, 20 permanent central incisors and 2 lateral incisors were treated, all in the maxillary arch (Figure 4.2.2).

Figure 4.2.2 Distribution of affected tooth



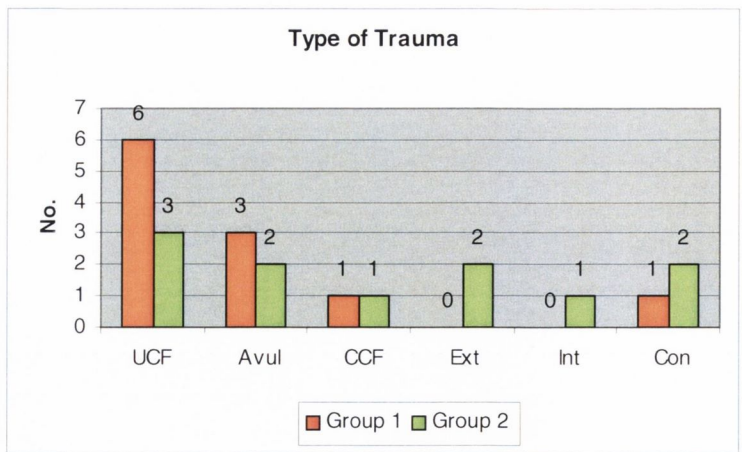
The average age that trauma was experienced was 7.8 years (± 1.1 years). The distribution is shown in Figure 4.2.3

Figure 4.2.3 Distribution of age of patient at time of trauma



Overall, uncomplicated crown fractures were the most common type of injury (40.9%), followed by avulsion (22.7%), concussion (13.6%), and finally complicated crown fractures (9.1%), extrusion (9.1%) and intrusion (9.1%). The distribution of injuries among the study groups is shown in Figure 4.2.4.

Figure 4.2.4 Distribution of type of trauma experienced



UCF= uncomplicated crown fracture, Avul=avulsion, CCF=complicated crown fracture, Ext=extrusion, Int=intrusion, Con=Concussion

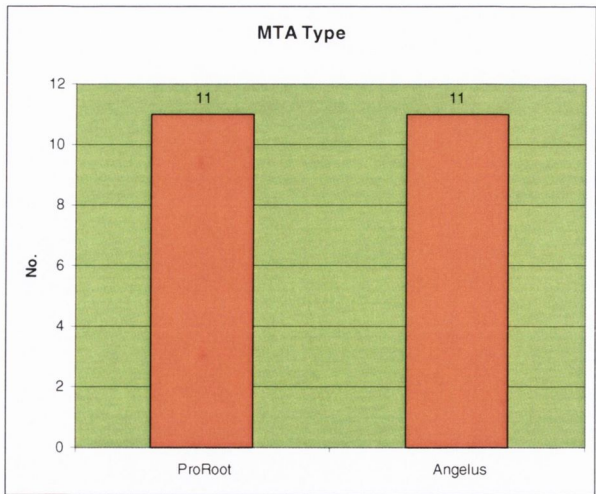
Dental anomalies were seen in 2 patients. Patient #11 in Group 1 had dens invaginatus of tooth 22. This tooth showed a successful clinical and radiographic outcome. Patient #21 in Group 2 had Amelogenesis Imperfecta, this tooth was recorded as a radiographic failure. Both these teeth had suffered concussion injuries.

4.3 Treatment Data

All 22 teeth were dressed with CH prior to MTA apexification. The average time of CH placement was 11.3 months.

WMTA ProRoot was placed in 11 teeth in alternate patients. WMTA Angelus was placed in 11 teeth (Figure 4.3.1)

Figure 4.3.1 Distribution of WMTA



Total treatment time was measured from MTA plug placement to canal obturation. The average treatment time overall was 59.2 days (8.5 weeks), with a range of 2.3-22 weeks (Table 4.3.1).

Table 4.3.1 **Distribution of total treatment time**

Group	No.	Min Tx time (weeks)	Max Tx Time (weeks)	Mean Tx time (weeks)	SD (weeks)
1	11	2.3	17	6.7	4
2	11	3.9	22	10.2	6.7

Prolonged treatment times of > 8.5 weeks were observed in 31.8% (7) teeth. Missed appointments accounted for the delay in Patients # 3,6, 7,10 & 12. Failure of the MTA plug to set occurred in Patient # 14 requiring another apexification visit. Pain in the contralateral incisor requiring treatment occupied the obturation visit of patient #20, extending the total treatment time.

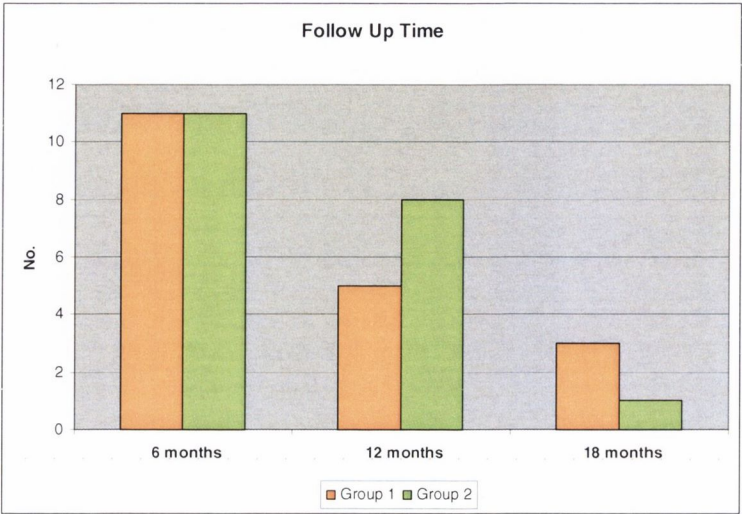
Overall the average follow up time was 10.8 months with a range of 6-18 months (Table 4.3.2 & Figure 4.3.2).

Table 4.3.2 **Distribution of follow up time**

Group	Min F/U months (n)	Max F/U months (n)	Mean F/U months	SD months
1	6 (11)	18 (3)	10.6	5.3
2	6 (11)	18 (1)	11.0	3.5

F/U = follow up

Figure 4.3.2 **Distribution of follow up times**



Overall, 100% of teeth were followed up for 6 months, 59.1% for 12 months and 18.2% for 18 months. Analysis of data was performed on the 6 month data as this was the longest follow up period recorded for the whole study sample.

Local anaesthetic was used at the MTA plug placement visit in 81.8% cases. LA was not used in 18.2% (4) cases, 13.6% (3) in Group 1 and 4.5% (1) in Group 2. Absolute success was recorded in case #19, absolute failure was shown in 2 cases (# 21 & 22) where LA was not used and 25% (1). The remaining case (#13) was of questionable outcome, scoring a relative failure in PAI outcome but a relative success in PARL outcome.

All patients showed excellent behaviour (++) on Frankl behaviour scale. Nitrous oxide sedation was employed in 1 patient (#13) to enable treatment provision.

Only 1 patient (# 4) experienced discomfort following MTA plug placement, not requiring analgesics. No patients reported discomfort following the obturation visit.

4.4 Clinical Parameters

4.4.1 TTP

One patient (#15) presented with tenderness to percussion at baseline. This symptom had resolved at 1 week review following treatment commencement and placement of calcium hydroxide dressing.

4.4.2 Mobility

Increased mobility was recorded in 7 patients at baseline: patients # 3,6,11,14,15,16 and 19. This increased mobility persisted at 3, 6, 12 and 18 month follow up only in patient #3. The increased mobility was found have returned to normal in all other teeth at 3 month review.

4.4.3 Tone

An increased tone on percussion was noted in a tooth in Group 1 (Patient #15) at baseline but did not persist to review appointments.

4.4.4 Sinus tract/swelling

A sinus tract or swelling was recorded in 7 patients at baseline (# 10, 14, 16, 17, 18, 21 & 22). Group 1 contained 2 teeth with baseline sinus or swelling, while 5 were in Group 2.

All affected teeth showed lesion resolution at 3 month review. A buccal sinus developed after 6 months in patient #3 in Group 1. In 4 teeth with baseline sinus or swellings, an absolute success of PAI score outcome was recorded, 1 was a relative success while 2 were scored as absolute failures (Figure 4.4.1).

Figure 4.4.1 Baseline Sinus or Swelling with relation to PAI outcome

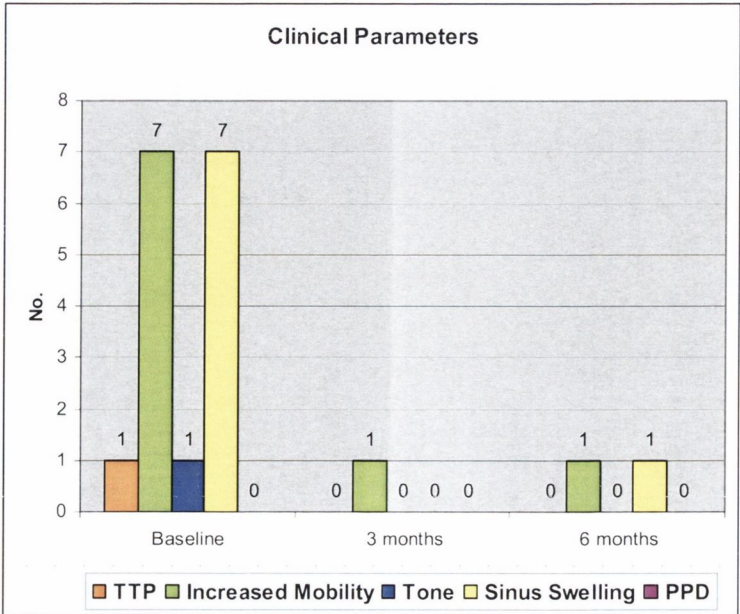
		PAI Outcome			
Group	Sinus/Swelling	AS	RS	AF	RF
1	2	1	0	1	0
2	5	3	1	1	0
Total		4	1	2	0

4.4.5 PPD

No increase in periodontal probing depths were recorded.

Figure 4.4.2 illustrates the presence of clinical parameters at baseline and follow up appointments.

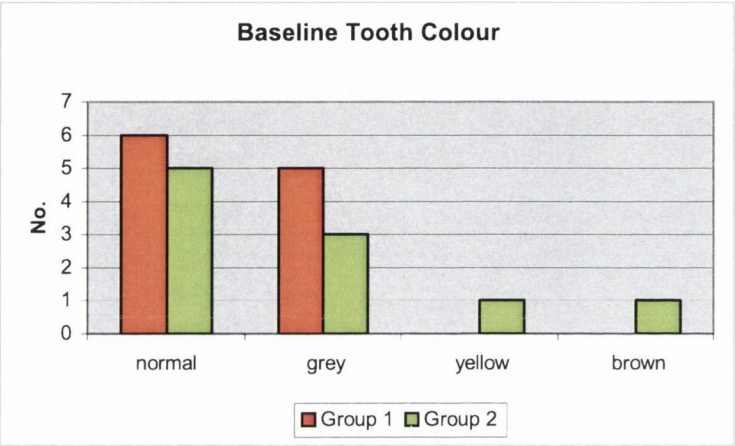
Figure 4.4.2 Clinical Parameters at baseline and follow up



4.4.6 Tooth colour

At baseline 50% of teeth were normally coloured; 6 teeth in Group 1 and 5 in Group 2 (Figure 4.4.3). Grey discolouration was recorded in 8 teeth; 5 located in Group 1 and 3 in Group 2. Yellow discolouration was seen in 4.5% (1) tooth, located in Group 2. Persistent brown discolouration due to Amelogenesis mperfecta was recorded in 1 patient, also located in Group 2.

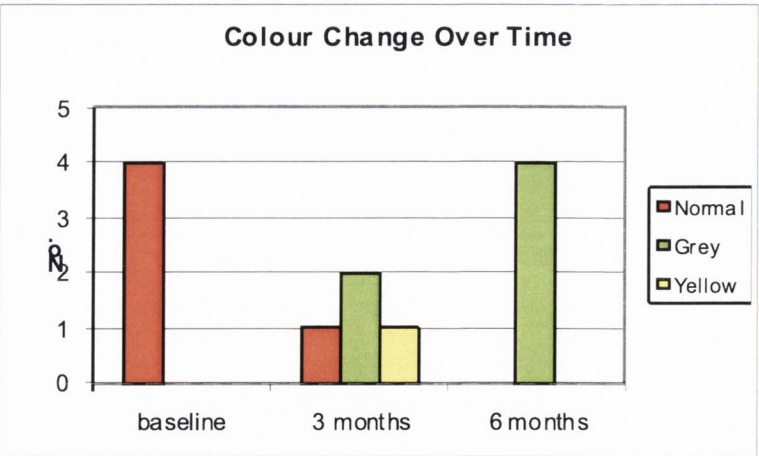
Figure 4.4.3 Distribution of tooth colour at baseline



4.4.7 Colour change

Overall, discolouration occurred in 18.2 % (4) teeth following MTA placement that were not discoloured at baseline (Figure 4.4.4).

Figure 4.4.4 Tooth colour change from baseline to 6 months



WMTA Angelus (Group 2) was the apexification material in 100% cases. The colour change was from normal to grey in 2 cases (# 4 and # 16) by 3 months. Another patient (#2) showed a yellow to grey colour

alteration by 6 months. The final case (#8) changed from normal to yellow at 3 months, then further discoloured to grey at 6 months review.

4.4.8 Clinical Success Rate

The clinical success rate is shown in Table 4.4.1. Only patient #3, in Group1, showed a clinical failure, with persistent mobility and development of a buccal sinus.

Table 4.4.1 Table showing Clinical Success Rate

	Clinical Success			
	Success		Failure	
Group	<i>n</i>	%	<i>n</i>	%
1	10	90.9	1	9.1
2	11	100	0	0
Total	21	95.5	1	9.1

4.5 Radiographic Parameters

4.5.1 Examiner calibration

Following the calibration exercises the examiners recorded baseline and follow up radiographic parameters for all patients. The results of each examiner were analysed to assess the examiner calibration.

4.5.1.1 Inter-Examiner

To assess agreement between examiner 1 and examiner 2 Kappa-Cohen test values were calculated for each of the radiographic variables shown in Table 4.5.1.

Table 4.5.1 Kappa-Cohen values of inter-examiner agreement

Radiographic Parameter	Kappa-Cohen Value (κ)
Stage Root Development	0.81
Apical Anatomy	0.78
PARL	1 (perfect agreement)
PAI	1 (perfect agreement)
Apical Barrier	0.77
MTA Plug	1 (perfect agreement)

The contingency tables of the variables are shown below (Tables 4.5.2-4).

Table 4.5.2 Root development scores: Examiner 1 vs. Examiner 2

	Examiner 2				
	Root development	2	3	4	5
Examiner 1	2	2	0	0	0
	3	0	7	0	0
	4	0	0	7	2
	5	0	0	1	3

Table 4.5.3 Apical Anatomy scores: Examiner 1 vs. Examiner 2

	Examiner 2			
	Apical Anatomy	Parallel	Divergent	Convergent
Examiner 1	Parallel	9	2	1
	Divergent	0	3	0
	Convergent	0	0	7

Table 4.5.4 Apical barrier formation: Examiner 1 vs. Examiner 2

	Examiner 2			
	Apical Barrier	Absent	Partial	Complete
Examiner 1	Absent	11	1	2
	Partial	0	5	0
	Complete	0	0	3

Substantial agreement is shown between Examiner 1 and Examiner 2 for all the radiographic parameters.

A Chi-square test of Pearson was used to compare the results for maximum diameter of the PARL recorded by examiner 1 and examiner 2. A significant difference was found between the groups ($P < 0.0001$). This should, however be interpreted with caution due to the small sample.

4.5.1.2 Intra-Examiner

To assess intra-examiner agreement between time 1 and time 2 for examiner 1 and examiner 2, Kappa-Cohen test values were calculated for each of the radiographic variables shown in Table 4.5.5.

Table 4.5.5 Kappa-Cohen Values of intra-examiner agreement

	Examiner 1	Examiner 2
Radiographic Parameter	κ Value	κ Value
Stage Root Development	0.93	0.87
Apical Anatomy (P/D/C)	0.85	0.79
PARL (Y/N)	1	1
PAI	1	1

Substantial intra-examiner agreement is shown for all variables by both examiners between the time periods.

The contingency tables for analysis of these variables are shown below (Tables 4.5.6-9).

Table 4.5.6 Root development scores: Examiner 1 time 1 vs. time 2

Ex 1	Time 2				
	Root development	2	3	4	5
Time 1	2	2	0	0	0
	3	0	7	0	0
	4	0	0	9	1
	5	0	0	0	3

Table 4.5.7 Root development scores: Examiner 2 time 1 vs. time 2

Ex 2	Time 2				
	Root development	2	3	4	5
Time 1	2	2	0	0	0
	3	0	7	0	0
	4	0	0	8	2
	5	0	0	0	3

Table 4.5.8 Apical Anatomy scores: Examiner 1 time 1 vs. time 2

Ex 1	Time 2			
	Apical Anatomy	Parallel	Divergent	Convergent
Time 1	Parallel	10	0	0
	Divergent	1	3	0
	Convergent	1	0	7

Table 4.5.9 Apical Anatomy scores: Examiner 2 time 1 vs. time 2

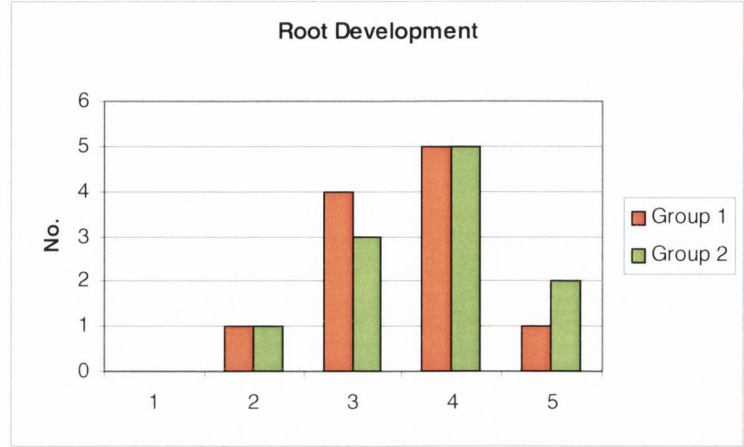
Ex 2	Time 2			
	Apical Anatomy	Parallel	Divergent	Convergent
Time 1	Parallel	8	1	1
	Divergent	1	4	0
	Convergent	0	0	7

A Chi-square test of Pearson was used to compare the results for maximum diameter of the PARL recorded by examiner 1 at time 1 and time 2. A significant difference was found between the time periods ($P < 0.0001$). An identical finding was shown for examiner 2 at time1 and time 2. These results suggest lack of intra-examiner agreement for measurement of the maximum diameter of PARL but should be interpreted with caution due to the small numbers.

4.5.2 Stage of root development

The distribution of stage of root development between both groups is shown in Figure 4.5.1. The root development distribution is similar in both groups. Stages 3 and 4 of root development were the most frequently observed, found in 77.3% of teeth overall.

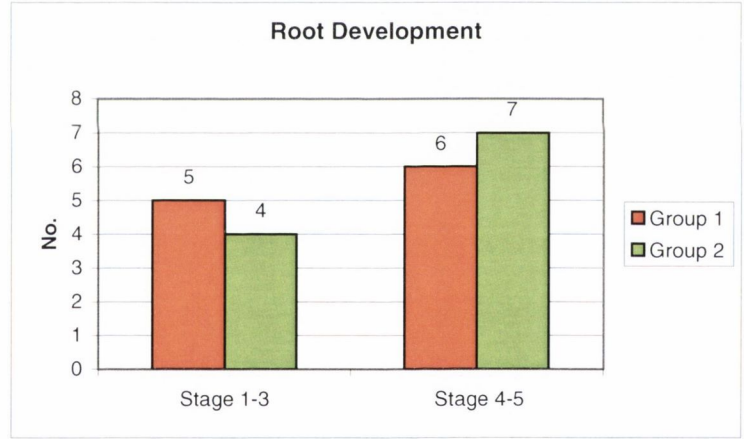
Figure 4.5.1 Distribution of Root Development



To simplify result analysis root development was divided into 2 categories (Figure 4.5.2):

- 1) Stage 1-3: very immature, wide open apices and
- 2) Stage 4-5: completed root length but open apices.

Figure 4.5.2 Simplified Distribution Of Root Development



The relationship between age and stage of root development is shown in Table 4.5.10. The younger age group (6-7 years) have 50% more teeth in the less mature category while the older age group tend to have more mature root development.

Table 4.5.10 Relationship of Root Development to Age of Trauma

	Age 6-7 years		Age 8-9 years		
Root Development	G1	G2	G1	G2	Total
1-3	3	3	2	1	9
4-5	2	2	4	5	13

G1=Group 1, G2= Group 2

Teeth with more mature root development were frequently associated with non-divergent apices in both study groups. A Fisher’s Exact Test was carried out to analyse the relationship between root development and apical anatomy in the total study population (Table 4.5.11). The two-sided P value was calculated as 0.055, which is considered as an almost significant relationship.

Table 4.5.11 Relationship of Root Development to Apical Anatomy

	Apical Anatomy					
Root Development	Divergent			Non-Divergent		
	G 1	G 2	Total	G 1	G 2	Total
1-3	1	2	3	4	2	6
4-5	0	0	0	6	7	13
Total	3			19		

G1=Group 1, G2= Group 2

Table 4.5.12 illustrates the relationship between root maturity and ideal or non-ideal plug position. An ideal MTA plug was more predictably seen related to a more mature root in both groups. A Fisher’s Exact Test was carried out to compare this relationship in the total study population (Table 4.5.12). The two-sided P value was calculated as 0.187, which is not considered to be a significant relationship.

Table 4.5.12 Relationship between Root Development and MTA Plug Position

	MTA Plug Position					
Root Development	Ideal			Non-ideal		
	G 1	G 2	Total	G 1	G 2	Total
1-3	2	2	4	3	2	5
4-5	6	4	10	0	3	3
Total	14			8		

G1=Group 1, G2= Group 2

The relationship of stage of root development and apical barrier formation is shown in Table 4.5.13. Teeth with more mature root development were more frequently associated with an apical barrier

overall. There does not appear to be a strong correlation, however an apical barrier is seen more frequently in more mature teeth in both groups (Table 4.5.13).

Table 4.5.13 Relationship of stage of root development with apical barrier presence

	Apical Barrier					
Root Development	Absent			Present		
	G1	G2	Total	G1	G2	Total
1-3	2	3	5	3	1	4
4-5	1	2	3	5	5	10
Total	8			14		

G1=Group 1, G2= Group 2

On examination, both groups demonstrated a larger number of more mature teeth with a successful PAI score outcome. A Fisher’s Exact Test was performed on the entire study population to investigate this relationship between root development and PAI score outcome. The relationship was found not to be significant (Table 4.5.14).

Table 4.5.14 Relationship between Root Development and PAI Outcome

	PAI Outcome									
Root Development	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
1-3	3	3	0	1	7	0	0	2	0	2
4-5	5	6	0	0	11	1	1	0	0	2
Total	17		1		18	2		2		4

G1=Group 1, G2= Group 2

Table 4.5.15 shows the relationship between root maturity and PARL outcome. Absolute and relative success occurred more frequently in more mature teeth, but a similar number of unsuccessful outcomes were seen in both root development groups.

Table 4.5.15 Relationship between root development and PARL outcome

Root Development	PARL Outcome									
	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
1-3	0	3	1	0	4	1	0	0	0	1
4-5	4	3	0	0	7	1	1	0	0	2
Total	10		1		11	3		0		3

G1=Group 1, G2= Group 2

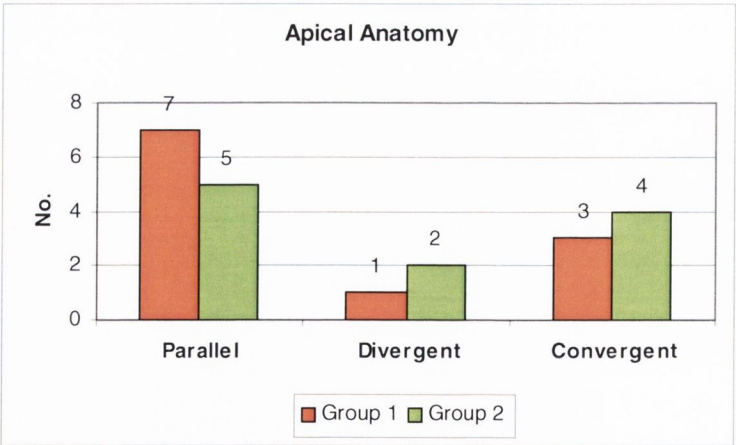
4.5.3 Apical anatomy

Parallel apical anatomy was recorded in 54.6 % teeth compared to only 13.6 % with diverging and 31.8% with a converging shape. Figure 4.5.3 depicts the distribution within the study groups.

In order to simplify result analysis and reflect clinical relevance, apical anatomy was arranged into 2 categories:

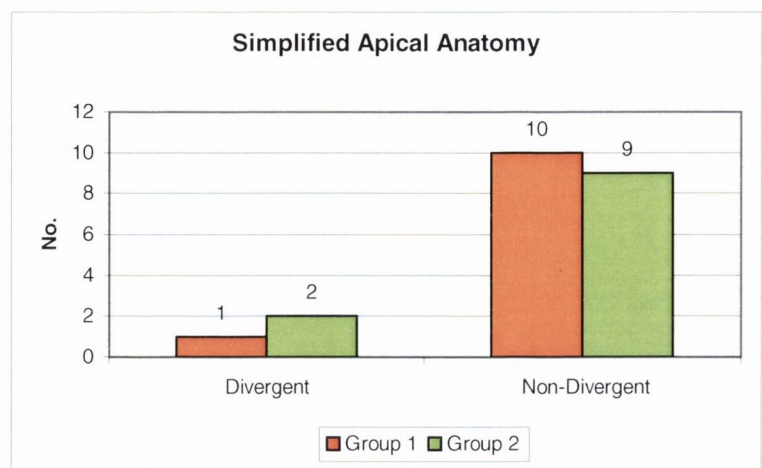
- 1) Divergent apices
- 2) Non-divergent. (Parallel and convergent apices combined)

Figure 4.5.3 Apical Anatomy Distribution within the Groups



The distribution of the simplified categories within the study groups is shown in Figure 4.5.4. Non-divergent apices occur much more frequently; 86.4% cases compared to only 13.6% with divergent apices.

Figure 4.5.4 **Distribution of simplified apical anatomy within the study groups**



On examination of the relationship between apical anatomy and root development, it was noted that non-divergent apices are more frequently associated with more mature roots (See Table 4.5.11).

The relationship between apical anatomy and the existence of a baseline PARL is displayed in Table 4.5.16.

Table 4.5.16 **Relationship between apical anatomy and baseline PARL**

Apical Anatomy	Baseline PARL					
	Absent			Present		
	G 1	G 2	Total	G 1	G 2	Total
Divergent	1	0	1	0	2	2
Non-Divergent	3	5	8	7	4	11
Total	9			13		

G1=Group 1, G2= Group 2

The presence of a PARL was observed more frequently in non-divergent apices than divergent, in both study groups. A Fisher’s Exact Test revealed a non-significant relationship between these variables with a P value of 1.

Table 4.5.17 represents the relationship of apical anatomy to the MTA plug position. A greater number of ideal plugs were associated with non-divergent apices in both study groups and no ideal plugs were observed in divergent apices in either group.

Table 4.5.17 Relationship of apical anatomy to MTA plug position

	MTA Plug Position					
Apical Anatomy	Ideal			Non-ideal		
	G 1	G 2	Total	G 1	G 2	Total
Divergent	0	0	0	1	2	3
Non-Divergent	8	6	14	2	3	5
Total	14			8		

G1=Group 1, G2= Group 2

A Fisher's Exact Test was performed out to compare this relationship between the apical anatomy and position of the MTA plug in the total study population (Table 4.5.17). The two-sided P value was calculated as 0.04, which is considered as a significant relationship.

Table 4.5.18 depicts the relationship between apical anatomy and the formation of an apical barrier. In both study populations an apical barrier formed more readily in teeth with non-divergent apices. A Fisher's Exact Test was performed to compare this relationship between the apical anatomy and apical barrier formation in the total study population (Table 4.5.18). The two-sided P value was calculated as 0.53, which is considered as non-significant relationship.

Table 4.5.18 Relationship between the apical anatomy and the presence of an apical barrier

	Apical Barrier					
Apical Anatomy	Present			Absent		
	G 1	G 2	Total	G 1	G 2	Total
Divergent	1	0	1	0	2	2
Non-Divergent	7	6	13	3	3	6
Total	14			8		

G1=Group 1, G2= Group 2

Table 4.5.19 shows the relationship between apical anatomy and the change in tooth PAI status between baseline and 6 months. Both groups show similar results. A successful PAI score change over time

was more frequently associated with non-divergent apices in both study groups. A Fisher's Exact Test was carried out to compare this relationship in the total study population (Table 4.5.19). The two-sided P value was calculated as 1.0, which is considered a non-significant relationship.

Table 4.5.19 Relationship between Apical Anatomy and PAI outcome

Apical Anatomy	PAI Outcome									
	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Divergent	1	1	0	1	3	0	0	0	0	0
Non-Divergent	7	8	0	0	15	1	1	2	0	4
Total	17		1		18	2		2		4

G1=Group 1, G2= Group 2

The relationship between apical anatomy and the fate of associated periapical radiolucencies is illustrated in Table 4.5.20. Both groups show similar results. Successful healing of peri-apical pathology is recorded more frequently in association with non-divergent apices in both study groups. A Fisher's Exact Test of the group as a whole calculated a two-sided P value of 1.0, representing a non-significant relationship between these variables.

Table 4.5.20 Relationship between Apical Anatomy and PARL outcome

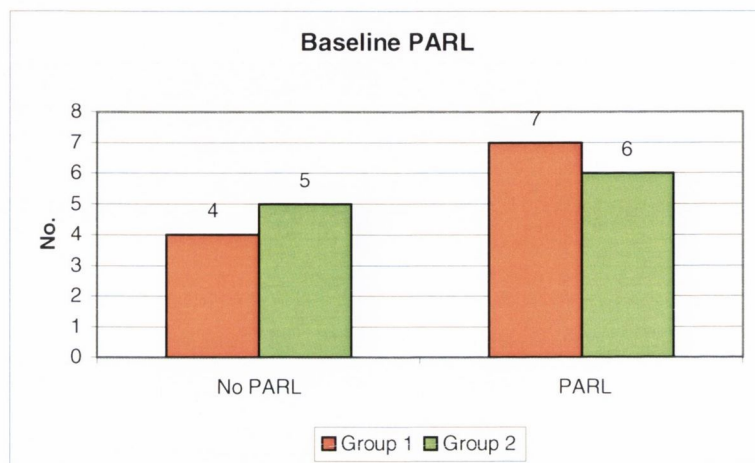
Apical Anatomy	PARL Outcome									
	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Divergent	0	2	0	0	2	0	0	0	0	0
Non-Divergent	4	4	1	0	9	2	1	0	0	3
Total	10		1		11	3		0		3

G1=Group 1, G2= Group 2

4.5.4 Baseline PARL

The overall presence of a PARL at baseline was recorded in 59.1% (14) teeth, while 40.9% (9) teeth had no PARL. This was distributed similarly between the study groups (Figure 4.5.5).

Figure 4.5.5 Presence of a PARL at baseline



The relationship between apical anatomy and the presence of a baseline PARL is displayed in Table 4.5.15. Existence of a PARL was associated more frequently in non-divergent apices than divergent, in both study groups. A Fisher's Exact Test revealed a non-significant relationship between these variables with a P value of 1 (see Table 4.5.15).

Table 4.5.21 depicts the relationship between presence or absence of a PARL at baseline with MTA plug placement. Group 1 showed more periapical radiolucencies related to ideal than non-ideal plugs, whereas the reverse is true of group 2. There were the same number of ideal MTA plugs regardless of PARL in the group overall. There were however overall, more periapical radiolucencies related to non-ideal plugs.

Table 4.5.21 Relationship between MTA plug position and baseline PARL

	MTA Plug Position					
Baseline PARL	Ideal			Non-ideal		
	G 1	G 2	Total	G 1	G 2	Total
absent	3	4	7	1	1	2
present	5	2	7	2	4	6
Total	14			8		

G1=Group 1, G2= Group 2

The effect of baseline periapical pathology seems to have little relationship to the formation of an apical barrier with similar rates of barrier formation in groups with and without a PARL overall. Within the groups, Group 2 demonstrated barrier formation more frequently in teeth without associated baseline pathology but this pattern was not observed in Group 1 (Table 4.5.22).

Table 4.5.22 Apical barrier formation with relation to baseline PARL status

	Baseline PARL					
Apical Barrier	Absent			Present		
	G 1	G 2	Total	G 1	G 2	Total
Absent	1	1	2	2	4	6
Present	3	4	7	5	2	7
Total	9			13		

G1=Group 1, G2= Group 2

The radiographic outcome as measured by the PAI index was related to the presence or absence of a PARL at baseline (Table 4.5.23). Overall absolute success and relative success occurred in equal numbers regardless of the presence of a baseline PARL. The distribution was similar among both study groups except in Group 1, 2 relative failures occurred in the presence of a baseline PARL, while no relative failures were observed in Group 2.

Table 4.5.23 PAI outcome in relation to baseline PARL status

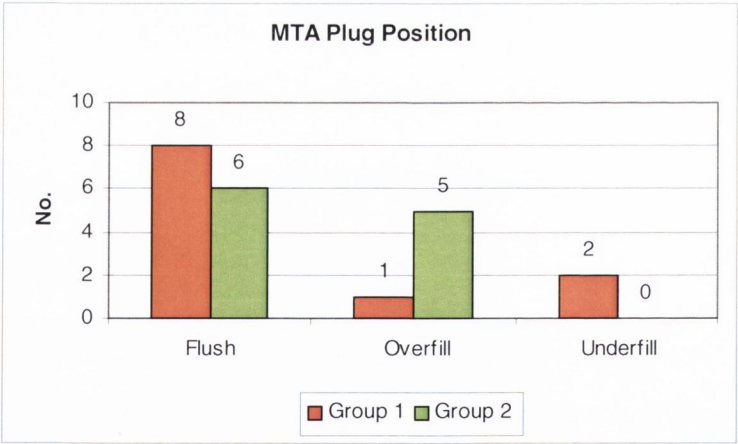
	PAI Outcome									
Baseline PARL	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Absent	4	5	0	0	9	0	0	0	0	0
Present	4	4	0	1	9	1	1	2	0	4
Total	17		1		18	2		2		4

G1=Group 1, G2= Group 2

4.5.5 MTA plug position

When the quality of the MTA plug was examined, overall 63.6 % (14) were scored as flush with the apex, 27.3% (6) were overfilled and 9.1% (2) underfilled. Figure 4.5.6 shows the distribution of plug position between the 2 study groups. In Group 1 72.7% plugs were ideal compared to 54.5% in Group 2. There were more overfilled teeth in Group 2 than Group 1 (5 vs. 1) but less underfilled teeth (0 vs. 2).

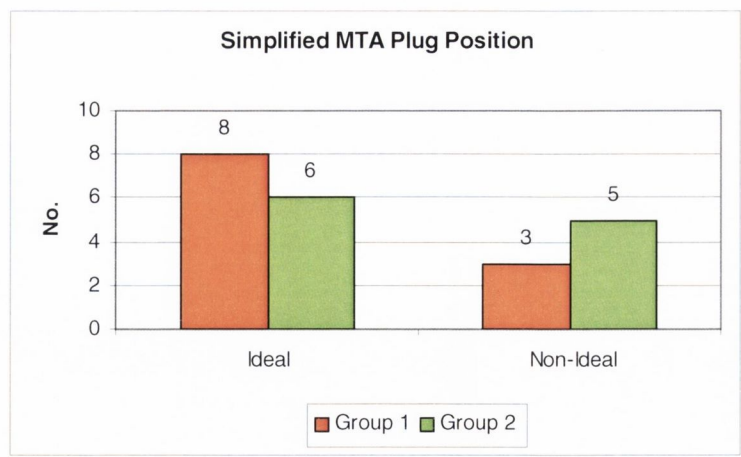
Figure 4.5.6 Distribution of MTA Plug Position



In order to simplify result analysis and reflect clinical relevance, plug position was placed into 2 categories (Figure 4.5.7):

- 1) Ideal (flush)
- 2) Non-ideal (overfilled + underfilled combined)

Figure 4.5.7 Distribution of Simplified MTA Plug Position



The relationship between root maturity and ideal or non-ideal plug position revealed an ideal MTA plug was more predictably seen related to a more mature root in both groups. This was not found to be a statistically significant relationship (see Table 4.5.12).

The relationship between the ideal or non-ideal plug position and a divergent or non-divergent apical pattern in both groups, with both groups showing a strong trend towards ideal plugs associated with non-divergent apices and no ideal plugs associated with divergent apices. In the group as a whole the relationship was statistically significant with a two-tailed P value of 0.04 (see Table 4.5.17).

The relationship between presence or absence of a PARL at baseline and MTA plug position was examined. There are the same number of ideal MTA plugs regardless of PARL in the group overall. There are however overall, more periapical radiolucencies related to non-ideal plugs (see Table 4.5.21).

The effect of MTA plug position on apical barrier formation is shown in Table 4.5.24. Overall, a larger number of cases showed apical barrier formation in the presence of an ideal MTA plug position. This is similar within both study groups. An apical barrier failed to form with similar frequency, regardless of plug position.

Table 4.5.24 Relationship of MTA plug position to apical barrier presence

	Apical Barrier					
MTA Plug	Absent			Present		
	G 1*	G 2	Total	G 1	G 2	Total
Ideal	2	2	4	6	4	10
Non-Ideal	1	3	4	2	2	4
Total	8			14		

A large number (13) of ideal MTA plug positions were associated with PAI absolute success in the sample as a whole compared to only 5 teeth with non-ideal plugs experiencing absolute success (Table 4.5.26). A similar trend was seen in both study groups. Overall more teeth showing less favourable outcomes were related to non-ideal plugs, with 3 out of 4 unsuccessful outcomes possessing non-ideal plug position (Table 4.5.25). The association was not statistically significant relationship with the numbers available.

Table 4.5.25 Relationship between MTA plug position and PAI outcome

	PAI Outcome									
MTA Plug position	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Ideal	7	6	0	0	13	1	0	0	0	1
Non-ideal	1	3	0	1	5	0	1	2	0	3
Total	17		1		18	2		2		4

G1=Group 1, G2= Group 2

Table 4.5.26 shows the influence of MTA plug position on the fate of periapical radiolucencies. Complete healing of a PARL was reported in relation to an ideal apical plug in 4 teeth in Group 1, compared to only 2 in Group 2. Healing in relation to non-ideal MTA plugs were seen in 4 teeth in Group 2 but none in Group 1. One case of PARL reduction without complete healing was noted with a non-ideal MTA plug. Both groups had an absolute failure related to a non-ideal plug, while Group

1 had a failure related to an ideal plug. There does not appear to be a strong relationship between the MTA plug position and healing of PARL.

Table 4.5.26 Relationship between MTA plug position and PARL outcome

	PARL Outcome									
MTA Plug position	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Ideal	4	2	0	0	6	1	0	0	0	1
Non-Ideal	0	4	1	0	5	1	1	0	0	2
Total	10		1		11	3		0		3

G1=Group 1, G2= Group 2

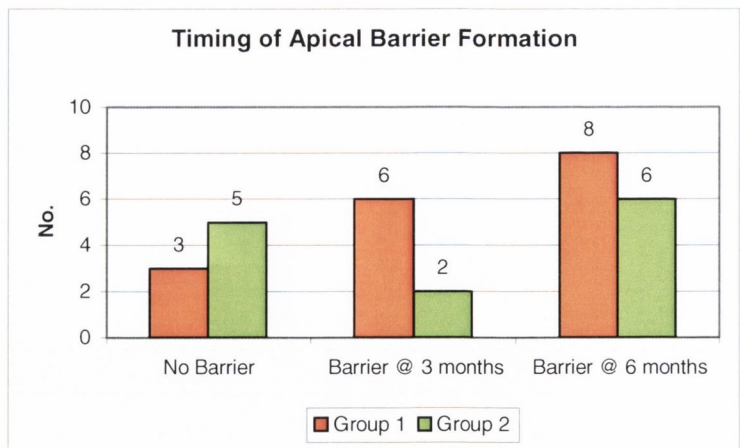
In 2 cases (#10 and 14) a separation was noted within the MTA plug following the obturation visit. Both of these cases were in Group 2. In one case (#14) the initial MTA plug placed had failed to set necessitating replacement.

4.5.6 Apical barrier

Group 1 showed superior barrier formation at both 3 and 6 month follow up. At 6 months 72.7% teeth in Group 1 demonstrated barrier presence compared to only 54.6% in Group 2. An apical barrier failed to form in 27.3% of teeth in Group 1 and 45.5% of teeth in Group 2 at 6 month follow up.

Overall an apical barrier was distinguished in 36.4% (8) of teeth by 3 months, 63.6% (14) by 6 months and 36.4% (8) did not form an apical barrier during the specified study period (Figure 4.5.8).

Figure 4.5.8 Timing of apical barrier formation



Apical barrier formation was shown to occur more often in teeth with more advanced root development (see Table 4.5.13).

Both study populations showed apical barrier formation more readily in teeth with non-divergent apices. A Fisher’s Exact Test was performed out to compare this relationship between the apical anatomy and apical barrier formation in the total study population (see Table 4.5.18). The relationship was not found to be statistically significant.

The presence of baseline periapical pathology seems to have little effect on the formation of an apical barrier, with similar rates of barrier formation in groups with and without a PARL overall (see Table 4.5.22).

The effect of MTA plug position on apical barrier formation was examined. Overall, a larger number of cases showed apical barrier formation in the presence of an ideal MTA plug position. This is similar amongst both study groups. An apical barrier failed to form with similar frequency regardless of ideal or non-ideal plug position (see Table 4.5.25).

In 92.9% (13) of cases (7 in Group1, 6 in Group 2) apical barrier formation was seen in the context of absolute success using the PAI score. No teeth with an outcome of absolute failure developed an apical barrier. Group 1 contained the only case (#3) of barrier formation in the presence of relative failure, where the PAI had remained stable >1(Table 4.5.27). In 76.5% cases absolute success was accompanied by apical barrier formation or continued root

development, 23.5% cases of AS failed to develop a barrier in the study time.

Table 4.5.27 Relationship between presence of an apical barrier and PAI outcome

Apical Barrier	PAI Outcome									
	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Absent	1	3	0	1	5	1	1	1	0	3
Present	7	6	0	0	13	0	0	1	0	1
Total	17		1		18	2		2		4

G1=Group 1, G2= Group 2

In 50% (7) cases (4 in group 1, 3 in group 2) an apical barrier formed in tandem with complete healing of a PARL. A solitary case (# 3) of apical barrier formation occurred in relation to increasing size of a PARL in Group 1(Table 4.5.28).

Table 4.5.28 Apical barrier formation in relation to PARL outcome

Apical Barrier	PARL Outcome									
	AS		RS		Total	AF		RF		Total
	G1	G2	G1	G2		G1	G2	G1	G2	
Absent	0	3	1	0	4	1	1	0	0	2
Present	4	3	0	0	7	1	0	0	0	1
Total	10		1		11	3		0		3

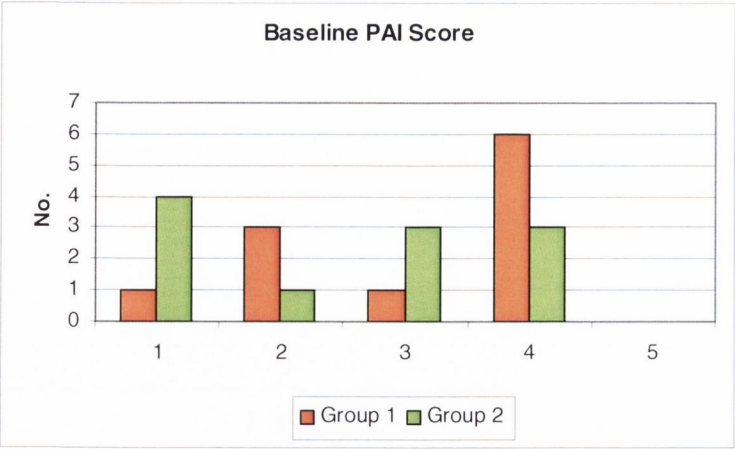
G1=Group 1, G2= Group 2

Continued root development was also noted in some cases (# 2, 5, 9, 10, 11, 17 and 19) contributing to the radiographic appearance of an apical barrier. In one of the cases of absolute failure (#3) an estranged root fragment developed separately to the root. This root fragment subsequently became infected, requiring surgical removal.

4.5.7 PAI

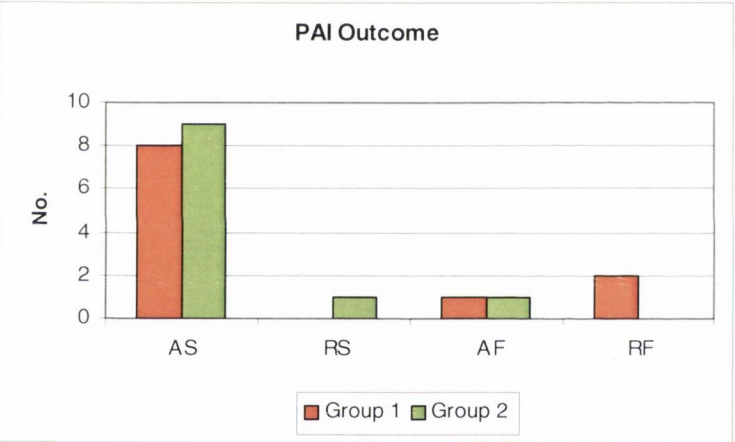
Baseline PAI scores in each study group are displayed in Figure 4.5.9.

Figure 4.5.9 Baseline PAI Score



The change in PAI from baseline to last review at 6 months is reflected in the increased success rates shown in figure 4.5.10.

Figure 4.5.10 PAI outcome at 6 month review



In Group 1, 72.7% (8) cases showed absolute success through achieving a PAI score of 1. In 1 case the PAI remained stable at 1, in the other 7 cases the PAI reduced to score 1. There were 2 cases (# 3 & 13) of relative failure where the PAI score remained stable >1. In 1 case (#22) an increase of PAI was noted which was scored as absolute failure (Table 4.5.29).

In Group 2, 81.8% (9) of cases had a PAI of 1, scored as absolute success. In 4 cases the PAI remained stable at score 1 while in the other 5 cases, healing occurred to achieve a score 1. In 1 case (#10) the PAI decreased from 4 to 2, a relative success. The increase of PAI

from 3 to 4 in patient #21 was scored as an absolute failure (Table 4.5.29).

Overall, 77.3% (17) of teeth were scored as absolute success (PAI 1). In 5 cases the PAI score had remained stable at PAI score 1, while 12 had shown a decrease in PAI score down to score 1. A single case (#10) was scored as relative success, 2 as absolute failure (# 21 & 22) and 2 (#3 & 13) as relative failure.

In this study the absolute rate corresponds to the absolute success or failure, while the relative rate is defined as the absolute rate plus the relative scores (Table 4.5.29).

Table 4.5.29 Success Rates using PAI score

	AS Rate		RS Rate		AF Rate		RF Rate	
	n	%	n	%	n	%	n	%
Group 1	8	72.7	8	72.7	1	9.1	3	27.3
Group 2	9	81.8	10	90.9	1	9.1	1	9.1
Total Group	17	77.3	18	81.8	2	9.1	4	18.2

AS= absolute success
AF= absolute failure

RS= relative success
RS= relative failure

Fisher’s Exact Tests were used to analyse the PAI outcome at 3 months and 6 months, between group 1 and group 2 to assess a difference in outcome between the materials. The two-sided P values were calculated as 1.000, considered not significant. This suggests no difference in PAI outcome between Group 1 and Group 2, from baseline to 3 months and baseline to 6 month follow up (Table 4.5.30).

Table 4.5.30 Comparison of PAI outcome between Group 1 and Group 2

	RSR 3/12	RFR 3/12	Total	RSR 6/12	RFR 6/12	Total
Group 1	7 (32%)	4 (18%)	11 (50%)	8 (36%)	3 (14%)	11 (50%)
Group 2	7 (32%)	4 (18%)	11 (50%)	10 (45%)	1 (5%)	11 (50%)
Total	14 (64%)	8 (36%)	22 (100%)	18 (82%)	4 (18%)	22 (100%)

RSR= relative success rate

RFR= relative failure rate

To analyse the change in PAI score over time in the group overall, a Repeated Measures ANOVA was applied (Figure 4.5.11). Over time the decrease was consistent between the two time intervals ($P=0.03$).

Figure 4.5.11 **Graph to show change in PAI score over time**

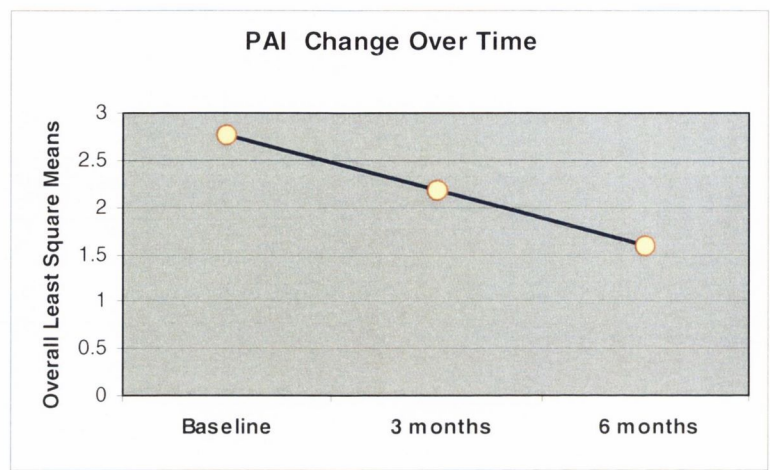


Table 4.5.31 contains the data on PAI scores recorded from study participants at each time point in the study.

Px	Baseline				3 Months			6 months			PAI		PARL	
	Grp	PAI	PARL	MaxDiam (mm)	PAI	PARL	Max Diam (mm)	PAI	PARL	Max Diam (mm)	PAI Change	PAI Outcome	PARL Change	PARL Outcome
1	1	2	0		2	0		1	0		-1	S		
3	1	4	1	5	4	1	5	4	1	7	0	RF	?	F
5	1	1	0		1	0		1	0		0	S		
7	1	4	1	4	2	0	-	1	0		-3	S	H	S
9	1	4	1	4	3	1	-	1	0		-3	S	H	S
11	1	2	0		1	0		1	0		-1	S		
13	1	4	1	7	4	1	4.5	4	1	5	0	RF	?	RS
15	1	2	0		1	0		1	0		-1	S		
17	1	4	1	6	1	0	-	1	0		-3	S	H	S
19	1	4	1	4	1	0	-	1	0		-3	S	H	S
22	1	3	1	-	3	1	-	4	1	5	+1	F	?	F
2	2	1	0		4	1	4	1	0		0	S	H	S
4	2	3	1	-	3	1	-	1	0		-2	S	H	S
6	2	3	1	-	3	1	-	1	0		-2	S	H	S
8	2	1	0		1	0		1	0		-2	S		
10	2	4	1	4	2	0	-	2	0		0	RS	H	S
12	2	1	0		1	0		1	0		-2	S		
14	2	4	1	4	2	0	-	1	0		-3	S	H	S
16	2	1	0		1	0		1	0		0	S		
18	2	4	1	4	3	1	-	1	0		-3	S	H	S
20	2	2	0		1	0		1	0		-1	S		
21	2	3	1	-	4	1	7	4	1	7	+1	F	?	F

Table 4.5.31

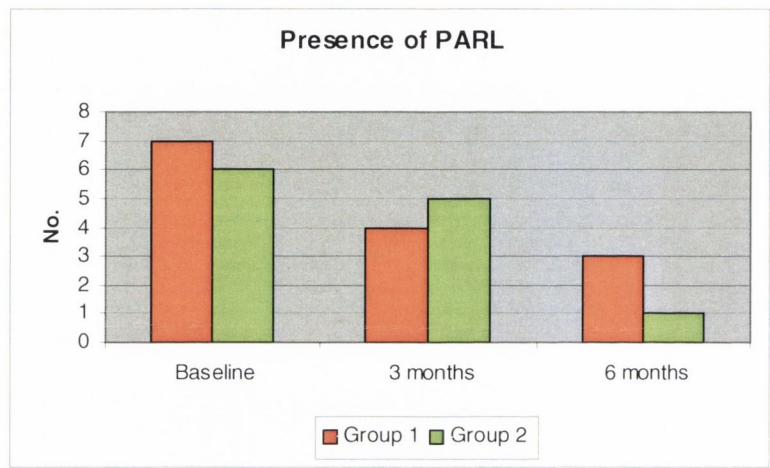
Data of PARL and PAI score change over time

4.5.8 PARL

Table 4.5.31 contains the data on PARL presence and diameter change in study participants at each time point in the study.

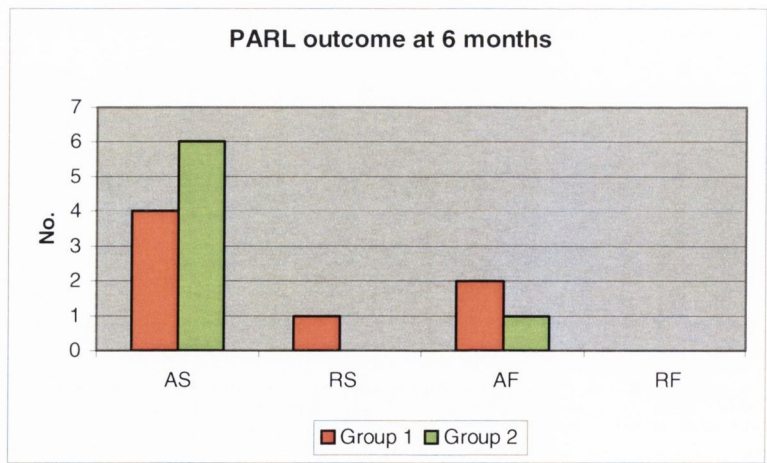
The presence of a PARL at baseline and follow up is represented in Figure 4.5.12.

Figure 4.5.12 PARL presence over time



The change in PARL activity over the study period is reflected by Figure 4.5.13, which shows the success rates within each group at 6 month follow up.

Figure 4.5.13 PARL outcome at 6 months



In Group 1, 63.6% (7) teeth were associated with a PARL at baseline. At 3 months, 42.9 % (3) had healed completely (AS), 14.3% (1) had decreased in size but was still present and 28.6% (2) had remained static (RF). At 6 months 57.1% (4) teeth showed complete healing (AS), 14.3% (1) tooth (#13) remained as a decreased lesion but still not

resolved (RS) and 28.6% (2) teeth (#3 & 22) changed from being static to increasing in size so were considered absolute failures.

In Group 2, 54.6% (6) teeth possessed an associated PARL at baseline. At 3 month review. In 33.3% (2) cases (#10 & 14) the PARL had resolved completely (AS), in 16.7% (1) case (#18) the pathology had decreased but not healed (RS) while 33.3% (2) cases showed no change (RF). In 16.7% (1) of the group (#21) an increase in radiolucency was noted (AF). Patient #2 had developed a radiolucency with a maximum diameter of 4mm, which was considered an absolute failure, At 6 month review, 85.7% (6) cases showed complete healing which was considered as absolute success. Only 1 case (#21) displayed a persistent large radiolucency, which was scored as an absolute failure (Table 4.5.32).

In the group overall, 35.7% (5) teeth with associated PARL showed complete healing by 3 months and, 71.4% (10) had healed by 6 months. In total 28.6% (4) cases did not fully resolve. Of these 1 tooth showed incomplete healing (#13) and 3 were absolute failures with an increase in PARL diameter (Table 4.5.32).

In this study the absolute rate corresponds to the absolute success or failure, while the relative rate is defined as the absolute rate plus the relative scores. Table 4.5.32 shows the success rates with relation to final PARL outcome.

Table 4.5.32 Success rates using PARL outcome at 6 months

	AS Rate		RS Rate		AF Rate		RF Rate	
	n	%	n	%	n	%	n	%
Group 1	4	57.1	5	71.4	2	28.6	2	28.6
Group 2	6	85.7	6	85.7	1	14.3	1	14.3
Total Group	10	71.4	11	78.6	3	21.4	3	21.4%

AS=absolute success RS=relative success
 AF=absolute failure RF=relative failure

Fisher’s Exact Tests were used to analyse the PARL change at 3 months and 6 months, between Group 1 and Group 2 to assess a

difference in outcome between the materials. The two-sided P values were calculated as 1.000, considered not significant. This suggests no difference in PARL healing between Group 1 and Group 2, from baseline to 3 months and baseline to 6 month follow up (Table 4.5.33).

Table 4.5.33 **Comparison of PARL outcome over time between the study groups**

	RSR 3/12	RFR 3/12	Total	RSR 6/12	RFR 6/12	Total
Group 1	5 (71.4%)	2 (28.6%)	7 (50%)	5 (71.4%)	2 (28.6%)	7 (50%)
Group 2	3 (42.9%)	4 (57.1%)	7 (50%)	6 (85.7%)	1 (14.3%)	7 (50%)
Total	8 (57.1%)	6 (42.9%)	22 (100%)	11 (78.6%)	3 (21.4%)	22 (100%)

RSR=relative success rate

RFR=relative failure rate

A Fisher's Exact Test was performed on the total group data to analyse the change in PARL status from baseline to 3 months. A significant change was found with a two-tailed P value of 0.031. The same test was performed to compare 3 month data to 6 month and again a significant decrease in PARL pathology was noted. In this case a two-tailed P value of 0.017 was calculated. These statistical tests represent a significant reduction in periapical pathology in the whole sample over the study period.

4.5.9 PAI and PARL

If both PAI and PARL outcome are taken in account the combined success rates can be calculated (Table 4.5.34).

Table 4.5.34 **Correlation between PAI outcome and PARL outcome**

	PAI Outcome															
PARL Outcome	AS				RS				AF				RF			
	G1	G2	Total	G1	G2	Total	G1	G2	Total	G1	G2	Total				
AS	8	9	17	0	1	1	0	0	0	0	0	0				
RS	0	0	0	0	0	0	0	0	0	1	0	1				
AF	0	0	0	0	0	0	1	1	2	1	0	1				
RF	0	0	0	0	0	0	0	0	0	0	0	0				
Total	8	9	17	0	1	1	1	1	2	2	0	2				

AS=absolute success

RS=relative success

AF=absolute failure

RF=relative failure

The addition of absolute success or failure of both criteria gives an overall absolute success or failure rate (Table 4.5.35).

In 3 cases there was no correlation between the outcomes:

In case #10 the PAI outcome was RS but the PARL was AS. In patient #3 the PAI outcome was RF and the PARL was AF. In case #13 the PAI outcome was RF as the PAI score stayed the same >1 and the PARL outcome was RS as the PARL decreased in size but did not heal. It was decided to score this case as a relative success as healing was in progress, which is a sign of endodontic success.

Table 4.5.35 depicts the combined outcome within the study groups.

Table 4.5.35 Outcome using combination of PAI and PARL outcomes

	AS Rate		RS Rate		AF Rate		RF Rate	
	n	%	n	%	n	%	n	%
Group 1	8	72.7	9	81.8	2	18.2	2	18.2
Group 2	10	90.9	10	90.9	1	9.1	1	9.1
Total Group	18	81.8	19	86.4	3	13.6	3	13.6

AS=absolute success

RS=relative success

AF=absolute failure

RF=relative failure

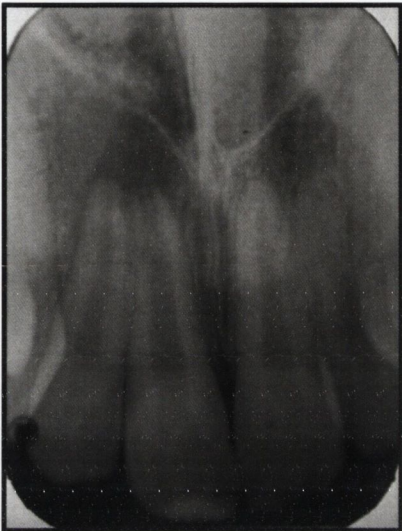
When absolute success in both outcomes is considered the combined absolute success rate in Group 1 is 72.7% and in Group 2 is 90.9%. The overall absolute success rate is 81.8%. The relative success rate is 81.8% in Group 1, 90.9% in Group 2 and 86.4% overall.

The combined absolute failure rate is 18.2% in Group 1 and 9.1% in Group 2. The overall absolute failure rate is 13.6%. The relative failure rate is the same as the absolute failure rate 18.2% in Group 1, 9.1% in Group 2 and 13.6% overall.

4.5.10 Radiographs of Cases

The case in Figure 4.5.14 from Group 2 shows a successful outcome, with resolution of the large PARL and formation of a hard tissue barrier on the white MTA Angelus plug.

Figure 4.5.14 Radiographs of Case #4 (Group 2)



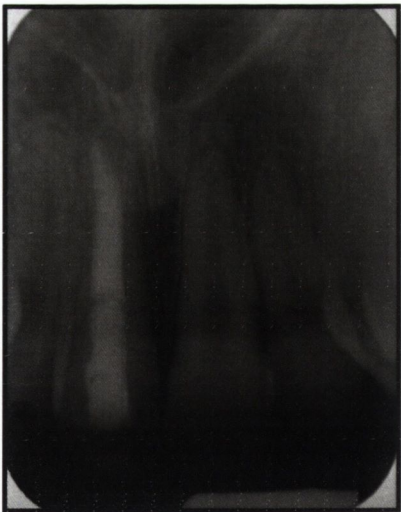
Baseline



3 month follow up



6 month follow up



12 month follow up

Figure 4.5.15 depicts radiographs of a case from Group 2. This case again shows a successful outcome, with reduction in apical pathology.

Figure 4.5.1 Radiographs of Case #8 (Group 2)



Baseline



3 month follow up



6 month follow up



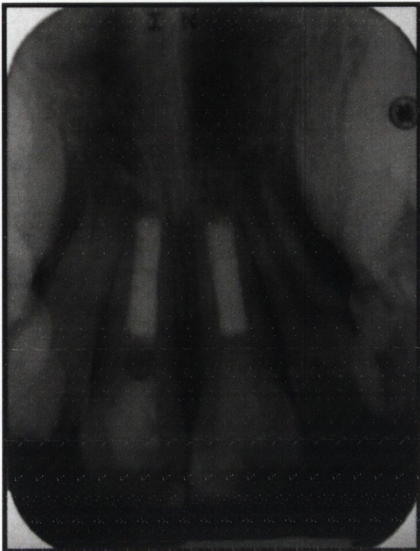
12 month follow up

Both the teeth in Figure 4.5.16 show successful outcomes at 6 month recall, with hard tissue barrier formation over both MTA plugs.

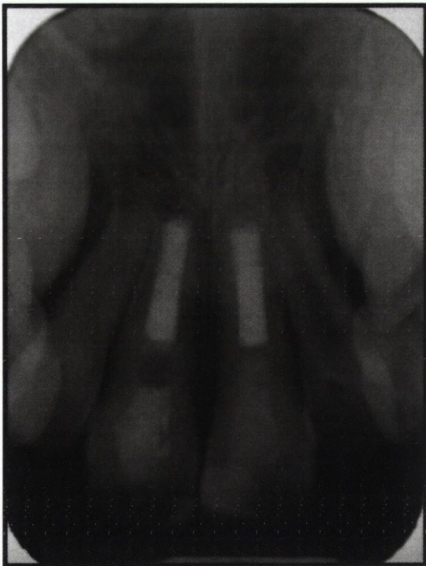
Figure 4.5.2 Radiographs of Cases #15 and #16 (Group 1 & Group 2)



Baseline



3 month follow up



6 month follow up

This case from Group 1 is interesting at it shows the development of an estranged root fragment which required surgical removal. This case is an example of absolute failure clinical due to sinus formation and absolute radiographic failure due to increased periapical pathology (Figure 4.5.17).

Figure 4.5.3 Radiographs of Case #3 (Group 1)



Baseline



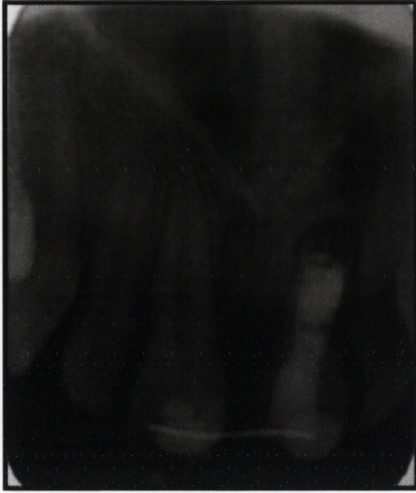
6 month follow up



12 month follow up



Immediately post-apicectomy



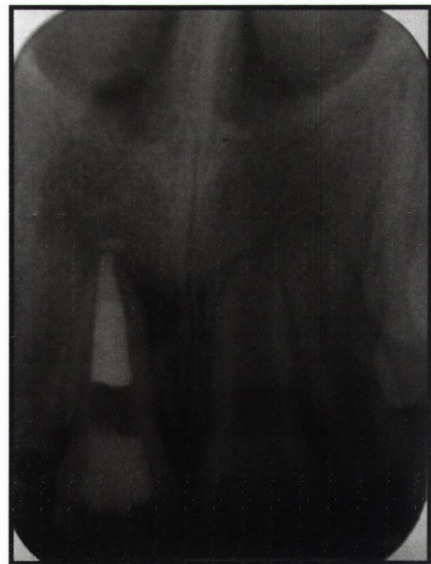
7 months following apicectomy

Figure 4.5.18 shows radiographs from case #21 in Group 2. This patient was affected by Amelogenesis imperfecta, accounting for the unusual radiographic appearance of the enamel layer and large pulp chambers. This case is an example of absolute radiographic failure as the PARL has increased in size over time. An apicectomy is required.

Figure 4.5.4 Radiographs of Case #21 (Group 2)



Baseline



3 month follow up



6 month follow up

5 Discussion

The vast majority of human studies using MTA as an apexification material are case reports, with only a handful of more extensive studies available. The use of white MTA ProRoot® is recorded in case reports by Karp *et al.* (2006) and D'Arcangelo *et al.* (2007) but in only one clinical trial (Simon *et al.* 2007), otherwise grey MTA has been used or the colour was not specified. There are no published reports on the use of white MTA Angelus. It would appear that there is only one pilot study addressing the clinical outcome of MTA apexification in immature permanent incisors (Sarris *et al.* 2008). Our study investigated the clinical and radiographic success of one step apexification of non-vital, immature permanent incisors in children using 2 types of white MTA. The objective was to establish the effectiveness of white MTA as an apexification material in immature permanent incisors and propose it as an adequate or superior replacement to calcium hydroxide.

The study population examined comprised of more than twice as many males as females with an average age of 10 years. All teeth included were maxillary incisors, the vast majority being central incisors. This patient profile matches that proposed by Andreasen *et al.* 2003, with trauma of maxillary central incisors experienced by more males than females in a peak age group of 9-10 years.

In this study an excellent clinical success rate was achieved of 90.9% in Group 1, 100% in Group 2 and 95.5% in the group overall. There was no statistically significant difference in clinical performance between the 2 types of white MTA. This excellent clinical outcome mirrors results in other similar studies using both grey MTA and calcium hydroxide (Dominguez Reyes *et al.* 2005, El Meligy and Avery 2006, Sarris *et al.* 2008). Only one patient showed clinical failure (case #3), presenting with a buccal sinus at 6 months review. This tooth received an apicectomy procedure but was subsequently lost. This tooth had suffered an avulsion injury when very immature (root development

stage 2) and presented with a history of recurrent sinuses and infections. Pace *et al.* (2007) similarly reported a failed case, thought to be related to severity of the initial injury and immature root status.

The radiographic outcome was evaluated in this study using the periapical index (PAI) and also the change in associated periapical radiolucencies (PARL).

The PAI is a scoring system for assessment of periapical periodontitis in dental radiographs developed by Orstavik *et al.* (1986). Inflammation of periapical tissues due to necrotic and infected pulps is accompanied by structural changes in the tooth and bone, the PAI scores these changes from category 1-5. Similar results were found in both groups and no statistical difference was calculated in the PAI score outcome between Groups 1 and 2, showing a similarly favourable outcome with both materials. An overall absolute success rate of 77.3% was found, with a relative success rate of 81.8%. The overall absolute failure rate was 9.1% and the relative failure rate 18.2%. The reduction in pathology over time was consistent and statistically significant.

The changes in PARL over time were also used to score the radiographic outcome of teeth in this study. A change in the activity of a PARL represents healing or increasing periapical pathology, therefore a successful or an unsuccessful endodontic outcome. Statistical analysis again revealed no difference in PARL outcome between Group 1 and Group 2 indicating similar performance of both materials. Overall, 59.1% of teeth showed the presence of a PARL at initial assessment, by 3 months, 35.7% of these teeth showed complete PARL resolution and 71.4% had healed by 6 months. A statistically significant decrease in periapical pathology was recorded from baseline to final follow up in both groups. In total, 28.6% of teeth did not show PARL resolution during the study period. One of these teeth (case #13) was in the process of healing, so may heal with advancing time and 13.6% were absolute failures, with increase or development of pathology. This result does not compare favourably to Pradhan *et al.*

(2006) who reported healing in 100% of periapical pathology. Simon *et al.* (2007) however, reported an absolute success rate of only 65% and an absolute failure rate of 5%, with the remaining lesions in the process of healing.

The combination of PAI and PARL outcomes is the most relevant outcome measure, as success must be considered in both variables to be a truly favourable result. The combined absolute success rate was 81.8% overall, the relative success rate was 86.4% and the absolute and relative failure rate was 13.6%. In both the PAI and PARL outcome measures, no statistical differences were found between the study groups, therefore it can be surmised no statistical difference exists in the combined outcome. This outcome is superior to that reported by Simon *et al.* (2007) whose corresponding findings were 77%, 81% and 16%. The absolute radiographic success rate of 81.8% is also higher than the 76.5% reported by Sarris *et al.* (2008) who was studying an almost identical study population. The success rate of apexification using calcium hydroxide has been reported to range from 74-100% (Sheehy and Roberts 1997). A recent, well designed study by Dominguez Reyes *et al.* (2005) reported clinical and radiographic success rates of 100% in 26 young permanent, necrotic incisors. El Meligy *et al.* (2006) reported an 87% success rate for calcium hydroxide apexification, compared to 100% in the MTA group. When success of calcium hydroxide and MTA apexification are compared within the recent literature, a range of 76-100% has been reported for both materials.

The PAI removes the subjective and un-reproducible nature of endodontic evaluation and reduces bias by allowing blinded evaluation of radiographs (Orstavik *et al.* 1986). In this study excellent intra-examiner and inter-examiner reliability was observed with the PAI which agrees with a previous report by Orstavik (1988).

Simon *et al.* (2007) noted difficulty in categorising immature teeth in some situations, feeling that sensitivity of radiograph interpretation of immature teeth did not allow for accurate scoring of PAI 1 or 2, which may affect success rates. Orstavik (by personal communication, 2008)

warned against the possible difficulty of scoring the periapical status in immature teeth.

In our research, we found this index was reliable and reproducible for scoring the apical area of immature teeth. However, the outcome of 1 case (#10) may have been affected by lack of sensitivity between PAI score 1 and 2. A PAI score of 4, which reduced to 2 at 3 month and 6 month follow up was considered a relative success. Despite extensive healing the PAI was not scored as healthy (score 1). This may be accounted for either by time to heal or lack of index sensitivity. In a PAI score of 2, the periodontal ligament is widened, this was scored as successful by Sarris et al (2008), who excused the residual pathology as scar tissue. Walton and Torabinejad (2001) also mentioned that a widened periodontal space or unusual trabecular pattern might be seen radiographically following significant periapical pathology, despite complete healing. This may mimic persistent pathology, leading to fallibility in radiographic interpretation.

When comparing the radiographic evaluation of the PAI in this study to that of Simon et al. (2007) it is obvious that interpretation of the PAI scores can vary. Simon *et al.* (2007) scored a stable PAI score of 1 as relative success while we felt this merited an absolute success score as 1 represents a healthy periodontium, which did not change following treatment. Also a decrease of PAI was scored as absolute success by Simon *et al.* (2007), while we scored a decrease as absolute success only if the penultimate PAI score was 1, but relative success if it was >1, requiring more time for complete healing. Despite these differences, the PAI was found to be a reliable index for evaluation.

There was excellent inter-examiner and intra-examiner agreement in this study, showing Kappa-Cohen test values of 0.77-1.0 for the radiographic values. The exception was the measurements of the maximum diameter of periapical radiolucencies, where there were inconsistent results. One reason may be that in the anterior maxillary area, many structures overlap, making a clear view of a PARL difficult. Other studies used the maximum horizontal or vertical diameter of a lesion without reporting difficulties (Pradhan *et al.* 2006, Simon *et al.*

2007). Pradhan *et al.* (2007) further categorised the pathology into line radiolucency, small, medium or large. These categories may have been useful in this research to decrease variations in measurements. It is important to remember however, that the accurate measurement of a lesion's diameter is not of major clinical importance, rather how the lesion changes over time, indicating healing or otherwise.

Most teeth were recorded as having stage 3 or 4 root development, with the more mature roots frequently associated with non-divergent apices. This is logical when the histological development of a root apex is considered, as at the apical end of the root the sheath bends inwards at a 45° angle to form the apical opening (Avery and Cheigo 2006). The stage of root development did not seem to be related to the outcome, which is surprising as an immature tooth with a short root, and thin dentinal walls may be expected to have a poorer prognosis than more mature teeth. It is however of note, that case #3, whose tooth ultimately required extraction, had only reached stage 2 of root development. Non-divergent apical anatomy was recorded in 86% cases, compared to 14% with divergent apices. Dominguez Reyes *et al* 2005 reported a higher frequency (31%) of teeth with divergent apices in a similar study group.

The position of the MTA plug was recorded as either ideal (flush with apex) or non-ideal (underfilled or overfilled). An ideal plug was present in 73% of teeth in Group 1, 54.5% teeth in Group 2 and 63.6% overall. Sarris *et al.* (2008) considered 76.5% of MTA plugs adequate, which is superior to the findings of this study. The relationship between stage of root development and MTA plug position was examined. A trend towards ideal plug position related to more mature apices was noted, however statistical analysis failed to show a significant significance. Sarris *et al.* (2008) also observed this pattern but their numbers were insufficient to allow statistical analysis. The increased number of more mature roots in their study may explain the superior frequency of ideal MTA plugs. If a much larger sample size could be attained, it would be interesting to stratify participants according to root development and

match these to the control to examine how root maturity affects apexification success and also long term predisposition to fracture.

A statistically significant relationship ($P=0.04$) was noticed between the position of the MTA plug and apical anatomy, with more ideal plug positions recorded in association with non-divergent apices and no ideal plugs in apically divergent teeth. This makes clinical sense as it is more difficult to control the MTA placement in blunderbuss apices, extrusion beyond the apex occurs easily or sometimes a root may be left a little underfilled due to over-cautious compaction force used by the operator. Other authors have discussed the difficulty of achieving a satisfactory apical seal in wide-open apices using an orthograde approach (Hachmeister *et al.* 2002, Martin *et al.* 2007). In these difficult cases a resorbable, physical barrier can be placed to provide a barrier to compact the MTA against and prevent extrusion. Several materials including Collatape (Centerpulse dental, Carlsband, CA, USA) and calcium sulphate (Class Implant, Rome, Italy) have been suggested for this purpose (Kratchman 2004). The relationship of MTA plug position to radiographic outcome did not reach statistical significance, however, ideal plugs were frequently associated with radiographic success. It is interesting to note that 2 out of the 3 absolute failure cases in this study had non-ideal MTA placement, which is clinically relevant. This relationship may reach statistical significant in a larger study population. Conversely, Sarris *et al.* (2008) could not find a clinical association between the plug position and treatment outcome, and plug extrusion was not found to be important for healing by Erdam and Sepet (2008).

A hard tissue apical bridge is thought to occur over MTA due to cementinduction (Thomson *et al.* 2003), osteoblast stimulation (Koh *et al.* 1997) and stimulation of alkaline phosphatase expression by fibroblasts (Bonson *et al.* 2004). Unlike apexification using calcium hydroxide, biological barrier formation is not clinically necessary before treatment can be completed, but its formation is interesting as is considered a sign of success (Simon *et al.* 2007). In the current study, the presence of an apical barrier was recorded in 64% cases. In 92%

of these a barrier was noted in the context of absolute success. In case #3 however, despite an unsuccessful outcome, a partial apical barrier was recorded. Not all cases of success (82%) were associated with an apical barrier. Simon *et al.* (2007) observed similar findings, with a periapical barrier recorded in only 26% cases, however 81% of cases were considered successful. This suggests the appearance of a radiographic barrier is not a prerequisite for clinical success and cannot be reliably used as a success indicator but rather as a factor when considering healing.

It is possible that all teeth experiencing a successful outcome do have some degree of apical hard tissue formation that is not detectable radiographically due to inadequate thickness. Unlike animal studies, the clinician can only rely on radiographic detection of a radio-opaque barrier over the MTA plug. Induction of hard tissue barrier formation has been confirmed histologically in 93-100% of teeth in animal studies, confirming rapid and reliable barrier formation using MTA (Shabahang *et al.* 1999, Felipe *et al.* 2006).

The time taken for apical barrier formation over MTA plugs, was investigated by Pradhan *et al.* (2006) who identified a barrier radiographically in 70% of cases in a mean time of 3 ± 2.9 months. Radiographic review was performed 4 weekly in their study. In the current research, our radiographic review was 3 monthly, not allowing for an accurate assessment of the time scale for barrier formation. It was felt that observation of the ALARA principle (as low as reasonably achievable) of radiation exposure was more important in this child population. The speed of barrier formation is superior to that reported by Dominguez Reyes *et al.* (2005) in calcium hydroxide apexification where the average barrier formation was >12 months. This has little clinical relevance however, as barrier formation over MTA is merely a sign of healing, while in calcium hydroxide apexification it is essential for treatment completion.

The relationship between barrier formation and some of the other variables were explored in this study. The formation of an apical barrier occurred more readily in non-divergent apices in the study population, however this relationship did not reach statistical significance. Studies

using calcium hydroxide as an apexification material have noted extended treatment times required for barrier formation in teeth with divergent apices (Yates 1988, Finucane and Kinirons 1999).

An ideal MTA plug was frequently seen with an apical barrier in this study, but surprisingly lack of barrier formation was not associated with non-ideal plug position. This is different to the findings of Pradhan *et al.* (2006) who noted a significant relationship between MTA extrusion and non-formation of a biological barrier. Similarly a dog study by Shabahang *et al.* (1999) noted failed radiographic barrier detection in relation to MTA overfill.

The average total treatment time in this study was 8.5 weeks, measured from MTA placement to final canal obturation. Under ideal conditions, the total treatment time of one step apexification could be 1 week which has been achieved by Simon *et al.* (2007) and El Meligy and Avery (2006), however Sarris *et al.* (2008) reported treatment spans of 7-70 days, and Pradhan *et al.* (2006) took on average 0.75 ± 0.49 months to complete treatment. Prolonged treatment times in the present study were due to missed appointments, failure of MTA plug set and in one case, pain in the contralateral tooth requiring attention on the obturation visit. It must be also remembered that a hospital environment can be inflexible with regard to treatment session availability and capacity. Despite the unforeseen delay in some cases, the average treatment time compares favourably to calcium hydroxide, with a recent prospective clinical trial reporting an average of 12.19 months for barrier formation (Dominguez Reyes *et al.* 2005). With MTA the barrier is immediate, removing the necessity to await biological apical closure.

The age group included in the present trial ranged from 8 to 14 years. All children demonstrated excellent behaviour (Frankl Scale ++) during the MTA visits. These visits were long and required a high level of patient compliance. Such a high level of co-operation may be a consequence of previous behaviour shaping sessions with a postgraduate student of paediatric dentistry (AM). One patient required

nitrous oxide sedation, which enabled stress free treatment completion. The acceptability of the one step apexification to the children is reflected in the behaviour records. The treatment was completed predictably in 3 visits, compared to multiple visits using calcium hydroxide with no definitive time frame. Despite the delays in treatment completion with MTA, the average time was still far superior to that reported for calcium hydroxide.

The most common type of trauma experienced in the study population was uncomplicated crown fracture, followed by avulsion, complicated crown fracture and finally luxation injuries. Uncomplicated crown fractures have been reported in the literature as the most commonly experienced traumatic dental injury of maxillary permanent incisors (Hamdan and Rajab 2003). Pulp necrosis is not a common sequelae to crown fractures. A frequency of 6% pulpal necrosis was reported, 5 years following uncomplicated crown fractures in a retrospective study of 84 permanent incisors (Cavalleri and Zerman 1995). The risk of pulp death has however, been shown to increase in the presence of a concomitant luxation injury. The stage of root development has also been shown to contribute to the likelihood of pulp necrosis, with immature teeth being more likely to show revascularisation and pulp healing (Robertson *et al.* 2000). The high number of non-vital teeth in the study population, with reports of crown fractures alone is therefore surprising, especially when considering the immature stage of root development. In this study many of the acute trauma records were obtained outside Dublin Dental Hospital, it is possible therefore that luxation injuries were not always reported in association with referred crown fractures. It is also difficult to control for treatment received prior to referral, some fractured teeth may not have received an adequate restoration, leaving the dentine tubules unsealed, allowing bacterial penetration of the pulp chamber.

Local anaesthesia (LA) was not used in 18.2% cases, when the operator did not deem it necessary for MTA placement. Treatment without anaesthesia did not seem to affect on the clinical or

radiographic outcome, suggesting that LA is not always a necessity for successful MTA placement if the clinician feels that administration may negatively affect behaviour and compromise treatment completion.

Only one patient (4.5%) in this study reported pain following MTA plug placement which did not require analgesia. Sarris *et al.* (2008) noticed a greater frequency of pain experience between treatments, with 6% of patients experienced tenderness to percussion (TTP) following MTA placement. Following obturation 17.6% teeth showed tenderness to palpation and 11.7% reported TTP, one of which required analgesia.

Chlorhexidine gluconate (0.2%) was used as an irrigant in this research protocol due to operator preference when treating open apices. The use of sodium hypochlorite during one step apexification is also recommended in the literature (Witherspoon and Ham 2001). Evidence shows that chlorhexidine gluconate (CHx) has equal or superior antibacterial quality to sodium hypochlorite and excellent substantivity, despite not dissolving necrotic debris (Ohara *et al.* 1993, Jeansonne and White 1994, Ercan *et al.* 2004). CHx has been shown to penetrate dentinal tubules and disinfect carious tubules (Komorowski *et al.* 2000) and has also demonstrated a harmless effect on the microhardness and roughness of root canal dentin (Ari *et al.* 2004). Therefore, its use may be advocated for endodontic irrigation in immature teeth with wide-open apices and thin dentine walls.

Failure of endodontic treatment is primarily caused by the persistence of bacteria (Sundqvist *et al.* 1998). Bacteria can penetrate the root canal from many avenues, the coronal access cavity, exposed dentine tubules, the blood stream (anachoresis) and via the gingival sulcus (Andreasen *et al.* 2003). The complex anatomy of the root canal provides an excellent environment for growth, proliferation and persistence of bacteria, especially in cases with long standing infection (Brystrom and Sundqvist 1981). In this study 3 teeth were absolute failures. One case (#3) had an extensive lesion secondary to external inflammatory resorption, which would have allowed communication between bacteria in the gingival sulcus and the root canal. The second

case (#21)) was affected by Amelogenesis imperfecta, the increased enamel porosity and tooth surface loss may account for the increase in endodontic pathology and there was a history of recurrent infections. The final failure (case #22) did not have any obvious pathway for persistence of bacterial invasion; however there was a long-term history of recurrent buccal sinus presence.

Some operators have reported difficulty working with MTA. Glickman and Koch (2000) found the sandy consistency of hydrated grey MTA hard to manipulate, while Sarris *et al.* (2008) reported problems with residual material adhering to and remaining on dentinal walls. In this study optimal visualisation of canal walls and the apical plug was achieved using a microscope. The white colour of the MTA made identification of the material within the root canal much easier compared to the grey MTA used previously. The difficulties experienced by Sarris *et al.* (2008) may be due to lack of microscopy and the use of grey MTA. There was little difference noted in the handling properties between the 2 types of MTA, however MTA Angelus did have a slightly creamier consistency compared to the grainier ProRoot®, which slightly enhanced the ease of mixing and also loading of the MTA carrier gun.

Tooth discolouration is an important aesthetic concern in this age group of children. In this study population 50% of teeth showed discolouration at baseline data collection. It has long been hypothesised that the blood pigment haematin is responsible for dentine and enamel staining following erythrocyte destruction (Marin *et al.* 1997). Other causes of tooth discolouration in endodontically treated teeth include obturating materials, remnants of pulp tissue in pulp horns, intracanal medicaments or the coronal restoration (Walton and Torabinejad 2002).

Tooth discolouration has been reported following the use of grey MTA (Torabinejad *et al.* 1995a, Sarris *et al.* 2008). White MTA was introduced in 2002 as a more aesthetic option, aiming to reduce cases of tooth discolouration (Witherspoon and Ham 2001). The reduction of

iron oxide in white MTA compared to grey is thought to be responsible for the colour change (Asgary *et al.* 2005). In the present study 18.2% teeth of normal colour at baseline showed discolouration at follow up. Interestingly, MTA Angelus was used for apexification in all these teeth. In such a small group this is unlikely to reach statistical significance but is worthy of note that all cases occurred in Group 2. This is the first report in the literature of discolouration associated with white MTA Angelus used for apexification.

It is unclear as to why this discolouration occurred. All pulp tissue remnants would have been thoroughly removed at this point. No sealer was used, the aesthetic MTA was placed apically, far from the cervical margin and in all cases a tooth coloured intra-canal and coronal restoration were placed. One suggestion is the leaching of MTA components through the dentine, may have resulted in a cervical stain. The teeth that experienced colour change were all of stage 4 and 5 root development, so root immaturity was not a contributing factor. It was observed following hydration, that the white MTA Angelus was slightly darker than white MTA ProRoot®. Both types of MTA used were from the same batch throughout the study. X-ray photoelectron spectroscopy or Energy Dispersive Analysis could be carried out on the batches used to assess differences in composition between the materials used. This may lead to a possible indication of the cause of tooth discolouration.

Another interesting occurrence during the study was the failure of an MTA plug to set. This occurred in case #14, using MTA Angelus. The MTA was placed, followed by a damp paper point sealed into the canal according to the treatment protocol. It is possible that the moist paper point was not in the direct contact with the MTA needed to induce setting in the presence of moisture. Another possible cause of the set failure is the accidental incorporation of some chlorhexidine irrigant into the MTA mix. An *in vitro* study showed the failure of MTA to set when combined with chlorhexidine gluconate (Kogan *et al.* 2006). This finding highlights the necessity to dry the canal well prior to MTA placement, place the damp paper point in direct contact with the plug

and also the importance of checking the integrity of the plug before proceeding with obturation.

Continued root development was noted in 7 cases in this study, subsequent to MTA placement, enhancing the apical barrier formation. Continued root development is rare in presence of a necrotic pulp due to damage of HERS, which dictates root development (Avery and Chiego 2006). It is possible however, that continued root length growth in non-vital teeth is due to elements of HERS that have survived inflammation and endodontic therapy (Foreman and Barnes 1990). The avoidance of over-preparation of immature teeth is thought to reduce scarring and HERS damage, encouraging continued apical development (Gupta *et al.* 1999). In one case (#3), an apical root fragment developed, estranged from the root and became infected, requiring surgical removal. There have been a small number of case reports documenting continued apical root development following calcium hydroxide apexification in non-vital immature teeth, but none following MTA apexification. Yang *et al.* (1990), Gupta *et al.* (1999) and Selden (2002) have all reported on cases of continued root formation in immature teeth following calcium hydroxide placement.

In addition to calcium hydroxide, various authors have noted continued apical root development in non-vital, immature teeth following dressing with antiseptic and antibiotic pastes (Cooke and Robotham 1960, Ball 1964, Frank 1966, Sato *et al.* 1996). Recently, cases have been reported where necrotic, immature teeth have undergone apexogenesis following dressing with a polyantibiotic paste (Thibodeau and Trope 2007, Huang 2008). The antibiotics are thought to disinfect the canal, allowing the root sheath mesoderm cells to form root components. This apical mesodermal tissue is now considered as the apical papilla (Sonoyama 2008). Stem cells from apical papilla (SCAP) may survive infection and allow root maturation (Sonoyama 2008). The discovery of SCAP cells and the possibility of apexogenesis in necrotic immature cases is leading to a paradigm shift in treatment approach, and merits further investigation in the future (Huang 2008).

The main limitation of the present research was the inability to recruit a large treatment group of non-vital, immature, traumatised permanent incisors in children. The sample size was limited by a recruitment and treatment window of just 12 months following ethical approval and also strict inclusion criteria. An attempt was made to increase recruitment by writing a letter regarding the study to junior hospital staff (NCHD's), who see the bulk of acute trauma through the A&E department in Dublin Dental Hospital. An announcement was also made, regarding the study and inclusion criteria at a conference for the community (HSE) dentists. These methods did increase referrals to the trauma clinic of immature traumatised teeth. Unfortunately, not all these teeth fulfilled the inclusion criteria. The small sample size did not allow conclusive statistical analysis of relationships between variables within the study groups. Most statistical analysis was carried out in the group as a whole. Little differences were noticed between the groups, but with larger numbers and in depth statistical analysis trends may have been identified between the MTA ProRoot® control and the MTA Angelus. The small patient count seems to be a limiting factor in many studies of this kind. Sarris *et al.* (2008) managed to recruit only 17 suitable cases from the Leeds Institute Trauma Clinic over a similar time period to the current research. Simon *et al.* (2007) recruited a large group of 43 teeth with 1 year follow up for MTA apexification, but this was over a 4 year research window and included a much broader age range and all tooth types. El Meligy and Avery (2006) succeeded in locating 15 children with 2 non-vital immature incisors each within a 12-month window. However, not all of the teeth had experienced trauma, with some losing vitality secondary to caries. Another study comparing calcium hydroxide to MTA apexification had just 10 in each group (Pradhan *et al.* 2006). A number of calcium hydroxide clinical trials contained larger patient groups but these were recruited over 3-11 year time periods (Ghose *et al.* 1987, Kleier and Barr 1991, Mackie *et al.* 1993, Dominguez Reyes *et al.* 2005).

Follow up time is also a limitation of this research. Orstavik *et al.* (1996) documented healing of chronic apical periodontitis in 599 teeth using the PAI. Complete healing took 4 years in some cases, but healing was evident in almost 90% cases after 1 year. Simon *et al.* (2007) performed MTA apexification on 57 teeth but only reported on 43 teeth that had received a one year follow up. The average time of follow up in this study was 10.8 months (± 3.5 months). One year recall was achieved for 60% of cases but results were evaluated at 6 months, so as all participants had an equal chance of healing or development of pathology. Endodontic success or failure can be scored once healing is observed or pathology develops. A limited observation time may not give teeth adequate time to heal, negatively affecting success rates. In this study only 2 teeth seem to be limited by the follow up time. Patient #10 had a PAI outcome depicting relative success as the PAI decreased but remained at 2, which is not considered entirely healed. Patient #13 received a PAI outcome of relative failure as the PAI had remained stable and >1 , while the PARL decreased but did not entirely heal, an outcome of relative success. This was scored as relative success as healing of the PARL was observed. With more time it is hoped that these teeth would change from relative success to absolute success if they continue on this trend. Staff of the Trauma Clinic will follow up the outcomes in the future and record the results.

As mentioned previously, the acute trauma had been managed by various dentists, preventing control of treatment protocol before enrolment in the study. The most significant of the uncontrolled treatment factors was the length of calcium hydroxide dressing. All the teeth had calcium hydroxide dressing placed prior to MTA apexification, as advised by the MTA manufacturers (Tulsa Dental and Angelus Dental Solutions). Unfortunately there was a wide range of dressing times with an average of 11 months. It is interesting to note that despite some teeth receiving prolonged periods of calcium hydroxide medication, none of the teeth possessed an apical barrier adequate enough to allow obturation, necessitating the use of MTA to create an apical stop. Other cases have been reported of successful MTA

apexification following failed attempts at calcium hydroxide induced barrier creation (Maroto *et al.* 2003, Karp *et al.* 2006). This failure of calcium hydroxide apexification, perhaps reflects a poorer prognosis and reduced chance of a successful outcome. As discussed before, long-term calcium hydroxide can be detrimental to root strength and also may predispose teeth to ankylosis (Lengheden *et al.* 1991, Cvek 1992, Andreasen *et al.* 2002, Andreasen *et al.* 2006). During the study period to date, none of the teeth have demonstrated any evidence of replacement resorption or root fracture, but this will be monitored in the future.

In summary, this research has shown that white MTA used as an apexification material in immature permanent incisors produces excellent clinical results. There was no difference in clinical or radiographic success rates between the 2 types of white MTA, ProRoot® or Angelus. A small number of teeth experienced tooth discolouration following placement of MTA Angelus, which warrants further investigation. A strong relationship was noted between non-divergent apices and the placement of ideal MTA plugs. The option of using resorbable barriers should be explored in divergent teeth to pre-empt this. Although calcium hydroxide has been the material of choice for more than 40 years, the shortened treatment time, predictable apical stop and acceptability of one step apexification using MTA, make it an attractive treatment alternative. In the future new developments such as SCAP regeneration may minimise the need for endodontic therapy, but in the mean-time MTA may be proposed as the current apexification material of choice. There is a need for larger, multi-centre clinical trials to allow conclusive statistical analysis and provide a more robust evidence base.

6 Conclusions

This research shows that one step apexification using white MTA is a predictable, time efficient and successful treatment in children with traumatised, non-vital permanent incisors.

Similar clinical and radiographic success was found in both types of white MTA, ProRoot® and Angelus. An excellent overall clinical success rate of 95.5% was achieved. When the PAI and PARL outcomes were combined, absolute radiographic success was 82% with a relative success rate of 86% and a failure rate of 14%. These results are comparable to other similar studies using grey MTA ProRoot®.

The procedure was acceptable to all children, some of whom were as young as 8 years of age. All participants displayed excellent co-operation and minimum distress during treatment visits. All patients returned for necessary follow up visits, indicating the compliance and enthusiasm for the treatment provided. The mean treatment time of 8.5 weeks, although longer than ideal, compares favourably to calcium hydroxide apexification, which may require 5-20 months before obturation can proceed. White MTA therefore produces a predictable barrier in a shorter time frame than calcium hydroxide.

Excellent examiner calibration was seen for all radiographic parameters except for diameter of PARL. Inaccurate measurement of PARL diameter would have made little difference to the outcome, as it is lesion resolution that counted as absolute success, but it would be interesting to explore other systems of scoring radiolucency diameters to allow greater agreement and increase reliability. The PAI index was good for use in scoring immature teeth and showed excellent calibration. Its use should be encouraged for future studies to allow comparisons.

The MTA plug position demonstrated a statistically significantly relationship to the apical anatomy, with ideal plugs being more

frequently associated with non-divergent apices. The identification of this relationship may enable future prediction of difficult plug placement from assessment of the apical anatomy prior to treatment. The use of a resorbable material such as calcium sulphate may aid MTA placement in cases with divergent apices.

An association was observed between unfavourable outcome and non-ideal plug position. The relationship did not reach statistical significance in this small sample but the trend is important clinically. There may be an indication to replace non-ideal plugs if outcome is affected. Apical barrier formation occurred in 64% of cases and in 92% of these teeth it was noted in the context of absolute success, but not all teeth with a successful outcome had radiographic evidence of an apical barrier. This suggests that the appearance of a barrier is not a prerequisite for success, but should be considered as one of multiple factors when assessing outcome.

Clinically, the lighter colour of the white MTA enhanced visibility within the root canal. This enabled detection of remnants on the canal walls and more efficient plug placement. Little difference was noted in the handling characteristics of either type of white MTA, except a slightly creamier consistency of the MTA Angelus.

Although the short term cost implications of MTA are high, with much decreased treatment time, it can be concluded that MTA apexification is a cost effective option long term.

A cervical discolouration of 18.2% of teeth in this study occurred following placement of MTA Angelus. The reason is unclear but warrants further investigation.

Continued root development can occur following apexification with MTA. Usually this development is a continuation of the apical portion, and adds to the appearance of an apical barrier. Usually continued apical root development is clinically uneventful, but on occasion an

apical root fragment may develop, estranged from the main body and cause pathology requiring removal.

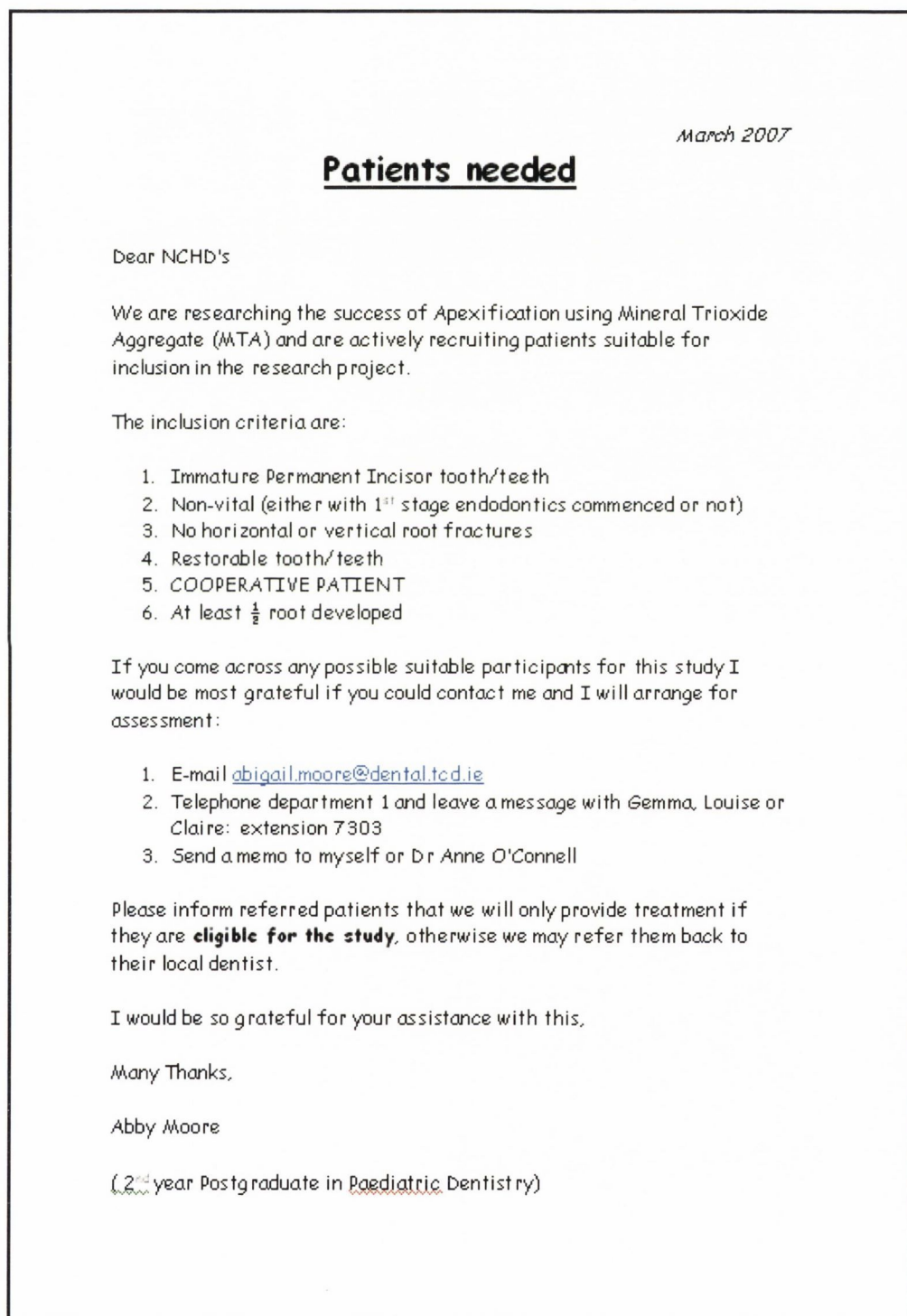
The main limitations of this study were inadequate sample size and limited follow up time. Despite these, our study does add some important information to the body of evidence base that exists on MTA apexification using white MTA in children. This study provides the first available evidence on the clinical and radiographic success using MTA Angelus for apexification. It is expected that all teeth will be followed up to the 18 month time point and be reported on subsequently.

The one step apexification procedure produced a more predictable and less time consuming apical barrier when compared to calcium hydroxide and the technique was acceptable to all participants. Both commercial brands of white MTA produced similar clinical and radiographic results. The clinical and radiographic success rates are comparable to existing studies using grey MTA and calcium hydroxide. In conclusion, white MTA ProRoot® and white MTA Angelus can be proposed as a suitable alternate apexification material to grey MTA and calcium hydroxide in children with non-vital immature permanent incisors.

7 Appendices

7.1 Appendix 1: Recruitment letter

Figure 7.1.1 Recruitment letter to junior hospital staff



7.2 Appendix 2: Copy of Ethical Approval

Figure 7.2.1 Ethical Approval



THE UNIVERSITY OF DUBLIN

TRINITY COLLEGE

SCHOOL OF MEDICINE

FACULTY OF HEALTH SCIENCES

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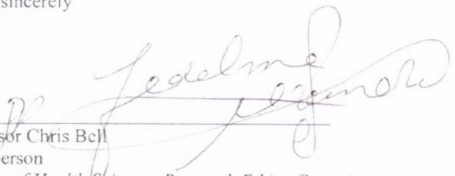
Thursday, 22 February 2007

Study: One step apexification using two types of mineral trioxide aggregate (MTA)

Dear Ms Moore

Further to a meeting of the Faculty of Health Sciences Research Ethics Committee 2006 - 2007, we are pleased to inform you that the above project has been approved without further audit.

Yours sincerely



Professor Chris Bell
Chairperson
Faculty of Health Sciences Research Ethics Committee

cc. Dr Anne O'Connell, Consultant, Snr Lecturer, Depart of Child & Public Health,
Dental School, College

Schools of the Faculty: Medicine, Dental Science, Nursing and Midwifery, Pharmacy and Pharmaceutical Sciences

7.3 Appendix 3: Information Sheet

Figure 7.3.1 Information Sheet Page 1

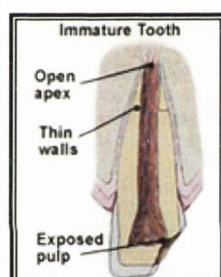
Patient Information Sheet (Study Group)

1-Step Apexification using 2 types of MTA

Department of Public and Child Dental Health, Dublin Dental School and Hospital

Introduction:

Your child has suffered a traumatic injury to his/her permanent tooth. Because their tooth was damaged while it was immature, the nerve of the tooth has died. This has interrupted the development of the root of the tooth.

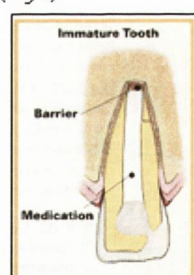


(Fig 1)

If a root does not complete development the end of the tooth, or apex may remain open and the walls may not develop to the full thickness. (Fig 1)

The thin walls make the tooth more susceptible to fracture. The open end makes it very difficult to complete a root canal filling.

A good root canal filling needs a hard stop at the end of the root.



(Fig 2)

Apexification is the procedure by which dentists are able to create an end stop in an immature tooth which provides a barrier for the permanent root filling.

(Fig 2)

In this research project we are using 2 types of a material to carry out apexification.

The material is called mineral trioxide aggregate or MTA. This is a tried and tested, CE approved product available routinely for dental care. It is very good at sealing the end of the root, is not toxic and is compatible with the tooth and surrounding structures. MTA also carries out the treatment more quickly than other materials so fewer visits are needed.

The 2 types of MTA we will use are essentially the same, except one material sets more quickly than the other. The 2 types of MTA will be assigned alternately to patients taking part in this study.

MTA is used regularly in our clinic for this procedure.

In this study we aim to compare:

- 1) The 2 types of MTA available
- 2) How MTA performs compared to another material, calcium hydroxide, which has been used traditionally. Calcium hydroxide has demonstrated good results in the past but MTA has been shown to have advantages over this material.

What does this involve?

Your child requires an apexification procedure.

This usually takes 3 visits.

On the 1st treatment visit we will be starting the root canal by removing unhealthy nerve tissue and placing a dressing in the tooth to kill bacteria. On the 2nd visit we will seal the end of the root (apexification) on the 3rd visit we will complete the root canal treatment.

We will review the success of the treatment at 3, 6 and 12 months by examining the tooth and taking a small x-ray. This is a standard recall protocol.

What happens after the study?

Your child will be reviewed and monitored by staff of the trauma Clinic in Dublin Dental School and Hospital as long as necessary.

Where will this take place?

All visits will take place in Clinic 1 on the 1st floor of Dublin Dental School and Hospital on Thursday afternoons.

Who will be treating my child?

One of 2 dentists will carry out treatment on your child during the study, Dr Abigail Moore or Dr Mary-Freda Howley.

You can contact either dentist at any time if you have questions or concerns.

7.4 Appendix 4: Consent Forms

Figure 7.4.1 Consent Form Page 1

Study Group Consent Form

1-Step Apexification using 2 types of MTA

*(Department of Public and Child Dental Health, Dublin Dental School and hospital,
Lincoln Place, Dublin 2.)*

This study and this consent form have been explained to me.

I understand that my child requires an apexification procedure on his/her tooth/teeth. I understand that the material being used for this is mineral trioxide aggregate (MTA). I agree to data being collected on the outcome of this treatment and used in this study.

I also give permission for the data from my child's treatment to be included in the overall findings of this research, which will be published in relevant dental literature.

My dentist has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree for my child to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this.

I understand that I may withdraw from the study at any time and that it will not affect my future treatment.

PARTICIPANT'S NAME: _____

PARTICIPANT'S SIGNATURE: _____

Date: _____

If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained: -

NAME OF CONSENTOR, PARENT or GUARDIAN: _____

SIGNATURE: _____

RELATION TO PARTICIPANT: _____

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when the participant gave consent to the dentist:

NAME OF FIRST WITNESS: _____	SIGNATURE: _____
NAME OF SECOND WITNESS: _____	SIGNATURE: _____

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Dentist's signature: _____

Date: _____

7.5 Appendix 5: Data Capture Forms

Figure 7.5.1 **Data capture form: Initial data**

Data Capture Form 1 - Initial Data

Hospital Number Sex

DOB Age

Medical History _____

Date of Trauma

Referral Source

Tooth Involved

Age at Trauma

Cause Injury _____

Type of Trauma:

Concussion/ <u>Subluxation</u>	☐	
Palatal <u>Luxation</u>	☐	
Buccal <u>Luxation</u>	☐	
Intrusive <u>Luxation</u>	☐	
Extrusive <u>Luxation</u>	☐	
Avulsion	☐	EODT _____
Crown Fracture	☐	Complicated ☐
		Uncomplicated ☐
Root Fracture	☐	

Figure 7.5.2 Data capture form: Initial data

Initial Clinical Presentation:

Tooth	TTP	Mobility	Percussion Tone	Colour	Sinus tract/abscess	PPD	EF	EPT

Management History:

Date RT commenced

Time Trauma - RT

Calcium Hydroxide placed: YES ☐ NO ☐

Total length of CH therapy

Figure 7.5.3 Data capture form: Follow up data

Data Capture Form 2 - Follow up Data

Hospital Number

Tooth Involved

Management:

Apexification material

WMTA ProRoot

WMTA Angelus

Local anaesthetic

Yes

No

Nitrous oxide

Yes

No

Behaviour (+, ++, -, --)

Total time of MTA-Obturation (days)

Restoration Provided

Clinical Presentation:

Review	Tooth	TTP	Mobility	Percussion Tone	Colour	Sinus tract/swelling	PPD	EF	EPT
3/12									
6/12									
12/12									
18/12									

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7.6 Appendix 6: Radiographic evaluation Forms

Figure 7.6.1 Examiner recording forms

Examiner: #2 (RL)
Time: t1
Data: Baseline

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Root development (1-6)																						
Apical Anatomy (P/D/C)																						
PAI (1-5)																						
PARL (Y/N)																						
Max Diameter PARL (mm)																						

Examiner: #1 (AOC)
Time: t1
Data: baseline

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Root development (1-6)																						
Apical Anatomy (P/D/C)																						
PARL (Y/N)																						
Max Diameter PARL (mm)																						
PAI (1-5)																						

Examiner: #1 (AOC)
Time: t1
Data: follow up

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
PAI (1-5)																						
PARL (Y/N)																						
Max Diameter PARL (mm)																						
Apical barrier (A/P/C)																						
MTA Plug (F/O/U)																						

Examiner: #2 (RL)
Time: t1
Data: follow up

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
PAI (1-5)																						
PARL (Y/N)																						
Max Diameter PARL (mm)																						
Apical barrier (A/P/C)																						
MTA Plug (F/O/U)																						

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