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AN *IN-VITRO* COMPARISON OF MICROLEAKAGE BETWEEN THREE CALCIUM SILICATE CEMENTS AND AMALGAM

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Dissertation submitted in partial fulfilment of the Degree of

MSc (Odont)

at the

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November 2014

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SUMMARY

Periapical endodontic surgery may be indicated when orthograde retreatment of failed endodontic therapy is unsuccessful, unfeasible or contraindicated. The sequence of procedures during the surgery includes exposure of the involved apex, root-end resection, root-end cavity preparation and placement of a root-end filling. The root-end filling is necessary in order to provide a hermetic seal, thereby preventing the egress of micro-organisms into the periradicular tissues from the root canal system.

The purpose of this *in-vitro* study was to compare the microleakage of three calcium silicate cements and amalgam when used as retrograde filling materials.

One hundred and twenty single rooted, single canal, human teeth with closed apices were collected and stored in Phosphate Buffered Saline after extraction. All root canals were instrumented using ProTaper rotary instruments and obturated by warm vertical condensation using gutta-percha with Topseal Root Canal Sealer. Access cavities were sealed with Fuji IX glass ionomer restorative material.

The apical 3mm of each root was resected perpendicular to the long axis of the root and root-end cavities were prepared to a depth of 3mm using ProUltra surgical ultrasonic tips. The teeth were randomly divided into four groups (n=30):

Group 1 - ProRoot MTA (Dentsply/Maillefer)
Group 2 - MTA Plus™ (Prevest Denpro Limited)
Group 3 – Biodentine™ (Septodont)
Group 4 – Permite Amalgam (SDI)

The materials were manipulated according to the manufacturer's instructions and used to fill the root-end cavities. The specimens were then coated with two layers of clear varnish, except the resected apical surface.

Teeth were stored in gauze, moistened with Phosphate Buffered Saline for 24 hours and thereafter submerged in Indian Ink dye for 48 hours. The excess dye was rinsed off the specimens under running water. Specimens were then sectioned horizontally in one millimetre increments from the apical end of the root. The extent of dye penetration was measured to the nearest millimetre using a stereomicroscope.

Data for different groups was collected and summarised in terms of percentage for the outcome vector (no leak, 1 mm leak, 2 mm leak and 3 mm leak). Furthermore pairwise comparisons between each of the calcium silicate materials to amalgam were done at the 0.017 level of significance, using Fisher's exact test.

Amalgam showed significantly more leakage than the calcium silicate materials ($p<0.001$). No significant differences were found among the calcium silicate materials, namely, Biodentine™ vs ProRoot ($p=0.776$), Biodentine™ vs MTA Plus™ ($p=0.667$) and ProRoot vs MTA Plus™ ($p= 0.350$).

DECLARATION

I Hussein Cassim Seedat, declare that this dissertation entitled, **"An *In-Vitro* Comparison of Microleakage between Three Calcium Silicate Cements and Amalgam"**, which I herewith submit to the University of Pretoria in partial fulfilment of the requirements for the degree MSc (Odont) is my own original work, and has neither been submitted for any academic award to this University, nor to any other institution of higher learning.

_____ _____

SIGNATURE **DATE**

'Seek knowledge from the cradle to the grave'

Prophet Muhammed

(Peace be upon him)

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to the following:

1. **Almighty Allah**, for granting me the strength and will to accomplish this task.
2. **Professor Peet van der Vyver**, Supervisor, Department of Odontology, University of Pretoria, for his mentorship, guidance, inspiration and for raising the standard of endodontics in South Africa.
3. **Professor FA de Wet**, Head of the Department of Odontology, University of Pretoria, for his time, guidance and mentorship.
4. **Mr Alan Hall** from the Laboratory for Microscopy and Microanalysis, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa.
5. **Professor PJ Becker**, Statistician, Biostatistics Unit, Medical Research Council of South Africa.
6. My parents, **Cassim and Zulekha Seedat** for their constant love and support, and for the sacrifices they made in helping me attain an education.
7. My loving wife **Shabnum**, and my dear children **Yusuf and Sanaa** for their encouragement, love, support, patience and for filling my life with the greatest of joy.
8. **Dr Hymie Herr**, for his mentorship, friendship, wisdom, and for guiding me toward the passion area in my career.
9. **Dr Imran Cassim**, my closest friend, who has helped me to reach heights in my career that would have been impossible without him.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Historical Highlights of Endodontic Surgery

Abulcasis is credited with the first case of intentional tooth replantation as a method of endodontic surgery in the eleventh century (Gutmann and Gutmann, 2010). Incision and drainage procedures to manage chronic sinus tracts were described by Heister in 1724 and Harris in 1839 (Gutmann and Gutmann, 2010).

In 1845, Hullihen described the process of surgical trephination, which consisted of creating a hole in the gum, the alveolar process and the root of the tooth into the root canal (Gutmann and Gutmann, 2010). A root-end resection procedure was first described by Smith in 1871, to manage a tooth with a necrotic pulp (Gutmann, 2014). In 1880, Farrar recommended that the diseased bone and soft tissue sac be removed, and he described the apex of the tooth as being dead and 'somewhat in the light of a foreign substance' (Gutmann and Gutmann, 2010). Farrar subsequently recommended the procedure of root-end resection in 1884 (Gutmann and Gutmann, 2010). The resection of the root-end with a fissure bur was endorsed by GV Black in 1886 (Gutmann, 2014). In 1890, Rhein presented a paper before the American Dental Association, describing the filling of the root, resecting the necrotic apex and then using a bur vigorously on surrounding pathologic tissue (Gutmann and Gutmann, 2010). Between the years 1896 and 1899, Carl Partsch of Germany, is credited with the methodical development of root-end resection and the Partsch incision, which became known as the semilunar incision (Gutmann and Gutmann, 2010; Gutmann, 2014). Partsch also supported the use of root-end fillings in 1898 (Gutmann and Gutmann, 2010).

Sir William Hunter, a British surgeon, delivered a lecture in 1910 at McGill University in Montreal entitled 'An address on the role of sepsis and antisepsis in medicine', which he published in London in 1911 (Gutmann and Gutmann, 2010). In his address, Hunter attacked the dental profession and promoted the belief that infections in other parts of the body owe their origins to oral sepsis (Castellucci, 2005). In 1916, Frank Billings, a physician, replaced the term 'oral sepsis' with 'focal infection', and claimed that dental extractions had cured diseases in distant parts of the body

(Gutmann, 2014). In 1919, Rosenow, a student of Billings, developed the theory of 'elective localization', which hypothesised that certain microorganisms had affinities for certain organs (Gutmann, 2014). These theories led to the belief that all diseases could be cured by extracting teeth, and consequently tooth extraction became the treatment of choice on all non-vital teeth, and even vital teeth with restorations, for almost forty years since Hunters remarks (Castellucci, 2005). According to Gutmann and Gutmann (2010), the focal infection theory was an attack on the field of endodontics and endodontic surgery from the 'ivory towers of the medical profession'. Teeth were unnecessarily removed by the thousands, as many dental schools did not permit the practice of endodontics, whilst others limited endodontics to anterior teeth only (Grossman, 1982). It was only in the late 1940's and early 1950's, that the theory of focal infection was dispelled, and faith was once again restored in endodontic treatment (Castellucci, 2005).

During the focal infection era, a few European practitioners, persevered in developing the field of surgical endodontics, despite Hunter's diatribe (Gutmann and Gutmann, 2010). From 1912 to 1921, Faulhaber and Neumann published several books describing anatomical considerations, guidelines for surgical access to the apices of maxillary and mandibular posterior teeth, and the use of amalgam as a root-end filling (Gutmann and Gutmann, 2010). Schuster advocated an apical slot preparation in 1913 (Gutmann, 2014). In 1914, von Hippel also advocated the creation of a vertical slot on the facial aspect of the root apex, and this was modified by Rudd in 1947 and brought into the contemporary endodontic era by Matsura in 1962, and was named the 'Matsura apical preparation (Gutmann and Gutmann, 2010). Buckley described the use of a bone chisel and mallet for resecting the root end in 1914, however a cross-cut fissure bur replaced this method of root-end resection in the 1920's (Gutmann and Gutmann, 2010). Ivy and Howe recommended the use of silver nitrate in 1917, to seal the dentinal tubules of the resected root-end (Gutmann and Gutmann, 2010). An article demonstrating the regeneration of cementum over the resected root-end was published by Blayney and Wach, in 1924 (Gutmann and Gutmann, 2010). In 1926, Neumann proposed a submarginal surgical flap, which was later claimed by Oschenbein and Leubke (Gutmann, 2014). Duclos, in 1934, presented methods detailing the management of the root apex, in which he favoured circular root-end preparation along the long

axis of the tooth for the placement of amalgam (Gutmann and Gutmann, 2010). In 1936, Karl Peter of the University of Ergeland provided a detailed review of the endodontic surgical procedure called 'Die Wurzelspitzenresektion der Molaren', which served as a reference text for contemporary endodontic surgical concepts (Gutmann and Gutmann, 2010). Gaining access to the root-end was identified as a difficulty, which led to the development of a small handpiece by Tangerud in 1939 (Gutmann and Gutmann, 2010). In 1939, Castenfeldt advocated the sealing of the entire resected root-end by creating a bevel that was cut into dentine from the canal to the cemental junction (Gutmann and Gutmann, 2010). Between 1941 and 1950, Jones published several articles on periapical surgery, and described the use of chloroform to soften the excess gutta percha to create a better seal (Gutmann and Gutmann, 2010). In 1958, Messing advocated the placement of amalgam into the root canal prior to root-end resection, and developed the Messing Gun to place the material deep into the canal (Gutmann and Gutmann, 2010). The recognition of endodontics as a speciality in the United States in 1963, led endodontic surgery into the contemporary era, with the further advancement of concepts, armamentarium and materials.

1.2 Introduction to Endodontic Surgery

Periapical pathosis occurs when the root canal system is breached and becomes exposed to the oral microflora, and these microbial invaders or their by-products egress into the periradicular area via the apical foramen or lateral canals (Kakehashi, Stanley and Fitzgerald, 1965; Nair, 2004; Ruddle, 2004). The objectives of non-surgical endodontic therapy are to entirely remove pulpal debris, microbes and microbial by-products from the root canal system and to three-dimensionally seal the space (Torabinejad, Watson and Pitt Ford, 1993).

Endodontic treatment failure may occur as a result of persistent intraradicular microbial infections or secondary infections and in some cases extraradicular infections (Siqueira, 2001). The failure of endodontic treatment may also be attributed to the inadequate shaping, cleaning and obturation of the root canal system, iatrogenic incidences during the endodontic procedure or re-infection of the root canal system due to a breakdown in the coronal seal subsequent to

endodontic therapy (Ruddle, 2004). The clinical treatment options available for failed endodontically treated teeth are non-surgical endodontic retreatment, periapical surgery or extraction (Ruddle, 2004). Dental extraction is considered when teeth are unrestorable, have non-treatable periodontal disease or root fractures (Ruddle, 2004). Nonsurgical retreatment of failed endodontic cases should be considered as the first treatment option and has proven to have a weighted average success rate of 95% (Von Arx, 2005).

Indications for endodontic surgery are (Chong and Rhodes, 2014):

1. Where periradicular disease process persists in an endodontically treated tooth, and retreatment is unsuccessful or unfeasible. A clinical example of unfeasibility is the risk of removing a large post, which may cause the root to fracture.
2. The correction of iatrogenic errors such as the retrieval of separated instruments or root filling materials that have passed beyond the apex of the root.
3. The requirement of a biopsy to investigate a suspicious lesion, or when a clinical investigation is required to directly visualise a possible vertical fracture.
4. When a combined nonsurgical and surgical approach is required such as treatment of a possible radicular cyst.

Contraindications of endodontic surgery include (Chong and Rhodes, 2014):

1. Dental factors which include an un-restorable tooth, inadequate root length or poor periodontal support.
2. Anatomical factors such as close proximity to a neurovascular bundle.
3. Surgical access factors such as limited mouth opening.
4. Patient factors such as psychological analysis or systemic diseases.
5. Clinician factors which include the level of training, skill and experience of the operator as well as the availability of appropriate equipment.

The procedure of surgery at the apex is commonly referred to as an apicoectomy. However, periapical or periradicular surgery are more preferable terms, as

'apicoectomy' only refers to one aspect (root-end resection) in the sequence of events required to carry out the procedure (Von Arx, 2005). The terms endodontic surgery or surgical endodontics are more appropriate as the objective of the procedure is to achieve three dimensional shaping, cleaning and obturation of the apical portion of the root canal system by accessing it via raising a surgical flap (Castellucci, 2003). Since this surgical procedure is now more routinely carried out using the surgical operating microscope it is also referred to as endodontic microsurgery (Kim and Kratchman, 2006). The sequence of procedures involved in periapical endodontic surgery are anaesthesia, flap design, incision and reflection of a full thickness flap, gaining access to the root apex, debridement of pathological periapical tissues, root-end resection, root-end cavity preparation, sealing off the root canal system with a root-end filling, flap replacement and suturing, postoperative instructions and care, removal of sutures and evaluation (Rubinstein and Torabinejad, 2004).

1.3 Use of the Operating Microscope

Microsurgery can be defined as a surgical procedure performed on small, complex structures using an operating microscope (Kim and Kratchman, 2006). The operating microscope was introduced to dentistry by Apotheker and Jako (1981). The first microscope was not widely accepted because it was poorly configured, ergonomically difficult to use, and had angled illumination rather than confocal illumination (Carr and Murgel, 2010).

In 1992, Dr Gary Carr introduced a dental operating microscope that had Galilean optics and was ergonomically configured for dentistry (Carr, 1992; Rubinstein, 2005). With Galilean optics, the light beams going to each eye are parallel and focused to infinity instead of convergent, so that the operators' eyes are at rest, as though looking into the distance (Rubinstein, 2005). Because the illumination is coaxial with the line of sight, there are no shadows when viewing the surgical site, and there is no eye fatigue during procedures that take several hours (Rubinstein, 2005).

When using surgical loupes and a headlamp, there is a tendency to bend over the patient resulting in head, neck and shoulder strain (Rubinstein, 2005). In addition to

the enhanced magnification and illumination with the use of the operating microscope, a more comfortable posture of the head, neck, spine and pelvis can be maintained by the operator, resulting in superior ergonomics (Rubinstein, 2005).
The benefits of using the operating microscope during endodontic surgery are (Kim and Kratchman, 2006):

1. The root apex can be examined under high magnification, making it possible to identify and manage anatomical complexities, perforations or fractures.
2. Diseased tissue can be precisely and completely removed.
3. The root tip can be easily distinguished from bone.
4. A smaller osteotomy can be made using magnification, resulting in quicker healing and less postoperative pain.
5. Fewer radiographs are required as the apex can be directly and precisely examined.
6. The procedure can be documented by video recording for educational purposes and communication with the referring dentist.

1.4 Hard and Soft Tissue Management

During endodontic surgery the cortical bone needs to be exposed by incision, elevation and reflection of a full thickness flap consisting of periosteum, gingival and mucosal tissues (Velvart, Peters and Peters, 2005). The correct management of the soft tissues is necessary to obtain complete, recession-free healing of the gingiva with the avoidance of scar formation in order to obtain an aesthetically pleasing result (Velvart and Peters, 2005).

Osteotomy involves the removal of the cortical and cancellous bone to gain access to the apical portion of the root (Hoskinson, 2005). Bone removal may be minimal or unnecessary should there be a fenestration of the root apex or a periapical lesion that has perforated the cortical plate (Hoskinson, 2005).

The process is carried out with an Impact-air 45 high speed handpiece (SybronEndo, Orange, California). The head of the turbine is at a 45^0 angle to the shaft making it easier to gain access to the apices of molar teeth (Rubinstein and Torabinejad,

2004). Furthermore, the water spray is directed toward the surgical site and the air stream is ejected from the back of the handpiece, thus eliminating the possible complications of air emphysema or air embolism (Rubinstein and Torabinejad, 2004; Kim and Kratchman, 2006).

A constant stream of water or saline is required on the cutting surface of the bur to avoid overheating of the bone (Hoskinson, 2005). Eriksson and Albrektsson (1983) found that bone is irreversibly damaged when its temperature is raised above 47^0C for one minute. They also found in a further study that the temperature threshold that would impair bone regeneration was between 44^0C and 47^0C when applied for a period of one minute (Eriksson and Albrektsson, 1984).

The use of a diamond bur is not recommended, as the diamond grit traps bone particles and therefore increases frictional heat (Hoskinson, 2005; Calderwood et al. 1964). A round, steel bur with widely spaced flutes to minimise with bone chips is recommended for bone removal (Hoskinson, 2005). The round bur will however be unsuitable for root-end resection. A Lindemann H151 (Brasseler USA, Georgia, USA) is a tapered steel surgical bur with widely spaced flutes and has been recommended for both osteotomy and root-end resection by several authors (Hoskinson, 2005). The selected bur should run parallel to the surface of the cortical plate, with a light brushing action to reduce friction (Gutmann and Harrison, 1985). The traditional endodontic surgical technique involved creating an osteotomy that is 8-10mm in diameter (Kim and Kratchman, 2006). Rubinstein and Kim (1999) found that the rate of healing was faster when the size of the osteotomy was smaller. The modern endodontic surgery advocates the osteotomy size to be 3-4mm in diameter, which is just large enough for a retrograde ultrasonic tip to access the bone crypt and vibrate freely (Kim and Kratchman, 2006). An advantage of using the microscope, is that it allows the operator to clearly distinguish the root tip from bone within a conservatively prepared osteotomy (Kim and Kratchman, 2006).

A sharp bone curette is then used for surgical curettage of periradicular soft tissue lesions, which should then be regarded as a biopsy that is sent for histopathological examination (Hoskinson, 2005).

1.5 Root-end Resection

The complex apical portion of the root canal system harbours microorganisms, unless it has been accessed by root canal instruments and chemically disinfected (Chong and Rhodes, 2014). This nidus of infection is removed by resecting the apical 3 mm of the root (Chong and Rhodes, 2014).

1.5.1 Indications for root-end resection (Stropko, Doyon and Gutmann, 2005)

1. Removal of pathologic processes such as foreign bodies, retained micro-organisms or firmly attached soft tissue lesion.
2. In order to remove anatomic variations, at least 3 mm of the root apex should be removed, as 93% of lateral canals and 98% of apical ramification are located in the apical 3 mm of the root (Kim and Kratchman, 2006).
3. Removal of iatrogenic errors such as separated instruments, ledges, blockages, zips and perforations.
4. To enhance the removal of deeply placed soft tissue lesions.
5. To gain access to the root canal system, that is inaccessible via orthograde treatment, and inspect the apical seal or lack thereof.
6. To create an adequate apical seal by enhancing access and vision.
7. Reducing of fenestrated root apices.
8. Evaluation of aberrant canals and root fractures by staining.

The process is carried out using a 170L tapered fissure bur in an Impact Air 45 turbine (Rubinstein and Torabinejad, 2004).

1.5.2 Bevel angle

Traditionally a bevel angle of 45^0-60^0 was advocated for the convenience of the operator so that the apex could be visualised and accessed for root-end preparation (Kim and Kratchman, 2006). The modern technique advocates that the root-end be resected perpendicular to the root, resulting in a 0^0-10^0 bevel angle (Kim and Kratchman, 2006; Chong and Rhodes, 2014). The advantages of not creating a bevel are that greater root length is preserved and less dentinal tubules are cut,

thereby reducing the leakage of microbes and their by-products from the root canal system (Chong and Rhodes, 2014; Gilheany, Figdor and Tyas, 1994). Further disadvantages of creating a bevel are the creation of a larger osteotomy, lingually positioned apices are missed, the root canal is elongated and the root is weakened because its diameter is reduced (Kim and Kratchman, 2006).

At this point the resected root-end is stained with methylene blue dye and inspected using the surgical operating microscope and micro-mirrors for isthmuses and for determining the canal morphology (Kim and Kratchman, 2006; Rubinstein and Torabinejad, 2004). This step was completely neglected during the traditional endodontic surgical technique (Kim and Kratchman, 2006). Methylene blue has the ability to stain organic material only, and is therefore able to define fractures, accessory canals, isthmus tissue and the periodontal ligament (Stropko, Doyon and Gutmann, 2005). The methylene blue should be applied for 10-15 seconds to allow its complete saturation, after which the surface should be rinsed and dried for inspection (Stropko, Doyon and Gutmann, 2005).

1.6 Root-end Cavity Preparation

The aim of preparing a root-end cavity is to remove root canal filling material and create a cavity that can be adequately filled (Kim and Kratchman, 2006). The prerequisites for root end cavity preparation include (Kim and Kratchman, 2006; Stropko, Doyon and Gutmann, 2005):

1. A thoroughly cleaned and shaped Class 1 cavity at least 3 mm deep into the root-canal system.
2. The walls of the preparation should be parallel to and coincident with outline of root canal space.
3. There should be adequate retention for the root-end filling.
4. Isthmus tissue should be completely removed.
5. The remaining dentinal walls should not be weakened.

Traditionally the root-end cavity was prepared using rotary burs in a micro-handpiece (Kim and Kratchman, 2006). The disadvantages of using these instruments to prepare the root-end cavity are (Kim and Kratchman, 2006):

1. Accessing the root-end is challenging especially when working space is limited.
2. The risk of perforation of the lingual root-end is high when the original pathway of the canal is not followed.
3. The 45^0 bevel required during resection exposes too many dentinal tubules.
4. Difficult to clean necrotic tissue in the isthmus area between canals.

The use of ultrasonics during endodontic surgery was first suggested by Richmond in 1957 by using an ultrasonic chisel to remove bone and to resect the root-end (Navarre and Steiman, 2002). In the early 1990s, Dr Gary Carr introduced specifically angulated ultrasonic retrograde tips for root-end cavity preparation (Carr, 1992). The modern technique for preparing a root-end cavity involves the use of these ultrasonic tips in a piezoelectric hand piece (Rubinstein and Torabinejad, 2004). When the piezoelectric crystals in the hand piece are activated by ultrasonic energy, the energy is transferred to the ultrasonic tip causing it to move forward and backward in a single plane (Rubinstein and Torabinejad, 2004). There are a variety of ultrasonic retrograde tips available to favour different surgical access situations.

The advantages of using ultrasonic tips rather than burs for cavity preparation are:

1. The apical preparation is deeper, cleaner and runs parallel to the long axis of the root (Wuchenich, Meadows and Torabinejad, 1994).
2. The operator experiences superior control and less fatigue with ultrasonic tips (Rubinstein and Torabinejad, 2004; Engel and Steiman, 1995).
3. There is a lower risk of root perforation due to a greater ability to stay central within the canal (Engel and Steiman, 1995).
4. The access to the root tip is easier (Rubinstein and Torabinejad, 2004).
5. Preparation of the isthmus area between canal exits is easier (Rubinstein and Torabinejad, 2004).

Saunders, Saunders and Gutmann (1994) found crack formation on the walls of the root-end cavities of extracted teeth prepared with ultrasonic tips and this finding was confirmed by Layton et al. (1996). However, Calzonetti et al. (1998) found no crack formation when ultrasonic root-end cavity preparation was performed on cadavers and this was confirmed by Gray et al. (2000), irrespective of a high or low ultrasonic intensity used. Min et al. (1997) suggested that the presence of a periodontal ligament would help dissipate stresses during ultrasonic preparation which would explain the absence of fractures in cadavers when compared to extracted teeth.

The original ultrasonic tips were made of smooth stainless steel and these are still widely used today (Stropko, Doyon and Gutmann, 2005). Diamond coated ultrasonic tips have a superior cutting efficiency and are useful for removing gutta-percha from the root-end cavity (Stropko, Doyon and Gutmann, 2005). Zirconium coated tips are not as effective as diamond coated tips in removing gutta-percha (Kim and Kratchman, 2006). The use of a rotary hand piece is no longer advocated with the advent of ultrasonic techniques for preparing root-end cavities (Stropko, Doyon and Gutmann, 2005).

The cutting tip should be applied lightly for optimal cutting efficiency using a gentle brushing motion with adequate water spray to cool the tip (Stropko, Doyon and Gutmann, 2005). It is advocated to routinely prepare an isthmus between two canals (Stropko, Doyon and Gutmann, 2005). Isthmus preparation is initiated by first scratching a tracking groove between the canals using an endodontic explorer (Stropko, Doyon and Gutmann, 2005). Alternately, a series of dots with a sharp ultrasonic tip can be created by placing it along the isthmus and activating it for an instant on those points (Stropko, Doyon and Gutmann, 2005). The dots are then joined to define the tracking groove, which is deepened slightly with the water switched off to aid with visibility. The water is then switched on and the isthmus is then prepared to a depth of 3mm (Stropko, Doyon and Gutmann, 2005). The root-end cavity is now prepared to receive the root-end filling.

1.7 Root-end Filling

The prepared root-end cavity is filled with a root-end filling material in order to provide a hermetic physical seal, thereby preventing the egress of micro-organisms or their by-products from the root canal system into the periradicular tissues (Chong and Pitt Ford, 2005). Periradicular curettage alone, without root-end filling, eliminates only the effect of the leakage from the root canal system into the surrounding tissues, but not the cause, as most periapical lesions are caused by a leaky apical seal (Kim and Kratchman, 2006). In order to ensure that healing that may occur does not regress, the root canal system should be resealed with an appropriate root-end filling.

1.7.1 Necessity of a root-end filling

It has been argued that a root-end filling may not be necessary should the existing root canal obturation appear well condensed radiographically (Harrison and Todd, 1980). However, the two dimensional radiographic appearance of white lines representing obturation may not indicate the presence of voids and cannot exclude the presence of micro-organisms within the root canal system (Nicholls, 1962; Rahbaran et al., 2001; Michiels, 2011). It is therefore mandatory to use a retrograde filling at the time of endodontic surgery (Friedman, 1991). Harty, Parkins and Wengraf (1970) concluded in a retrospective study of 1016 apicoectomy cases that the most important factor in achieving successful surgical endodontics was the apical seal.

1.7.2 Properties of an ideal root-end filling material

The ideal root-end filling material should be (Chong and Pitt Ford, 2005; Gartner and Dorn, 1992; Parirokh and Torabinejad, 2010a; Asgary, Eghbal and Parirokh, 2008):

1. Capable of preventing the leakage of bacteria and their by-products by adhering to the dentine walls and sealing the root end three-dimensionally.
2. Non-toxic.
3. Non-genotoxic.
4. Non-carcinogenic.
5. Biocompatible with host tissues and must not cause an inflammatory reaction.
6. Insoluble in tissue fluids.

7. Dimensionally stable.
8. Unaffected by moisture during setting and when set.
9. Radiopaque.
10. Able to inhibit or not promote the growth of micro-organisms.
11. Able to stimulate the regeneration of the periodontium, especially cementogenesis directly over the root-end filling.
12. Non-corrosive and not electrochemically active.
13. Non-staining to the tooth or periapical tissues.
14. Easy to use and have a long shelf-life.

1.8 Root-end Filling Materials

Various materials have been suggested and tested for use as root-end filling materials in the quest to attain the material that fulfils all of the ideal requirements.

1.8.1 Amalgam

Farrar has been credited with first using amalgam as a root-end filling material in 1884 (Priyanka, 2013). Since then silver amalgam has been the most widely used retrograde filling material and has served as a standard to which other materials are compared (Friedman, 1991).

The advantages of amalgam are that it is inexpensive, readily available, easy to manipulate, has good radiopacity and is insoluble in tissue fluids (Chong and Pitt Ford, 2005; Priyanka, 2013).

1.8.1.1 The disadvantages of amalgam as a root-end filling are:

(a) Initial microleakage

When an amalgam restoration is placed there is an initial gap between the amalgam and the dentine which becomes filled with corrosion products with time (7 days) (Tronstad et al. 1983). The use of a cavity varnish to line the root-end cavity significantly improves the sealing ability of amalgam (Tronstad et al. 1983).

(b) Electrochemical corrosion

Corrosion products of amalgam may have an undesirable effect on periapical tissues has been reported as a possible cause of failure of periapical surgery (Hohenfeldt, Aurelio and Gerstein, 1985).

(c) Biocompatibility

Chong, Pitt Ford and Kariyawasam (1997) found that amalgam root-end fillings displayed a persistent localised focus of inflammation immediately adjacent to amalgam root-end fillings. Torabinejad et al. (1997) also established that periradicular inflammation was present adjacent to amalgam retrofillings in monkeys five months after placement. Baek, Plenk and Kim (2005) confirmed that an inflammatory response was present adjacent to amalgam retrograde fillings in beagle dogs after a five month period.

(d) Undercut preparation

Since amalgam does not bond to dentine, it is necessary to prepare an undercut for retention, thereby weakening the apical portion of the root (Friedman, 1991; Priyanka, 2013).

(e) Amalgam tattoo formation

Amalgam scattering during placement results in the iatrogenic implantation of amalgam particles into the soft tissues resulting in tissue disfigurement (Chong and Pitt Ford, 2005; Mirowski and Waibel, 2002). The corrosion of these particles results in the formation of a black, blue or grey pigmented lesion on the mucosa (Chong and Pitt Ford, 2005; Mirowski and Waibel, 2002). Amalgam tattoo formation is referred to as 'focal argyrosis' due to the silver content of dental amalgam (Mirowski and Waibel, 2002).

(f) Mercury toxicity

Skoner et al. (1996) found no significant increase in blood mercury levels at 7 and 30 days after placing retrograde amalgam fillings when compared to preoperative blood mercury levels. However, the introduction of mercury into the periapical tissues still remains a concern.

(g) Zinc toxicity

The release of zinc from amalgam is cytotoxic to the surrounding tissues, therefore zinc free amalgams are less undesirable (Kaga et al. 1988; Wataha et al. 1994).

(h) Delayed expansion

Moisture contamination causes delayed expansion and deterioration of the physical properties of low copper amalgam, but not with high copper amalgam (Yamada and Fusayama, 1981).

In 1991, Friedman identified amalgam as the material of choice for retrograde filling (Friedman, 1991). However, newer materials have since been developed to challenge the use of amalgam. According to Chong and Pitt Ford (2005), the use of amalgam as a root-end filling should now be confined to history.

1.8.2 Gutta-Percha

Gutta-percha is derived from the sap of trees mostly of the genus *Palaquium* and in particular from the *palaquium gutta* (Prakash, Gopikrishna and Kandaswamy, 2005). Gutta-percha is the trans-isomer of polyisoprene and is often compared to natural rubber which is the cis-isomer of polyisoprene (Friedman et al. 1975). The difference between the two materials is that natural rubber is amorphous, whereas gutta-percha is 60% crystalline and therefore has a higher modulus of elasticity and tensile strength than natural rubber (Friedman et al. 1975).

Gutta-percha was introduced by Bowman in 1867 to fill the root canal space (Grossman, 1982). Gutta-percha for endodontic use contains 20% gutta-percha as a matrix, 66% zinc oxide as a filler, 11% heavy metal sulphates as radiopacifiers and 3% waxes or resins as a plasticizer (Friedman et al. 1975). In 1942 C.W Bunn discovered that gutta-percha exists in two crystalline phases and named them the alpha and beta phases (Goodman, Schilder and Aldrich, 1974). The alpha phase is the original form that is derived from the tree. When the alpha phase is heated above 65^0C it melts and when it is allowed to cool slowly (0.5^0C) the alpha phase recrystallizes (Goodman, Schilder and Aldrich, 1974). However, if it is routinely cooled the Beta phase recrystallizes (Goodman, Schilder and Aldrich, 1974). Most commercial gutta-

percha including dental gutta-percha exists in the Beta crystal form (Goodman, Schilder and Aldrich, 1974).

Gutta-percha is the most commonly used material to seal the root canal system during non-surgical endodontic treatment. When condensed, gutta-percha undergoes compaction and not compression, as a result there is no molecular spring-back to aid in the seal between the dentine-gutta-percha interface, thus making it necessary to use an endodontic sealer as a luting agent between dentine and gutta-percha (Schilder, Goodman and Aldrich, 1974). Both heat sealed and thermoplastic gutta-percha, used without sealer, displayed clinically unacceptable levels of leakage when tested as retrograde fillings (Abdal, Retief and Jamison, 1982; Vertucci and Beatty, 1986). It is necessary to use an endodontic sealer as a luting agent between gutta percha and dentine to reduce leakage even when thermoplastic gutta-percha is used (Skinner and Himel, 1987). Cold burnishing of gutta percha at the time of root end resection has been a proposed technique for sealing the root-end, however evidence shows that this technique results in significantly more leakage than amalgam and IRM (Smee et al. 1987; Vertucci and Beatty, 1986; Shaw, BeGole and Jacobsen, 1989). The use of gutta-percha as a root-end filling cannot be advocated due to its poor sealing ability.

1.8.3 Cavit (3M ESPE, St Paul, Minnesota, USA)

Cavit is a calcium sulphate based temporary restorative material, and is available in a premixed state that is simple to manipulate and apply to a root end cavity (Friedman, 1991). Cavit is available in a premixed state and it is simple to manipulate and apply to a root end cavity (Friedman, 1991). It is a hygroscopic material that undergoes linear expansion and sets when permeated with water, resulting in good marginal adaptation provided, a minimum thickness of 3,5mm of Cavit is placed (Friedman, 1991; Naoum and Chandler, 2002; Webber et al. 1978). However, Cavit is soluble and disintegrates when in contact with tissue fluids and therefore cannot be recommended as a root-end filling (Vasudev, Goel and Tyagi, 2003).

1.8.4 Glass-ionomer Cements

There are three types of glass-ionomer cements: Conventional, metal-reinforced and resin modified (Cho and Cheng, 1999). Conventional glass-ionomer cement was introduced in 1972 as a restorative material (Cho and Cheng, 1999). It is formed by a reaction between fluoro-aluminosilicate glass particles and an aqueous solution of polyalkanoic acid such as polyacrylic acid (Cho and Cheng, 1999). In 1977 metal-reinforced glass-ionomer was introduced by adding silver-amalgam alloy powder to the conventional glass ionomer to improve the mechanical properties (Cho and Cheng, 1999). In 1992 resin modified glass-ionomers were introduced which contain a monomer as well as a photo initiator, allowing it to be light-cured (Priyanka, 2013).

The advantage of glass-ionomer cements is that they are able to form a chemical bond to dentine thus providing a superior seal (Friedman, 1991). Initially they cause an intense inflammatory reaction which subsides completely (Chong and Pitt Ford, 2005). Glass-ionomers are slow setting, difficult to handle and the setting reaction is adversely affected by moisture (Friedman, 1991; Priyanka, 2013). Silver released from the metal-reinforced glass-ionomer can cause discolouration and the corrosion products are toxic (Chong and Pitt Ford, 2005). The resin modified glass-ionomers such as Vitrebond have better handling properties and the setting reaction can be controlled by light-curing (Priyanka, 2013). As it is not possible to assure that the surgical site will be moisture free during the setting reaction, glass-ionomer cements cannot be recommended as ideal root end fillings.

1.8.5 Reinforced Zinc-Oxide Eugenol Cements

In 1962, Nichols mentioned that he preferred zinc-oxide eugenol as a retrograde filling because of its good handling properties and satisfactory postoperative results (Nicholls, 1962). However, early zinc-oxide eugenol cements were weak, had a long setting time and were soluble (Chong and Pitt Ford, 2005). Two modifications of zinc-oxide eugenol cement have been recommended as root-end fillings:

(a) IRM (Dentsply, Maillefer)

In IRM, 20% poly-methyl methacrylate by weight has been added to the zinc-oxide powder and the eugenol liquid remains unaltered (Chong and Pitt Ford, 2005).

(b) Super EBA cements

a. Stailine Super EBA (Staident International, Staines, Middlesex, UK) is the original Super EBA cement developed (Yaccino et al. 1999). The powder consists of 60% zinc-oxide, 34% silicon dioxide and 6% natural resin. The liquid comprises 62.5% ortho-ethoxy benzoic acid (EBA) and 37.5% eugenol (Yaccino et al. 1999).
b. Super EBA (Harry J. Bosworth Co., Skokie, Illinois, USA). In the powder component of this version of the cement the silicon dioxide is replaced with 34% Alumina, but the liquid component is exactly the same as the Stailine Super EBA (Yaccino et al. 1999).

Super EBA was first suggested as a retrograde filling in 1970 (Hendra, 1970). Oynick and Oynick (1978) recommended the use of Super EBA as a root-end filling as it is easy to manipulate and place due to its plasticity, adheres to the dentinal walls in moist conditions, has an adequate mixing time and sets quickly once in contact with tissues. Super EBA has the ability to bond to itself unlike IRM, and can therefore be placed incrementally (Vasudev, Goel and Tyagi, 2003). Under SEM, Super EBA displayed good marginal adaptation and collagen fibres were observed growing over the material and in cracks within it (Oynick and Oynick, 1978). Compared to traditional zinc-oxide eugenol cements, Super EBA has a high compressive strength, high tensile strength, neutral pH and low solubility (Civjan and Brauer, 1964). A solubility study by Poggio et al. (2007) proved that both Super EBA and IRM were minimally soluble in water after 24 hours and two months.

Eugenol is believed to be the major cytotoxic component of ZOE cements as free eugenol trapped in the set mass of zinc eugenolate is released by hydrolysis of the cement surface (Chong et al. 1994). The modified reinforced ZOE cements are able to resist dissolution thereby reducing the release of eugenol. The eugenol in IRM may have an affinity for the Polymethylmethacrylate thereby limiting the release of

eugenol (Chong et al. 1994). Both IRM and Super EBA exhibit cytotoxicity when freshly mixed, however this is rapidly diminished as the cements set (Chong and Pitt Ford, 2005). In a histological study of root end fillings in dogs, Trope et al. (1996) confirmed the excellent tissue response to Super EBA and IRM, with EBA performing better than IRM but not statistically significant. A cytotoxicity study by Al-Sa'eed, Al-Hiyasat and Darmani (2008) showed that super EBA was more cytotoxic than IRM even though more eugenol is released from the latter. It was thus concluded that the zinc released from zinc-oxide eugenol cements is the major toxic element in zinc oxide eugenol cements (Al-Sa'eed, Al-Hiyasat and Darmani, 2008). Zinc toxicity when released from amalgam has been previously reported (Wataha et al. 1994).

Super EBA and IRM displayed excellent sealing ability when compared to amalgam, gutta-percha and glass ionomer cement with Super EBA displaying a more superior seal than IRM (Smee et al. 1987; Beltes et al. 1988; Higa et al. 1994; Szeremeta-Browar, VanCura and Zaki, 1985; Bondra et al. 1989). Based on the evidence Super EBA and IRM can be recommended as a root end filling materials.

1.8.6 Composite resin

Composite resins are composed of aromatic or aliphatic dimethacrylate monomers such as bisphenol-A-glycidyl methacrylate (BisGMA), triethylglycol dimethacrylate (TEGDMA) and urethane dimethacrylate(UDMA) (Chong and Pitt Ford, 2005).

Retroplast (Retroplast Trading, Rønne, Denmark) is a chemically cured flowable resin composite comprising of BisGMA and TEGDMA (Chong and Pitt Ford, 2005; Rud, Rud and Munksgaard, 1996). A technique using Retroplast bonded with the dentine bonding agent GLUMA (Heraus Kulzer, Werheim, Germany) was introduced as a root-end filling in 1984 (Rud, Rud and Munksgaard, 1996). The advantage of using GLUMA instead of other bonding agents is that it contains gluteraldehyde which provides a disinfecting ability (Rud, Rud and Munksgaard, 2001).

The benefit of using this technique is that no traditional cylindrical root-end cavity preparation is required, which can be particularly advantageous in difficult to access roots such as mandibular molars (Rud, Rud and Munksgaard, 2001). The advocated cavity design is a shallow, concave saucer shaped one with a

cavosurface angle close to 180^0 (Rud, Rud and Munksgaard, 1996). This preparation design allows for less volume of composite against the dentine surface and prevents contraction gaps forming between dentine and composite during the polymerisation (Rud, Rud and Munksgaard, 2001). EDTA is used to remove the smear layer after preparation of the root-end prior to the application of Gluma (Rud et al. 1991).

An *in-vitro* apical dye leakage study demonstrated that composite with dentine bonding agent showed the least leakage when compared to amalgam, Cavit and gutta-percha (McDonald and Dumsha, 1987). In a long-term follow up study of Retroplast-Gluma bonded retrograde fillings 32 out of 33 cases maintained complete bone healing when evaluated between 8 and 9 years postoperatively (Rud, Rud and Munksgaard, 1996). The regeneration of alveolar bone, periodontal ligament fibres and cementum over composite bonded retrograde fillings has been reported in case studies involving both monkeys and humans (Andreasen et al. 1993).

The use of bonded composite as a root-end filling is technique sensitive and dependant on the maintenance of a completely dry field during placement (Chong and Pitt Ford, 2005). In cases where haemostasis was unsuccessful, healing was incomplete probably due to bond failure between composite and dentine (Rud et al. 1991).

1.8.7 Compomer

Compomers are polyacid-modified composite resins that have a glass-ionomer component (Chong and Pitt Ford, 2005). The inflammatory response of compomer after four weeks was comparable to that of Super EBA when implanted in rat femurs, and bone healing was observed around both materials at 12 weeks (Pertot et al. 1997). A clinical study comparing compomer and glass-ionomer cement as root-end filling, showed that a significantly higher success rate was observed in the compomer group (89%) than in cases in the glass-ionomer group(44%) (Platt and Wannfors, 2004). An electrochemical study by Park et al. (2004) found that there was no significant difference between MTA, Super EBA and Dyract-flow compomer.

1.8.8 Gold Foil

The first reported use of gold foil as a retrograde filling was by Schuster in 1913 and Lyons in 1920 (Vasudev, Goel and Tyagi, 2003). Gold foil exhibits excellent marginal adaptation and biocompatibility (Goel et al. 1983). Improvement of biting forces was recorded following intentional replantation, and the group in which gold foil was used as a root-end filling material found to be superior to amalgam and polycarboxylate cement (Goel et al. 1983). No significant difference was observed in bone healing when gold-leaf and amalgam were used as root-end fillings (Waikakul and Punwutikorn, 1991). A moisture free environment is required for the placement of gold foil, making it impractical to use as a root-end filling (Vasudev, Goel and Tyagi, 2003).

1.8.9 Diaket (3M ESPE)

Diaket is a polyvinyl resin that is formed between zinc oxide and diaketone, and was originally developed for use as a root canal sealer when used in the ratio 1:1 powder to liquid (Chong and Pitt Ford, 2005; Regan, Gutmann and Witherspoon, 2002). A thicker consistency made from two or three parts powder to one part liquid is recommended for use as a root-end filling (Regan, Gutmann and Witherspoon, 2002). Diaket has good radiopacity and a working time of more than thirty minutes (Chong and Pitt Ford, 2005).

When tested as a retrograde filling, Diaket proved to have a superior sealing ability than amalgam and glass-ionomer cement (Kadohiro, 1984), as well as IRM and EBA (Walia, Newlin and Austin, 1995). Gerhards and Wagner (1996) found Diaket to have a similar sealing ability to amalgam and an inferior sealing ability to glass ionomer cement.

Nencka, Walia and Austin (1995) reported excellent handling characteristics and biocompatibility of Diaket when implanted in rat bone. Complete regeneration of the periodontium was observed when MTA and Diaket were used as retrograde fillings in dogs and Diaket was reported to have superior handling properties than MTA (Regan, Gutmann and Witherspoon, 2002). A histological evaluation of the tissue response to Diaket showed a hard tissue matrix with periodontal ligment and

cementum formation over Diaket indicating that it is a bio-inductive material (Williams and Gutmann, 1996). Diaket compared to gutta-percha displayed a better healing response with bone formation, periodontal ligament regeneration and cementum formation (Witherspoon and Gutmann, 2000).

1.8.10 Polycarboxylate Cement

Zinc polycarboxylate cement was introduced in 1968 by Dr Dennis Smith and is made up of powder and liquid components that hardens when mixed via an acid base reaction (Ladha and Verma, 2010). The powder comprises modified zinc-oxide with fillers and the liquid is an aqueous solution of polyacrylic acid (Ladha and Verma, 2010). Polycarboxylate cement has a stronger bond to enamel, and a far weaker bond to dentine due to a chelation reaction between the carboxyl groups of the cement and the calcium in tooth structure (Ladha and Verma, 2010). It is used as a luting cement and restorative material; and due to its low water solubility, was considered as a root-end filling material (Friedman, 1991). In a dye-penetration study, Barry et al. (1976) showed that polycarboxylate cement leaked significantly more than amalgam when used as a root-end filling. Due to its viscosity and accelerated setting time in a warm environment, application as root-end filling is difficult (Friedman, 1991). The difficult handling properties and poor sealing ability as a root-end filling render polycarboxylate cements unsuitable for use in this circumstance.

1.8.11 Calcium Silicate Cements

Mineral Trioxide Aggregate (MTA) can be described as a hydraulic cement because it sets and is stable underwater, and is primarily dependant on hydration reactions for its setting, which is in contrast to the usual acid base reactions of other dental materials (Darvell and Wu, 2011). The main constituent of MTA is calcium silicate (Camilleri, 2010). A generic term has been proposed to classify this category of material with the emergence of different versions of MTA, especially after the expiration of its patent (Darvell and Wu, 2011). The terms 'hydraulic silicate cements' or 'calcium silicate cements' have been proposed, with the latter having gained more popularity (Darvell and Wu, 2011; Camilleri, 2010). Current calcium silicate cements include:

1. ProRoot MTA (Dentsply/Maillefer, Ballaigues, Switzerland).
2. MTA Plus™ (Prevest Denpro Ltd., Jamu, India).
3. Biodentine™ (Septodont Ltd., Saint Maur des Fausse's, France).
4. MTA Angelus (Angelus Solucoes Odontologicas, Londrina, Brazil).
5. MTA Bio (Angelus Solucoes Odontologicas, Londrina, Brazil).
6. MM-MTA (MicroMega, Besançon, France).
7. MTA-CPM (EGEO SRL, Buenos Aires, Argentina).
8. Bioaggregate (Innovative BioCeramix, Vancouver, Canada).
9. EndoSequence Root Repair Material (Brasseler USA, Savannah, Georgia, USA).

1.9 ProRoot MTA (Dentsply/Maillefer, Ballaigues, Switzerland)

MTA was developed for use as a root-end filling material at Loma Linda University by Professor Mahmoud Torabinejad and colleagues in the early 1990's (Roberts et al. 2008; Camilleri and Pitt Ford, 2006). The first description of MTA in the scientific literature was in 1993 and by 1998 the U.S Food and Drug Administration approved MTA's use for endodontic treatment (Roberts et al., 2008; Lee, Monsef and Torabinejad, 1993; Schwartz et al. 1999).

1.9.1 MTA: The Powder

MTA is a fine hydrophilic powder derived from a Portland cement parent compound (Roberts et al. 2008). Portland cement is a basic ingredient of concrete used in the construction industry, and was first used as a root canal filling in 1878 by Witte (Schembri, Peplow and Camilleri, 2010; Gandolfi et al. 2014).

According to the patent, MTA is a Type 1 ordinary Portland cement with a Blaine number of 4500-4600 cm^2/g which indicates its fineness (Camilleri and Pitt Ford, 2006; Torabinejad and White, 1998). The raw materials used to manufacture Portland cement are calcium oxide (CaO) 60-66%, silica (SiO2) 19-25%, alumina (Al2O3) 3-8% and ferric oxide 1-5% (Camilleri, 2007). The calcium oxide (lime) is derived from the breakdown of limestone, and the other oxides are derived from shale (Camilleri, 2007). These raw materials are blended in a rotary kiln at 1450 ^{o}C producing a clinker, which is ground with gypsum (3-6%) to produce cement (Camilleri and Pitt Ford, 2006; Schembri, Peplow and Camilleri, 2010; Camilleri, 2007; Torabinejad and

White, 1998). The gypsum or calcium sulphate dihydrate(CaSO4.2H2O) is added to retard the setting time of the cement (Steffen and van Waes, 2009; Torabinejad and White, 1998). The final clinker comprises 55% tricalcium silicate, 20% dicalcium silicate, 10% tricalcium aluminate and 10% tetracalcium aluminoferrite (Camilleri, 2007). Bismuth is a heavy metal and is added to the cement in the form of Bismuth oxide (Bi_2O_3) in the ratio of 4:1 to provide radio-opacity to MTA for radiological diagnosis (Camilleri, 2007; Kim et al., 2008; Torabinejad and White, 1998).

1.9.2 Hydration of MTA

MTA cement is prepared by mixing its powder with sterile water using a 3:1 powder to liquid ratio (Parirokh and Torabinejad, 2010a). Upon hydration calcium hydroxide and a calcium silicate hydrate gel are formed and this solidifies into a hard structure in approximately 165 minutes (Camilleri, 2007; Parirokh and Torabinejad, 2010a). The hydration reaction of MTA is exothermic, and is assumed to be similar to that of Portland cement (Chedella and Berzins, 2010).

1.9.2.1 Analysis of hydration reactions of Portland Cement (Chedella and Berzins, 2010):

1. Tricalcium silicate setting:
   $2Ca_3SiO_4 + 4H20$ -----> $3CaO.2SiO_2.3H_2O + 3Ca(OH)_2$

2. Dicalcium silicate setting:
   $2Ca_2SiO_4 + 4H_2O$ -----> $3CaO.2SiO_2.3H_2O + Ca(OH)_2$

   The essential products of these two reactions are calcium silicate hydrates and calcium hydroxide.

3. Tricalcium aluminate forms ettringite in the presence of gypsum(CaSO4) and water:
   $Ca_3(AlO_3)_2 + 3CaSO_4 + 32H_20$ -----> $Ca_6(AlO_3)_2 + (SO_4)_3.32H_20$

4. After all gypsum is consumed the tricalcium aluminate reacts with ettringite to form monosulphates:
   $Ca_6(AO3)_2(SO_4)_3.32H20 + Ca3(AlO_3)2 + 4H20$ -----> $3Ca_4(AlO_3)_2(SO_4).12H_20$

5. Tetracalcium alluminoferrite hydration in the presence of gypsum:
   $2Ca_2AlFeO_5 + CaSO_4 + 16H_20$ -----> $Ca_4(AlO_3/FeO_3)_2(SO_4).12H_20 + 2Al/Fe(OH)_3$

1.9.2.2 Stages in the hydration reaction of Portland Cement (Camilleri, 2007):

I) Pre-induction phase (Initial few minutes)

The ionic species undergoes a rapid dissolution. The hydrolysis of tricalcium silicate results in the precipitation of a calcium silicate hydrate phase at the surface of the cement. Ettringite forms and precipitates on the surface of the cement by the dissolution of tricalcium aluminate and its reaction with calcium and sulphate ions.

II) Induction Phase (Initial few hours)

The calcium silicate hydrate coating on the unreacted cement acts as a barrier between the non-hydrated material and the bulk solution, thereby retarding further hydration. This lasts for one to two hours and is regarded as a 'dormant' phase during which the cement is plastic and manipulable. The calcium silicate hydrate coating then breaks up resulting in the hydration process continuing and an initial set occurring. The spaces between the cement grains are filled with hydration products and after about one hour a calcium silicate hydrate gel. Stiffening of the cement occurs with intergrowth of calcium silicate hydrate and the porosity of the cement decreases with an increase in solid material. The tricalcium aluminate forms more ettringite and once the sulphate ions are consumed, the ettringite is broken down to monosulphates.

III) Acceleration Phase (3-12 hours after mixing)

The rate of tricalcium and dicalcium silicate hydration increases. The calcium ion concentration decreases from the liquid phase as more crystalline calcium hydroxide (Portlandite) is precipitated.

IV) Post acceleration phase

The hydration process slows down and becomes diffusion controlled. Tricalcium and dicalcium silicate hydration continues and calcium sulphate is depleted resulting in ettringite conversion into monosulphates.

1.9.3 Differences between Grey MTA and White MTA

Grey ProRoot MTA was introduced to the market in 1998 and in 2002 white ProRoot MTA became available (Bozeman, Lemon and Eleazer, 2006). An electron probe microanalysis revealed that the dominant compounds present in both Grey and White MTA are lime (CaO), Silica (SiO_2) and bismuth oxide (Bi_2O_3) (Asgary et al. 2005). The same study also revealed that white MTA has 54.9% less Al_2O_3, 56.5% less MgO and 90.8% less FeO (Asgary et al. 2005; Roberts et al. 2008). A qualitative x-ray analysis of grey and white MTA using compositional imaging by Asgary confirmed that aluminium, magnesium and predominantly iron are less in white MTA (Asgary et al. 2006). White MTA was also found to have smaller particles and therefore a finer texture than grey MTA (Asgary et al. 2006). The significant reduction of iron in white MTA infers that iron is responsible for the grey colouration of grey MTA (Asgary et al. 2005; Asgary et al. 2006). The development of white MTA was intended to address the cosmetic concerns of the tooth discolouration potential of grey MTA (Asgary et al. 2006).

Calcium and phosphorous were described to be the major constituents of the original MTA described by Torabinejad et al. (1995b). Neither grey nor white MTA tested by Asgary et al. (2006) contained phosphorous, which implies that the formula of the original ProRoot MTA developed has been changed.

1.9.4 Differences between MTA and Portland Cement

Joseph Aspdin patented Portland cement in 1824, and named it after Portland, England where he had obtained the limestone (Viola, Filho and Cerri, 2011). Grey and white varieties of Portland cement are available. The raw materials used to manufacture grey Portland cement are heated to between 1400 ^{0}C-1450 ^{0}C by burning coal, which is responsible for the introduction of heavy metals into grey Portland cement (Steffen and van Waes, 2009). White Portland cement is

manufactured with purer raw materials that have a low iron content and requires being heated up to 1600^0C which can only be achieved by using gas and not coal (Steffen and van Waes, 2009).

An electron probe microanalysis by Asgary et al. (2004) concluded that composition of white Portland cement and white MTA was the same, except for the absence of Bismuth Oxide in Portland cement, and the overall finer particle size of white MTA. Dammashcke et al. (2005) demonstrated that Portland cement had a wider range and size of particles whereas white Proroot MTA had smaller, more uniform particles.

Oliveira et al. (2007) also confirmed that the components of Portland cement and MTA are similar, except for the absence of bismuth oxide in Portland cement and lack of Alumina in ProRoot MTA . A Scanning electron microscopy study comparing the hydration of white Portland cement and MTA by Camilleri (2007), showed that the tricalcium and dicalcium silicate composition of white Portland cement and MTA were similar. In the same study MTA was found to be deficient of the aluminate phase that is present in Portland cement. The aluminate phase acts as a flux and is necessary for sufficient clinkering or partial fusion of the raw materials in Portland cement (Camilleri, 2007). The lack of Alumina in MTA suggests that it is prepared in a laboratory and not a rotary kiln (Camilleri, 2007). This may imply that MTA is derived from a medical grade Portland cement rather than that used in the construction industry.

1.9.5 Presence of arsenic and other heavy metals in MTA

Arsenic is a metallic element that is present in soil, rock and may be a contaminant of water in certain areas (Duarte et al. 2005). Arsenic is absorbed by mucosa and enters erythrocytes, from where it is deposited mainly in the tissues of the liver, kidneys and lungs, and is mainly toxic to the hepatic, renal and peripheral nervous system (Duarte et al. 2005). There is a possibility that arsenic may be carcinogenic, as it binds to certain proteins resulting in the inhibition of DNA repair (Mandal and Suzuki, 2002). Amongst the heavy metals incorporated into Portland cement during the clinkering process of Portland cement, are arsenic, chromium and lead (Schembri, Peplow and Camilleri, 2010). The arsenic content of MTA is a concern because the material is placed in close proximity to hard and soft tissues (Schembri,

Peplow and Camilleri, 2010). The maximum values for dental restorative materials specified by ISO 9917-1 of arsenic is 2mg/kg and lead is 100mg/kg (ISOEN9917–1, 2007).

Duarte et al. (2005) analysed the release of arsenic from white Portland cement, grey Portland cement, Proroot MTA and MTA Angelus using atomic absorption spectrophotometry. They concluded that all the materials contain arsenic, however the values were well below those considered harmful, thus justifying the use of MTA and Portland cement clinically (Duarte et al., 2005). In a letter to the editor, Carolyn Primus, the inventor of white ProRoot MTA (Primus, 2004), disagreed with the findings of Duarte et al. (2005), stating that the composition and properties of Proroot MTA are controlled during the manufacturing process, thus rendering it free of contaminants (Primus, 2006). Monteiro Bramante et al. (2008) used atomic absorption spectrophotometry to investigate the presence of arsenic in grey ProRoot MTA, grey MTA Angelus, White MTA Angelus, grey Portland cement, white Portland cement, CPM, CPM Sealer, MTA Obtura and an experimental MTA. They found that only MTA Obtura, MTA Angelus and white Portland cement have arsenic levels below those specified in ISO 9917-1 (Monteiro Bramante et al. 2008). Schembri, Peplow and Camilleri (2010), used graphite furnace atomic absorption spectrophotometry, and found that white ProRoot MTA and MTA Angelus contain levels of arsenic higher than that specified by ISO 9917-1. They also found that chromium, arsenic and small quantities of lead are leached from hydrated MTA in simulated body fluids, however, after fifteen days no further leaching of the metals was observed, indicating that there is no risk of continuous release of these metals into the body (Schembri, Peplow and Camilleri, 2010). The arsenic compounds within MTA and Portland cement may be stable, as neither MTA nor Portland cement were found to be genotoxic or cytotoxic (Ribeiro et al., 2006a; Ribeiro et al., 2006b).

1.9.6 MTA: The Crystal

Following the hydration phase in which the anhydrous material dissolves, the hydration products crystallise forming an interlocking mass (Lee et al. 2004). The microstructure of the hydrated mass of MTA is composed of cubic crystals and needle-like crystals (Lee et al. 2004). The cubic crystals interlock with each other at a constant angle to form the basic framework of set MTA (Lee et al. 2004). The needle-

like crystals have sharp extremities and form thick bundles that fill the intergrain space between cubic crystals (Lee et al. 2004; Wang et al. 2010).

1.9.7 Factors affecting the hydration and structure of MTA

1.9.7.1 Type of liquid used to hydrate MTA Powder

a) Sterile water

Sterile water is the recommended vehicle by the manufacturer to hydrate MTA in a 3:1 powder to liquid ratio (Parirokh and Torabinejad, 2010a).

b) Distilled water

When MTA was mixed with sterile water and placed in a distilled water environment to complete its hydration process, the cubic crystals were found to dominate the structure and the needle-like crystals filling the intergrain space were sparser resulting in an irregular, uneven structure (Lee et al. 2004; Wang et al. 2010; Lee et al. 2007).

c) Saline

MTA mixed with sterile water and placed in saline to complete its hydration process resulted in a larger crystal structure with significantly more needle-like crystals filling the intergrain space between the cubic crystals due to the Na^+ and Cl^- ions in saline providing more nucleation sites for needle like crystals (Lee et al. 2004; Wang et al. 2010; Lee et al. 2007). The microhardness of MTA samples placed in in saline and distilled water was not significantly different (Lee et al. 2004; Lee et al. 2007). The compressive strength of MTA hydrated directly with saline was found to be higher than MTA mixed with sterile water (Kogan et al. 2006).

d) Local Anaesthetic

2% Lidocaine HCL with adrenaline used to hydrate MTA resulted in a significant increase in compressive strength when compared to MTA hydrated with sterile water, however the local anaesthetic mixture was found to delay the setting time (Kogan et al. 2006).

e) Sodium Hypochlorite

MTA hydrated with sodium hypochlorite was found to set quicker than when sterile water was used, however the compressive strength of the sodium hypochlorite mixture was significantly lower (Kogan et al., 2006).

f) Ethylenediaminetetraacetic Acid (EDTA)

MTA hydrated with EDTA resulted in a granular structure with an absence of crystal formation (Lee et al. 2007). EDTA chelates calcium ions released from the tricalcium complex of MTA thereby inhibiting the hydration of MTA (Lee et al. 2007).

g) Chlorhexidine gluconate

MTA mixed with 2% chlorhexidine gel did not achieve a complete set in a study by Kogan et al. (2006). Stowe et al. (2004) found that using 0.12% chlorhexidine gluconate instead of sterile water enhanced the antimicrobial effect of MTA and no difficulty in achieving a set was reported.

h) Calcium Chloride

5% Calcium chloride used to hydrate MTA instead of sterile water resulted in a faster setting time but lower compressive strength of MTA (Kogan et al. 2006).

1.9.7.2 Powder to liquid ratio

When MTA powder is mixed with water, an amorphous, porous capillary structure within the MTA mass is observed under SEM (Fridland and Rosado, 2003). Water is absorbed from the surrounding aqueous environment into the capillaries and pores, becomes saturated with calcium hydroxide which dissolves from hardened MTA and is released into the surrounding environment (Fridland and Rosado, 2003). Water re-enters the capillary matrix and becomes saturated with calcium hydroxide and this osmotic process continues, resulting in the continuous release of calcium hydroxide into the surrounding environment (Fridland and Rosado, 2003). MTA can therefore be described as a hygroscopic material as it has the ability to attract and hold water molecules from the surrounding environment (Storm et al. 2008).

A powder to liquid ratio of 3:1 as recommended by the manufacturer for hydration resulting in a higher value of porosity and solubility of MTA when compared to less

water used (Fridland and Rosado, 2003; Fridland and Rosado, 2005). The MTA structure does not dissolve as only the calcium hydroxide component of the MTA is soluble and not the Silica matrix (Fridland and Rosado, 2005). Should the 3:1 powder to liquid ratio result in a soupy mix, moist gauze can be placed over it until the mixture acquires the desired consistency to work with (Fridland and Rosado, 2003).

1.9.7.3 Environmental pH

Lee et al. (2004) found that the microstructure of MTA displayed underdeveloped cubic crystals and an absence of needle like crystals when MTA was hydrated and stored in pH 5, in contrast to well-formed cubic and needle like crystals when hydrated MTA samples were placed in a pH 7 solution. The microhardness of MTA was also found to be significantly lower in samples stored in pH 5 solution compared to those stored in the pH 7 solution (Lee et al. 2004). Namazikhah et al. (2008) found that an acidic environment impaired the surfaces hardness of MTA and that porosity of MTA samples increased with increasing acidity of the storage solution.

1.9.7.4 Method of mixing

MTA powder is manually mixed with sterile water to form a slurry. Mechanical mixing of MTA and water in a plastic mixing capsule using an amalgamator resulted in a higher compressive strength of MTA compared to manual mixing (Basturk et al. 2013). According to Nekoofar, Aseeley and Dummer (2010), triturating MTA and water in a mixing capsule containing a plastic pestle, produces an MTA slurry which is standardised, consistent and more manageable.

1.9.7.5 Condensation pressure

Aminosharaie, Hartwell and Moon (2003) found that hand condensation was preferable to ultrasonic condensation of MTA as it resulted in better adaptation and less voids. Use of higher condensation pressure resulted in less voids and microchannels within MTA and lower compressive strength when compared to lower condensation pressure (Nekoofar et al. 2007). Well-formed crystalline structures around microchannels were observed under SEM when lower condensation pressure was used compared to a reduction in crystalline formation when high condensation pressure was used (Nekoofar et al. 2007). When heavy condensation pressure is used

it results in the material being compacted too closely resulting in pores and broken microchannels which does not allow water to readily diffuse within the cement and thus impairs the hydration process (Nekoofar et al. 2007).Yeung, Liewehr and Moon (2006) compared fill density of MTA condensed by hand and by indirect ultrasonic activation and concluded that the latter produced a denser fill. A higher compressive strength of MTA was recorded regardless of method of mixing technique (manual & mechanical) when indirect ultrasonic agitation was applied to MTA (Basturk et al. 2013). Nekoofar, Aseeley and Dummer (2010) concluded that direct ultrasonic agitation of MTA resulted in higher microhardness of MTA.

1.9.7.6 Blood contamination

Torabinejad et al. (1994) investigated the effect of blood contamination on leakage and concluded that it did not affect leakage significantly. Vanderweele, Schwartz and Beeson (2006), found that MTA uncontaminated by blood had a higher displacement resistance than blood contaminated samples.

Nekoofar, Stone and Dummer (2010) found that the compressive strength of MTA is reduced when it comes into contact with blood, thereby compromising its physical properties. In a study examining the microstructure of MTA, specimens contaminated with blood were found to have an absence of calcium hydroxide crystals in the early phase of hydration (Nekoofar et al. 2011). In the same study, specimens contaminated partially or entirely with blood displayed an absence of acicular crystals which are characteristic of ettringite, whereas these crystals were abundant in those specimens uncontaminated with blood (Nekoofar et al. 2011).

1.9.7.7 Storage temperature

Saghiri et al. (2010) investigated the effect of storage temperature on White MTA and found that the surface hardness of the groups stored at 25 ^{0}C and 40 ^{0}C were significantly higher than those stored at 4 ^{0}C. At higher temperatures the hydrates were distributed more homogenously and needle-like crystals formed, resulting in better interlocking of the crystal structure (Saghiri et al. 2010). At lower temperatures the hydrates were distributed heterogeneously and weak interlocking of the solids were observed because of short needle-like crystals (Saghiri et al. 2010). Based on

these findings it is recommended not to store MTA in a refrigerator, but rather at or above room temperature.

1.9.8 Sealing ability of MTA as a root-end filling material

The sealing ability of MTA as a root-end filling material has been tested by examining the microleakage and marginal adaptation of the material (Torabinejad and Parirokh, 2010).

1.9.8.1 Microleakage

Microleakage can been defined as the passage of bacteria, fluids and chemical substances between the root canal filling material and the tooth (Timpawat, Vongsavan and Messer, 2001). The various methods used to examine microleakage of MTA are dye penetration, fluid filtration, bacterial leakage and protein leakage (Torabinejad and Parirokh, 2010).

a) Dye penetration

Bacteria and there by-products are larger than dye molecules, therefore, if a root-end filling material resist the penetration of dye molecules, it infers that it would prevent the egress of bacteria and their by-products from within the root canal system into the periapical tissues (Verissimo and do Vale, 2006; Torabinejad, Watson and Pitt Ford, 1993; Aqrabawi, 2000). Dyes that have been used to test the microleakage of MTA include rhodamine B, methylene blue, India ink, Pelikan ink, fuchsin and silver nitrate (Torabinejad and Parirokh, 2010). Dye leakage studies revealed that MTA shows less leakage than amalgam, Super EBA and IRM (Torabinejad et al. 1994; Torabinejad, Watson and Pitt Ford, 1993; Aqrabawi, 2000; Martell and Chandler, 2002; Davis et al. 2003; Pereira, Cenci and Demarco, 2004; Asgary, Eghbal and Parirokh, 2008).

b) Fluid filtration

The fluid filtration technique measures the transport of fluid through specimens with the use of pressure (Kontakiotis, Chaniotis and Georgopoulou, 2007). Fluid filtration studies show that MTA provides a far superior seal than amalgam, Super EBA and IRM (Bates, Carnes and del Rio, 1996; Yatsushiro, Baumgartner

and Tinkle, 1998; Wu, Kontakiotis and Wesselink, 1998; Fogel and Peikoff, 2001; Karlovic et al. 2005).

c) Bacterial Leakage

Microleakage studies using bacterial penetration showed that MTA displays a higher resistance to bacterial penetration than amalgam and Super EBA (Torabinejad et al., 1995c; Fischer, Arens and Miller, 1998; Maltezos et al. 2006). MTA showed more resistance to leakage of endotoxin than amalgam, Super EBA and IRM (Tang, Torabinejad and Kettering, 2002).

d) Protein Leakage

The ideal thickness of MTA that would best resist penetration was tested by Valois and Costa (2004) using a protein leakage technique and was found to be four millimetres. Saghiri et al. (2008) tested the effect of environmental pH on protein leakage of MTA and found that the more acidic the environmental pH, the least resistant to leakage the MTA was.

1.9.8.2 Marginal adaptation

Studies using scanning electron microscopy show that MTA exhibits better marginal adaptation than glass-ionomer cement, amalgam, IRM and SEBA (Camilleri and Pitt Ford, 2008; Torabinejad et al. 1995d; Gondim et al. 2003; Shipper et al. 2004).

1.9.9 Biocompatibility

Biocompatibility is an important property of root-end filling and endodontic repair materials as they are in close contact with the periodontium, and should be non-toxic toward the host tissues (Torabinejad and Parirokh, 2010).

MTA has been found to be non-mutagenic (Kettering and Torabinejad, 1995), non-neurotoxic (Asrari and Lobner, 2003), and anti-nociceptive against neurogenic and inflammatory pain (Abbasipour et al., 2009). With regard to the effects on vascular tissues Masuda et al. evaluated the revascularisation of connective tissue using the improved rabbit ear chamber and established that MTA produces no adverse side effect on microcirculation (Masuda et al. 2005). Furthermore, Tunca et al. (2007) found that MTA has a vasoconstrictor effect due to calcium influx which may help in

controlling haemorrhage. MTA was shown to be one of the least cytotoxic materials using various cell culture studies, and has been confirmed to stimulate the production of cytokines and signalling molecules (Torabinejad and Parirokh, 2010). MTA is non-toxic toward progenitor cells (Torabinejad and Parirokh, 2010).

1.9.10 Bioactivity

A biomaterial may be defined as a non-drug substance that is suitable for inclusion in systems which augment or replace the function of bodily tissues or organs (Heness and Ben-Nissan, 2004). A biomaterial may be described by three terms depending on the tissue reaction it illicits when placed in contact with human tissues (Heness and Ben-Nissan, 2004):

a) **Bioinert:** Minimal interaction occurs with the surrounding tissues when these materials are placed in the human body; for example, titanium and stainless steel.

b) **Bioresorbable**: When placed within the human body a bioresorbable material starts dissolving and is replaced by advancing tissue such as bone; for example tricalcium phosphate, calcium oxide and calcium carbonate.

c) **Bioactive**: When placed in contact with living bone, a bioactive material undergoes a surface modification via an ion-exchange reaction between the material and the surrounding body fluids which results in a layer of biologically active calcium carbonate apatite on the material's surface that is equivalent to the mineral phase of bone; for example Bioglass, glass ceramic and synthetic hydroxyapatite.

One of the characteristics of calcium silicate biomaterials is apatite formation (Gou et al. 2005; Zhao et al. 2005). MTA is composed mainly of calcium and silicate (Camilleri et al. 2005), and has been described as a bioactive material (Enkel et al. 2008).

I) MTA was used to obturate root canals and placed in contact with Phosphate Buffered Saline (PBS) for two months to simulate being in tissue fluid (Sarkar et

al. 2005). It was found that MTA dissolves to a certain extent and leaches mostly calcium and to a lesser extent silica, bismuth, iron, aluminium and magnesium. Furthermore, SEM revealed a white crystalline deposit between MTA and Dentine (Sarkar et al. 2005). Energy dispersive x-ray analysis showed this white structure to have a similar composition to hydroxyapatite namely calcium, phosphorous and oxygen (Sarkar et al. 2005). The hydroxyapatite is probably formed by the interaction between the calcium released from the MTA and the phosphate from the PBS (Sarkar et al. 2005). The formation of hydroxyapatite on the surface of MTA enhances the chemical bond between MTA and dentine, and can promote the remineralisation of the surrounding hard tissues (Sarkar et al. 2005).

II) Bozeman, Lemon and Eleazer (2006) compared White MTA and Grey MTA when placed in PBS and also found hydroxyapatite crystal growth on the surfaces of both materials, with the hydroxyapatite crystals being more predominant over grey MTA than white MTA even though they both released a similar amount of calcium into the solution. It was proposed by Bozeman, Lemon and Eleazer (2006) that the voids in the form of pores and capillaries observed by Fridland and Rosado (2003) may fill with hydroxyapatite as calcium ions are released into the voids.

III) When white Portland cement was immersed in PBS, an amorphous calcium phosphate was formed, and this transformed into a calcium deficient carbonated apatites within thirty minutes, which is equivalent to the apatite phases of bone, cementum and dentine (Tay et al. 2007).

IV) Dentine discs filled with ProRoot MTA, MTA Branco, MTA Bio and white Portland cement were placed in PBS (Reyes-Carmona, Felippe and Felippe, 2009). A white precipitate formed on the surface of all materials within the first hour of immersion, forming an interfacial layer between the materials and dentine which was identified as carbonate apatite, based on its elemental composition of calcium and phosphate. Furthermore, these carbonated apatite crystals formed tag-like structures that extended into the dentine tubules which

resulting in an intra-tubular mineralization process (Reyes-Carmona, Felippe and Felippe, 2009).

V) Gandolfi et al. (2010) confirmed the apatite forming ability on the surface of MTA when placed in PBS, using environmental scanning electron microscopy.

VI) A bacterial leakage investigation using MTA as a root-end filling on extracted teeth Parirokh et al. (2009) compared the samples when placed in normal saline and phosphate buffered saline. The MTA samples that were placed in PBS exhibited less bacterial leakage and a white crystalline layer over MTA (Parirokh et al. 2009).

VII) The apatite forming ability of MTA was compared to an experimental calcium silicate cement that contains phosphate ions (Asgary et al. 2008). Hydroxyapatite crystals were formed and deposited on the surface of both materials when placed in PBS. However when placed in normal saline only the experimental calcium silicate cement was able to stimulate the formation hydroxyapatite probably because of its endogenous content of calcium and phosphate ions (Asgary et al. 2008). MTA depends on releasing its calcium ions that react with extrinsic phosphate ions in the surrounding environment in order to form hydroxyapatite, as it lacks intrinsic phosphate ions (Asgary et al. 2008).

1.9.11 Cementum formation (Cementogenesis)

A histological study performed on beagle dogs by Torabinejad et al. (1995a) showed that when MTA and amalgam were used as a root end fillings, cementum formed directly over MTA, whereas no cementum formed over amalgam. In a similar histological study carried out on monkeys using MTA and amalgam as root end fillings, a thick layer of cementum was found over the MTA that continued over the resected dentine and joined the cementum on the side of the root (Torabinejad et al. 1997). In addition to the cementum formation, blast cells were found on the surfaces of the cementum and periodontal fibres inserting into the cementum in some areas (Torabinejad et al. 1997). Torabinejad et al. (1997), deduced that the cementum formation was derived from ingrowing connective tissue from the bone

rather than from remaining periodontal ligament from the sides of the roots because of:

a) The presence of blast cells on the surface of the cementum.
b) Parallel incremental lines in the cementum which would have been expected to be diagonal had the cementum been derived from existing periodontal ligament.
c) Certain sections showed islands of cementum formation directly against the MTA that did not continue onto the dentine surface.

In an animal study using dogs, the periapical tissue response to amalgam, Super EBA and MTA used as root end fillings and found that cementum formed over the MTA samples only (Baek, Plenk and Kim, 2005). Regan, Gutmann and Witherspoon (2002) performed a histological study comparing MTA and Diaket as root-end fillings and found that both materials were able to stimulate cementum formation over them, as well as regeneration of periodontal ligament and adjacent bone.

The combination of the physical bond that MTA forms with dentine, and the regeneration of cementum results in the formation of a double seal (Regan, Gutmann and Witherspoon, 2002).

1.9.12 Osteogenesis

MTA and Super EBA were found to be osteoconductive as it stimulated osteogenesis when implanted in bone (Moretton et al. 2000). The promotion of osteoblastic activity by MTA in bone has been well established (Baek et al. 2010; Torabinejad et al. 1998; Koh et al. 1998).

1.9.13 Mechanism of action of MTA

According to Parirokh and Torabinejad (2010b), from the time MTA is placed in contact with human tissues, it forms calcium hydroxide and releases calcium ions for cell attachment and proliferation, creates an alkaline environment which is antibacterial, modulates the production of cytokines, encourages the differentiation and migration of hard tissue producing cells, and forms hydroxyapatite on its surface

and provides a biological seal with dentine. MTA is a bioactive material, that is hard tissue conductive and hard tissue inductive, as it stimulates bone formation and cementum regeneration (Parirokh and Torabinejad, 2010b).

1.9.14 Drawbacks of MTA

The drawbacks of MTA are (Parirokh and Torabinejad, 2010b; Bortoluzzi et al. 2006):

1. Long setting time.
2. Potential to cause tooth discolouration.
3. Presence of toxic elements within the material.
4. Difficult handling properties.
5. MTA is expensive.
6. No known solvent.
7. Difficult to remove once set.
8. Washout in the early stages of placement.

Despite the drawbacks, the introduction of MTA as a root-end filling and endodontic repair material has revolutionised the field of endodontics due to its ability to set in a moist environment, its excellent sealing ability and the bioactive nature of MTA when it comes in contact with tissue fluids.

1.10 MTA Plus™ (Prevest Denpro Limited, Jamu, India)

MTA Plus™ is a novel mineral trioxide aggregate material which has a finer particle size than MTA (Camilleri, Formosa and Damidot, 2013). The MTA Plus™ powder is supplied with a proprietary salt-free polymer gel and water, either one of which can be used as mixing vehicles (Gandolfi et al. 2014). The finer particle size improves the handling and placement of MTA Plus™, and the purpose of the gel is to provide an anti-washout property to the material (Gandolfi et al. 2014; Formosa, Mallia and Camilleri, 2013a).

It is necessary to irrigate the osteotomy site prior to closing a periapical flap to avoid complications (Formosa, Mallia and Camilleri, 2013a). One of the drawbacks of MTA is washout, which may be defined as the tendency of a cement to disintegrate

upon early contact with blood and other fluids (Bortoluzzi et al. 2006; Khayat, 1995). Washout resistance is an important quality of a root-end filling as the final irrigation and resuming of blood flow to the area may result in loss of some of the material placed in the root-end cavity, thereby compromising the apical seal (Formosa, Mallia and Camilleri, 2013a; Formosa, Mallia and Camilleri, 2013b).

During the construction of underwater structures a water-soluble polymer is added to concrete in order to make it resistant to washout, by modifying its rheological properties (Khayat, 1995). This is achieved by increasing the viscosity of the liquid used to mix the cement powder, thereby increasing the resistance of the cement to segregation by an external washing action (Formosa, Mallia and Camilleri, 2013b). A similar concept was employed in the development of the gel additive to MTA Plus™ (Formosa, Mallia and Camilleri, 2013b). According to Wang et al. (2007) an ideal anti-washout agent should have the following properties:

a. Prevent decay of cement in liquid.
b. Should not interfere with hydration reaction or bioactivity of the cement.
c. Should not decrease the mechanical strength of the set cement.
d. Should improve and not worsen the handling properties of the cement.
e. Should not extend the setting time of the cement.
f. Should not reduce the radiopacity of the cement.

1.10.1 Composition of MTA Plus™ powder

An X-ray diffraction analysis of the unhydrated powder of MTA Plus™ demonstrates that its components are tricalcium silicate, dicalcium silicate and bismuth oxide for radiopacity (Formosa, Mallia and Camilleri, 2013b). MTA Plus™ was found to have a higher specific surface area than ProRoot MTA thereby confirming the smaller particle size of MTA Plus™ (Formosa, Mallia and Camilleri, 2013b; Camilleri, Formosa and Damidot, 2013). Unpublished data by the inventor, CM Primus states that 50% of the particles are finer than 1µm (Qi et al. 2012).

1.10.2 Composition of the anti-washout gel

In the construction industry, commonly used anti-washout admixtures are cellulose derivatives, such as hydroxypropyl methyl cellulose, and microbial polysaccharides, such as Welan gum (Khayat, 1995). A quantitative analysis of the MTA Plus™ anti-washout gel shows that it comprises 97.8% water, 2.08% silicon, 0.08% Potassium, 0.02% chlorine and 0.01% calcium (Formosa, Mallia and Camilleri, 2013b). However, the silicon content could be as a result of the prolene film used during the chemical analysis of the gel and the other components could be impurities, therefore, it is difficult to accurately identify the constitution of the proprietary gel (Formosa, Mallia and Camilleri, 2013b).

1.10.3 Mixing and setting times

The powder: liquid ratio for standard mixing is 3:1, however more gel may be added to modify the rheological properties and setting time of the cement (Gandolfi et al. 2014). The setting time of MTA Plus™ mixed with water (180 mins) was longer than MTA Plus™ mixed with the anti-washout gel (115mins) by 65minutes (Formosa, Mallia and Camilleri, 2013b). According to the manufacturer the working time is 12 minutes and the setting time is less than one hour when mixed to a thick consistency (Avalon Biomed Inc.). Qi et al. (2012) found that 3:1 mixture set within 1.2 hours when mixed with the gel.

1.10.4 Hydration of MTA plus™

The anti-washout mixture results in the formation of a branched polymer network within the cement which controls water movement and reduces the possibility of dilution by external water (Formosa, Mallia and Camilleri, 2013b). MTA Plus™ mixed with anti-washout gel displays less fluid uptake from the surrounding environment than MTA Plus™ with water, (Formosa, Mallia and Camilleri, 2013b).The increased surface area, due to the finer particles would result in greater cohesion of particles with each other and also assist in resisting to washout before the calcium silicate hydrate network develops strength (Formosa, Mallia and Camilleri, 2013a). The increased adhesion between the cement grains may be reason for the increased viscosity of the cement paste (Formosa, Mallia and Camilleri, 2013b). A quantitative mass loss assessment experiment proved that MTA Plus™ with anti-washout gel

resulted in significantly less anti-washout than MTA Plus™, MTA Angelus and White Portland cement mixed with water (Formosa, Mallia and Camilleri, 2013a).

1.10.5 Properties of set MTA Plus™

Upon hydration of MTA Plus™ powder with the anti-washout gel or water, calcium silicate hydrate forms (Formosa, Mallia and Camilleri, 2013b). MTA Plus™ mixed with anti-washout gel produces a denser, more compact and less porous structure that is less sensitive to environmental conditions than MTA Plus™ mixed with water (Formosa, Mallia and Camilleri, 2013b). The MTA Plus™ with anti-washout gel showed less porosity, sorption and solubility when compared to ProRoot MTA and MTA Plus™ mixed with water (Gandolfi et al. 2014). The MTA Plus™ mixed with anti-washout gel has a greater compressive strength than MTA Plus™ mixed with water (Formosa, Mallia and Camilleri, 2013b).

1.10.6 Bioactivity

MTA Plus™ mixed with antiwashout gel and water results in high calcium release and pH (Formosa, Mallia and Camilleri, 2013b; Gandolfi et al. 2014). The bioactivity of MTA Plus™, mixed with water or antiwashout gel, was confirmed by the formation of a thick precipitate of calcium phosphate spherules when soaked in simulated body fluids (Gandolfi et al. 2014). MTA Plus™ is a bioactive cement in that it displays high calcium ion release which forms hydroxyapatite in a physiologic solution (Formosa, Mallia and Camilleri, 2013b; Gandolfi et al. 2014).

1.11 Biodentine™ (Septodont LTD., Saint Maur des Fausse's, France)

Biodentine™ is a synthetic tricalcium silicate based cement that is advertised as a 'bioactive dentine substitute', and has been commercially available since January 2011 (Pawar, Kokate and Shah, 2013; Rajasekharan et al. 2014). The production of Biodentine™ is based on 'Active Biosilicate Technology™', which results in the production of pure tricalcium silicate that is free of metallic impurities (Septodont).

1.11.1 Composition of Biodentine™

It has a powder component in a capsule and liquid packaged in a pipette. The powder is made up of tricalcium silicate (main core material), dicalcium silicate (second core material), calcium carbonate and calcium oxide (filler materials), iron oxide(colouring agent) and zirconium oxide(radiopacifier) (Priyalakshmi and Ranjan, 2014). The liquid consists of a hydrosoluble polymer (water reducing agent) and calcium chloride(setting accelerator) (Septodont). The hydrosoluble polymer may also be described as a superplasticizer and maintains the flowability of the mixture in a low water to solid ratio (Camilleri, Sorrentino and Damidot, 2013). The advantage of synthesising pure tricalcium silicate compared to purifying natural tricalcium silicate is that the mineralogy is not altered by sintering conditions or variable composition of raw materials (Camilleri, Sorrentino and Damidot, 2013). The absence of metallic impurities has been confirmed by analysing acid extracts and leached trace elements of Biodentine™ (Grech, Mallia and Camilleri, 2013; Camilleri et al. 2012). The particle size of the Biodentine™ powder was found to be much finer than that of MTA (Camilleri, Sorrentino and Damidot, 2013).

1.11.2 Mixing and hydration of Biodentine™

The aqueous solution is mixed with the powder within the capsule in a triturator for thirty seconds at a speed of 4000-4200 rotations per minute (Septodont). The hydration reaction results in the formation of a calcium silicate hydrate gel and the release of calcium hydroxide (Camilleri et al. 2012). According to Camilleri, Sorrentino and Damidot (2013) the calcium carbonate acts a nucleation site for the calcium silicate hydrate; as a result there is a shorter induction period and therefore an initial set within 12 minutes. The final setting time of Biodentine™ was found to be 45 minutes (Grech, Mallia and Camilleri, 2013).

1.11.3 Physical properties of Biodentine™

Set biodentine has a dense microstructure with almost all the porosities filled with calcium silicate hydrate and calcium hydroxide (Rajasekharan et al. 2014). Biodentine™ is more dense and less porous compared to MTA (Rajasekharan et al. 2014). The flexural strength, elastic modulus and Vickers hardness are similar to

dentine and higher compared to MTA (Camilleri, Sorrentino and Damidot, 2013). The pH of the leachate of Biodentine™ was found to be 11.3 after one day and 12.3 after 28 days, indicating the alkalinity of the cement (Camilleri et al., 2012).

1.11.4 Effect of environmental pH on Biodentine™

Poplai, Jadhav and Hegde (2012) demonstrated that the pushout bond strength at pH 7.4 was far higher than at pH 4.4). They concluded that at low pH conditions, the bond strength of biodentine is lower than at high pH conditions (Poplai, Jadhav and Hegde, 2012).

1.11.5 Effect of blood contamination

When contaminated with blood the pushout bond strength of biodentine was higher than that of MTA at 24 hours, however after seven days there was no difference in pushout bond strength between Biodentine™ and MTA (Aggarwal et al. 2013). It was concluded that there was no difference in pushout bond strength of biodentine with respect to setting duration or blood contamination (Aggarwal et al. 2013).

1.11.6 Effect of smear layer removal

The smear layer is caused by the action of root canal instruments against the dentine wall and comprises organic and inorganic material (El-Ma'aita, Qualtrough and Watts, 2013). It ranges between 1-5µm thick and is made up of dentinal shavings, remnants of necrotic pulp, bacteria and bacterial byproducts. There are conflicting opinions on whether to remove the smear layer or not prior to obturation of the root canal. The smear layer may promote micro leakage as it is loosely adherent to dentine and may harbour bacteria or serve as a substrate for remaining bacteria within the root canal system (Violich and Chandler, 2010). Furthermore, the smear layer may prevent irrigants and medicaments from penetrating the dentinal tubules thereby hampering their disinfection (Violich and Chandler, 2010). Possible advantages of the smear layer are that it may entomb any surviving bacteria within the dentinal tubules or prevent bacterial penetration into the tubules (El-Ma'aita, Qualtrough and Watts, 2013).

A literature review of microleakage studies concluded that removal of the smear layer results in an improved sealing ability of gutta percha and sealer to dentine due to enhanced penetration of sealer into dentinal tubules (Yildirim, Orucoglu and Cobankara, 2008). Conversely, a significant reduction in the sealing ability of calcium silicate cements is observed with the removal of the smear layer (Yildirim, Orucoglu and Cobankara, 2008; Yildirim et al. 2010). This may be attributed this to the large particle size of the calcium silicate cements (2.44-3.05µm) compared to dentinal tubules (0.9-2,5µm) (El-Ma'aita, Qualtrough and Watts, 2013; Komabayashi and Spangberg, 2008). Furthermore, the smear layer plays an important role in the formation of an interfacial layer of hydroxyapatite (layer resembling hydroxyapatite) between calcium silicate cements and radicular dentine (El-Ma'aita, Qualtrough and Watts, 2013). Atmeh et al. (2012) described the formation of a 'mineral infiltration zone' which is a tag like structure alongside the interfacial layer. The high alkalinity of calcium silicate cements causes the 'caustic etching' of the collagen rich interfacial dentine, thus allowing the infiltration of Ca^{2+}, OH^{-}, and CO_3 ions into the porosities and increasing mineralisation in this region (Atmeh et al. 2012).

1.11.7 Sealing ability of Biodentine™

A dye leakage study comparing MTA, Biodentine™ and glass-ionomer as root-end fillings found that all materials leaked, but biodentine leaked significantly less than the others (Kokate and Pawar, 2012). A marginal adaptation study using Confocal Laser Scanning Microscopy showed that Biodentine™ had the lowest marginal gaps followed by MTA, with glass ionomer cement having the highest marginal gaps (Ravichandra et al. 2014). Biodentine™ and IRM exhibited the lowest degree of porosity when compared to Bioaggregate and a prototype radiopacified tricalcium cement (TCS-20-Zr). The study also revealed that dry storage of biodentine resulted in cracks at the Biodentine™-root dentine interface, as well as shrinkage and cracks within the bulk material (Ravichandra et al. 2014). A scanning electron microscope, marginal adaptation study comparing Biodentine, MTA and IRM revealed that MTA and IRM had a superior marginal adaptation to Biodentine™ when used as root-end filling materials (Soundappan et al. 2014).

1.11.8 Biocompatibility of Biodentine™

Biodentine™ was found to be non-cytotoxic and non-genotoxic to dental pulp fibroblasts (Laurent et al. 2008). Both Biodentine™ and MTA were found to be non-cytotoxic to human gingival fibroblasts (Zhou et al. 2013).

1.11.9 Bioactivity of Biodentine™

The deposition of hydroxyapatite crystals on the surface of Biodentine™ were demonstrated when exposed to simulated body fluid (Camilleri, Sorrentino and Damidot, 2013). In the same study, Camilleri, Sorrentino and Damidot (2013) found that the calcium hydroxide peak in set Biodentine™ was observed after one day, whereas the calcium hydroxide peak in set MTA was observed after 28 days, possibly due to the slow ongoing crystallisation process of MTA. Han and Okiji (2011) used wavelength dispersive spectroscopy to study the ultrastructure of the dentine interface between Biodentine and white ProRoot MTA. Both Biodentine™ and MTA caused the uptake of calcium and silicone (Si) into the root canal dentine, with the uptake of elements being more predominant with biodentine (Han and Okiji, 2011). In another study Han and Okiji (2013) found that a precipitate comprising calcium and phosphate was observed on the surface of Biodentine™ when placed in PBS, as well as a calcium and phosphate rich, tag like structure formed by Biodentine™ within dentinal tubules. Gandolfi et al. (2013) concluded that Biodentine™ is a biointeractive material after observing a coating of calcium phosphate spherulites that formed over it when placed in Hank's Balanced Salt Solution. The calcium phosphate spherulites were smaller and denser over biodentine, and larger and less dense over ProRoot MTA (Gandolfi et al. 2013).

1.12 Clinical Applications of Calcium Silicate Cements

The first description of MTA in scientific literature in November 1993, recommended it for use as an endodontic repair material during lateral root perforation (Lee, Monsef and Torabinejad, 1993). In December 1993, Torabinejad, Watson and Pitt Ford recommended MTA for use as a root-end filling.

The field of endodontics has been revolutionised by the introduction of MTA and subsequent calcium silicate cements such as MTA Plus™ and Biodentine™ due to

their ability to set in a moist environment, and their excellent biocompatibility, sealing ability and bioactive nature.

Apart from being an excellent material of choice for root-end filling, calcium silicate cements can also be used for the repair of root and furcation perforations, root canal direct pulp capping, pultpotomy, the repair of root resorption defects and apical barrier formation in teeth with open apices, apexification and to establish a coronal seal during regenerative endodontics (Parirokh and Torabinejad, 2010b; Rajasekharan et al., 2014; Garcia-Godoy and Murray, 2012). In addition to these clinical applications, Biodentine™ is also indicated as a temporary enamel restorative material and a permanent dentine restorative material (Septodont); and MTA Plus™ may be used as a root canal sealer when mixed to a thinner consistency (AvalonBiomed Inc.).

1.13 Success Rate of Endodontic Surgery: Past and Present

Tsesis et al. (2006) compared the outcomes of surgical endodontic treatment, when performed using the traditional and modern technique, but using IRM as a root-end filling in both techniques. Complete healing was observed in 91.1% of cases using the modern technique compared to 44.2% when the traditional technique was used (Tsesis et al., 2006). In a prospective clinical study using modern microsurgical techniques and MTA as a root-end filling by Saunders (2008), the success rate was 88.8%.

Tsesis et al. (2009) carried out a meta-analysis of the literature, and found that a successful outcome was achieved in 91.6% of cases more than one year postoperatively, when using the modern endodontic surgical technique.

Setzer et al. (2010) carried out a meta-analysis of the literature, comparing the traditional root-end surgery technique with the modern endodontic microsurgery technique. The weighted pooled success rates were 59% for traditional root-end surgery, and 94% for modern endodontic microsurgery.

In conclusion, endodontic surgery using modern surgical techniques, armamentarium and root-end filling materials significantly improves the treatment outcome compared to the traditional technique.

CHAPTER 2: AIM AND OBJECTIVES

2.1 Aim

The purpose of this *in-vitro* study was to compare the sealing ability of White ProRoot MTA, MTA Plus™, Biodentine™ and Permite Amalgam with each other, when used as root-end filling materials.

2.2 Objective

The broad objectives of this study were to:

i) Perform root canal treatment on extracted teeth.
ii) Resect the apical 3 mm of the teeth.
iii) Prepare root end cavities and fill them with Permite Amalgam (control), White ProRoot MTA, MTA Plus™ and Biodentine™.
iv) Immerse the specimens in dye and section three 1mm slices from the apical end.
v) Examine the slices under stereomicroscopy for dye penetration at the root-end filling-dentine wall interface.

2.3 Hypothesis

White ProRoot MTA, MTA Plus™ and Biodentine™ would display better sealing ability than Permite Amalgam.

2.4 Statistical Null/Zero Hypotheses

There will be no difference in the sealing ability of White ProRoot MTA, MTA Plus™, Biodentine™ and Permite Amalgam.

CHAPTER 3: MATERIALS AND METHODS

3.1 Collection of Material

One hundred and twenty single rooted, human, extracted teeth were collected from the out-patient dental extraction clinic of the Oral and Dental Hospital, School of Dentistry, Faculty of Health Sciences, University of Pretoria. Each patient or patient's legal guardian, in the case of a minor, that attends the facility for dental extraction is asked to complete an informed consent from (Addendum A). Patients that give written consent grant, permission for their extracted teeth to be used for the purpose of scientific research.

Every aspect of this research project was conducted in line with the ethical and safety standards for handling human tissues and conducting laboratory research, as prescribed by South African law: the Health Profession Act 56 of 1974 (South African National Health Bill, 2003)

3.2 Selection and Storage of Teeth

The teeth were stored in Phosphate Buffered Saline (PBS) immediately after extraction and stored at room temperature. The teeth were stored for a period of two weeks before being used for the experiment. The prerequisites for the sample selection were similar to those used by Pichardo et al. (2006):

1. Root formation should be complete.
2. There should be a single canal, which should be straight, and root canal therapy should not have been previously performed on the teeth. This was verified radiographically.
3. The teeth should not have any fractures. This was verified using a surgical operating microscope (D.F. Vasconcellos, São Paulo, Brazil)(Fig. 3.1)
4. There should be no root caries or root resorption.

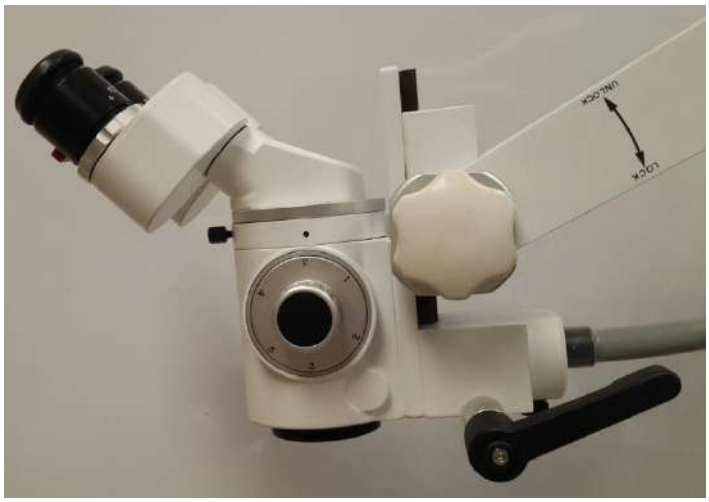

Figure 3.1: D.F. Vasconcellos Surgical Operating Microscope (São Paulo, Brazil).

3.3 Preparation of Root Canals

The crowns of all teeth were sectioned with a flat, cylindrical diamond bur (Komet, Lemgo, Germany) with a high speed hand piece (W&H, Bürmoos, Austria), perpendicular to the long axis of the root at a standard measurement of 18mm from the apex as suggested by Pichardo et al. 2006.

All the root canals were prepared to within 0.5mm of the canal apex with ProTaper Universal (Dentsply/Maillefer, Ballaigues, Switzerland) rotary instruments (Fig. 3.2) using the X-Smart Plus endodontic motor (Dentsply/Maillefer)(Fig. 3.3), according to the manufacturer's instructions. A size 15 K-file (Dentsply/Maillefer) was used to establish a reproducible glide path (Fig. 3.4). Glyde Root Canal Conditioner (Dentsply/Maillefer) (Fig. 3.5) was used as a lubricant on the files prior to insertion into the canals.

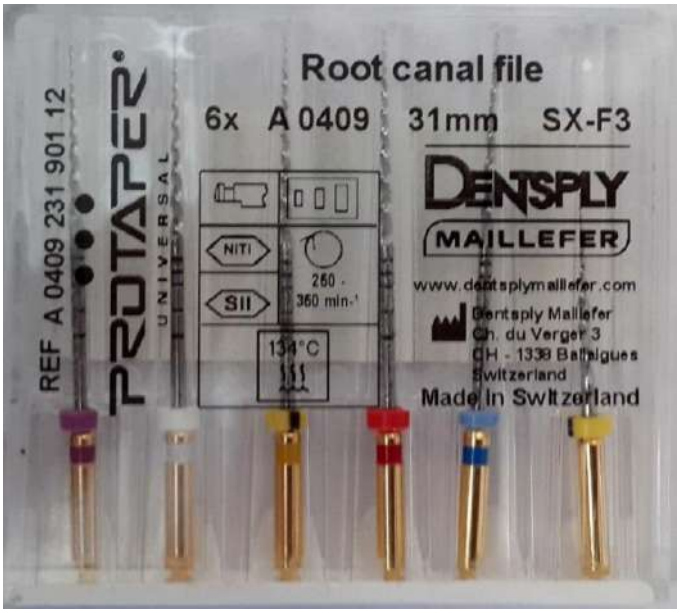

Figure 3.2: ProTaper Universal (Dentsply/Maillefer) rotary instruments.

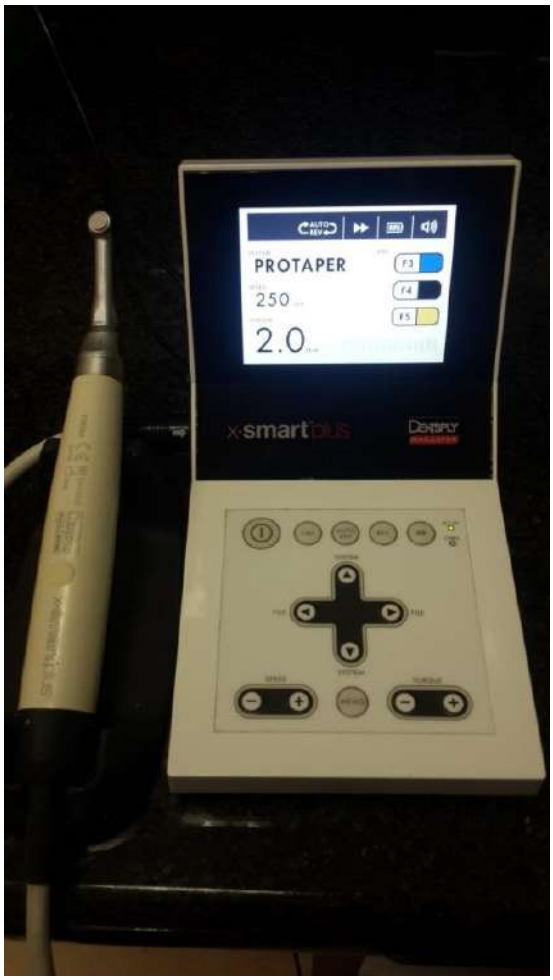

Figure 3.3: The X-Smart Plus endodontic motor (Dentsply/Maillefer).

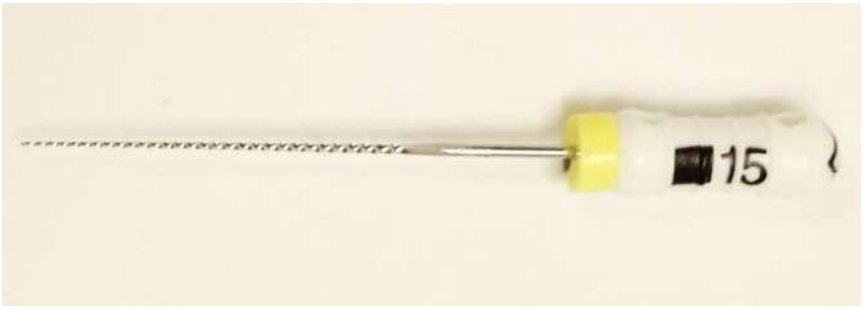

Figure 3.4: Size 15 K- file (Dentsply/Maillefer) used to establish a reproducible glide path in the root canals.

Figure 3.5: Glyde Root Canal Conditioner (Dentsply/Maillefer).

A size 10 K-file (Dentsply/Maillefer)(Fig. 3.6) was used to maintain apical patency between rotary file insertions. A 6% sodium hypochlorite solution (Nordiska Dental, Angelholm Sweden)(Fig. 3.7) was used for irrigation between rotary instrument insertion. The canals were prepared with ProTaper Universal instruments (Dentsply/Maillefer), up to a size F3 instrument. The canals were finally rinsed after preparation with 17% EDTA (Topclear, Dental Discounts, Johannesburg, Sandton, South Africa)(Fig. 3.8). F3 ProTaper Paper Points (Dentsply/Maillefer) were used to dry the canals.

Figure 3.6: Size 10 K-file (Dentsply/Maillefer) used to maintain apical patency between rotary file insertions.

Figure 3.7: 6% sodium hypochlorite solution (Nordiska Dental, Sweden).

Figure 3.8: 17% EDTA solution (Topclear, Dental Discounts).

3.4 Obturation of Root Canals

All the prepared root canals were obturated using the continuous wave, warm vertical condensation technique. ProTaper F3 Gutta-Percha-Points (Dentsply/ Maillefer)(Fig. 3.9) were 'buttered' with Topseal Root Canal Sealer (Dentsply/ Maillefer)(Fig. 3.10) and fitted into the prepared root canal of each tooth, ensuring adequate tugback.

Figure 3.9: ProTaper F3 Gutta-Percha-Points (Dentsply/Maillefer).

Figure 3.10: Topseal Root Canal Sealer (Dentsply/Maillefer).

The Calamus Dual Obturation Unit (Dentsply/Maillefer)(Fig. 3.11) was used to complete the obturation of the root canals. The electric heat plugger of the Calamus Dual system was activated and placed to within four to five millimetres of the apex to create an apical plug of gutta-percha. The rest of the canal was backfilled with thermoplastic gutta-percha using the flow handpiece of the Calamus Dual system. The access cavities were sealed coronally using Fuji IX glass-ionomer restorative material (GC Corporation, Tokyo, Japan)(Fig. 3.12). The specimens were then stored in Phosphate Buffered Saline solution for 48 hours.

Figure 3.11: Calamus Dual Obturation Unit (Dentsply/Maillefer).

Figure 3.12: Fuji IX glass ionomer restorative material (GC Corporation).

3.5 Root-end Resection and Cavity Preparation

The apical 3 mm of all the teeth were resected perpendicular to the long axis of the tooth using a straight carbide fissure bur (Komet, Lemgo, Germany) on a high speed hand piece (Fig. 3.13).

Root-end cavities were prepared on all teeth to a depth of 3 mm using an ultrasonic ProUltra Surgical Tip (Dentsply/Maillefer) (Fig.3.14) in an NSK Ultrasonic Scaler Unit (NSK, Nakanishi, Japan) (Fig. 3.15). The ultrasonic tip was used with light pressure in a brushing motion, and the Class I cavity created was parallel to the long axis of the tooth (Fig. 3.16).

Figure 3.13: The apical 3 mm of a specimen being resected perpendicular to the long axis of the tooth using a straight carbide fissure bur, in a high speed hand piece.

Figure 3.14: ProUltra Surgical Tip (Dentsply/Maillefer).

Figure 3.15: NSK Ultrasonic Scaler Unit (NSK, Nakanishi, Japan).

Figure 3.16: Root end cavity prepared, parallel to the long axis of the tooth.

3.6 Filling of the Root-end Cavities

All the specimens were coated with two layers of clear nail varnish (Estee Lauder, Paris, France), except for the resected apical portion to seal all other possible portals of communication with the root canal (Fig. 3.17).

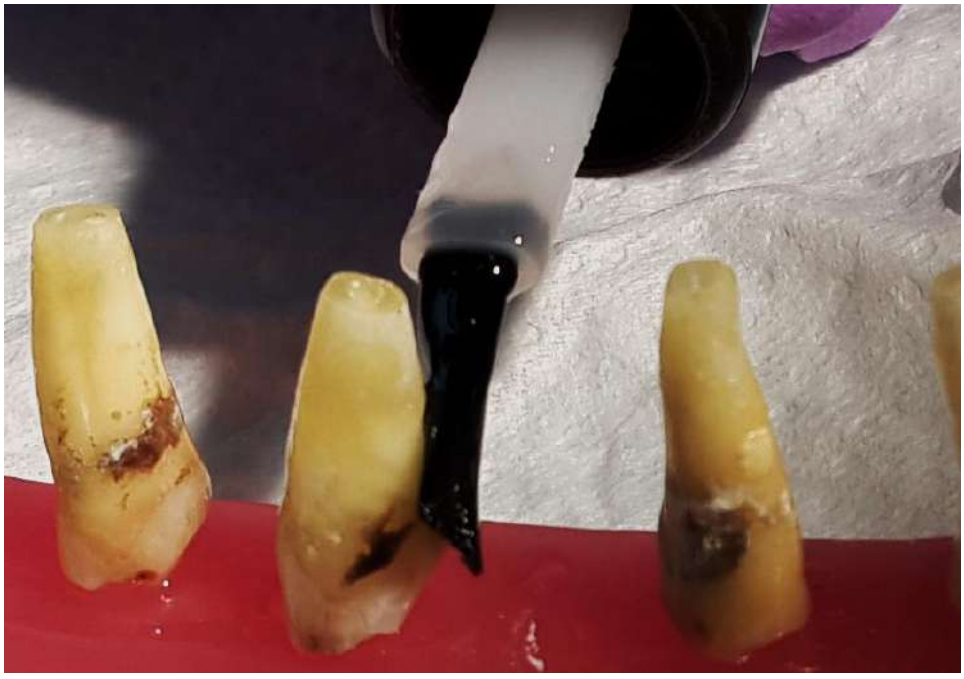

Figure 3.17: All the specimens were coated with two layers of clear nail varnish, except for the resected apical end.

The one hundred and twenty specimens were randomly divided into four groups (n=30) and treated as follows:

Group 1: **White ProRoot MTA** (Dentsply/Maillefer) (n=30) (Fig. 3.18)

The material was hand mixed according to the manufacturer's instructions and placed into the root-end cavity preparation, using the MAP system (Dentsply/Maillefer) (Fig. 3.19).

Figure 3.18: White ProRoot MTA (Dentsply/Maillefer).

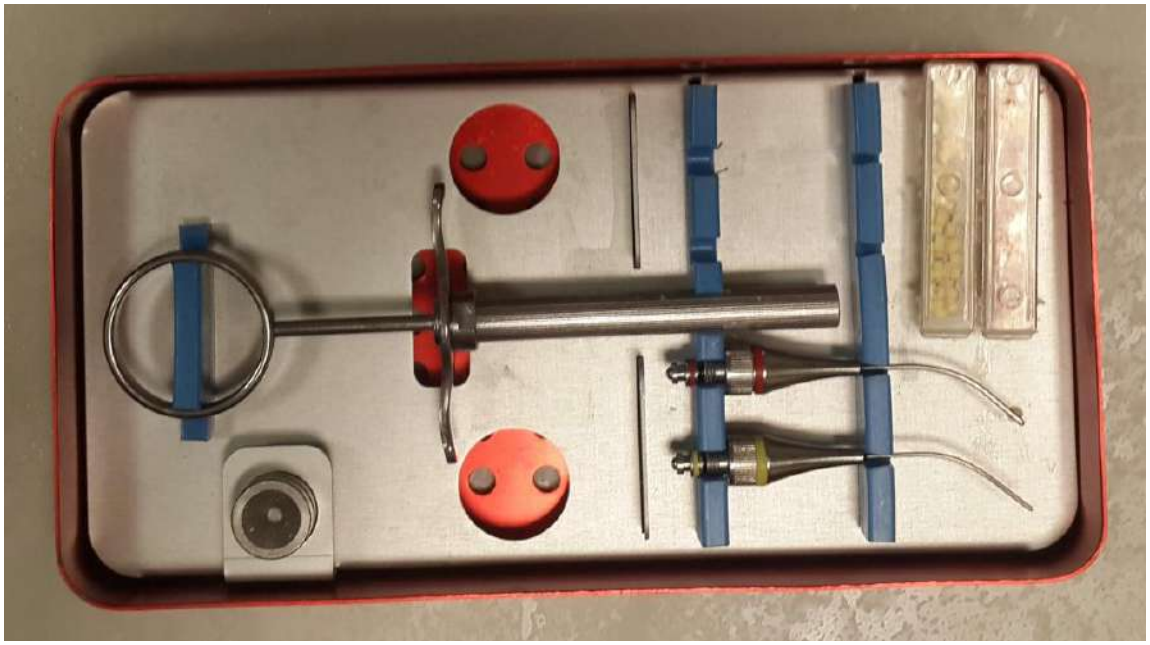

Figure 3.19: The MAP system (Dentsply/Maillefer).

Group 2: **MTA Plus™** (Prevest Denpro Ltd., Jamu, India)(n=30)(Fig. 3.20)

The material was hand mixed according to the manufacturer's instructions and placed into the root-end cavity preparation, using the MAP system (Dentsply/Maillefer).

Figure 3.20: MTA Plus™ powder and gel (Prevest Denpro Ltd., Jamu, India).

Group 3: **Biodentine™** (Septodont Ltd., Saint Maur des Fausse's, France)(n=30) (Fig. 3.21)

The material was mixed in an amalgamator (TPC Advanced Technology, California, USA)(Fig. 3.22) according to the manufacturer's instructions and placed into the root-end cavity preparation, using the MAP System (Dentsply/Maillefer).

Figure 3.21: Biodentine capsule and liquid pipettes (Septodont Ltd., Saint Maur des Fausse's, France).

Group 4: **Amalgam** (Permite, SDI, Victoria, Australia) (n=30) (Fig. 3.22)

The material was mixed in an amalgamator (TPC Advanced Technology, California, USA) (Fig 3.23) according to the manufacturer's instructions and placed into the root-end cavity preparation, using a retrograde amalgam carrier (Medesy, Pordenone, Italy) (fig 3.24).

Figure 3.22: Amalgam capsules (Permite, SDI, Victoria, Australia).

Figure 3.23: Amalgamator (TPC Advanced Technology, California, USA).

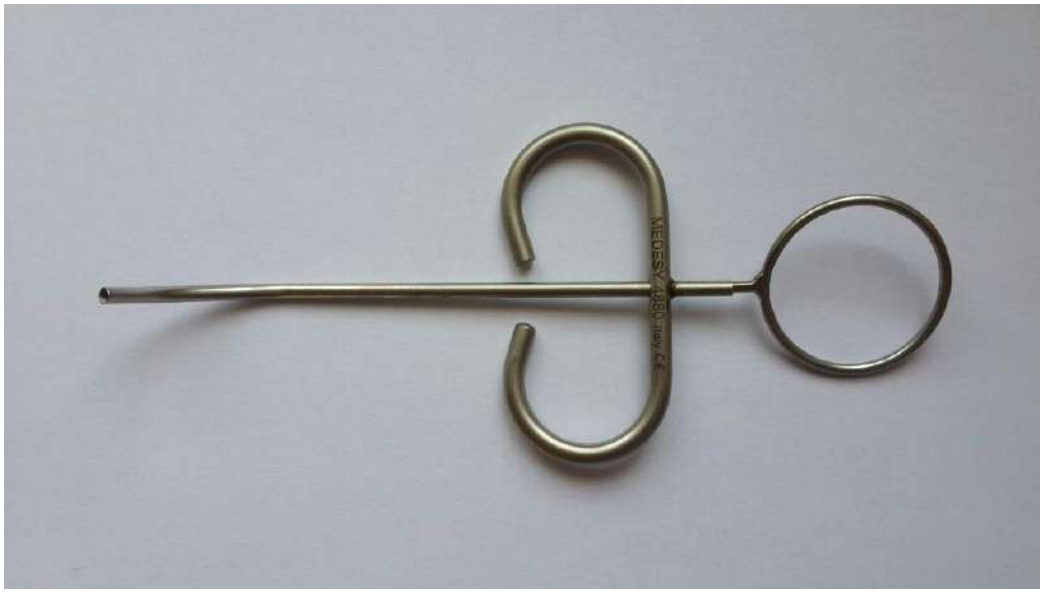

Figure 3.24: Retrograde amalgam carrier (Medesy, Pordenone, Italy).

Gauze moistened in PBS was used to wrap the specimens, and they were stored in sealed containers for twenty four hours to allow the complete set of the materials.

3.7 Dye Penetration

The specimens were immersed in Indian Ink dye (Winsor & Newton, London, England) (Fig. 3.25), and remained immersed in the dye reservoir for forty eight hours. The specimens were then removed from the dye reservoir, and excess dye was rinsed off with running water for fifteen minutes.

Figure 3.25: Black Indian Ink (Winsor and Newton, London, England).

The specimens were sectioned transversely in 1 mm increments from the apical end with a wafering blade in an Isomet™ Low Speed Saw (Buehler, Lake Bluff, Illinois, USA) under continuous water irrigation (Figs. 3.26 and 3.27). Three, 1 mm slices of each root-end were obtained in this manner, and packaged in a labelled sealed packet, identifying the sample as being 1 mm, 2 mm or 3 mm from the apex.

Figure 3.26: Isomet™ Low Speed Saw (Buehler, Lake Bluff, Illinois, USA)

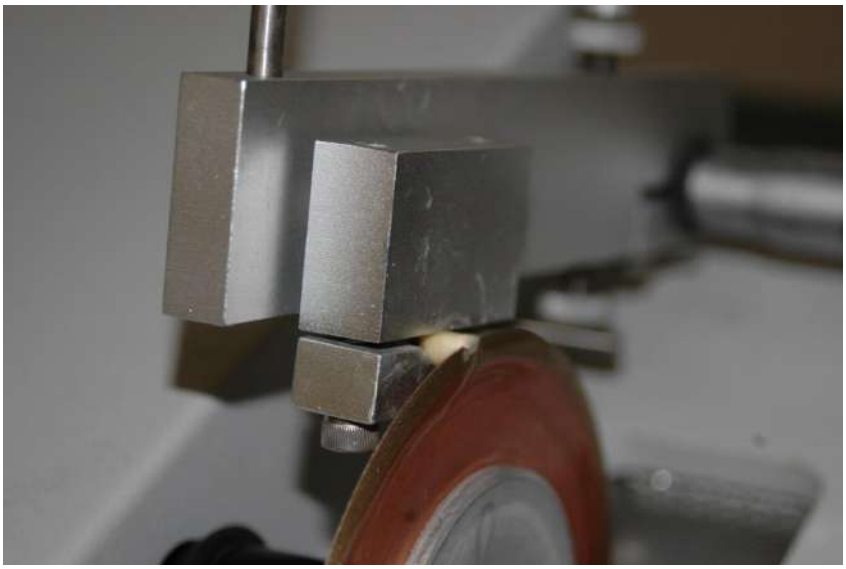

Figure 3.27: Specimens sectioned transversely with a wafering blade in 1 mm increments.

The sections were then mounted on microscopic glass slides (Fig. 3.28) and examined under a stereomicroscope (Carl Zeiss, Jena, Germany)(Fig. 3.29) by two independent, blinded and calibrated examiners who were unaware of the materials used, and have experience in performing measurements under stereomicroscopy. The extent of dye penetration was measured to the nearest millimeter using the stereomicroscope, based on the presence of visible dye between the root-end filling and dentinal wall interface.

Figure 3.28: Sliced apical transverse sections mounted on a glass slide for stereo-microscopic examination.

Figure 3.29: Stereomicroscope (Carl Zeiss, Jena, Germany).

3.8 Data Analysis

Data was summarized in terms of percentage for the outcome vector (no leak; 1 mm leak; 2 mm leak; 3 mm leak). Furthermore, pairwise comparisons between each of the new generation filling materials to amalgam would be done at the 0.017 level of significance using Fisher's exact test. The latter test could also be used at the 0.05 level of significance to assess the four filling materials in one analysis.

CHAPTER 4: RESULTS

The measurement of the depth of dye penetration between the root-end filling material and the dentinal wall interface was of interest.

4.1 Group 1: Dye penetration of White ProRoot MTA specimens

In this group, 26 specimens showed no leakage, four specimens leaked to a depth of 1 mm and only three specimens leaked to a depth of 2 mm (Fig. 4.1). None of the specimens in this group leaked further than 2 mm.

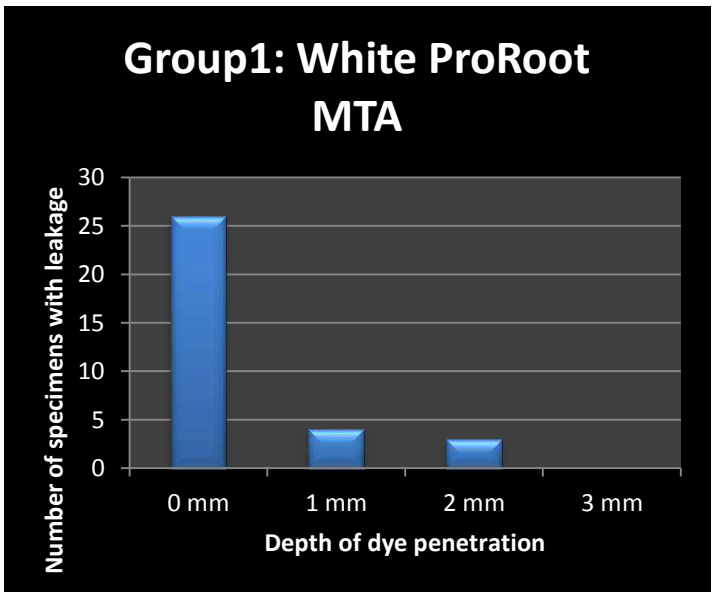

Figure 4.1

Figure 4.2 shows transverse sections of a representative ProRoot MTA specimen at the 1, 2 and 3 mm levels that demonstrated no leakage.

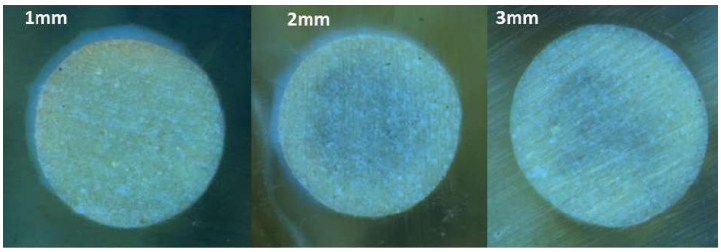

Figure 4.2

Figure 4.3 depicts a transverse section of one of the three ProRoot MTA specimens that displayed leakage up to the 1 mm and 2 mm levels.

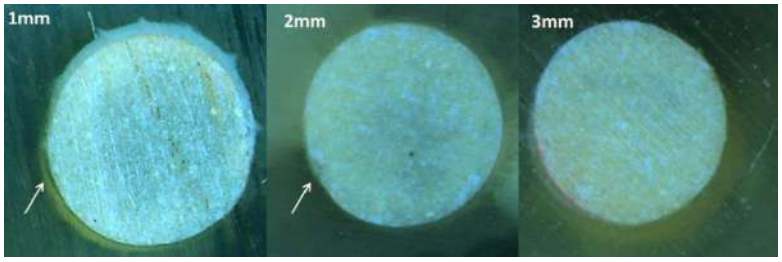

Figure 4.3

4.2. Group 2: Dye penetration of MTA Plus™ specimens

Twenty two of the specimens in this group showed no leakage, eight specimens leaked to a depth of 1 mm, and three specimens leaked to a depth of 2 mm (Fig. 4.4). None of the specimens in this group leaked further than 2 mm.

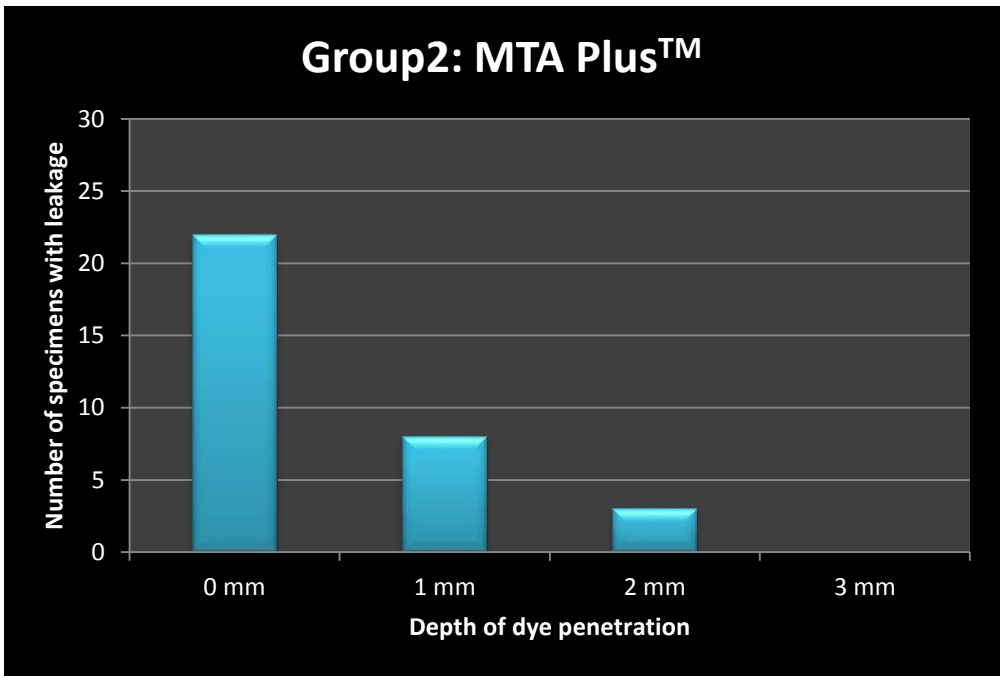

Figure 4.4

Figure 4.5 exhibits transverse sections of a representative MTA Plus™ specimen at the 1, 2 and 3 mm level that displayed no leakage.

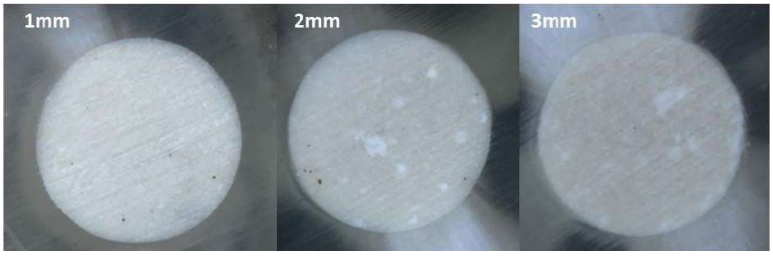

Figure 4.5

Figure 4.6 depicts a transverse section of one of the eight MTA Plus™ specimens that demonstrated leakage only up to the 1 mm level.

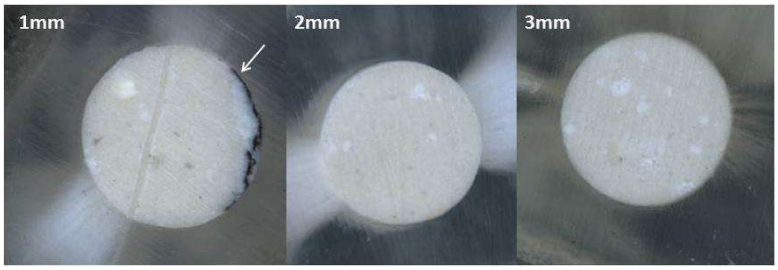

Figure 4.6

4.3 Group 3: Dye penetration of Biodentine™ specimens

No leakage was observed in twenty four specimens of this group, six specimens leaked to a depth of 1 mm, and four specimens leaked to a depth of 2 mm (Fig. 4.7). None of the specimens in this group leaked further than 2 mm.

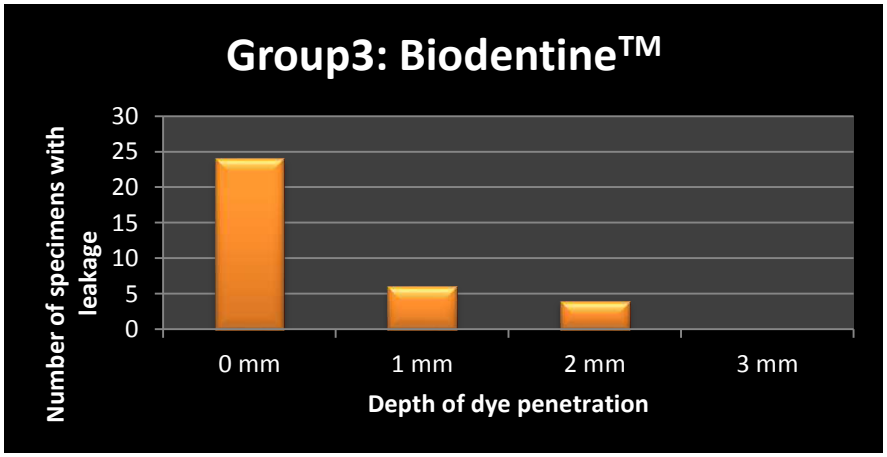

Figure 4.7

Figure 4.8 shows a transverse section of a representative Biodentine™ specimen at the 1, 2 and 3 mm level displaying no leakage.

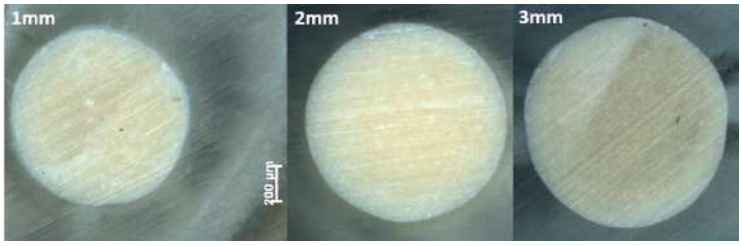

Figure 4.8

Figure 4.9 illustrates transverse sections of one of the four Biodentine™ specimens that displayed leakage at the 1 mm and 2 mm level.

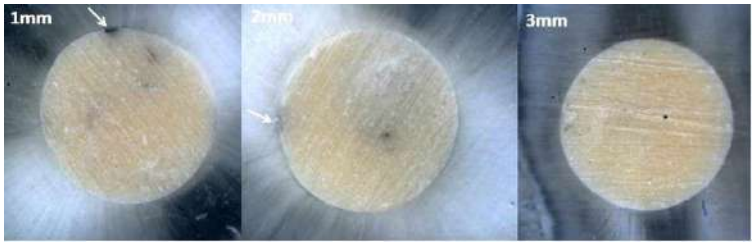

Figure 4.9

4.4 Group 4: Dye penetration of Permite Amalgam specimens

All thirty of the specimens in this group showed leakage to a depth of 1 mm, twenty seven specimens leaked to a depth of 2 mm, and twelve specimens leaked to a depth of 3 mm (Fig. 4.10).

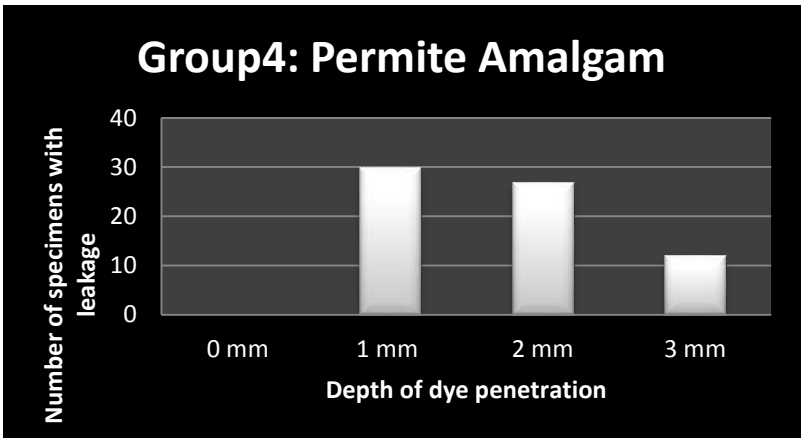

Figure 4.10

Figure 4.11 depicts a transverse section of a representative Amalgam specimen that displayed leakage at the 1, 2 and 3 mm levels.

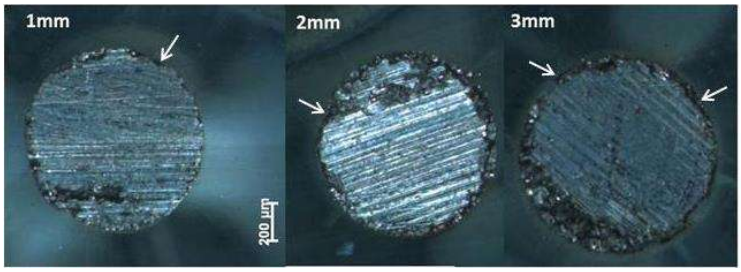

Figure 4.11

4.5 Comparison of Data for Dye Leakage of Specimens in the Different Groups.

Figure 4.12 illustrates the results of all the specimens from the different groups combined before statistical analysis. The specimens from the Permite Amalgam group showed the most leakage while the White ProRoot MTA group showed the least amount of leakage.

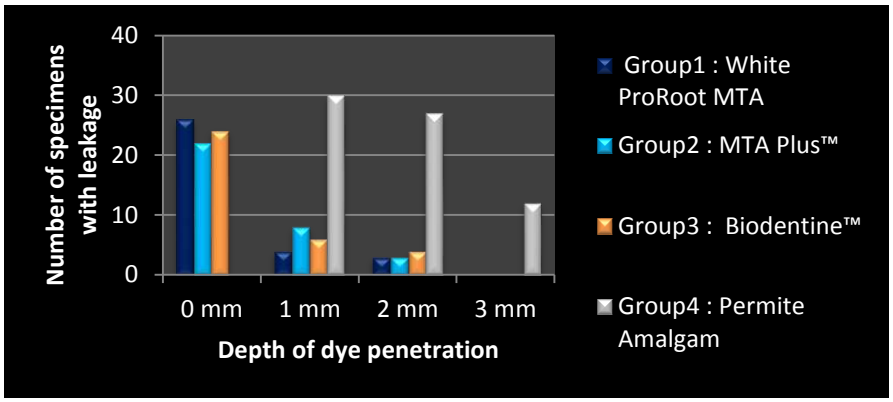

Figure 4.12

4.6 Statistical analysis

The outcomes of the failure vector are reported for each treatment group in Table 4.1 and Figure 4.13. The outcome distribution for each of the calcium silicate root-end filling materials (ProRoot MTA, MTA Plus™ and Biodentine™) is significantly different to that of amalgam (Permite amalgam). In particular, amalgam has significantly more leakage than any of the calcium silicate root-end filling materials.

Those differences are very clear if we simplify the leakage outcome as present or absent. We then note that leakage of amalgam samples were 100% (30/30), and that 20% (6/30) of Biodentine™ samples, 13.3% (4/30) of ProRoot MTA samples and 27.6% (8/30) of MTA Plus samples leaked. It is also important to note that none of the calcium silicate retrograde filled teeth demonstrated leakage up to 3 mm. In contrast 40% (12/30) of the amalgam filled teeth showed leakage up to the 3 mm level (Fig. 4.13).

Furthermore, no significant differences were found among the new generation root-end fillings, namely Biodentine™ vs ProRoot MTA ($p = 0.776$), Biodentine™ vs MTA Plus™ ($p = 0.667$), and ProRoot MTA vs MTA Plus™ ($p = 0.350$).

Table 4.1: Outcome distribution (%) of the calcium silicate filling materials and significant difference from amalgam.

Material	**No Leakage**	**Leakage** 1 mm	**Leakage** 2 mm	**Leakage** 3 mm	**Fisher's exact test p-value***
ProRoot MTA	86.7%	13.3%	10%	0%	<0.001
MTA Plus™	73.3%	26.7%	10%	0%	<0.001
Biodentine™	80%	20%	13.3%	0%	<0.001
Permite Amalgam	0%	100%	50%	40%	

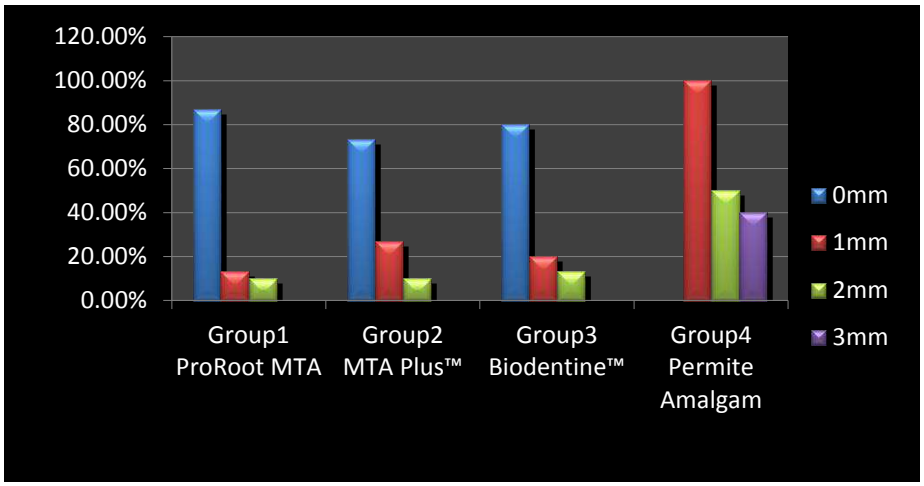

Figure 4.13

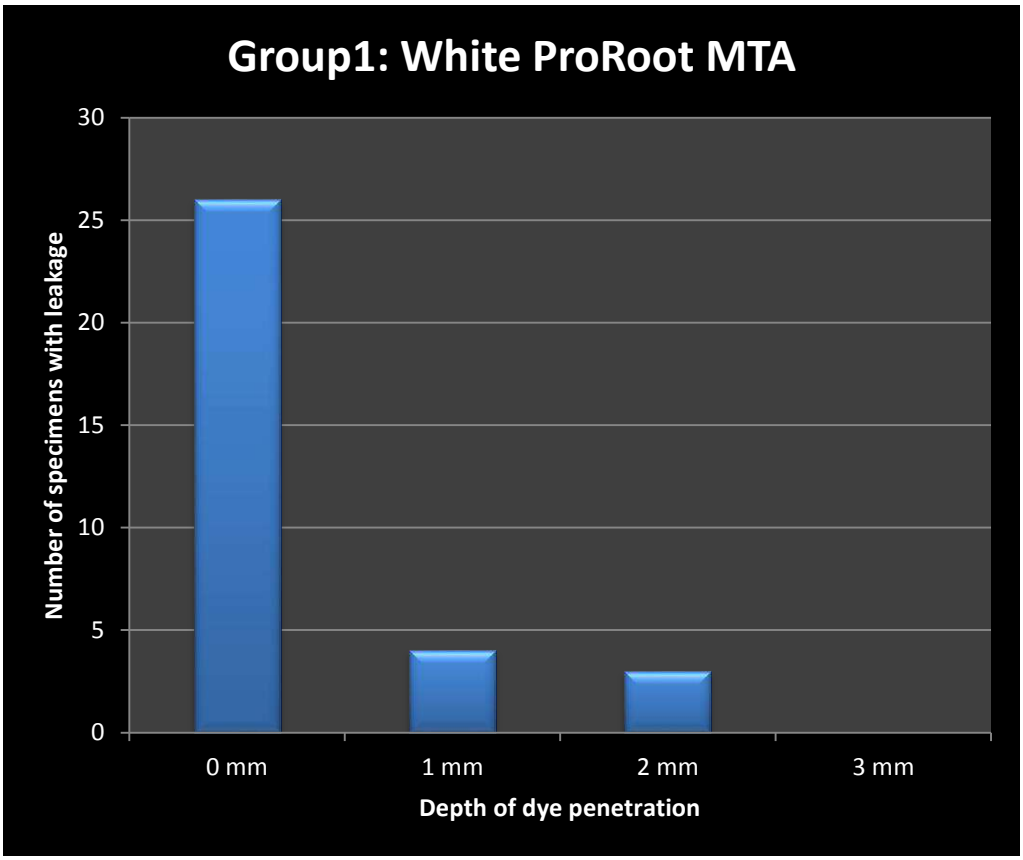

Figure 4.1: Dye penetration of specimens with White ProRoot MTA retrograde fillings.

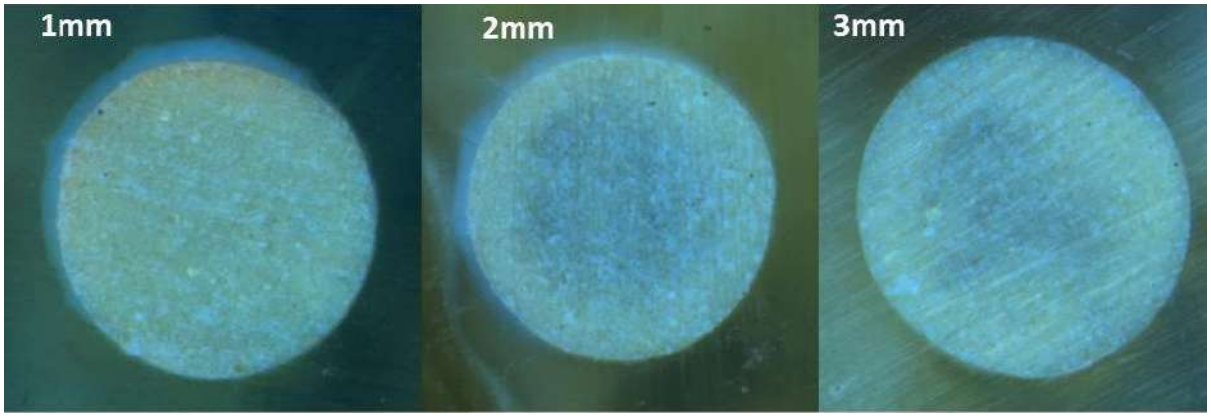

Figure 4.2: Stereomicroscopic view of transverse sections of a representative specimen of the White ProRoot MTA group. No leakage was visible in this specimen at all three levels investigated.

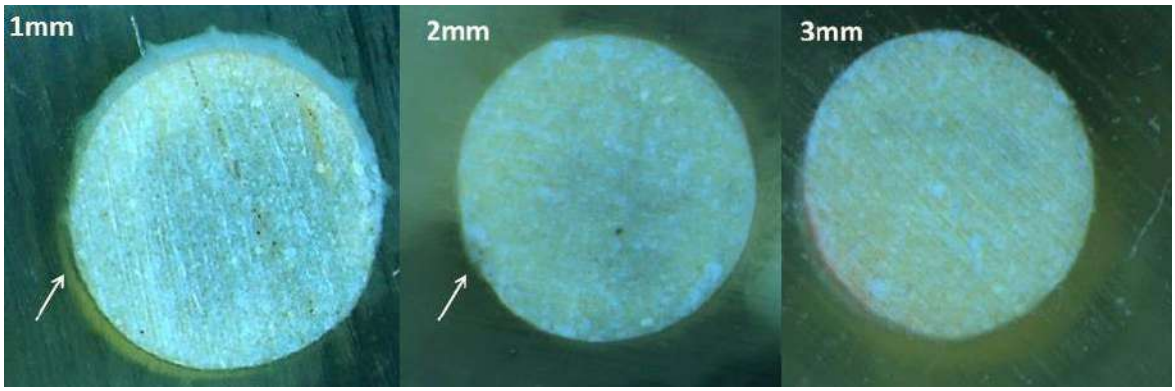

Figure 4.3: Stereomicroscopic view of transverse sections of a representative specimen of the White ProRoot MTA group. Note that leakage is visible in this specimen at the 1 mm and 2 mm level (arrows).

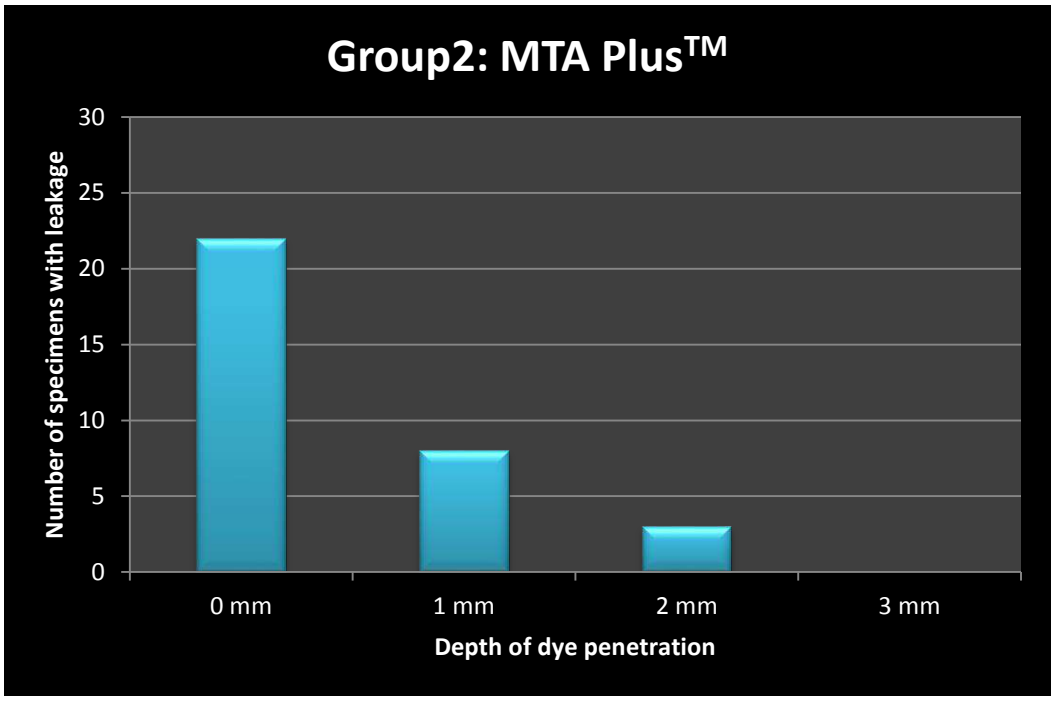

Figure 4.4: Dye penetration of specimens with MTA Plus™ retrograde fillings.

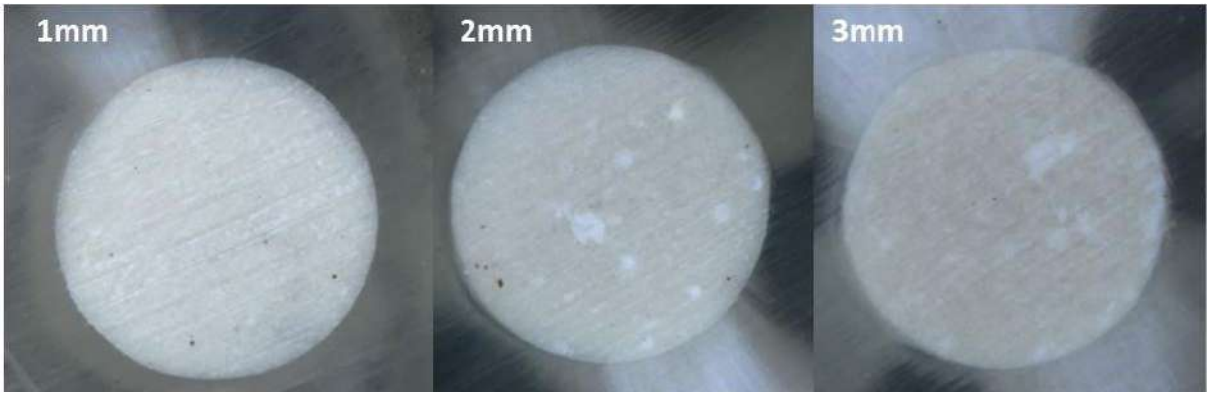

Figure 4.5: Stereomicroscopic view of transverse sections of a representative specimen of the MTA Plus™ group. No leakage was visible in this specimen at all three levels investigated.

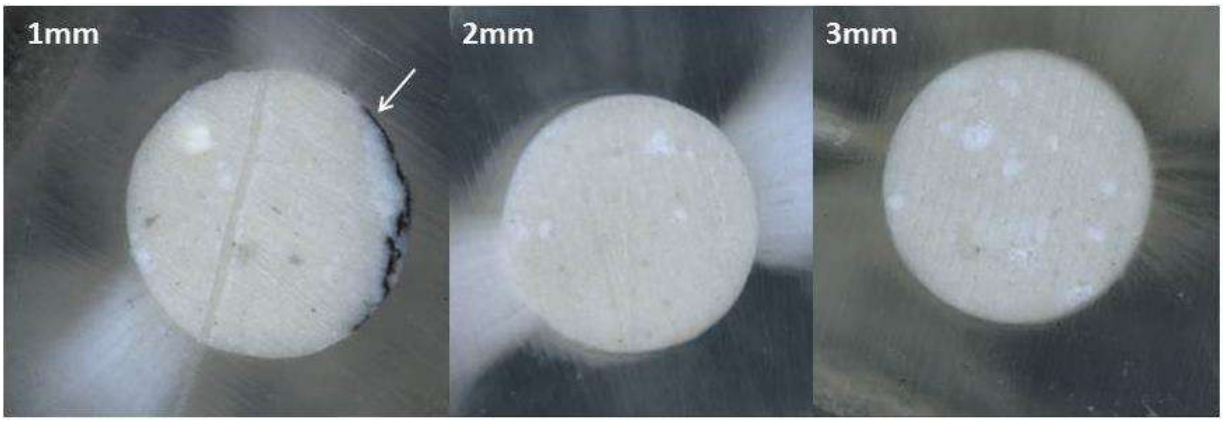

Figure 4.6: Stereomicroscopic view of transverse sections of a representative specimen of the MTA Plus™ group. Note that leakage is visible in this specimen only at the 1 mm level (arrow).

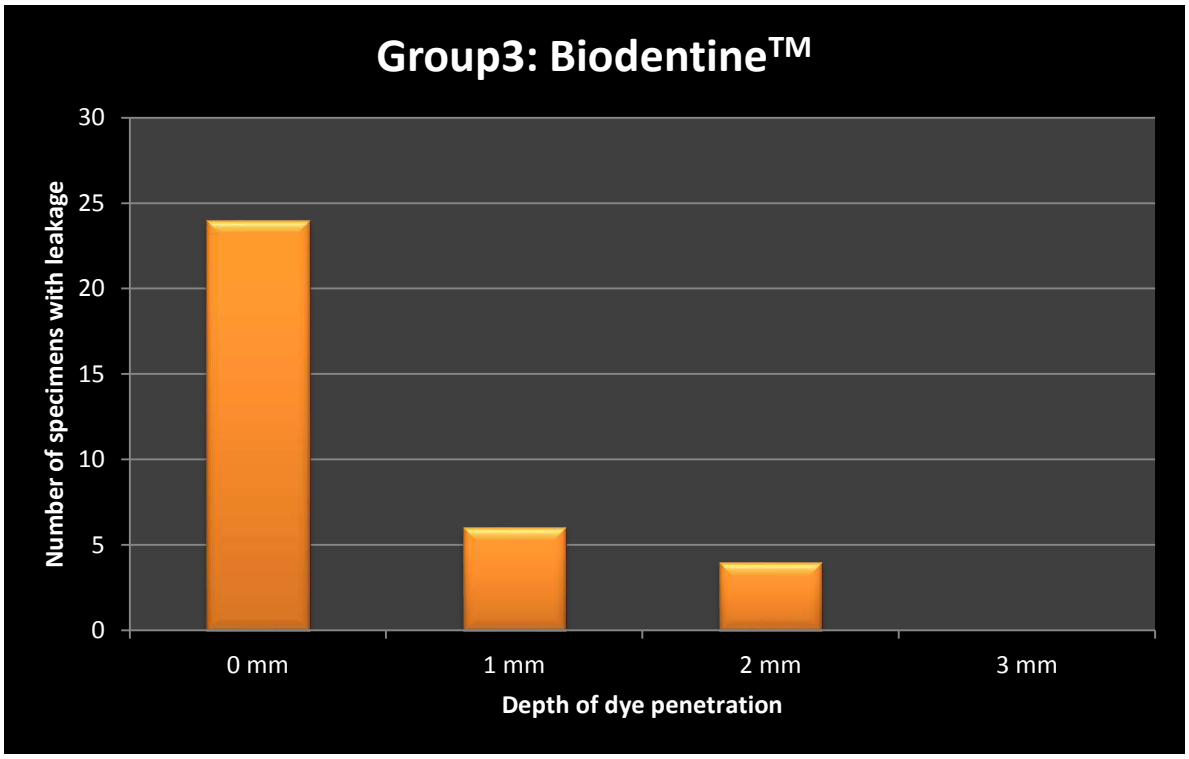

Figure 4.7: Dye penetration of specimens with Biodentine™ retrograde fillings.

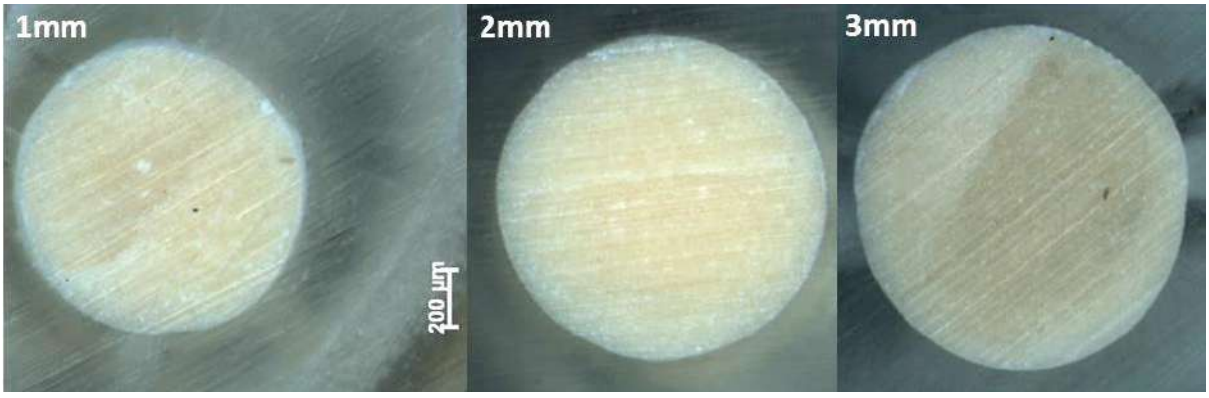

Figure 4.8: Stereomicroscopic view of transverse sections of a representative specimen of the Biodentine™ group. No leakage was visible in this specimen at all three levels investigated.

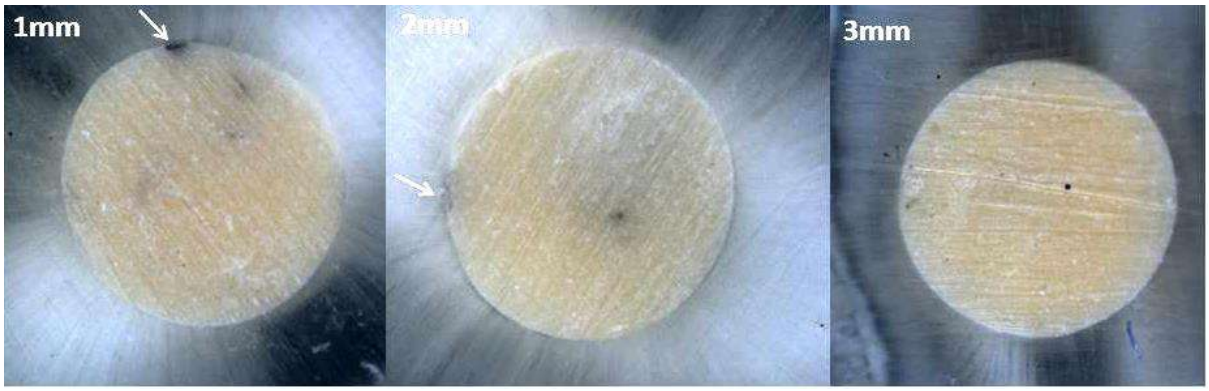

Figure 4.9: Stereomicroscopic view of transverse sections of a representative specimen of the Biodentine™ group. Note the leakage in this specimen at the 1 mm and 2 mm levels (arrows).

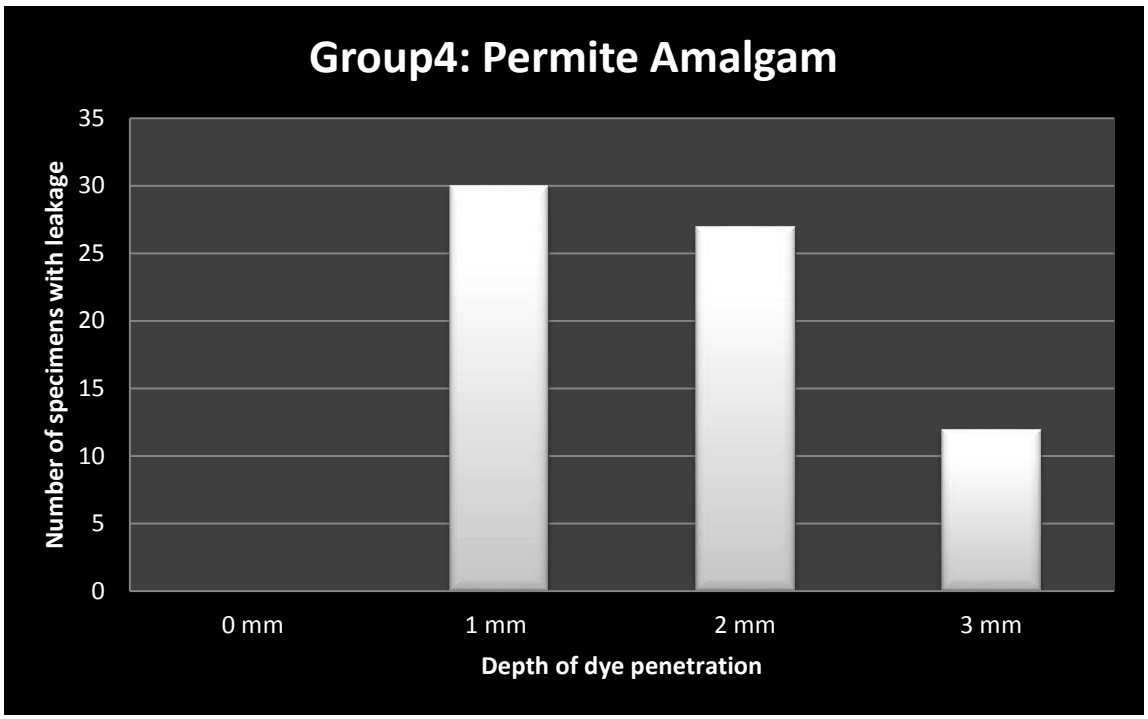

Figure 4.10: Dye penetration of specimens with Permite Amalgam retrograde fillings.

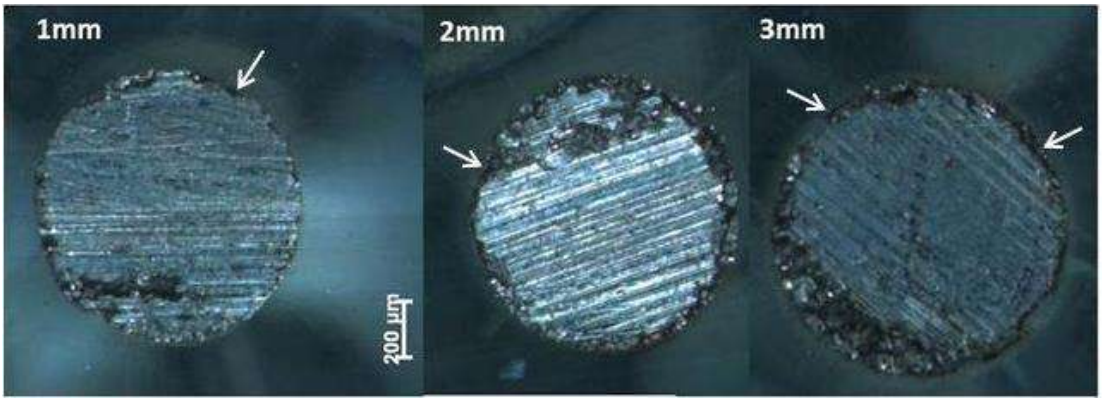

Figure 4.11: Stereomicroscopic view of transverse sections of a representative specimen of the Permite Amalgam group. Note the extensive leakage up to the 3 mm level (arrows).

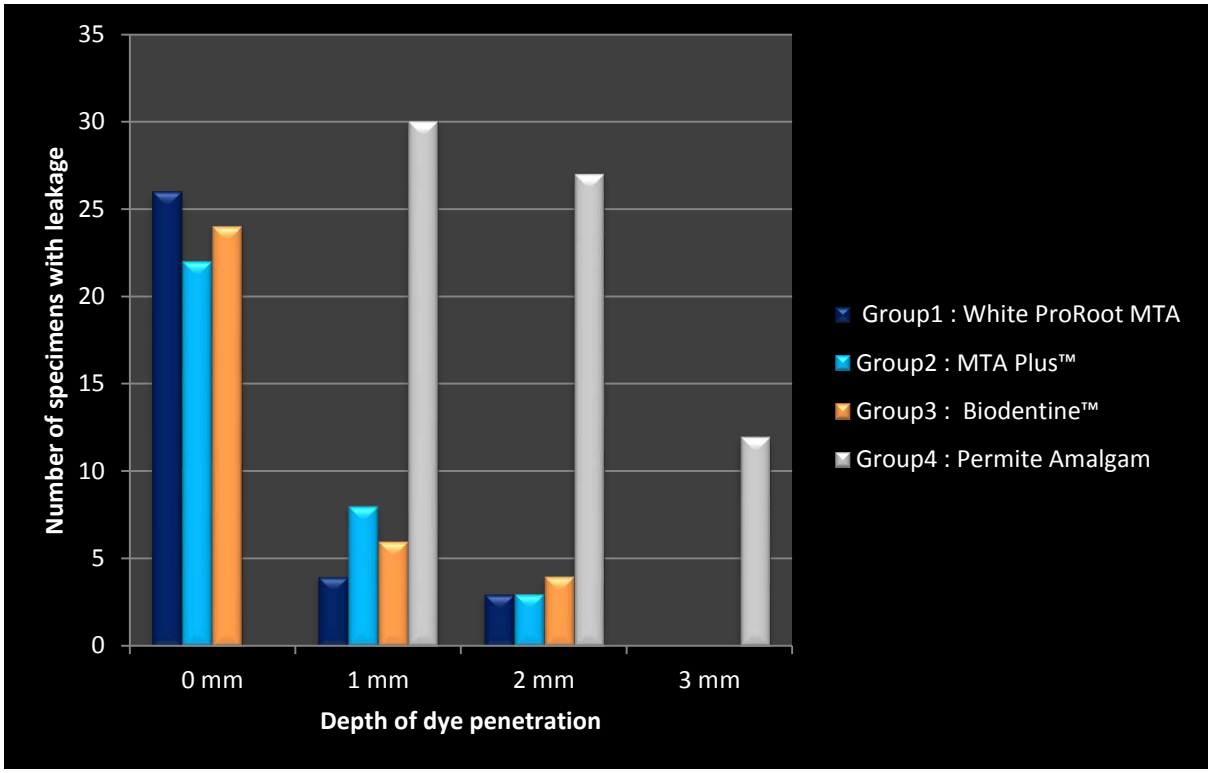

Figure 4.12: Comparative graph showing the dye leakage of all the specimens in the different groups.

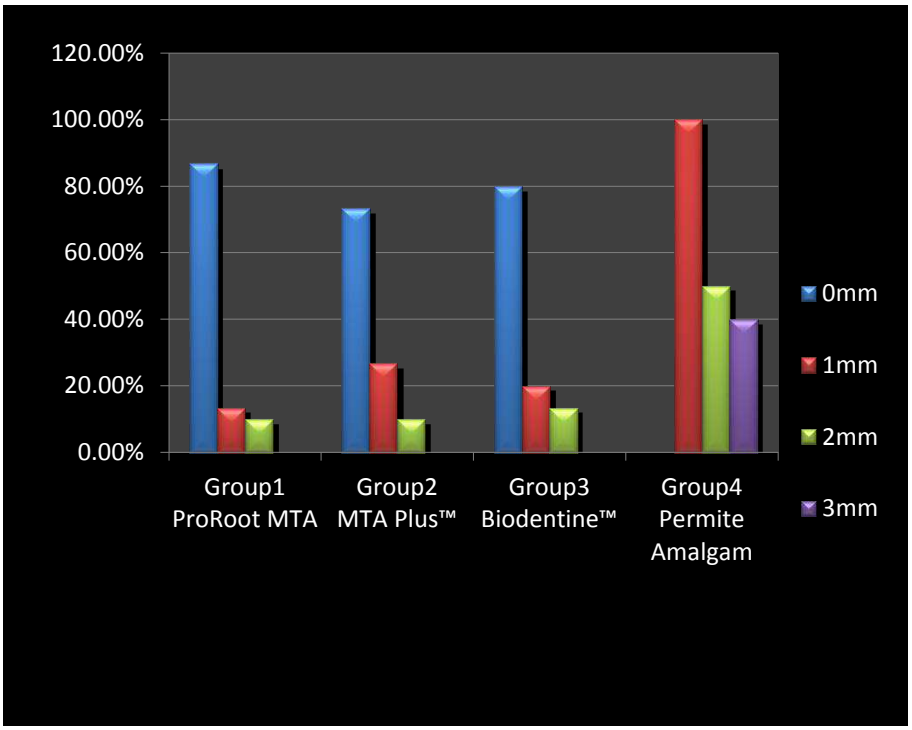

Figure 4.13: Graph displaying the outcome distribution percentage for each of the filling materials.

Table 4.1: Outcome distribution (%) of the calcium silicate filling materials and significant difference from amalgam.

Material	**No Leakage**	**Leakage** 1 mm	**Leakage** 2 mm	**Leakage** 3 mm	**Fisher's exact test p-value***
ProRoot MTA	86.7%	13.3%	10%	0%	<0.001
MTA Plus™	73.3%	26.7%	10%	0%	<0.001
Biodentine™	80%	20%	13.3%	0%	<0.001
Permite Amalgam	0%	100%	50%	40%	

CHAPTER 5: DISCUSSION

The purpose of this *in-vitro* study was to compare the sealing ability of the root-end fillings. White ProRoot MTA, MTA Plus™, Biodentine™ and Permite Amalgam were compared, by measuring the linear dye penetration of Indian ink at the interface between the root-end filling and dentinal wall. The achievement of a hermetic seal by a root-end filling is a critical factor that impacts on the long-term success of endodontic surgery.

Microleakage is an estimate of the sealing quality, and can be measured by allowing a tracer to penetrate the filled root-end cavity (Verissimo and do Vale, 2006). Tracers that are commonly used include dyes, radioisotopes, bacteria and their by-products (Verissimo and do Vale, 2006). Other methods of determining sealing ability include fluid filtration, electrical methods and marginal adaptation using scanning electron microscopy (Asgary, Eghbal and Parirokh, 2008).

The dye-immersion technique was introduced by Grossman in 1939, and is widely used because it is easy to perform, reproducible, safe, inexpensive, quantifiable and dyes are readily available (Verissimo and do Vale, 2006; Youngson et al. 1998). This technique is a passive method that depends on the phenomenon of capillarity, whereby the dye penetrates any space between the root-end filling and the canal wall (Verissimo and do Vale, 2006; Camps and Pashley, 2003). According to Torabinejad et al. (1994) when a filling material is able to resist the penetration of small molecules such as dye, it would have the potential to resist the penetration of bacteria and their by-products which are larger molecules than dye. Some of the dyes that may be used to assess microleakage include methylene blue, fuchsin, rhodamine B, silver nitrate, Indian Ink and Pelikan ink (Torabinejad and Parirokh, 2010). Methylene blue has been used as a tracer frequently to assess the microleakage of MTA and amalgam. Wu, Kontakiotis and Wesselink (1998) found that 1% methylene blue decolours to a certain extent when in contact with alkaline materials such as MTA and calcium hydroxide. It is important to use a tracer that mimics the clinical situation (Youngson et al. 1998). Indian ink was used in the present study as it is unlikely that bacterial leakage can occur in root canal spaces where this dye is unable to penetrate (Schafer and Olthoff, 2002). Chong et al. (1995)

compared the penetration of tracers and assessment methods of the sealing of root-end fillings, and found that bacterial penetration and Indian ink penetration provided similar results.

The linear dye penetration is then measured after the teeth are either made transparent by demineralisation, or sectioned transversely or longitudinally (Verissimo and do Vale, 2006). The disadvantage of longitudinal sectioning is that leakage may be underestimated due to the random selection of the cutting axis, resulting in the possibility that deepest dye penetration point may not be visible (Camps and Pashley, 2003). A possible disadvantage of the clearing technique is that dissolution of the dye may occur should the teeth be left in certain acids or alcohol for long periods of time (Ahlberg, Assavanop and Tay, 1995). Tamse, Katz and Kablan (1998) compared the extent of penetration of different dyes using the transverse sectioning technique and the clearing technique, and found that the extent of dye penetration of Indian ink was significantly greater in the transverse section technique. Transverse sectioning was performed in the present study, because it allows the examiner to observe the entire circumference of the retrograde filling - dentine interface, and the depth of dye penetration at each level (Verissimo and do Vale, 2006).

When using the dye immersion technique, a concern is that entrapped air would prevent the penetration of dye molecules (Spangberg, Acierno and Yongbum Cha, 1989). The use of negative pressure or a vacuum has been suggested to remove entrapped air (Spangberg, Acierno and Yongbum Cha, 1989). However, Roda and Guttman (1995), using Indian ink, concluded that using a vacuum to produce 'active dye penetration' does not enhance repeatability, may produce significant artefacts and offers no apparent clinical relevance. Furthermore other authors have found no difference in dye penetration, between a vacuum or passive immersion (Dickson and Peters, 1993; Katz, Rosenwasser and Tamse, 1998). Therefore, in the present study the samples were passively immersed into the Indian ink solution.

The removal of the smear layer results in better penetration of plastic filling materials and sealers into dentinal tubules, thus providing an improved seal (White, Goldman and Lin, 1984). Kubo, Gomes and Mancini (2005) studied the influence of removing

the smear layer on the sealing ability of MTA as a root-end filling by Rhodamine B dye penetration, and found that the least dye penetration occurred when the smear layer was not removed. The superior sealing ability of MTA in the presence of the smear layer was also confirmed by microleakage studies using bacterial leakage and fluid filtration (Yildirim, Orucoglu and Cobankara, 2008; Yildirim et al. 2010). The smear layer of the root-end cavities was not removed in the present study, so that the sealing ability of the calcium silicate materials was not adversely affected.

In the present study White ProRoot MTA, MTA Plus™ and Biodentine™ showed significantly better sealing ability when compared to Permite Amalgam. There were no statistically significant differences in sealing ability between the three calcium silicate cements.

Amalgam was considered as the material of choice for root-end filling for over a century, despite the many disadvantages of its use (Friedman, 1991). Most materials that were developed or considered for root-end filling were often compared with amalgam. The use of a cavity varnish in conjunction with amalgam has been shown to improve its sealing ability (Abdal, Retief and Jamison, 1982; Tronstad et al. 1983). However, Vertucci and Beatty (1986) found no significant difference in apical dye penetration, with or without the use of varnish. In the present study, no varnish was used in order to get a true appraisal of the sealing ability of the amalgam itself. An additional factor that improves the sealing ability of amalgam with time is the formation of corrosion products between itself and the dentine wall (Vertucci and Beatty, 1986). Tronstad et al. (1983) found that corrosion products start to fill the gap between amalgam and dentine within seven days. However, the generation of corrosion products is an undesirable goal, especially in the periradicular region (Hohenfeldt, Aurelio and Gerstein, 1985). In the present study a high copper non-Gamma 2 Amalgam was used because it results in minimal corrosion (SDI Limited).

Prior to the introduction of MTA, the reinforced zinc oxide eugenol cements, IRM (Dentsply/Maillefer) and Super EBA (Harry J. Bosworth Co., Skokie, Illinois, USA) were investigated as an alternative to amalgam for retrograde filling. The zinc oxide eugenol cements demonstrated superior sealing ability compared to amalgam (Smee et al. 1987; Beltes et al. 1988; Higa et al. 1994; Szeremeta-Browar, VanCura

and Zaki, 1985; Bondra et al. 1989). The disadvantages of Zinc Oxide Eugeneol cements are moisture sensitivity, irritation to vital tissues, possible solubility and difficult handling properties (Torabinejad, Watson and Pitt Ford, 1993). The presence of moisture rapidly accelerates the setting process of Zinc Oxide Eugenol cements, and there is a risk of the material setting before it is adapted to the root-end cavity wall (Torabinejad et al. 1994).

ProRoot MTA was found to have a significantly superior sealing ability to amalgam in the present study. The application of MTA in endodontics was first investigated in 1993 (Lee, Monsef and Torabinejad, 1993). In the first study to test MTA as a root-end filling Torabinejad, Watson and Pitt Ford (1993) measured the penetration of Rhodamine B dye in longitudinally sectioned teeth by confocal microscopy, and found that MTA leaked significantly less than amalgam and Super EBA. Methylene Blue dye penetration in longitudinally sectioned samples was not significantly different when the root-end fillings; amalgam, Super EBA, IRM and MTA were tested with and without blood contamination (Torabinejad et al. 1994). Furthermore, MTA was found to have significantly less dye penetration in the presence or absence of blood than amalgam, Super EBA and IRM (Torabinejad et al. 1994). MTA was found to have a superior seal than amalgam and Super EBA by Aqrabawi (2000), when the penetration of 1% methylene blue dye was measured on longitudinally sectioned samples. Davis et al. (2003) measured the linear penetration of Indian ink in decalcified and cleared teeth. MTA, Super EBA and amalgam were used as root-end fillings after the root-end cavities were irrigated with either saline, citric acid or doxycycline. They found that amalgam leaked significantly more than MTA and Super EBA, irrespective of irrigant used, but there was no statistically significant difference in leakage between MTA and Super EBA (Davis et al. 2003). Pereira, Cenci and Demarco (2004) evaluated the microleakage of amalgam, MTA, Super EBA and Vitremer by measuring linear dye penetration in transversely sectioned apical slices. They found that MTA leaked significantly less than Vitremer and Super EBA, and amalgam leaked significantly more than all the materials (Pereira, Cenci and Demarco, 2004). The present study concurs with other dye penetration studies that amalgam displays significantly more microleakage than ProRoot MTA.

Biodentine™ and MTA Plus™ are relatively new calcium silicate materials, introduced commercially in 2011 and 2012 respectively. No other published study, beside the present one has investigated the sealing ability of MTA Plus™ as a root end filling to date.

Kokate and Pawar (2012) compared the marginal seal of Biodentine™, MTA and Glass ionomer cement as root-end fillings, by examining the penetration of 1% Methylene blue in longitudinally sectioned samples with stereomicroscopy. They found that Biodentine™ displayed significantly less leakage than MTA and glass-ionomer cement, with glass-ionomer cement exhibiting the most microleakage (Kokate and Pawar, 2012). Ravichandra et al. (2014) examined the marginal adaptation of Biodentine™, MTA and glass-ionomer cement as root-end fillings by measuring the area stained by Rhodamine blue dye in transverse sections using confocal laser scanning microscopy. The study revealed that Biodentine™ showed the lowest marginal gaps and best marginal adaptation followed by MTA, and the highest marginal gaps were found in glass-ionomer cement. The results of the present study differed from those of Kokate and Parwar (2012) and Ravichandra et al. (2014). The present study found no significant differences between the sealing ability of Biodentine™ and MTA. A possible reason for the differences could be related to the sample size chosen by the authors of the abovementioned studies. Both studies used a sample size of 10 specimens per group while in the present study, a sample size of 30 specimens per group was used. Other possible differences may be attributed to the different types of dyes used in the studies. Furthermore, Kokate and Pawar (2012) sectioned their specimens longitudinally while in the present study, specimens were sectioned transversely. Ravichandra et al. (2014) used confocal laser scanning microscopy while in the present study stereomicroscopy was used to assess the specimens.

The phenomenon of hydroxyapatite formation over MTA, MTA Plus™ and Biodentine™ (Camilleri, Sorrentino and Damidot, 2013) when immersed in simulated body fluids such as PBS or Hank's Balanced Salt Solution, is well documented (Camilleri, Sorrentino and Damidot, 2013; Han and Okiji, 2011; Han and Okiji, 2013; Gandolfi et al. 2013; Gandolfi et al. 2014). In the present study the specimens were stored in PBS moistened gauze after placement of the retrograde fillings. The

bioactivity of the calcium silicate cements during storage, possibly improved their sealing ability due to chemical bonding of hydroxyapatite crystals to the radicular dentine. Amalgam, however does not bond to dentine, and is reliant on the preparation of an undercut in the root-end cavity for its retention (Friedman, 1991).

In the clinical scenario, MTA Plus™ and Biodentine™ may have certain advantages over ProRoot MTA, due to certain additional properties. MTA plus mixed with antiwashout gel would prevent the loss of the material from the root-end cavity when the surgical is site is rinsed, or when blood flow is resumed. This would probably improve the sealing ability of MTA Plus™, as more filling material would be present within the root-end cavity. Due to the addition of the setting accelerator Calcium Chloride, Biodentine™ reaches an initial set within 12 minutes which allows the operator to observe that the root-end filling material has set prior to closure of the surgical site. The observation of the initial set of the material would not be possible with MTA Plus™ or ProRoot MTA due to their significantly longer setting times. Further studies are necessary to verify the advantages that MTA Plus™ and Biodentine™ may have over ProRoot MTA, with regard to their sealing ability when used *in-vivo*.

CHAPTER 6: CONCLUSIONS

Within the limitations of the present study the following can be concluded:

1. Calcium silicate cements showed a significantly better sealing ability than Permite Amalgam when use as root-end filling material ($p<0.001$).
2. There was no significant difference in the sealing ability of White ProRoot MTA, MTA Plus and Biodentine ($p<0.001$).
3. Based on the findings of the present study, amalgam can be regarded as unsuitable for use as a root-end filling material.
4. Based on the findings of the present study, calcium silicate cements can be recommended as the materials of choice for root-end filling.

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Addendum A

ORAL AND DENTAL HOSPITAL

Dear Patient,

The personnel and students of Oral and Dental Hospital of the University of Pretoria (which is a teaching hospital) appreciate your confidence in us for dental treatment. Although we strive to complete treatment as speedily as possible, our primary task is the training of students. For this reason, the treatment of patients will necessarily be more time-consuming compared to the private sector.

The student who is responsible for your treatment is dependent on your promptness, co-operation and availability. While we strive to train students to treat you in the best possible manner, we kindly request your indulgence with the progress of your treatment plan. We would appreciate you informing us of any unfavourable circumstances. In this way we shall be able to improve our service to you.

Refusal by a patient to be treated by a particular student/dentist to whom he/she is allocated, is not acceptable. In such circumstances, further routine treatment for a patient will be refused. We appreciate your co-operation in this regard. If you have any enquiries, please discuss it with a lecturer/dentist on duty.

The Oral and Dental Hospital is a service rendering unit which is part of the University of Pretoria. Research, apart from teaching, is aimed at the continuous improvement of dental treatment and dental materials. The teaching and research that is done, is partly dependent on obtaining suitable material from our patients. We kindly request that you study the consent form below and if you approve, to please complete it. Should you have any enquiries, please feel free to discuss it with a lecturer/ dentist on duty.

CONSENT

By this I (full names and surname) _____
_____ patient/parent/guardian) grant permission to be treated/that _____ be treated by the Oral and Dental Hospital. I realise that the Oral and Dental Hospital of the University of Pretoria is a teaching hospital and, as such, part or all of my treatment may be given by a student. I realise that information and materials obtained from me during dental procedures may be used for dental training and/or research. I understand that, in the case of my information or materials being used in research, that research will be approved in advance by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria. In the event of such research being of a genetic nature (ie. if my genetic material is studied in the research), the researchers will ask my permission to use my material before they do so. I understand that, if I refuse to give permission for my information or materials to be used in research, this will not in any way affect the treatment I receive at the Oral and Dental Hospital. I understand that, in addition to research, the Oral and Dental Hospital from time to time will audit its patient records as part of the clinical audit process. My permission is granted on the understanding that in all circumstances my identity will remain confidential (secret) and that all my personal details will be dealt with confidentially.

Name: _____

Signature: _____

File No: _____

HOSPITAAL VIR TAND- EN MONDHEELKUNDE

Geagte Pasiënt,

Die personeel en studente van die Hospitaal vir Tand- en Mond-heelkunde (HTM) van die Universiteit van Pretoria, (wat ‘n Opleidings-hospitaal is) waardeer die vertroue wat u in ons stel deur hier vir u tandheelkundige behandeling aan te meld.

Alhoewel ons daarna streef om behandeling so spoedig moontlik af te handel, is ons primêre taak om studente op te lei. Daarom verloop pasiënte se tandheelkundige behandeling noodwendig stadiger as byvoorbeeld in die privaatsektor.

Die student wat verantwoordelik is vir u behandeling, is afhanklik van u samewerking en daarom versoek ons u vriendelik om gereeld, getrou en betyds u afsprake na te kom. Terwyl ons poog om studente op te lei om u as pasiënt reg te hanteer en te benader, vra ons u begrip indien dinge van tyd tot tyd nie ideal verloop soos u dit sou verkies nie. Ons sal dit egter waardeer indien u ons in kennis sal stel indien dinge nie heeltemal aan u verwagtinge voldoen nie. So kan ons die diens aan u verder verbeter.

'n Beroep word op u bereidwilligheid gedoen om behandel te word deur die student/tandarts aan wie u toegeken word. Indien 'n pasiënt nie bereid is om behandeling te ondergaan by die student/tandarts aan wie hy/sy toegewys is nie, sal verdere roetine behandeling vir die pasiënt geweier word. Indien u enige navrae het, bespreek dit asseblief met 'n dosent/tandarts aan diens.

Die HTM vorm deel van die Universiteit van Pretoria as 'n diens-leweringseenheid. Benewens opleiding, word navorsing om voortgesette verbetering van tandheelkundige behandeling en materiale te verseker ook gedoen. Die opleiding en navorsingswerk wat gedoen word is deels afhanklik van beskikbare pasiënt materiaal. Ons versoek u dus vriendelik om die toestemmingsvorm hier onder te bestudeer en as dit u goedkeuring wegdra, dit asseblief te voltooi. Indien u enige navrae het, bespreek dit asseblief met 'n dosent/tandarts aan diens.

TOESTEMMING

Hiermee verleen ek, (volle name en van) _____
_____ as pasiënt/ouer/voog toestemming om behandel te word/dat _____ behandel mag word deur die HTM. Ek besef dat die HTM van die Universiteit van Pretoria 'n opleidingshospitaal is en daarom mag my volledige behandeling of gedeelte daarvan deur 'n student uitgevoer word. Ek besef dat inligting en materiaal wat tydens tandheelkundige behandelingsprosedures van my verkry word, vir tandheelkundige opleiding en/of navorsing gebruik mag word. Ek verstaan dat in die geval dat my inligting of materiaal vir navorsing gebruik word, sal enige navorsing eers deur die Navorsingsetiekkomitee van die Fakulteit Gesondheidswetenskappe van die Universiteit van Pretoria goedgekeur moet word. In geval die navorsing van 'n genetiese aard is (bv. indien my genetiese materiaal bestudeer word in die navorsing), die navorsers eers my toestemming sal verkry. Ek verstaan dat, indien ek toestemming weier dat my inligting of materiaal vir navorsing gebruik word, dit op geen wyse my tandheelkundige behandeling wat ek by die HTM ontvang nadelig sal beïnvloed nie. Ek verstaan, dat bykomend tot die navorsing, die HTM van tyd tot tyd pasiëntrekords sal oudit as deel van die kliniese ouditproses. My toestemming word verleen met dien verstande dat my identiteit onder geen omstandighede openbaar gemaak sal word nie en dat al my persoonlike besonderhede vertroulik hanteer sal word.

Naam: _____

Handtekening: _____

Lêer nr: _____