The Sealing ability of Mineral Trioxide Aggregate (MTA®) used as a Retrograde Filling agent in roots with filled and unfilled root canals – an *in vitro* Comparative Study

By

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A thesis submitted in fulfilment of the requirements for the degree of Magister Scientae Dentium in the Department of Restorative Dentistry, University of the Western Cape

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Keywords

Mineral Trioxide Aggregate (MTA®)

Root-end Filling

Microleakage

Sealing the root canal

***In Vitro* study**

Abstract

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The aim of this *in vitro* study was to determine whether the presence or absence of the root canal seal had any influence on the retrograde sealing ability of MTA®.

In the methodology two sample groups were used, one group with the root canal sealed and another group with the root canal not sealed. All the roots used for the experiment were apicectomised and subjected to root-end cavity preparation. All the root-end cavities were retrofilled with MTA® and examined for microleakage by implementing a methylene blue staining technique. The observations on microleakage in the two sample groups were analysed statistically.

The results indicated no significant difference in the sealing ability of the MTA® when used in the two sample groups.

It can be concluded that the presence or absence of the orthograde filling in the root canals had no significant effect on the retrograde sealing ability of the MTA® at the level of the root-end cavities of apicectomised dental roots.

March 2007

Declaration

I declare that "**The Sealing ability of MTA® used as a Retrograde Filling agent in roots with filled and unfilled root canals – an *in vitro* Comparative Study"** is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Eduard Alexandru Marain **March 2007**

Signed:……

Ethical Statement

In order to retain the impartiality of the study, no financial or material support was requested or used from the MTA® manufacturer or from any other commercial institution. The entire financial, material and technical support was ensured by the UWC and the dental practice where Dr.E.A.Marian works, exclusively.

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Abbreviations, Glossary and Definition of Terms

AH 26® — A Root Canal Sealer

AH Plus® — A Root Canal Sealer

CRCS® — Calciobiotic Root Canal Sealer
(A Calcium Hidroxid-based Sealer)

GP — Gutta-Percha

IRM® — Intermediate Restorative Material

MTA® — Mineral Trioxide Aggregate

Super-EBA® — Reinforced zinc oxide cement

Chapter One

Introduction and Literature Review

1.1 Introduction

Mineral Trioxide Aggregate (MTA®) is presented as a hydrophilic powder, which has to be mixed with distilled water to a creamy consistency in order to be used. The main components of MTA®, such as calcium phosphate, calcium oxide and silica are common to Portland cement (Saidon, He, Zhu, *et al*, 2003). Duarte, Demarchi, Yamashita, *et al* (2003) specified that MTA®'s composition is: 80% Portland cement and 20% bismuth oxide. Due to their close similarities and behaviour, Portland cement is often spoken about as the cheaper alternative to MTA®. Although more attractive from a cost point of view, the original Portland cement has a higher arsenic content than MTA®, according to Duarte, Demarchi, Yamashita, *et al* (2003). They point out that another difference is the fact that the MTA® contains bismuth oxide, to make it radio opaque and visible on radiographs (Duarte, Demarchi, Yamashita, *et al*, 2003). The absence of this component in the Portland cement makes it relatively radio lucent and more difficult to assess on radiographic images.

From its introduction, MTA® was presented as a material with remarkable biological tolerance, a property confirmed by numerous subsequent studies. Numerous studies researching the biological characteristics of MTA® were made *in vitro* as well as *in vivo,* and assessed its effect on the different types of cells, as well as on the surrounding tissues as a whole.

Most of the studies were made comparative to other retrograde filling materials most commonly used, such as amalgam, Intermediate Restorative Material (IRM®) and Reinforced zinc oxide cement — Super-EBA®. Fewer comparative studies with glass ionomers and composite resins were made, as the quality of such fillings is very technique sensitive. It is very difficult to achieve the required technical accuracy under the difficult working conditions in a real surgical wound. When amalgam is used, it is customary to employ zinc-free amalgams as

retrograde filling material. Asrari (2003) reaffirms that zinc in amalgam might cause tissue damage "… zinc contributed to the precipitation of toxic zinc carbonate in the tissue associated with the root-end amalgam filling; since then, it has been standard to use zinc-free amalgam for root-end fillings".

1.2 Literature Review

Asrari & Lobner (2003) note that "… the most commonly used methods for studying toxicity of dental materials have been to determine mitochondrial function or measure a zone of inhibition of cell growth". For example, following cell colonization, the surface of Super-EBA® will present less extensive cell-free areas than that of amalgam (Zhu, Safavi & Spangberg, 1999). One can also determine the presence or absence of by-products of cell degeneration or lysis, such as lactate dehydrogenase (Asrari & Lobner, 2003). Neurons are highly sensitive cells used to study the cytotoxicity of different materials. A study of the neurotoxic effect of the MTA® compared to amalgam, Super-EBA® and Diaket was performed by Asari & Lobner (2003). They found that MTA® was the only material that did not manifest significant neurotoxicity in either fresh or set form. It was interesting to observe that all three other materials maintained their neurotoxic effect for an entire week.

The calcium oxide from the composition of MTA® reacts with water from the surrounding tissues and forms calcium hydroxide, a strong base that creates a high pH (Daurte, Demarchi, Yamashita, *et al*, 2003).

It must be noted that although MTA®'s high pH during its setting phase induces necrosis in the cell layer adjacent to it, surprisingly, this does not compromise the overall healing process of the surrounding tissues. This high pH in the setting stage, as well as maintaining an alkaline pH after setting, although lower, might account partially for its antibacterial characteristics. This is confirmed by Haglund, He, Jarvis, *et al* (2003), "… because of its high pH value during its fresh

and setting stage, MTA® caused adjacent cell lyses and medium protein denature, but after setting, MTA® showed favourable biocompatibility, with no effect on cell morphology and limited impact on cell growth".

Torabinejad, Hong, Lee, *et al* (1995) were of the first to show that when MTA® was used as retrograde filling material, during an animal study, it induced a slight inflammatory reaction in the surrounding tissues, favouring the formation of a fibrous capsule and of cementum.

Gingival and periodontal fibroblasts, as well as osteoblasts, are less sensitive to cytotoxic agents comparative to neural cells. However, they are used in dental research because they are the most relevant cells to be used for the assessment of periodontal tissue reaction towards different dental materials and pharmacological agents.

Haglund, He, Jarvis, *et al* (2003) stressed that the influence exerted by various materials used as root-end fillings on the microscopic morphological characteristics, capacity to function and ability to proliferate, of macrophages and fibroblast are very significant, as these cells are critical components in the inflammatory and proliferation processes accompanying the healing of a wound. They refer to two previous studies, namely, Koh, McDonald, Pitt Ford, *et al,* 1998 and Koh, Torabinejad, Pitt Ford, *et al,* 1997, regarding cellular response to MTA®. The former found the expression of macrophage colony stimulating-factor, as well as of IL-1beta and IL-6 by the macrophages and the osteoblasts, under the stimulating influence of MTA®. The expression of the IL-8 – which activates angio-genesis as part of the normal healing process, has also been shown in the presence of the MTA® (Regan, Gutmann & Witherspoon, 2002).

Pistorius, Willershausen, Briseno, *et al* (2003) demonstrated that fibroblasts contiguous to MTA® showed an almost unaltered protein synthesis, which resembles the biological tolerance for titanium – "MTA® (91.2 +/- 5.9%) and titanium (92.4 +/- 4.7%)", as well as an unhindered proliferation; "contrary, a continuous reduction in the rate of cell proliferation was observed for cells in contact with amalgam (61.0 +/- 2.5%) after 96 h".

The opinion exists that IRM® has a very good biological tolerance. Considering the much lower cost price of the IRM®, this could constitute an important alternative to MTA®. Chong, Pitt Ford & Hudson (2003) confirmed that success rates following treatment with IRM® and MTA® were very close, but they mentioned that for IRM® "the tissue response is one of toleration rather than bio acceptability". MTA® instead exerts a beneficial stimulating action on the function and proliferation of the periodontal cells. Bonson, Jeansonne & Lallier (2004) noted that proliferation of the fibroblasts on MTA® was superior; "MTA® preferentially induced alkaline phosphatase expression and activity in both PDL and gingival fibroblasts". The fibrous repair layer that forms on top of the MTA® surface, in the subsequent healing phase, is stimulated by the same MTA® to mineralise and form hard tissue; this hard tissue formation does not occur at the contact with the IRM® (Haglund, He, Jarvis, *et al,* 2003).

Keiser, Johnson & Tipton (2000) concluded, in their study, that MTA® should be recommended as root-end filling material. They showed that whether in its set state or freshly mixed and at different concentrations, MTA® showed lower citotoxicity on human PDL cells, than Super-EBA®; Super-EBA® is considered to be another material with remarkable biological tolerance. It is important to know this when the resected set MTA® is left as sealer for the resected root-end.

MTA® offers an adequate surface for the adhesion of the fibroblasts. It is also a medium that allows normal function of the osteoblasts and favours their multiplication, with the spread of osteoblast colonies on its surface. These facts evidentiated by Zhu, Haglund, Safavi, *et al* (2000) indicate the compatibility of the osteoblasts with MTA®.

Remarkably, on the surface of the MTA® retrograde filling, cementum, periodontal ligament fibres and osseous tissue are formed during the healing period (Torabinejad, Pitt Ford, McKendry, *et al,* 1997; Torabinejad, Hong, Lee, *et al,* 1995). As so suggestively presented by Witherspoon & Ham (2001) "…MTA® provides scaffolding for the formation of hard tissue and the potential of a better biological seal".

Economoies, Pantelidou, Kokkas, *et al* (2003) described how MTA® stimulates the adjacent fibroblasts to secrete collagen fibres that will form a dense fibrous layer at the MTA® surface. This forms as soon as 1-2 weeks after the treatment. From this dense fibrous layer, some collagen fibres leave towards the bone, in a manner similar to that of the periodontal ligament fibres. In the following healing period this fibrous structure may become partially or fully mineralised – "new bone at the site of the resected apices was evident in all MTA®-filled roots after 2-5 weeks" (Haglund, He, Jarvis, *et al*, 2003). Haglund, He, Jarvis, *et al* (2003) concluded that the simulation of post surgical repair of periradicular tissues recommends MTA® as being biocompatible.

Measuring the cytotoxicity on L-929 cells and human gingival fibroblasts, *in vitro*, Osorio, Hefti, Vertucci, *et al* (1998) demonstrated that MTA® showed lower toxicity than root canal sealers and root-end filling materials such as Endomet®, AH 26® and Amalgam, Gallium GF2, Ketac Silver, Super-EBA®, respectively.

Tang, Torabinejad & Kettering (2002) remind us that materials such as amalgam, Super-EBA® or IRM® allow healing with repair of the periapical tissues, while MTA® allows healing with the regeneration of the periapical tissues.

The intraosseous surgical placement of small implants of MTA®, Super-EBA®, IRM® and amalgam in guinea pig tibia and mandible, in contact with the bone marrow, revealed that MTA® did not induce inflammatory reaction in either tibial or mandibular sites, while inducing bone apposition directly on its surface (Torabinejad, Hong, Pitt Ford and Kaijawasam, 1995; Torabinejad, Ford, Abedi, *et al*, 1998).

The regeneration of cementum over MTA® root-end fillings is a remarkable healing result. In an experiment performed on monkeys, the histological examination of the periapical tissues, five months after the placement of the retrograde MTA® filling, revealed formation of new cementum over the MTA®, in five of six treated roots (Torabinejad, Pitt Ford, McKendry, *et al*, 1997).

Torabinejad, Hong, Pitt Ford and Kettering (1995) aligned the most used filling materials for the root-end, according to their increasing cytotoxicity, as follows: MTA® (the least cytotoxic), followed by zinc-free amalgam (more cytotoxic then MTA®), then by Super-EBA® and then by IRM®.

A very important aspect in the study of MTA® was to assess its mutagenicity. Kettering & Torabinejad (1995) tested the potential of MTA® to express mutagenicity directly or indirectly and showed negative results, demonstrating that MTA® has no mutagenic potential. Their result is important, as absence of mutagenicity is a prerequisite of any acceptable retrograde filling material.
In 2006, a study performed by Braz et al on human peripheral lymphocytes indicated that MTA® did not induce lesions in their DNA.

In addition to its remarkable biological characteristics MTA®offers an effective marginal seal that prevents microleakage. This hypothesis will be investigated in this study.

The dimensional stability during and after setting is a very important characteristic of MTA®. This is a major contributing factor to MTA®'s excellent marginal sealing ability.

Sealing ability is the property of a material to establish and maintain an intimate contact with the cavity walls, with the contact capable of effectively preventing microleakage at the interface.

In general the putty fillers that have to be compacted into a cavity accomplish a poorer marginal seal than their lower viscosity counterparts, which adhere to the cavity walls. Consequently, as expected, MTA® showed a superior sealing ability to that of amalgam (Yatsushiro, Baumgartner & Tinkle, 1998).

When the compaction of amalgam inside root-end cavities was preceded by the application of a cavity liner, the sealing ability of such liner-amalgam combinations was found to be inferior to that of MTA® or Super-EBA®. These

results were obtained by Bates, Carnes & del Rio (1996) by using a fluid filtration measurement system in order to measure microleakage.

MTA® also showed a sealing ability superior to that of Super-EBA® (Wu, Kontakiotis & Wesselink, 1998). Adamo, Buruiana, Schertzer, *et al* (1999) stated that, despite its advantages, Super-EBA® is "…moisture sensitive, gives eugenol irritation of the periapical tissues, is partially soluble in the oral fluids, technique sensitive and radio lucent".

Fischer, Arens & Miller (1998) determined experimentally that MTA® offered superior apical seal and prevented the penetration of S.marcescens with higher efficiency when it was used for filling of root-end cavities, compared to IRM®, Super-EBA® and amalgam. They drew the attention to the fact that this superior sealing ability could be attributed to the setting expansion that takes place when MTA® sets in the presence of moisture.

Tang, Torabinejad & Kettering (2002) demonstrated that the seal offered by MTA® is effective enough to prevent leakage, not only at bacterial, but also at sub-bacterial molecular level, preventing the marginal penetration of bacterial endotoxins more efficiently than amalgam, IRM® or even Super-EBA®.

The difference in the capability of various filling materials (including MTA®) to prevent bacterial leakage, is reduced by using an effective dentin bonding agent under the filling (Adamo, Buruiana, Schertzer, *et al*, 1999). However, to do bonding in a root-end cavity is very difficult, and may even be impossible to perform in the conditions of a real periapical surgical wound.

The concern regarding the long-term mechanical resistance of MTA® under the stress of masticatory forces, was addressed by Peters & Peters (2002). In an experiment simulating five years of occlusal load of retrograde filled teeth, they showed that the degree of marginal integrity of root-end fillings examined stayed high, despite minimal chipping. Yatsushiro et al 1998 noted that a thickness of half a millimetre at the surface of the root-end filling becomes dissolved, reducing the surface level accordingly.

The positive results from the studies regarding the biological integration of MTA® to periapical tissue, as well as its very good sealing ability, led to its acceptance as retrograde filling material, and attracted further research to determine the various factors which could influence the quality of the MTA® retrograde seal.

Torabinejad & Chivian (1999) confirmed that along with its implementation for pulp capping, root perforation repairs, root canal sealing and the creation of a barrier for internal bleaching, the use of MTA® for retrograde fillings is one of its main applications.

Davis, Jeansonne, Davenport, *et al* (2003) studied the effect of irrigation of retrograde cavities with different acidic solutions. Their results showed that although irrigation with acidic solutions reduced the microleakage when amalgam was used as filling material (probably due to the removal of the smear layer), it did not improve the marginal seal for MTA®. However, Davies, Jeansonne, Davenport, *et al* (2003) stressed in their conclusion that demineralising the retrograde cavities lead to improved cementogenesis and overall periapical healing. They also mentioned that the slowing down of the setting of MTA® was observed following the irrigation of the root-end cavities with acidic solutions.

It is speculated that the quality of the marginal seal could be affected by the technique used for the preparation of the root end cavity. The root end cavity can be prepared conventionally with rotary instruments (burs mounted in a slow speed hand piece) or with ultrasound instruments. It is feared that preparation with ultrasound instrumentation might result in cracks in the cavity walls, which might negatively influence the quality of the marginal seal. Gondim, Zaia, Gomes, *et al* (2003) mentioned that, consistent with previous studies analysed by them, their experiment performed in 2003 showed that the majority of the root-end cavities prepared by ultrasound instrumentation, exhibited marginal chipping. However, they indicated that it was not clear if such marginal chipping would affect the periapical healing in any way. They also noted that the marginal chipping could be reduced or removed by finishing the sealed root-end surface with a carbide burr, although the marginal adaptation of the MTA® stayed good with or without

being seconded by the finishing procedure. Their conclusion was that the root-end cavities may be better accessed and aligned by the use of the ultrasound retrotips, resulting in an overall superior retrograde preparation.

Andelin, Browning, Hsu, *et al* (2002) showed that the sealing ability of resected and non-resected MTA® were very similar, unaffected by the resection of set MTA®. They also noted that the inductive effect towards the formation of cementum on the surface of freshly placed MTA® was evident more often than on that of set resected MTA.

The pH of infected areas is lower than that of healthy tissue. Roy, Jeansonne & Gerrets (2001) measured the sealing ability of MTA® set respectively in acidic (pH 5.0) and in neutral (pH 7.4) environments 24 hours after setting. The results showed that the acidic pH 5.0 surrounding did not negatively affect the sealing ability of the MTA®, as compared to the pH 7.4 (Roy, Jeansonne & Gerrets, 2001).

Torabinejad, Rastegar, Kettering, *et al* (1995) showed in a study that the contamination of the root-end cavity with blood did not significantly affect the sealing ability of MTA®, IRM®, amalgam or Super-EBA®. This is an important finding that may influence the choice of filling material to be used in surgical wounds where it is difficult to ascertain dry, clean working conditions inside the root-end cavity.

A revision of the latest publications with regard to MTA® supports previous findings about this multipurpose material and will be discussed below.

The experience accumulated in the clinical area indicates that MTA® is the preferred material for endodontic surgery, as well as for other procedures such as direct pulp capping, perforations of the root and pulp chamber, internal resorbtion and stripping, transportations and lacerations of the apex (Casella and Ferlito, 2006).

Clinical success in performing direct pulp cappings with MTA®, on young patients, that suffered coronal fractures with pulp exposures, on teeth with the root still in formation, were also reported by Karabucak, Li, Lim, *et al* (2005). They indicated the efficiency of the MTA® in maintaining the vitality of the dental pulp, that further allowed normal development of immature roots.

MTA® helps in the difficult task of obtaining a higher success rate in the pulpotomy treatments of primary teeth, in the quest to substitute formocresol and its high tissue toxicity (Holan, Eidelman & Fuks, 2005). The replacement of formocresol with MTA®, due to better clinical results in pulpotomized primary teeth, is also recommended by Farsi, Alamoudi, Balto *et al* (2005).

Maroto, Barberia, Planells, *et al* (2003) showed that more than half of the teeth treated in their study demonstrated radiological signs resembling dentine formation over the MTA®, while also more than half of the teeth showed hypermineralization or calcification of the canals. Remarkably, MTA® may be used to achieve apexification without prior use of calcium hydroxide as pre-obturational canal medication (Fellipe, Fellipe & Rocha, 2006).

According to Jaramillo, Fernandez & Villa (2006), complete periapical healing with bone formation at the site of the lesion was achieved in the treatment, of periapical areas associated with dens invaginatus, with MTA®. Tait, Ricketts and Higgins described in May 2005 how an immediate and permanent apical seal could be formed with the use of-MTA®, saving months of waiting, otherwise necessary in alternative apexification procedures. This approach allows immediate intracanal resin bonding of fiber posts. In an experiment executed by Xavier, Weismann, de Oliveira, *et al* (2005), MTA® demonstrated a better marginal adaptation when compared to Super EBA® and Vitremer®.

MTA®'s antibacterial effect was reconfirmed again by Eldeniz, Hadimli, Ataoglu, *et al* (2006) when in a comparative study with other root-end filling materials (amalgam, Intermediate Restorative Material (IRM®), Super Bond C&B®, Geristore®, Dyract®, Clearfil APX® composite with SE Bond®), the mineral

trioxide aggregate showed a stronger antimicrobial effect than the others. Although, the study shows that the IRM® also showed a strong antibacterial action, just as MTA®, one must remember the superior biocompatibility of the MTA®.

The antimicrobial effect of MTA® towards bacteriae such as *Enterococus faecalis, Micrococcus luteus, Stephylococcus aureus, Staphylococcus luteus, Staphylococcus epidermis* and *Pseudomonas aeruginosa* was demonstrated by Sipert, Hussne, Nishiyama, *et al* (2005). They, however, mentioned that MTA® and Portland cements did not inhibit *Escheritia coli*, while EndoRez® did not show an antimicrobial effect at all.

The antibacterial as well of the antifungal effect of the MTA® was demonstrated by Sipert, Hussne, Nishiyama, *et al*, 2005. In 2006, the experiments of Al-Hezaimi, Naghshbandi, Oglesby, *et al* (2006) pointed that MTA® is an efficient antifungal agent against *Candida albicans*. They stressed the importance of the MTA® concentration for the antifungal effect.

The dispute whether the cheaper Portland cement can be considered and used as a substitute for the MTA®, continues in literature. The basis for such rationale is studies that show that materials such as Portland cement (Islam, Chang &Yap, 2005) and IRM® (Lindeboom, Frenken, Kroon, *et al*, 2005) display very similar sealing efficiency and clinical effectiveness as MTA®. Studying the biocompatibility properties of the two cements, Camilleri, Montesin, Di Silvio, *et al* (2005) showed similar behaviour of MTA® and Portland cements. The competition between MTA® and Portland cement was again brought to attention by comparative studies by Danesh, Dammaschke, Gerth, *et al* (2006). Their work revealed that MTA® presents higher microhardness and radio-opacity, as well as lower solubility than its challenger, namely Portland cement.

Following their experimental work, Dammaschke, Gerth, Zuchner & Schafer (2005) stated that it would be wrong to consider Portland cement to be the equal though cheaper substitute for MTA®. They emphasize objective differences between the two cements eg. Cu, Sr, Mn are heavy metals found in a larger

quantity in Portland cement, MTA® exhibits less aluminium species and Fe+ chromophores than its counterpart, while the particle size is larger in Portland cement. These differences do non cancel the major similarities existing between the two cements.

The ongoing research of MTA®'s biocompatibility, confirms its safety.
In a project studying the genotoxicity and cytotoxicity of MTA®, Ribeiro, Sugui, Matsumoto, *et al* (2006) showed that both MTA® and Portland cement did not provoke apoptosis, nor were genotoxic. The selective positive effect of MTA® on the proliferation of cementoblasts was put in evidence by Oviir, Pagoria, Ibarra, *et al* (2006). They demonstrated experimentally that when placed in contact with OCCM.30 cementoblasts and OKF6/TERT1 keratocytes, MTA® selectively exhibited a higher stimulation of the OCCM.30 cementoblasts.

Super EBA® is one of the best biologically tolerated retrograde filling materials, just as MTA®. However, a histological study done during an animal trial, performed by Yildirim, Gencoglu, Firat, *et al* (2005), stressed a major difference in the biological reaction provoked by the two materials. They showed that the inflammatory reaction induced by MTA® was smaller than that induced by Super EBA®. They also showed that while Super EBA became covered with connective tissue, MTA® was covered by new cementum, during the healing process.

When tested for its influence on fibroblasts and granulocyte-macrophages, MTA® again showed the lowest cytotoxicity when compared to the effect of other materials such as amalgam, glass-ionomer Super EBA®, N-Ricket® and gutta-percha (Souza, Justo, Oliveira, *et al*, 2006). At a time when the physiology of a tissue cannot be interpreted without reference to the cytokines involved, a study by Rezende, Vargas, Cardoso, *et al* (2005) showed during a bacterial test envolving Fusobacterium nucleatum and Peptostreptococcus anaerobius, that MTA® had no influence on the expression of IL-12 and IL-10 during the M1 and M2 macrophage response.

The importance of the surrounding conditions on the setting and the end quality of the set MTA® as a filling material was stressed again, when Walker, Diliberto and

Lee (2006) revised the influence that moisture has on setting MTA® and confirmed that as a general rule moisture increases the flexural strength of the set MTA®.

An excellent biological property of MTA® is that it induces the formation of a calcified hard tissue barrier around the MTA® exposed by the root end cavity to the surrounding tissues, as well as around any MTA® extruding beyond the apex. Actually, Fellipe, Fellipe and Rocha (2006) demonstrated that a hard tissue calcified barrier forms more efficiently and frequently on the surface of the MTA®, extruding from the canal beyond the apex, periapically, than inside the apical portion of the canal, when the canal is sealed just short of the canal opening on the surface of the apical root surface.

Due to the importance of the vascular proliferation in the healing tissues, De Deus, Ximenes, Gurgel-Filho, *et al* (2005) studied the effect of MTA® on endothelial cells *in vitro*. Their results indicated that after 48-72 hours, the cytotoxicity displayed by MTA® at the beginning of its setting, declined sufficiently to allow the regeneration of the initially damaged cellular colonies. They suggest that this shift in the biotolerance of MTA® during its setting may explain the appearance of the vascularization of the tissues contacting the MTA® only towards the end of its setting.

A very interesting study is that of Yan, Peng, Fan, *et al* (2006) who recorded a weaker adherence of MTA® to dentine following the use of Glyde File Prep®, during the mechanical treatment of the root canal, if compared to the plain use of chlorhexidine (2%) or sodium hypochlorite (5,25%) on the dentinal surface.

The importance of the manner of preparation of the root-end cavity for the sealing ability of MTA®, was stressed once again by an experiment performed by Karlovic, Pezelj-Ribaric, Miletic, *et al* (2005). They showed that the implementation of the Er:YAG laser for the preparation of the root-end cavities lead to lower microleakage for any of the three retrograde filling materials used by them (MTA®, IRM and Super-EBA), as compared to their sealing ability in cavities prepared with ultrasonic retrotips. It is interesting to note that when

attempting to seal a perforation in the furcation area, it is difficult to dry the dentine limiting the perforation, in order to allow efficient adherence of the repair material to the hard structure surrounding the perforation area. However, moisture in the perforation area is not a problem when MTA® is used as repair material, as the sealing effectiveness of the MTA® is not negatively affected by the humid environment. While MTA® may be used alone for the repair of such perforations, even the use of a very adhesive material such as Vitremer® requires the pretreatment of the furcation with a haemostatic collagen sponge prior to the application of the cement (Tsatsas, Meliou & Kerezoudis, 2005).

Research and reporting on mineral trioxide aggregate is an ongoing process. This process is driven by the need to know as much as possible about the material that already offers so much and holds even more promise for the future of dental therapeutics. The wide variety of studies dedicated to the research of MTA® and the positive results obtained, leave us with no doubt about the suitability of the MTA® for an apical seal.

Amongst the multitude of research studies dedicated to the research of the factors that could influence the quality of MTA® retrograde fillings, there were none indicating the influence that the presence, or absence, of the conventional orthograde root canal seal inside the canal, might have on the sealing ability of the MTA® at the root-end cavity level.

The information generated by the results of the present study will offer evidence-based support for the decision of whether the sealing of a root canal prior to an apicectomy (with a retrograde MTA® filling) is imperative or only recommendable.

Chapter Two

Comparison of Retrograde Sealing Ability of MTA® in Roots with Sealed and Not Sealed Canals

2.1 Materials And Methods

2.1.1 Ethical Considerations

Although this is an *in vitro* study a few remarks have to be made. From the start it must be mentioned that the teeth used in the present study were not extracted for the purpose of the study, nor intended for it. The reason for their extraction was, invariably, for therapeutic purposes. In each case the odontectomy was considered to be the last resort option and the only reasonable treatment solution, after other treatment options were explored together with the patient. The strict implementation of this ethical principle made the collection of 50 suitable premolars extracted for orthodontic or periodontic reasons, as intended for the study, practically impossible. The premolars extracted for periodontal reasons were scarce, while most of the premolars extracted for orthodontic reasons exhibited wide root canals with large incomplete apical foramina – inadequate for retrograde filling. In addition, part of the suitable extracted premolars had to be directed to other academic projects. After carefully considering the facts (reality), it was decided that for the present study it is reasonable to accept, not only premolar roots, but also suitable roots of other single and multi- rooted teeth, as long as 50 selected teeth will offer 50 root-end cavities suitable for the final microscopic examination and evaluation. This would not alter

the aim, relevance and the significance of the experiment. The presence or the absence of the coronal part of the tooth, was also assessed relevantly for the experiment. It was concluded that, although the presence of the coronal part played a role in the preparation stages of the tooth for microscopy, it is of no significant relevance during the microscopic examination stages. As the experiment was interested in the root-end/apical area of the tooth, it was more justified and precise to talk about roots instead of the tooth as a whole.

2.1.2 The Odontectomy

As always, the extractions were carried out atraumatically for the tooth and the supporting periodontal structures. The gentle subluxation of the tooth (from its dento-alveolar ligament) with a periotome preceded the actual exarticulation of the tooth. This manoeuvre was performed by forcing the periotome as deep as possible into the periodontal space, in order to sever the periodontal ligament and mobilize the tooth, thus facilitating the subsequent completion of the luxation with the forceps. The continuation of the luxation procedure with the forceps lead to the further and more ample widening of the alveolus, increasing the intra-alveolar mobilization of the tooth. Ultimately, the well-mobilized, luxated tooth was removed from its alveolus. This exarticulation technique allowed the extraction of the teeth without radicular or coronal fractures.

2.1.3 Root Surface Preparation of the Extracted Teeth

Before being placed in the storage container, the extracted teeth were scaled and polished and then washed with saline solution. The scaling was performed with a sickle shaped hand-scaler (Hu-Fredy) with the purpose of removing any tarter, epithelial or/and osseous fragments attached to the radicular or/and coronal surfaces. It also removed the periodontal ligament and planed the cementum surface of the

root, so that it does not impair the efficient seal of the nail varnish on the lateral root surface. The surfaces of the teeth were then polished with soft nylon brushes, activated in a slow speed contra-angle hand piece with pumice/saline slurry. This was done only for those teeth selected for the experiment. Polishing was performed to remove plaque and micro-debris sufficiently from the coronal and radicular surfaces, as well as to smooth and polish these surfaces, but performed gently enough, to prevent over-abrasion of the root surface and thinning of the radicular walls.

2.1.4 The Selection of the Roots to be used in the Experiment

After an initial visual inspection of one hundred and three extracted teeth, sixty-five single- and multi-rooted, caries free teeth were reinspected visually and radiologically and considered suitable for the present experiment. The selection was made by adopting the criteria enunciated by Andelin, Browning, Hsu, *et al* (2002): "root length of at least 12mm, no evidence of previous root canal therapy, an initial apical size no greater than ISO size 20". The roots were also inspected under magnification to rule out the presence of root fractures (Hachmeister, Schindler, Walker, *et al*, 2002). From a lot of sixty-five teeth, fifty-three were further selected to offer one hundred roots suitable for the actual experiment, in which the root-end fillings would be prepared. The one hundred roots were then re-polished extremely lightly with a pumice/saline slurry with rotating soft nylon brushes mounted in a slow speed contra-angle hand piece, and re-inspected to confirm their suitability for the experiment. The area of interest for the experiment was the root-end area, with the root-end cavity. When the terms tooth or root are used, one must therefore see them as the bearers of the root canal, root-end and root-end cavity, the relevant areas for this experiment.

2.1.4.1 The Storage of the Teeth selected for the Experiment

While collecting the extracted teeth, in the period prior to the experiment, their preservation or storage could be done using a few methods.
Yatsushiro (Yatsushiro, Baumgartner & Tinkle, 1998) specified that the purpose of the storage medium is to inhibit bacterial growth and to keep the teeth hydrated. They used a solution of PBS (phosphate-buffered saline) with 2% sodium azide.

Torabinejad, Smith, Kettering, *et al* (1995), used 10% buffered formalin as storage medium for the extracted teeth; the storage was carried out at a low temperature. The same storage and preservation medium was used by Fischer, Arens & Miller (1998) and Hachmeister, Schinkler & Walker (2002). Valois & Costa (2004) kept the teeth in a 1% sodium hypochlorite (NaOCl) solution overnight for surface disinfection and later stored them in a phosphate-buffered saline solution until use.

Gondim, Zaia, Gomes, *et al* (2003) stored the teeth extracted for their experiment in a solution of 2% formaldehyde in distilled water for not more than three months prior to the root-end filling procedure. Adamo, Buruiana, Schertzer, *et al* (1999) stored the teeth in a 0.05% NaOCl solution while Peters & Peters (2002) stored the exarticulated teeth in thymol solution.

In the present project, the storage medium used for the preservation of the extracted teeth was a solution of thymol in saline, at dilution of 1 gram of thymol crystals (supplied by Scala Pharmacy, Claremont Medical Centre, Higgs, 2005) in 1000ml saline (SABAX® POUR SALINE 0,9%, **Adcock Ingram Ltd**, Lot 503R102). The new 1,5 *l* plastic container/jar and its lid used for the storage, were first washed thoroughly with detergent and then rinsed copiously to remove all the detergent. The interior part of the container and lid were then disinfected with a 90% alcohol solution and then rinsed off with sterile saline. The container with the teeth in the thymol solution was kept in a refrigerator, for low temperature (5^{o}C) storage. The preservation period for the teeth was not longer than six weeks.

All fifty teeth were subjected to mechanical root canal preparation procedures. Following, is a description of the root canal treatment technique implemented. These are actually techniques described by Stock & Nehammer (1990) in their book "Endodontics in practice" and by Prof. Louw (Louw, 2002) as well as by Prof. Cantatore (Cantatore, 2004) in lectures which the researcher (EAMarian) had the privilege of attending.

2.1.4.2 The Access into the Pulp Chamber

A high speed rotating round diamond bur was positioned on the occlusal or palatal surface of each tooth and advanced towards the pulp chamber until it was opened. The widening of the access and the complete removal of the roof of the pulp chamber was then performed with a high speed rotating ***Endo-Z***® bur. The pulp chamber was opened until good access to the orifices of the canals was achieved. The content of the chamber was then removed. The pulpal tissue and the eventual loose pulpolites were removed with a suitable hand instrument - a Blake spoon or a small size excavator. If a strongly attached pulpolite was encountered, it was mobilised and removed with the help of the vibrating tip of an ultrasound scaler, moved circumferentially on the pulpolite surface, at the interface between the pulpolite and the walls of the chamber. When undercuts were present on the lateral walls of the pulp chamber, these were removed with an ***Endo-Z***® bur at high speed under irrigation, opening and flaring the cavity in order to create good access towards the root canal. After its mechanical debridement, the pulp chamber was washed with a 2.5% NaOCl solution. Once the clean pulpal floor was exposed, the orifice opening of the root canal was identified visually and checked with the palpatory action of the tip of a dental probe.

The mechanical treatment of the canals could now commence. The purpose of the mechanical treatment of the root canals was to debride them completely, to shape

them as a funnel pointing apically and to create apical rests. This would create conditions for efficient irrigation, medication and sealing of the root canals.

2.1.4.3 Establishing the Patency of the Canal and Apex and Irrigation

The manner/technique in which the patency of a canal must be achieved could be called filing exploration, indicating that the most important aspect of this manoeuvre is the exploration of the canal and only secondly, the filing of the walls that would facilitate the work of the file inside the canal. It could also be described as palpatory engagement and filing of the root canal or as exploratory advancement and filing into the root canal in an apical direction. This is, again, to express/stress the exploratory gentleness of the instrumentation technique to be used.

The irrigation of the canal: After each filing incursion, the canal was irrigated in order to remove dentinal debris, preventing it from accumulating in the apical area. The irrigation also lubricated the canal during instrumentation with files. Irrigation was performed with a 2,5% solution of NaOCl, with the help of a plastic syringe fitted with a gauge 30 needle or an endodontic irrigation needle. In this experiment, the needle was inserted into the canal until it was locked by the walls of the canal being prepared or until it reached the apical rest of the sufficiently prepared canals. From there, the needle was retrieved approximately 1mm until looseness of the needle inside the canal was achieved, in order to allow free backflow of the irrigation solution in a coronal direction, preventing its injection through the apical isthmus, into the periapical area. Only then the irrigation solution was gently injected into the canal until the backflow solution appeared to be free of dentinal filings/debris.

Patency of the canals along the entire length as well as the patency of the apices was achieved by using the balanced force technique. The canals were loaded with irrigation solution as far as possible without pressure, so that it did not extrude through the apex. To start, a No.10 Hedstrom file was introduced into each canal with a light/gentle pressure in an apical direction, until the file tip started to engage

the canal walls. At this moment the file was pressed lightly/gently in an apical direction concomitant with a clockwise rotation of not more than 90 degrees. If the grip of the file by the fingers was released, while the file was in its rotational clockwise tension, the file tended to de-rotate (rotate back) slightly. This meant that once the grip of the file was released the file recoiled slightly anticlockwise. At this moment the grip of the file was re-established with the fingers and the file was gently turned anticlockwise, while slightly pulled coronally in order to facilitate the disengagement of the file tip from the canal. The file was retrieved 2-3mm and then the earlier performed step was repeated to the same depth (recapitulation), where the weaker/looser engagement of the tip of the file by the canal portion that just has been engaged by the previous manoeuvre, now allowed easy anticlockwise rotation of 360 degrees. This 360 degrees anticlockwise rotation was performed concomitant with a feather- light pressure on the file in an apical direction. The file was then retrieved 1mm coronally and circumferential filing of the canal walls was performed by in-out movements of the file, but without rotating it and without trying to file any of the dentine engaging the tip of the file (in order to prevent its fracture).

These steps were repeated while pushing the file (with feather-light pressure) slightly more apically every time, just enough for the file to become inserted into the canal. This was repeated until the apex was reached and patented. Once the patency of the canal and the apex was established with the Hedstrom file No.10, this patency was widened by repeating all the manoeuvres, with a No.15 file. Once the complete patency of the canal and the apex were established by sliding a No.15 file freely through them, the root was ready for the canal length determination and next stage of the root canal treatment.

Usually the apical patenting is furthered to file size 15, with good clinical results. However, the guiding rule should be that the apical patenting should stop at the first size of file that engages/bites the apical canal walls (depending on the width of the apical isthmus), but not less than size 10. The adequate patenting of the canal in the

apical area creates conditions for its effective seal at the end of the root canal treatment.

2.1.5 The Mechanical Treatment of the Canal

The mechanical treatment of the canals involved the creation of an apical rest and shaping of the canals, while preserving their patency.

Due to the fact that the experimental teeth were extracted teeth, the canal length determination was made under direct visual and palpatory control on the external surface of the root. A No.15 file was slid through the canal (in an apical direction) until the tip of the file extruded 0,5-1mm from the canal. The file tip was then pushed back into the canal (in a coronal direction) with the help of a spatula, until the tip of the file became flush with the root/apex surface and did not protrude from the canal. With the file in this position the rubber stopper of the file was slid and brought against the coronal landmark from where the canal length was to be measured. The file was then pulled out from the canal and the canal length measured on the file. The working length was calculated to be 1mm shorter than the full canal length.

In this experiment the following instruments were used to prepare the canals: *K Flex*® files No.10-40 (Kerr, USA) (Item No 60147, 60148, 60149, 60150, 60151 and 60152), Peeso Engine Reamers No.1,3 (CE, Swiss) (Lot No 9708 EJ and 3774 MD).

The preparation of the apical rest was started by inserting a No.20 file into the canal and engaging and filing the lateral walls of the canal in the same manner as was done with the No.15 file. The difference was that the No.20 file was advanced and stopped 1mm short of the full canal length, which is the working length. This time more time was dedicated to the filing of the lateral walls. The walls were filed with an "in-and-out" movement against and around the entire circumference of the canal wall and in a

clockwise circumferential filing movement. At the same time the instrumentation/filing was done predominantly with an anticurvature orientation. This meant that at the entrance to the canal filing was directed more to the walls opposite to the direction in which the canal/apex curved, in order to create a straighter access towards the apical area. The filing with the No.20 file was continued until it could glide freely, strictly to the working length, where the apical rest started to form. Concomitant, a light funnelling of the canal started to form in the coronal part of the canal, in an anticurvature direction.

The No.25 file was then implemented, in a similar manner to the No.20 file. The apical rest became established and the coronal funnelling of the canal increased slightly. Once the first engagement of the No.25 file and implicitly enlargement of the canal took place down to the apical rest, the further instrumentation had to be continued with extreme care and respect for the apical rest, with just a feather-light touch of the apical rest every time the file reached it. The patency of the canal in the apical area was then recapitulated with the No.15 file, in order to preserve the patency; this was once again done extremely gently, in order not to harm the existing apical rest.

At this stage a ***Gates Glidden***® No.1 bur was engaged at slow speed in the coronal quarter of the canal, to a depth of approximately 3-4 mm. The entrance to the canal was then widened with a ***Gates Glidden***® bur No.3, inserted into the canal for only about 2 mm. The passage between the canal area instrumented with the burs and the canal area instrumented manually were then smoothened by recapitulating the hand filing of the coronal half of the canal with the No.25 file, which was kept at least 1-2 mm away from the apical rest during this filing. The filing was once again in a circumferential clock-wise and anticurvature manner. The funnel shape of the canal was now established.

Further widening of the canal was now achieved by using the next size file (No.30), in the same manner as used for the No.20 and No.25 files.

For the present study the preparation of the canal was continued to file No.35. The patency of the apical area was enforced by gliding file No.15 to the full canal length and barely passing it, to allow an extrusion of 0.1mm of the patenting file from the canal. This was done by "gliding, not filing" the file, without engagement and/or alteration of the lateral walls of the apical canal.

When the mechanical treatment of the canal was finished, the canal was filled with NaOCl solution, which was left there for five minutes. The canal was then re-irrigated with the syringe and dried thoroughly with the help of paper points and air spray. The instrumented canal was now ready to be sealed.

All the canals were prepared as described from 2.1.4.3 up to this point.
However, not all the canals were sealed. The fifty teeth were separated into two groups:

Group 1: 50 roots had the canal sealed by means of Gutta-Percha (GP) points and sealer; also called the **Sealed Group.**

Group 2: 50 roots were left with the canal unsealed;
also called the **Not Sealed Group.**

This means that the root canal preparation for the roots in **Group 2**, stopped here.
For the roots in **Group 1** the root canal preparation continued with a third stage – the root canal seal, as follows:

2.1.5.1 The Sealing of the Prepared Root Canals, in Group 1

The canals were sealed using the lateral condensation technique, as this technique is most broadly used in clinical practice. The root canal sealer used was AH Plus® (DENTSPLY DeTrey GmbH, D-78467 Konstanz, Lot 0410001362).

The sealer was mixed according to the instructions of the manufacturer and a small quantity was deposited into the canal with the help of a **lentulo** carrier activated at

slow speed. The small quantity of sealer was deposited at the apical area of the canal with a few light pumping motions of the activated **lentulo**, but keeping the spiral tip of the instrument 1-2 mm short of the working length, away from the apical rest.

The sealer was then carried with the tip of a file No.15 with a few light, pumping movements through the apical portion of the canal, beyond the apical rest down to the portal of exit, to cover the full extent of the canal length. The placement of the sealer paste in the canal was then recapitulated with the lentulo carrier in order to ensure its placement in the apical area, and replace the sealer possibly removed by the No.15 file used earlier. A calibrated GP point (Lot No 844205, MDS, Cape Town), of same size as the last file used for the canal preparation, was selected. Its tip was loaded with a small quantity of sealer and then inserted into the canal to contact the apical rest. The lateral condensation was performed with a finger spreader (DENTSPLY Maillefer, Flat tip tapered finger spreader, Lot No A 0182 0025 000D), followed by the insertion of secondary GP points (DENTSPLY Maillefer, Lot No F-78180, Montigny) after having their tips coated in sealer, until a good compaction of the sealing materials was achieved.

The GP points extruding coronally from the canal were sectioned inside the pulpal cavity with a hot ball burnisher. The remaining warm, sectioned GP points, adjacent to the canal were then compacted against the canal orifice and the pulpal floor, with the cold ball burnisher.

From this stage onwards the roots of the two groups were treated in a similar way, undergoing coronal temporary filling and retrograde filling with MTA®.

In order to preserve the anatomo-histological characteristics of dentine similar to a functional tooth, the root surfaces were kept moist with saline solution, throughout the entire procedure. This was done by wrapping the roots in an impregnated/wet gauze, by irrigation or by immersion in such a solution. The roots were very lightly re-polished for cleaning purposes. The coronal access cavities were cleaned from the

sealer excess with sponge pellets and then filled with a temporary filling material, namely, zinc phosphate cement.

2.1.5.2 The Root-end Resection

The resection of the apical end of the roots was performed 3 mm from the apex, with a cylindric diamond bur, 1mm in diameter, at high speed under copious saline irrigation. A diamond bur with coarse grit was used in order to have increased cutting efficiency and reduce the friction with the root structure. The sectioning was performed with a feather-light pressure of the bur on the root under continuous irrigation. The sectioning was performed perpendicularly on the long axis of the root. Once the apical end was removed, the sectioned surface of the root was finished with a carbide bur, also under irrigation, in order to remove irregularities and plane it.

2.1.5.3 The preparation of the root-end cavity

The root-end cavities were prepared with the help of a round carbide bur at slow speed, under continuous irrigation with saline solution. The preparations were directed along the root canal to a depth of 3 millimetres from the sectioned surface. A minute quantity of flowable light-curing composite resin material was placed onto and around the carbide bur at a distance of 3mm from its tip and then cured. This composite resin stopper/marker created on the active part of the bur acted as a guide, indicating the depth to which the activated bur had to be inserted into the root-end canal, in order to obtain a constant depth of the root-end cavities for all the roots. The prepared cavities were rinsed with saline solution dispensed under pressure from a syringe fitted with a needle. The well-irrigated root-end cavities were then dried with paper points and air blown through a needle from a syringe.

2.1.5.4 The placement of the retrograde filling

The preparation of the MTA® (DENTSPLY Tulsa Dental, Tulsa, Ok; Lot 05002015) was made by mixing MTA® powder with the distilled water supplied by the manufacturer on a sterile glass slab. The powder and the water were incorporated to each other in small increments, in a proportion of 3:1, until a creamy consistency was reached. The obtained MTA®creamy mixture was then used, immediately following its preparation, to fill the root-end cavities. This was inserted into the root end cavities with the help of a ball burnisher. The excess MTA® extruding from the cavity was removed, bringing the filling material to the same level with the cavity margins. Individual care and attention was given to each and every filling. If required the MTA® mixture was rehydrated by adding minute quantities of water and remixing it. However, the MTA® was not handled after (subsequently) 4-5 minutes from the start of its initial mixing. After this period of time the MTA® mixture was discarded and a new quantity prepared. Once the root-end filling was completed, the tooth was placed in a container at 100% humidity. The setting time of the MTA® mixture is 4-5 hours. The teeth were therefore left in the storage container overnight to allow for effective setting of the material. The next morning the root surfaces were varnished.

2.1.5.5 The sealing of the root surface

The sealing of the root surfaces was done by covering it with a double layer of nail varnish (Rimmel 60 seconds nail polish 541 Highland, Reference No 545 (Made in Spain)). The purpose of this was to cover and obliterate any lateral canals that might open on the root surface. Such lateral canals could constitute a communication path for the dye solution from the root surface towards the MTA®-cavity wall interface, at the root-end cavity level. The teeth were washed with saline solution and then dried thoroughly. The entire coronal part and lateral surfaces of the roots were covered with a first layer of varnish, leaving the root-end section surface entirely unvarnished. The varnishing also covered the temporary coronal filling, but on the lateral surface

of the root it extended strictly to the limit/margins of the sectioned surface, without transgressing onto the sectioned surface. Once the first coat dried, a second layer of varnish was applied in a similar manner as the first layer. The nail varnish used was a quick drying varnish (10 seconds). Two different colours were used for the two separate layers. This allowed an easier and better assessment of the covering of the first coat by the second coat.

2.1.5.6 The Staining Procedure

The prepared teeth were immersed fully in methylene blue 5% solution for 24 hours, at a constant temperature of 37^{o}C (similar to the homeostatic intraosseous temperature), inside a thermo-regulated bath. The methylene blue 5% solution was continuously agitated mechanically for even distribution of the dye and the temperature. At the end of the staining cycle the roots were removed from the dying bath, copiously rinsed with water and then dried.

2.1.5.7 The Preparation of the Roots for Microscopy

The coats of nail varnish were removed, by scraping it off the lateral surfaces of the root with a scaler. During the varnish removal, care was taken not to damage the root-end section surface. The longitudinal cutting of the apical area of the roots in order to create optimal section surfaces for microscopic examination could have been impaired by the different angulations of the roots of multi-rooted teeth. Thus, the roots of the multi-rooted teeth were separated by sectioning their crowns longitudinally, in continuation to the furcation depressions, to facilitate the optimal sectioning for microscopy.

The cleaned roots were then embedded in resin in order to prevent damage to the specimens during the sectioning of the roots in preparation for microscopy. The translucent resin used for the embedding of the roots was Fobroglass® (Foukes Bros.Ltd, Cape Town), a chemically cured resin. The resin blocks obtained were then

trimmed to a smaller size, to fit the retainer latch of the microtome (Minitom, Strauers, Switzerland). Care was taken to orientate the side walls of the reduced blocks parallel to the long axis of the root in order to facilitate the longitudinal sectioning of the roots. The reduced resin block was then immobilized in the latch of the microtome, positioned so that, in a horizontal plane, the longitudinal axis of the root-end cavity aligned parallel to the blade surface. With the stopper loosened, the micrometer was then rotated in order to move the arm holding the resin block horizontally to the left or to the right until, in a vertical plane, the centre of the round opening of the root-end cavity on the root-end section surface, fell precisely above the edge of the microtome blade. Once the cutting edge of the blade became aligned exactly with the middle of the root-end cavity and the underlying root canal, the microtome arm was blocked in this position by tightening the micrometer stopper and thus locking the micrometer. The starting position indicated by the micrometer was noted down. The microtome was fitted with a diamond blade with a thickness of 350 microns (Strauers, Switzerland). The blade was now activated to a slow rotation of 300 r/min, under continuous water-cooling. The odonto-tome was allowed to move slowly down allowing the resin block to lean lightly on the water-cooled, slowly rotating diamond blade edge, allowing the gravity to bring the resin block in a free-fall downwards into the rotating blade, at a very light pressure. Following the first central sectioning of the root/block, the sectioned surface of that part of the resin block that remained fixated to the microtome arm, was inspected visually to see if it exposed the interface of the longitudinal aspect of the root-end cavity walls and the MTA® filler adequately, as well as the contiguous root canal. Once it was determined that the first sectioned surface exposed the necessary internal root details satisfactorily, the micrometer stopper was loosened and the arm holding the resin block was advanced horizontally 700 microns, by rotating the micrometer correspondingly. The next sectioning was made at 700 microns. The microtome arm holding the resin block was blocked again, in this new position, by locking the micrometer with a tightening movement of the stopper. A new section was now cut. Considering that the microtome blade itself has a thickness of 350 microns, it means that a section slice obtained, will have a thickness of 350 microns. This sectioned

specimen is the one to be placed on a microscopy glass and examined under a microscope. Before its placement on the microscopy glass, the specimen slice was rubbed extremely gently against a flat ultra fine glass-paper covered with water and then rinsed under water, in order to remove the eventual smear layer formed during the sawing process.

2.1.5.8 The Microscopy Methodology

Each specimen was examined under a stereomicroscope (Wild/M5-81911, Heebrugg, Switzerland). The illumination of the specimen was ensured by a bi-directional complementary incandescent light source (part of the microscope complex), directed towards the microscopy field. The dye penetration was measured in quarters of a millimetre, from 0.0 mm to 3.0 mm. The image of an endodontic ruler, with divisions to the level of quarters of a millimetre, printed on a transparency, was used for the measurements. The prepared section was placed in the examination field of the microscope. The optical complex of the microscope was then moved vertically towards and from the specimen surface until optimal clarity of the microscopic image was achieved. The endodontic ruler transparency was then superimposed on top of the specimen surface in the long axis of the root-end cavity, with the 0.0 mm mark at the root-end section surface, at the very entrance into the root-end cavity and the 3.0 mm mark at the bottom of the root-end cavity. The printed surface of the transparency was always placed down towards, and in contact with the examined specimen surface, in order to avoid the double imaging of the printed markings due to their reflection on the non-printed surface of the transparency. The examiner visually appreciated to what extent the blue dye penetrated the interface of the lateral cavity wall and the MTA® filler. It was then determined to which quarter millimetre mark, on the superimposed endodontic ruler transparency, the end of the blue die penetration corresponded. This level was read on the superimposed ruler starting from its 0.0 mark at the entrance into the cavity and then noted down as the level up to which the die penetrated. This measurement was made on both left (mesial) and right (distal) aspects of the cavity section; each level was appreciated, marked and

recorded separately. All the specimens were examined under two successive magnifications (10x25; 10x50) in order to reduce error possibility during the verification of the extent of dye penetration.

Each root-end cavity was positioned in the central area of the microscopic field, at the point of maximum focal illumination, with the vertical axis of the cavity oriented perpendicularly onto the microscopic field, in order to create similar examination conditions for all the specimens. The intensity of the light was adjusted to a level that gave the highest contrast between the darker blue dye and the light surrounding the dentine and MTA®, in order to offer optimal conditions for the visual assessment of the microleakage.

Chapter Three

Research Findings and Analysis

3.1 Introduction

Drs Marian and Saayman measured the leakage depth in the treated roots on the mesial and distal sides, for the groups sealed with GP and without any GP or sealant. From these two raw measurements two new dependent measurements were established namely, the average and maximum of the mesial and distal measurements. Before studying these four measurements, the differences between the two readers had to be investigated to utilise the benefit of having two independent readers. After analysing the differences of measurement of the two readers the relevant dependent measurements were combined to create a set of four multivariate measurements for each root. The group sealed with GP contained 49 roots and the group without any GP or sealant 46 roots.

The four measurements were studied in the following order: Firstly, Mesial and Distal, then Deepest and Average. The Mesial and Distal leakage depths were related because they belonged to the same root and the correlation and regression structure thereof would be studied after these two measurements were combined. The primary Mesial and Distal measurements were used to determine the Maximum or the so-called Deepest leakage depth of each root. These two primary measurements were also used in a simpler mathematical function to calculate the Average (or Mean) leakage depth. For the preceding reasons the primary measurements would be discussed before the investigation of the Maximum and Average leakage depths.

3.2 Comparing the measurements of the two readers for the Not Sealed group

When comparing two readers it is necessary to study possible **differences** between their determinations. In this case they determined the depth of penetration of the staining which indicated the extent of leakage separately on the mesial and distal sides of the roots. The distribution of these **differences** was investigated by means of Stem-and-Leaf Diagrams.

Difference of the Mesial Leakage Depth as measured by the two Readers		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50	00	2
-1.25		
-1.00		
-0.75		
-0.50	0	1
-0.25	00000 00	7
0.00	00000 00000 00000 00000 00000 0000	29
0.25	00000	5
0.50	0	1
0.75		
1.00		
1.25		
1.50		
1.75	0	1
2.00		
		46

Figure 3.1
Stem-and-Leaf Diagram of the **Differences** in the Mesial Depth of Leakage for the **Not Sealed Group**, as measured by Drs Marian and Saayman

In the above figure most of the observations were zero, see the twenty-nine (29) differences that were equal to zero implying that there was no difference between the readings (they were identical) of the two observers, this was more than half of the 46 repeat observations. These 29 identical measurements indicated that there was a

strong correspondence between the two readers. The observation at '1.75' showed that the two readings were 1.75mm apart and that the depth measurement as taken by Dr Marian was 1.75mm larger than that of Dr Saayman. Due to the structure of this statistical difference distribution it was unlikely for the two readings to be so far apart. Furthermore, there were two differences at '-1.50mm' that indicated that, in this case, the measurements taken by Dr Marian were 1.50mm smaller than that of Dr Saayman. As stated above 29 of these pairs of repeat observations were identical, for ten observations Dr Saayman gave a larger depth reading and in seven cases Dr Marian gave a larger reading than Dr Saayman. Three of these differences were excessive (-1.50, -1.50 and 1.75), which represented 6.5% of the 46 pairs of observations.

The stem-and-leaf diagram below displays the distribution of the Difference of the Distal Leakage Depth as measured by the two readers. There was a heavy concentration of observations (differences) in the vicinity of 'zero'

Difference of the Distal Leakage Depth as measured by the two readers		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50	0	1
-1.25	0	1
-1.00		
-0.75		
-0.50		
-0.25	000	3
0.00	00000 00000 00000 00000 00000 0000	29
0.25	00000 0000	9
0.50	00	2
0.75		
1.00		
1.25		
1.50		
1.75	0	1
2.00		
		46

Figure 3.2
Stem-and-Leaf Diagram of the **Differences** in the Distal Depth of Leakage for the **Not Sealed Group**, as measured by Drs Marian and Saayman

The observations in the vicinity of 'zero' were of little concern because one cannot expect the two readers to hardly have any differences. However, the three substantial differences mentioned with respect to Figure 3.1 were still visible in Figure 3.2 and originated from the same reader pairs as before.

Difference between the Maximum of the Mesial and Distal Leakage Depths		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50	0	1
-1.25	0	1
-1.00		
-0.75		
-0.50		
-0.25	00000	5
0.00	00000 00000 00000 00000 00000	25
0.25	00000 00000 00	12
0.50	0	1
0.75		
1.00		
1.25		
1.50		
1.75	0	1
2.00		
		46

Figure 3.3
Stem-and-Leaf Diagram of the Maximum of the Mesial and Distal Depth of Leakage for the **Not Sealed Group**, as measured by Drs Marian and Saayman

As mentioned before there was a heavy concentration of differences around ‘zero’ and three unusual differences away from ‘zero’ were present. The next stem-and-leaf diagram supplied the distribution of the Differences between the Averages of Mesial and Distal leakage depths (with respect to the readers).

Difference between the Averages of the Mesial and Distal Leakage Depths		
Stem	***Leaves***	**Frequency**
-2.000		
-1.875		
-1.750		
-1.625		
-1.500	0	1
-1.375	0	1
Scale		
Break		
-0.750		
-0.625		
-0.500		
-0.375	0	1
-0.250	0	1
-0.125	00000	5
0.000	00000 00000 00000 00000 00000	25
0.125	00000 0	6
0.250	0000	4
0.375		
0.500	0	1
0.625		
0.750		
Scale		
Break		
1.375		
1.500		
1.625		
1.750	0	1
1.825		
2.000		
		46

Figure 3.4
Stem-and-Leaf Diagram of the Average of the Mesial and Distal Depth of Leakage for the **Not Sealed Group**, as measured by Drs Marian and Saayman

From the four figures above it was clear that there was a high number of observations for which the differences were equal to zero. Due to this deviation from normality (Gaussian distribution) it was decided not to apply the Paired t-Test. Furthermore, these figures showed a strong measure of central tendency around zero. However, the negative tail contained two extreme observations, which was due to the exchange of the numbering of two roots. A less extreme outlier occurred in the positive tail. All

three of these outliers were so unlikely, taking the shape of the distribution into account, that it was decided to omit these measurements by the two readers from the data set.

The scale (or precision) on which Drs Marian and Saayman have made their measurements (0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00) influenced the distribution of all four measurements. The precision of the measurements of both readers influenced the respective **differences** as well. This was well displayed in the four corresponding stem-and-leaf diagrams above.

Table 3.1
Summary of Differences between the measurements of the Depth of Leakage for the **Not Sealed Group**, by Drs Marian and Saayman

	Mesial Leakage Depth	Distal Leakage Depth	Maximum of Mesial and Distal Leakage	Average of Mesial and Distal Leakage
Number of Cases where the Measurement by Dr Marian was Smaller	5	10	7	9
Number of Cases where there was No Difference between the Measurements by Drs Marian and Saayman	29	29	25	25
Number of Cases where the Measurement by Dr Marian was Larger	12	7	14	12
Total Number of Specimens	46	46	46	46

To compare the differences between the two readers, eg the row where Dr Marian made a smaller measurement compared to that of Dr Saayman and the row where Dr Marian's depth measure was larger than that of Dr Saayman, the counts within these two rows must be statistically similar. The Exact McNemar Test was used to compare these two sets of counts. The outcome of these tests was that none of these

pairs were significantly different. After the three observations (comparisons) of specific roots were omitted from the data set of Not Sealed Group the differences were tabulated for each measure such as mesial depth, distal depth, deepest depth and the average depth.

Table 3.2
Not Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Mesial side of each root as measured by the two readers, as well as the Average Difference between the Leakage Depth on the Mesial side of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	Total
Yes	Count of Root#	24	3	27
	Average of Differences	-0.031	-0.250	-0.056
No	Count of Root#	4	12	16
	Average of Differences	0.250	0.000	0.063
Count of Root#		28	15	43
Average of Differences		0.009	-0.050	-0.012

The margins of the summary measures of Dr Marian were contained in the last row and he had 15 roots with no leakage, whereas the margins of the summary measures of Dr Saayman were contained in the final column and she had 16 roots with no leakage. The 'No – No' cell contains 12 observations (differences) that were equal to 'zero'. The 'Yes – Yes' cell contains 24 observations that were not equal to 'zero'. The second line of this cell contains the average of those 24 differences and it was equal to –0.031. The two off-diagonal cells of the two-by-two table above contained three and four differences respectively. In these cells the one reader stated that there was no leakage and the other that there was some leakage, and *vice versa*.

Table 3.3
Not Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Distal side of each root as measured by the two readers, as well as the Average Difference of the Leakage Depth of the Distal side of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	25	1	26
	Average of Differences	0.050	-0.250	0.038
No	Count of Root#	6	11	17
	Average of Differences	0.250	0.000	0.088
Count of Root#		31	12	43
Average of Differences		0.089	-0.021	0.058

The explanation of the above two-by-two table was similar compared to Table 3.2 and the frequencies of the off-diagonal cells were equal to one and six. These counts were not sufficiently different to say that there was any bias present in that the one reader indicated more 'zeros' than the other ($p>0.10$).

Table 3.4
Not Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Deepest Leakage side of each root as measured by the two readers, as well as the Average Difference between the Deepest Leakage Depth of the two sides of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	28	2	30
	Average of Differences	0.036	-0.250	0.017
No	Count of Root#	7	6	13
	Average of Differences	0.250	0.000	0.135
Count of Root#		35	8	43
Average of Differences		0.079	-0.063	0.052

In the above two-by-two table the frequencies of the off-diagonal cells were equal to two and seven. Even these counts could not warrant significant bias in that the one reader indicated more 'no leakages' than the other ($p>0.10$).

Table 3.5
Not Sealed Group - Frequency table of Observations classified by Any or No Leakage on both sides of each root as measured by the two readers, as well as the Average Difference of the Average Depth of the two sides of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	28	2	30
	Average of Differences	0.000	-0.125	-0.008
No	Count of Root#	7	6	13
	Average of Differences	0.179	0.000	0.096
Count of Root#		35	8	43
Average of Differences		0.036	-0.031	0.023

The above table did not show any differences between the two readers, as was the case with the preceding three tables.

3.3 Comparing the measurements of the two readers for the Sealed Group

The measurements of the two readers were compared in the same way as in paragraph 3.2 which contains the comparison for the Not Sealed group.

Difference of the Mesial Leakage Depth as measured by the two Readers		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50		
-1.25		
-1.00		
-0.75		
-0.50		
-0.25	0000	4
0.00	00000 00000 00000 00000 00000 00000 00000 0000	39
0.25	00000	5
0.50		
0.75		
1.00		
1.25	0	1
1.50		
1.75		
2.00		
		49

Figure 3.5
Stem-and-Leaf Diagram of the **Differences** in the Mesial Depth of Leakage for the **Sealed Group**, as measured by Drs Marian and Saayman

A strong central tendency around ‘zero’ was present in the stem-and-leaf diagram above and one unusual difference (equal to 1.25), which may indicate a mistake made by one of the readers.

Difference of the Distal Leakage Depth as measured by the two Readers		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50		
-1.25		
-1.00		
-0.75		
-0.50		
-0.25	0000	4
0.00	00000 00000 00000 00000 00000 00000 000	33
0.25	00000 00000 0	11
0.50		
0.75		
1.00		
1.25		
1.50	0	1
1.75		
2.00		
		49

Figure 3.6
Stem-and-Leaf Diagram of the **Differences** in the Distal Depth of Leakage for the **Sealed Group**, as measured by Drs Marian and Saayman

The appearance of Figure 3.6 was similar to that of Figure 3.5.

Difference between the Maximum of the Mesial and Distal Leakage Depths		
Stem	***Leaves***	**Frequency**
-2.00		
-1.75		
-1.50		
-1.25		
-1.00		
-0.75		
-0.50		4
-0.25	0000	36
0.00	00000 00000 00000 00000 00000 00000 00000 0	8
0.25	00000 000	
0.50		
0.75		
1.00		
1.25		
1.50	0	1
1.75		
2.00		
		49

Figure 3.7
Stem-and-Leaf Diagram of the Maximum of the Mesial and Distal Depth of Leakage for the **Sealed Group**, as measured by Drs Marian and Saayman

The appearance of Figure 3.7 was similar to that of Figures 3.5 and 3.6.

Difference between the Average of the Mesial and Distal Leakage Depths		
Stem	***Leaves***	**Frequency**
-2.000		
-1.875		
-1.750		
-1.625		
-1.500		
-1.375		
Scale		
Break		
-0.750		
-0.625		
-0.500		
-0.375		
-0.250	0	1
-0.125	00000 0	6
0.000	00000 00000 00000 00000 00000 000	28
0.125	00000 00000	10
0.250	000	3
0.375		
0.500		
0.625		
0.750		
Scale		
Break		
1.375	0	1
1.500		
1.625		
1.750		
1.825		
2.000		
		49

Figure 3.8
Stem-and-Leaf Diagram of the Average of the Mesial and Distal Depth of Leakage for the **Sealed Group**, as measured by Drs Marian and Saayman

As before, the four figures above had a high number of observations for which the difference where equal to 'zero'. Due to this deviation from normality it was decided not to apply the Paired t-Test. For the four figures directly above there was only one unusual value present in the positive side of the stem-and-leaf diagram. It was

unlikely that this difference was part of the usual measurements of the two readers and therefore, it was omitted.

Table 3.6
Summary of Differences between the measurements of the Depth of Leakage for the **Sealed Group**, by Drs Marian and Saayman

	Mesial Leakage Depth	**Distal Leakage Depth**	**Maximum of Mesial and Distal Leakage**	**Average of Mesial and Distal Leakage**
Number of Cases where the Measurement by Dr Marian was Smaller	4	4	7	4
Number of Cases where there was No Difference between the Measurements by Drs Marian and Saayman	39	33	28	36
Number of Cases where the Measurement by Dr Marian was Larger	6	12	14	9
Total Number of Specimens	49	49	49	49

For the table above the Exact McNemar Test was used to compare the top and bottom rows of counts. The outcome of these tests was that the pairs of column: Mesial Leakage Depth, Maximum of Mesial and Distal Leakage and Average of Mesial and Distal Leakage were not significantly different. However, the two readers differed slightly on the measurement: Distal Leakage Depth ($p<10\%$).

Table 3.7
Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Mesial side of each root as measured by the two readers, as well as the Average Difference between the Leakage Depth on the Mesial side of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	Total
Yes	Count of Root#	25	2	27
	Average of Differences	-0.010	-0.250	-0.028
No	Count of Root#	4	17	21
	Average of Differences	0.250	0.000	0.048
Count of Root#		29	19	48
Average of Differences		0.009	0.026	-0.026

Nothing unusual was observed in Table 3.7, as was the case for the remaining three tables.

Table 3.8
Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Distal side of each root as measured by the two readers, as well as the Average Difference of the Leakage Depth of the Distal side of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	29	2	31
	Average of Differences	0.034	-0.250	0.016
No	Count of Root#	5	12	17
	Average of Differences	0.250	0.000	0.074
Count of Root#		34	14	48
Average of Differences		0.089	0.066	-0.036

Table 3.9

Sealed Group - Frequency table of Observations classified by Any or No Leakage on the Deepest Leakage side of each root as measured by the two readers, as well as the Average Difference between the Deepest Leakage Depth of the two sides of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	32	2	34
	Average of Differences	0.016	-0.250	0.000
No	Count of Root#	4	10	14
	Average of Differences	0.250	0.000	0.071
Count of Root#		36	12	48
Average of Differences		0.079	0.042	-0.042

Table 3.10

Sealed Group - Frequency table of Observations classified by Any or No Leakage on both sides of each root as measured by the two readers, as well as the Average Difference of the Average Depth of the two sides of the roots in the four groups

		Leakage as indicated by Dr Marian		
Leakage as indicated by Dr Saayman		Yes	No	All
Yes	Count of Root#	32	2	34
	Average of Differences	0.016	-0.125	0.007
No	Count of Root#	4	10	14
	Average of Differences	0.188	0.000	0.054
Count of Root#		36	12	48
Average of Differences		0.036	0.035	-0.021

In the Not Sealed group the measurements on the roots with numbers 25, 47 and 24 were removed from the data set. The table overleaf compares the measures taken by Dr Marian on the Mesial and Distal sides after the information on the three roots was removed.

Table 3.11
Not Sealed Group - Frequency table of the Presence of Leakage on the Mesial and Distal sides of each root as measured by Dr Marian

	Mesial Leakage as indicated by Dr Marian		
Distal Leakage as indicated by Dr Marian	Yes	No	Total
Yes	24	7	31
No	4	8	12
Total	28	15	43

The interpretation of the entries in the diagonal cells was as follows: Twenty-four roots had leakages on both sides (Mesial and Distal); eight of the 43 roots displayed no leakage on either side, according to Dr Marian. For seven of the roots there were leakages on the Distal side and no leakages on the Mesial side. For four roots he identified no leakage on the Distal side and some leakage on the Mesial side.

The symmetry of this table indicated that there was no bias (to leak more on a certain side) with respect to one of the two sides (mesial and distal). The agreement between the two sides was 74% (32/43).

Table 3.12
Not Sealed Group – Frequency table of the Presence of Leakage on the Mesial and Distal sides of each root as measured by Dr Saayman

	Mesial Leakage as indicated by Dr Saayman		
Distal Leakage as indicated by Dr Saayman	Yes	No	Total
Yes	23	3	26
No	4	13	17
Total	27	16	43

For the table above the discordance with respect to the identification of leakage between the two sides was insignificant. For this table a strong correspondence between the Mesial and Distal sides existed in that the counts on the diagonal was

equal to 23 plus 13 (36). The agreement between the two sides was 84% (36/43). Therefore, a difference with respect to the agreement between the Mesial and Distal sides existed between the two readers. The next set of tables compare the presence of leakage on the mesial and distal sides for the Sealed group.

Table 3.13
Sealed Group - Frequency table of the Presence of Leakage on the Mesial and Distal sides of each root as measured by Dr Marian

	Mesial Leakage as indicated by Dr Marian		
Distal Leakage as indicated by Dr Marian	Yes	No	Total
Yes	27	7	34
No	2	12	14
Total	29	19	48

The agreement with respect to leakage for the table above was equal to 81% (39/48). No bias occurred with reference to the leakage of the two sides (p>0.10).

Table 3.14
Sealed Group - Frequency table of the Presence of Leakage on the Mesial and Distal sides of each root as measured by Dr Saayman

	Mesial Leakage as indicated by Dr Saayman		
Distal Leakage as indicated by Dr Saayman	**Yes**	**No**	**Total**
Yes	24	7	31
No	3	14	17
Total	27	21	48

The identification of leakage agreement for the table above was equal to 79% (38/48). For the measurements of Dr Saayman no bias occurred with reference to the leakage of the two sides (p>0.10).

3.4 The Comparison of the two groups after the readings of the two observers were *combined*

The depth readings were combined after the information of three roots in the Not Sealed group and a single root of the Sealed group were removed.

Average Mesial Depth					
	Not Sealed			**Sealed**	
Stem	**Leaves**	**Frequency**	**Stem**	**Leaves**	**Frequency**
0.0	00000 00000 00	12	0.0	00000 00000 00000 00	17
0.1	33333 33	7	0.1	33333 3	6
0.2	55555 55555	10	0.2	55555 55555 55	12
0.3	88	2	0.3	8	1
0.4			0.4		
0.5	00	2	0.5	00000	5
0.6	3	1	0.6	3	1
0.7	555	3	0.7	5	1
0.8	8	1	0.8		
0.9			0.9		
1.0	0	1	1.0	00	2
Hi	1.75; 2.63; 3.00; 3.00	4	Hi	1.13; 2.75; 3.00	3
		43			**48**

Figure 3.9
Stem-and-Leaf Diagram of the Mesial Leakage Depth comparing the **Not Sealed Group** to the **Sealed Group**

From the above Stem-and-Leaf Diagram it was evident that for the Not Sealed group there were four unusually deep leakages present (listed under Hi). For the Sealed group there were three uncommonly deep leakages present (listed under Hi). A further phenomenon in this diagram was a peak of observations at 'zero' (or No Leakage) and then a slight dip followed by other modes (of higher frequencies). Both empirical distributions displayed a long tail towards the larger values. The percentage of roots that had leakages of 0.25mm or more was 56% for the Not Sealed group and 52% for the Sealed group.

Table 3.15
Descriptive Statistics of the Mesial Leakage Depth within the two groups

Group	Not Sealed	Sealed
Medians	0.250	0.250
Averages	0.471	0.352
Standard Deviation	0.752	0.603

From the above table it could be observed that the two calculated Medians were equal and the Average of the Not Sealed group was slightly larger than that of the Sealed group. The locality (medians) of the two distributions was compared by means of the Wilcoxon Rank Test and it was found that there was no difference between the two groups ($p>0.10$).

Average Distal Depth					
	Not Sealed			**Sealed**	
Stem	**Leaves**	**Frequency**	**Stem**	**Leaves**	**Frequency**
0.0	00000 00000 0	11	0.0	00000 00000 00	12
0.1	33333 33	7	0.1	33333 33	7
0.2	55555 555	8	0.2	55555 55555 55555 5	16
0.3	88	2	0.3	88888	5
0.4			0.4		
0.5	00000 00	7	0.5	00	2
0.6	3	1	0.6		
0.7			0.7		
0.8			0.8	888	3
0.9			0.9		
1.0	000	3	1.0	00	2
Hi	1.88; 2.38; 2.75; 3.00	4	Hi	2.75	1
		43			48

Figure 3.10
Stem-and-Leaf Diagram of the Distal Leakage Depth comparing the **Not Sealed Group** to the **Sealed Group**

As was the case with the Mesial Leakage Depth multi-modality could be observed. The percentage of roots that had leakages of 0.25mm or more was 58% for the Not Sealed group and 60% for the Sealed group.

Table 3.16
Descriptive Statistics of the Distal Leakage Depth within the two groups

Group	Not Sealed	Sealed
Medians	0.250	0.250
Averages	0.483	0.315
Standard Deviation	0.719	0.447

For the Distal side the Medians were equal, but the Average Leakage Depth was slightly larger for the Not Sealed group than the Sealed group ($p>0.10$). The Standard Deviation for the Not Sealed group was larger than that of the Sealed group.

Average Deepest Leakage					
	Not Sealed			**Sealed**	
Stem	**Leaves**	**Frequency**	**Stem**	**Leaves**	**Frequency**
0.0	00000 0	6	0.0	00000 00000	10
0.1	33333 3333	9	0.1	33333 3	6
0.2	55555 55555	10	0.2	55555 55555 55555	15
0.3	888	3	0.3	8888	4
0.4			0.4		
0.5	000	3	0.5	00000	5
0.6	333	3	0.6		
0.7	55	2	0.7		
0.8	8	1	0.8	88	2
0.9			0.9		
1.0	00	2	1.0	000	3
Hi	1.88; 2.63; 3.00; 3.00	4	Hi	2.75; 2.75; 3.00	3
		43			48

Figure 3.11
Stem-and-Leaf Diagram of the Average Deepest Leakage comparing the **Not Sealed Group** to the **Sealed Group**

For the Not Sealed group the multi-modality was not so prominent as for the Sealed group. The proportion of roots with leakages more than 0.25mm was 65% for the Not Sealed group and 67% for the Sealed group.

Table 3.17
Descriptive Statistics of the Deepest Leakage Depth within the two groups

Group	Not Sealed	Sealed
Medians	0.250	0.250
Averages	0.538	0.453
Standard Deviation	0.743	0.679

Again the Medians of the two groups were equal, but the Average Leakage Depth of the Not Sealed group was slightly larger than for the Sealed group ($p>0.10$). The magnitude of the Standard Deviation was considerable with respect to the scale of measurement due to the skewness towards the larger values.

Average of the Average Leakage at the Mesial and Distal Sides					
	Not Sealed			Sealed	
Stem	Leaves	Frequency	Stem	Leaves	Frequency
0.0	00000 06666 66	12	0.0	00000 00000 6666	14
0.1	33333 333	8	0.1	33333 99999 9	11
0.2	55555 5	6	0.2	55555 5555	9
0.3	1188	4	0.3	1888	4
0.4	4	1	0.4	4	1
0.5	006	3	0.5	06	2
0.6	33	2	0.6		
0.7			0.7	5	1
0.8	8	1	0.8	8	1
0.9	4	1	0.9	4	1
1.0	0	1	1.0	0	1
Hi	1.81; 2.50; 2.88; 3.00	4	Hi	1.63; 1.69; 1.94	3
		43			48

Figure 3.12
Stem-and-Leaf Diagram of the Average of the Average Leakage Depth comparing the **Not Sealed Group** to the **Sealed Group**

For both groups the multi-modality was not so prominent as before. The reason for the disappearance of most of the modes was that the Average of the Mesial and Distal sides was calculated, as well as the Average of the measurement of the two readers. The calculation of the two sets of Averages created a smoother empirical distribution. The proportion of roots with leakages more than 0.25mm was 53% for the Not Sealed group and 48% for the Sealed group.

Table 3.18
Descriptive Statistics of the Average of Average Leakage Depth within the two groups

Group	Not Sealed	Sealed
Medians	0.250	0.188
Averages	0.477	0.333
Standard Deviation	0.731	0.444

The fact that the two Medians were not equal was not of great importance because the Leakage Depth observations were smoothed by the double calculation of Means ($p>0.10$). As before the Average Leakage Depth of the Not Sealed group was larger than that of the Sealed group. The Standard Deviations were similar as for the three measurements above.

In the next four tables it was investigated whether the two groups differed with respect to any leakage.

Table 3.19a
Frequency Table of **Any Leakage** for the Two Treatment Groups

Average *Mesial* Leakage Measurement	Group: Not Sealed	Group: Sealed	Total
Leaked	31	31	62
Percentage Roots Leaking	72.1%	64.6%	
Not Leaked	12	17	29
Total	43	48	91

Table 3.19b
Frequency Table of **Any Leakage** for the Two Treatment Groups

Average *Distal* Leakage Measurement	Group: Not Sealed	Group: Sealed	Total
Leaked	32	36	68
Percentage Roots Leaking	74.4%	75.0%	
Not Leaked	11	12	23
Total	43	48	91

Table 3.19c
Frequency Table of **Any Leakage** for the Two Treatment Groups

Average *Deepest* Leakage Measurement	Group: Not Sealed	Group: Sealed	Total
Leaked	37	38	75
Percentage Roots Leaking	86.0%	79.2%	
Not Leaked	6	10	16
Total	43	48	91

Table 3.19d
Frequency Table of **Any Leakage** for the Two Treatment Groups

Average of *Average* Leakage Measurement	Group: Not Sealed	Group: Sealed	Total
Leaked	37	38	75
Percentage Roots Leaking	86.0%	79.2%	
Not Leaked	6	10	16
Total	43	48	91

In the above four tables it was observed that the proportion of roots with any leakage in the Not Sealed group varied between 72% and 86%. The proportion of roots with any leakage in the Sealed group varied between 64% and 80%. However, there was more leaking within the Not Sealed group compared to the Sealed group for each measurement as could be observed in Tables 3.19a – 3.19d. These proportions of leakage were never statistically significant different ($p>0.10$) within the two groups.

Table 3.20a
Comparison of the Frequency of Leakages on the Mesial and Distal sides for the same roots, of the **Not Sealed Group**

	Mesial - Leaked?		
Distal - Leaked?	**Leaked**	**Not Leaked**	**Total**
Leaked	26	6	32
Not Leaked	5	6	11
Total	31	12	43

Table 3.20b
Comparison of the Frequency of Leakages on the Mesial and Distal sides for the same roots, of the **Sealed Group**

	Mesial - Leaked?		
Distal - Leaked?	**Leaked**	**Not Leaked**	**Total**
Leaked	29	7	36
Not Leaked	2	10	12
Total	31	17	48

From the above tables it appeared that there was no preponderance to leak on either the Mesial or Distal side of the roots, for both groups (Exact McNemar Test, $p>0.10$).

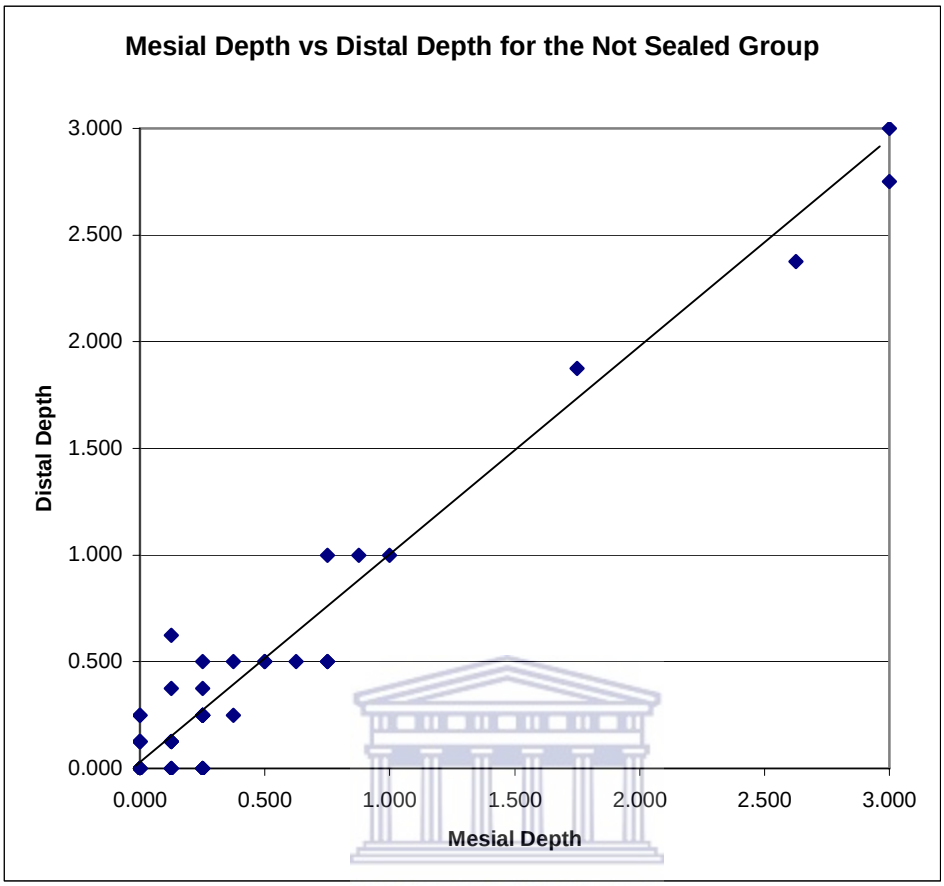

Figure 3.13
Comparison of the Leakage Depths of the Mesial and Distal sides, for the **Not Sealed Group**

Ten observations were situated in the position (0.0, 0.0), this is the set of roots where no leakage has taken place. Nine observations were located on the edges of the graph, because only one of the dimensions was without leakage. Twenty-nine roots had leakages on the Mesial and the Distal sides and was visible in the body of the graph. The Pearson Correlation between the Mesial and Distal measurements was equal to 0.975 and this was due to the strong concentration of observations near to the line of equality (where one would expect the observations to be located if there was an exactly equal leakage between the two sides). If the Pearson Correlation Coefficient is near to 'one' (its maximum), it indicates that there is a strong correspondence between the two leakage measurements, as is the case for the Not Sealed group. Ninety-five percent of the variation of the Leakage Measurement on

the Mesial side was explained by the Leakage Measurement on the Distal side, or *vice versa.* This was understandable because the two sides were so near to each other.

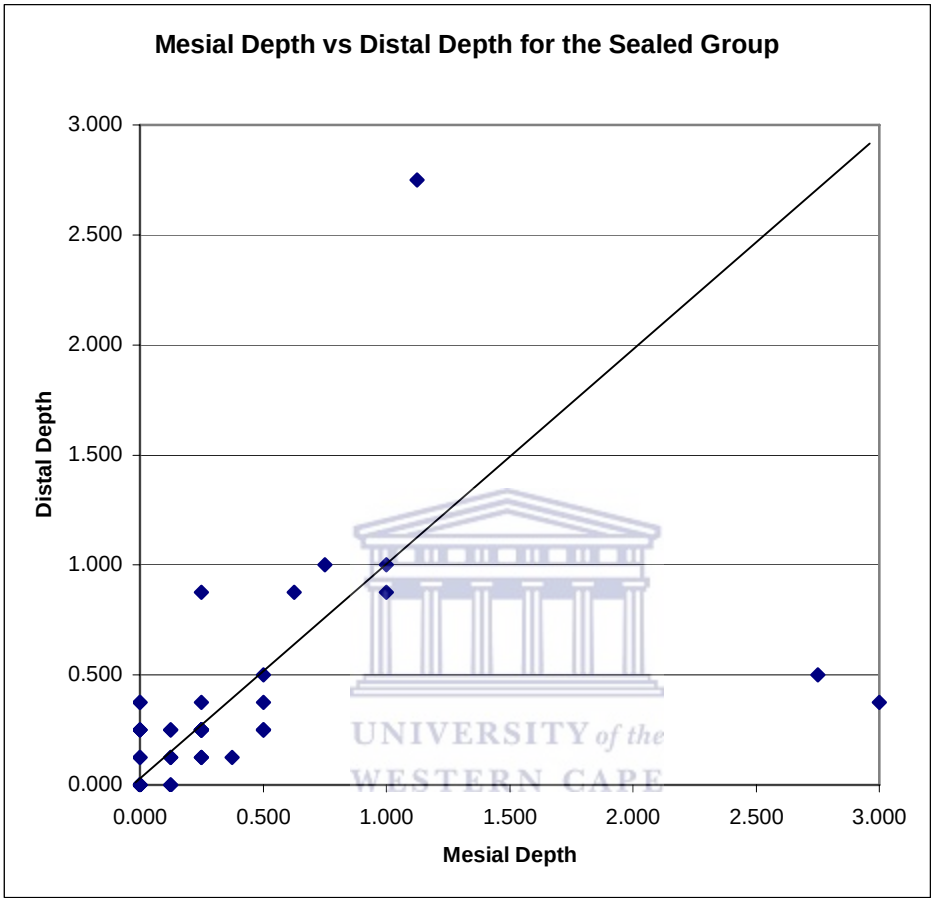

Figure 3.14
Comparison of the Leakage Depths of the Mesial and Distal sides, for the **Sealed Group**

A high number of observations were situated in the position (0.0, 0.0), this is the set of roots where no leakage has taken place (six observations). This would be invisible for the reader because all six observations were on the same position and eleven observations were located on the edges of the graph, because one of the dimensions was without leakage. Twenty-six observations were in the body of the graph due to leakages on both sides. For the Sealed group the Pearson Correlation between the Mesial and Distal measurements was equal to 0.416 and this was due to the dispersion of observations away from the line of equality (where one would expect

the observations to be located if there was an exactly equal leakage between the two sides). Especially the three data points with one leakage measurement considerably different from the other, contributed to lower the Pearson Correlation away from 'one'. For the Sealed group only 17% of the variation of the Leakage Measurement on the Mesial side was explained by the Leakage Measurement on the Distal side, or *vice versa*. Removing these three unusual observations resulted in an improved correlation coefficient of 0.784. After removing these observations, the proportion of the variation of the Leakage Measurement on the Mesial side explained by the Leakage Measurement on the Distal side (or *vice versa),* was equal to 61%. These three outliers had a detrimental effect on the Pearson Correlation, however, the presence of the outliers was only partially explained by the exchange of the numbering of two roots.

3.5 Conclusions from the Statistical Analysis of Leakage Measurements

The data collection was performed thoroughly and recorded by two dental scientists. The data (leakage measurements) was recorded to the nearest quarter of a millimetre. Although this was practical, it created data concentrations on some of the measurements, especially on the small distances. A low number of inconsistencies between these two readers occurred.

The paired measurements by the two readers were compared meticulously. For the Not Sealed group the accuracy (the difference between the readers) equal to 0.25mm was 89% on the Mesial side and 91% on the Distal side. For the Sealed Group the accuracy between '-0.25mm to +0.25mm' (as measured by differences) was 98% for both the Mesial and Distal sides. This was extraordinary for two readers to have readings so alike.

It was to be expected that the Leakage Depths on both sides to be similar, therefore, graphical and statistical comparisons were made between the Mesial and Distal sides. In the two bivariate graphs the strong correspondence between the leakages for the two sides was observed. Exceptionally high correlations (near to one) were found for both groups (the Not Sealed group showed all the way a better correspondence between the measured leakages).

The Two Treatment groups were similar with respect to the Leakage that has taken place for the two original measurements (Mesial and Distal Depth of Leakage), as well as for the two derived measurements (Deepest and Average Depth of Leakage), ($p>0.10$ for all measurements). The distributional shape of the Two Treatment groups was similar, which completed the comparison. The shape of the two compared distributions was equivalent. Therefore, the leakage for both treatments was low and not different for the Two Treatments investigated.

Chapter Four

Discussion and Conclusion

4.1 Summary of Study

Correct decisions regarding the choice of optimal pre-surgical preparations, can often make the difference between the success or failure of the subsequent surgical procedure.

The aim of this study was to determine whether the presence or absence of the root canal seal had any influence on the retrograde sealing ability of MTA®.

An *in vitro* study was devised with this objective. One hundred roots underwent mechanical preparation of the canals. Subsequently, half of the total number of the roots had the prepared canal sealed, while the other half remained with the prepared canal unsealed. This was considered to be the differentiated pre-apicectomy treatment of the root canals. Following this, all the roots were apicectomised and retro-filled with MTA®. After the full setting of the MTA® and the placement of orthograde temporary fillings, the corono-radicular units had their entire external surfaces sealed with varnish, except the root-end section surface. They were, then, merged into a bath with methylene blue for 24 hours.

Once dyed, the roots were sectioned longitudinally and assessed microscopically, with a stereomicroscope, at different magnifications. The examiners observed/assessed the interface between the lateral canal walls and the MTA® filling for the absence or presence of microleakage and the extent thereof, indicated by the degree of dye penetration in this area. It was done in order to appreciate the sealing ability of MTA® in the two different (sealed/not sealed) given conditions of the root canals. The techniques devised and implemented for the experiment were standardized and reproducible, in order to ensure the scientific character of the methodology. Two well-experienced researchers made

visual observations on the comparative end-results of the experiment. These results were statistically analysed and a valid comparison made.

The direct visual control during the endodontic, as well as during the root-end treatment procedure, allowed the researchers the opportunity to correlate the tactile sensations during the instrumentation of the canals, with direct visual observation of roots. As the number of roots was substantial (one hundred roots), this allowed the researchers to increase their understanding and proficiency in these two areas, with a unique insight that only an extensive *in vitro* study can offer.

In the roots with the canals sealed, the MTA® was placed against a continuous floor of the root-end cavity, as the root canal sealer obliterated the canal perimeter on the floor of the root-end cavity. In contrast, in the roots with the canals not sealed, the MTA® was placed against a discontinuous floor, with a void in its central area represented by the empty canal.

It was expected that a filling material compacted in a cavity against a continuous cavity floor will compress more efficiently towards the floor and then, implicitly, towards the lateral walls of the cavity. This was supposed to result in a more efficient seal at the level of the lateral cavity walls and consequently, less microleakage, in the group with sealed canals. In contrast, in the roots with not sealed canals, it was expected that when the filling material was compacted in a cavity against a discontinuous cavity floor, it would compress less efficiently towards the floor and then, implicitly, less efficiently towards the lateral walls of the cavity. This was supposed to result in a less efficient seal at the level of the lateral cavity walls and consequently, more microleakage. However, these expectations prior to the start of the project were not fulfilled by the results obtained at the end of the experiment.

Both observers recorded the depth of microleakage on the mesial and distal sides of the roots for the two treatment groups. These two measurements were combined as the Maximum (Deepest) Leakage, as well as the Average Leakage. The readings of the two researchers were evaluated to investigate whether any

bias occurred, say for instance that the one researcher provided a higher reading than the other, in most cases. No bias was detected for any of the two raw readings (Mesial and Distal Leakage Depths) and the two deducted measurements (Deepest and Average Leakage Depths). A set of four dependable measurements was obtained by averaging the original measurements made by the two researchers.

The findings following the microscopic assessment of the microleakage on both mesial and distal aspects on the MTA® filled root-end cavities were analysed statistically. The average microleakage in the group with sealed canals was 0.333mm, with the median at 0.188mm. In the group with not sealed canals the average microleakage was 0.477mm, with a median situated at 0.250mm. The good sealing ability of the MTA®, in cavities with continuous floor due to the sealed canal, was expected. But, the good sealing ability of the MTA® in the cavities with a discontinuous floor came as a surprise. Although the average microleakage and the median determination, in the group with sealed roots, was lower than those determined in the group with not sealed canals, the difference between the two groups was extremely small (less than a quarter of a millimetre). This difference was statistically not significant ($p>0.10$).

The MTA® showed good sealing ability in both types of root-end cavities, namely the roots with sealed canals, as well as in those with not sealed canals.
A differentiation in the interpretation of the results has to be made. The results obtained indicate that the sealing of the canal prior to the retrograde filling with MTA®is not compulsory, but this does not imply that it is unnecessary.

The sealing of the canal prior to the retrograde filling with MTA ®, still remains necessary, for reasons unrelated to the sealing ability of the retrograde filling. The support offered by the orthograde filling to the retrograde instrumentation and the placement of the retrograde MTA®, offers procedural guidance, comfort and safety, hence, making the entire retrograde procedure easier.

The literature review part of this paper reflects the effort to align the present study to other existing studies with common elements, technical details or scope.

The 3mm retrograde preparation depth used by the present study, is similar, for example, to the experiments performed by Karlovic, Pezelj-Ribaric, Miletic, et al (2005), who had a similar aim, to study the microleakage of retrograde fillings performed with MTA®. Although beyond the scope of the present work, it is interesting to note that they achieved positive results (similar to the present research) related to the sealing ability of MTA®, which was in turn related to the preparation of the root-end cavities with either the Erbium: YAG laser or ultrasound instruments.

This experiment took into consideration ideas such as that of Davis, Jeansonne, Davenport, et al (2003), who researched the effects of conditioning the root-end cavity walls with acidic solutions, such as doxycycline or citric acid, on the sealing ability of MTA®. Although there were no significant differences in the microleakage of the MTA® retrograde fillings placed by them in cavities rinsed with doxycycline, citric acid or saline, the present experiment refrained from using acidic rinsing solutions, as they tended to delay the MTA® setting time, unnecessarily extending the initial superficial "wash off" of the freshly placed material.

Work by Tang, Torabinejad and Kettering et al (2002), emphasizing the need to use a sub-bacterial dispersed phase in the solution used for the disclosure of microleakage around the MTA® root-end fillings, guided the selection of the staining solution in the present study. The good sealing ability of the MTA® used as retrograde filling demonstrated by them, is consistent with the findings of the present study, as well as with the findings of the other works mentioned above, on the subject.

It was already known, from experiments such as that of Aqrabawi J (2000), that the sealing ability of the MTA® is very good in retrograde cavities of roots that were already root canal sealed in orthograde manner, prior to retrofilling. His results are in agreement with the findings of the present study.

The closest to the scope of the present experiment, was the study of Al-Kahtani, Shostad, Schifferele and Bhambhani (2005) who studied the microleakage of the

MTA® in the presence and in the absence of the orthograde root canal sealing. The key difference from the present experiment, is that Al-Kahtani performed the MTA® fillings, also in an orthograde manner.

Unfortunately there is no similar experiment in the existing literature to compare the results with, as this was an original project. Once published, the results of this experiment will become of reference in this sense, namely the effect of the presence or absence of the intracanal seal on the sealing ability of MTA® placed in a retrograde way.

Influence from outside parties in this study was nonexistent due to the fact that no financing or other kind of support was used from manufacturers, or from any other interested commercial institution, for the execution of this study. The entire financial/material support and scientific/academic guidance originated only from the Dental Schools of University of Stellenbosch and University of the Western Cape, as governmental organizations, and from the private practice where the researcher performs his clinical activity, as an independent contribution from the private sector. Thus, the present research project is an independent study, based strictly and only on scientific and ethical principles.

The importance of the present study resides in the fact that the results obtained at the end of this study, represent evidence-based information that constitutes another aid for the clinician, in the decision-making process involved in the planning of an apicectomy.

4.2 .Clinical Significance

The findings of the present study will help the dental practitioner in situations when it is difficult to synchronize an apicectomy procedure, which has to be performed in the operating theatre, with the sealing procedure of the root canal. The difficulty arises from the availability of theatres, especially when large cysts are to be removed.

4.3 Conclusion

The presence or absence of the root canal filling did not significantly influence the sealing ability of the MTA®, when placed into root-end cavities of apicectomized roots in a retrograde fashion. In other words, the support offered by the orthograde filling of the root canal prior to the apicectomy did not significantly lessen the leakage of the MTA® root-end fillings. The sealing ability of the MTA® proved to be good in both instances. Therefore, it can be concluded that the presence or absence of the orthograde filling in the root canals had no significant effect on the retrograde sealing ability of the MTA® at the level of the root-end cavities of apicectomised dental roots during the first 24 hours.

Further studies should be done to evaluate the microleakage for longer periods of time as well as the leakage of the root between the coronal seal and the orthograde filling. In future this could give us an answer to what the maximum time lapse could be between performing an apicectomy using MTA and sealing the canal with GP from the coronal side when it is impossible to synchronize the two procedures.

Addendum

Here are a few practical technical recommendations, emanating from the experience generated by the project. Special attention has to be paid to the following:

- the proper debridement of the root-end cavity (as a dentinal smear layer could prevent the intimate contact and efficient adherence of the MTA® to the cavity walls), ensures that no rests of gutta-percha points used for the root canal seal remained attached to the cavity walls, as these may constitute access paths for future microleakage,

- good irrigation of the root-end cavity will reduce the residual microbial population inside the root-end cavity and the possibility of their later redevelopment. This is recommended in spite of the fact that it is very improbable that any microbial population will survive the low pH of the MTA® during its initial setting stage, at the moment when it is placed into the cavity,

- once the MTA® powder and water is mixed, this must be inserted immediately and rapidly into the root-end cavity, as it desiccates and sets fast in its initial setting stage, and its re-mixing may lead to altered internal structuring of the set MTA® cement, which could allow increased microleakage and its infiltration in the future,

- avoid the sequential placement of the same MTA® mixture into the root-end cavity (the sequential placement must not be confused with the

incremental placement, which is done in one stage; the sequential placement refers to the sequencing of the placement of the same quantity of mixed MTA® in two separate stages). This is caused by the failure to insert the MTA® into the cavity rapidly and in full, immediately after it was mixed; due to such a delay the superficial section of the cavity remains unfilled at the time when the MTA® already started its desiccation and initial setting; the subsequent rehydration and re-mix of this MTA® will lead to the filling of that portion of the cavity that remained unsealed in the first stage, with an MTA® poorly structured and less adherent to the cavity walls, at subsequent second stage,

- if the sequential placement of MTA® into the cavity cannot be avoided, each sequence must use a new quantity of MTA® powder in a fresh mix and not re-use the existing MTA® partially set, by rehydrating and remixing it,

- using different colours of nail polish for the different layers of varnish applied to seal the external surfaces of the roots, will help the researcher to observe whether the previous layer of varnish will be fully and continuously covered by the next coating,

- in the specimens where the determination of the microleakage level was difficult, the use of different magnifications and illumination intensities made the precise reading and determination of the level of die penetration possible.

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