An *In-Vitro* Study Assessing the Effect of Smear Layer on Root Canal Microleakage

A minithesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dental Sciences in Restorative Dentistry at the Faculty of Dentistry, University of the Western Cape

By

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Key words

Smear layer

Root canal

Microleakage

AH Plus Endodontic sealer

Summary

Coronal leakage is now recognized as an important cause of failure of root canal treatment. This pathway of leakage may be affected by the presence or absence of the smear layer that in turn may affect the close adaptation between the root canal filling material and the root canal walls. This may result in subsequent coronal leakage and failure of treatment.

Aim:

The aim of this study was to compare the sealing ability of AH Plus sealer to the canal wall in the presence and absence of the smear layer.

Methodology:

Forty five extracted teeth with fully developed apices were selected. The pulp of each tooth was removed and the root canal was instrumented using the step back technique. All the canals were prepared to a size 50 endodontic file at the working length. During instrumentation, the forty four root canals were irrigated with 3 ml of 3% sodium hypochlorite solution (NaOCl) using a 27-gauge needle after each instrument. Throughout the study, the teeth were kept moist, using sterile gauze soaked in deionized water. Prior to obturation, the forty four teeth were randomly divided into two groups of 22 teeth each identified as Group A and Group B. The two groups were irrigated in different ways to either preserve or remove the smear layer, and the remaining tooth was prepared without irrigation and served as the control for the SEM examination. Group A was irrigated with 18% Ethylene diamine tetra-acetic acid (EDTA) and 3%

sodium hypochlorite (NaOCL). Group B was irrigated with 3% NaOCL only. The canal was dried with "extra-fine" and "medium" sized paper points at the working length. AH plus sealer was mixed according to the manufacturer's instructions and the canals were filled with a sealer using the single cone gutta-percha technique. The obturated roots were stored at 37 ºC and the root apices were sealed with wax and two layers of nail varnish, except for 1mm around the opening of the canal. The roots were placed in 2% methylene blue dye. The teeth were then removed from the dye and sectioned. The roots were cross-sectioned at the coronal, middle and apical thirds so that the extent of dye penetration could be measured with a light microscope at magnification of 100 times. Two teeth from both groups A and B were selected for scanning electron microscopic examination. The roots were grooved longitudinally, they were then split into two halves by placing a blade in the groove and applying gentle pressure. Both fractured halves of each root were mounted on an aluminum stub, vacuum-dried, coated with 20 nm of gold and then examined under the scanning electron microscope (SEM) as a control to determine whether the smear layer was removed with the procedure undertaken for that tooth.

Results:

The results showed that the coronal and middle thirds of group A (NaOCL + EDTA) had the cleanest surface, while the cleaning of the coronal and middle thirds of group B (NaOCL only) was not as efficient when compared to group A. The worst cleaning occurred in the apical third in both groups. The comparative analysis of the groups in this study using a Fisher's exact test revealed no statistically significant

differences in the levels of coronal leakage of the two experimental groups (p value < 0.05).

Conclusion:

The use of EDTA and NaOCL efficiently removes the smear layer and caused clear opening of the dentinal tubules in the coronal and middle thirds, but less so in the apical third of the root canals. The use of NaOCL only did not remove the smear layer of the root canal walls as efficient in the coronal and middle thirds.

There was no statistically significant difference in coronal leakage when using AH Plus sealer in the presence or absence of the smear layer.

DECLARATION

I hereby declare that An *In-Vitro* Study Assessing the Effect of Smear Layer on Root Canal Microleakage is my own work, that it has not been submitted before for any degree or examination in any University, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Mutasim Hassan Elnour

October 2008

Signed.....

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DEDICATION

This work is dedicated to:

To my Parents and family for their constant support and love

AND TO

My supervisor whose; guidance, encouragement, help and support made this project successful.

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CHAPTER 1

INTRODUCTION

The fundamental principle of conventional root canal treatment is to rid the root canal system of bacteria and their by-products, and to prevent re-contamination of the root canal space (Kayaoglu *et al* 2005, Young, Parashos and Messer 2007). Three dimensional sealing of the root canal is one of the main goals of endodontic treatment and it is essential for preventing reinfection of the canal and for preserving the health of the periapical tissues, thereby ensuring the success of root canal treatment (De Almeida *et al* 2000, Sevimay and Kalayci 2005). Successful root canal treatment can be achieved with complete obturation of the root canal system with an impervious, biocompatible and dimensionally stable filling material (Ahlberg and Tay 1998). Complete seal of the root canal system is almost impossible with currently available materials and obturation techniques using a combination of gutta percha and root canal sealer (Gutmann 1993).

Coronal leakage is now recognized as an important cause of failure of root canal treatment (Torabinejad, Ung and Kettering 1990, Saunders and Saunders 1994). It has been shown that most leakage occurs between the wall of the root canal and the sealer (Hovland and Dumsha 1985). This path of leakage may be affected by the presence of a smear layer (Saunders and Saunders 1992). The smear layer is a layer of organic and inorganic debri created as a result of mechanical instrumentation of the root canal wall (McComb and Smith 1975, Saunders and Saunders 1994). Coronal leakage occurs at one of the interfaces: the gutta-percha-sealer interface or at the sealer-dentin

interface. The presence of the smear layer complicates the dentin-sealer interface (Saunders and Saunders 1994).

The smear layer resulting from root canal instrumentation acts as a physical barrier interfering with the adaptation and penetration of the sealer into the dentinal tubules, which might contribute to the increasing occurrence of microleakage (Oksan *et al* 1993, De Almeida *et al* 2000). Use of chemically active, adhesive root canal sealers may play an important role in minimizing apical leakage (De Almeida *et al* 2000, Sevimay and Kalayci 2005).

Micro-organisms present inside the root canals may remain active in the dentinal tubules even after vigorous chemomechanical preparation (Pommel, Jacquot and Camps 2001, Young, Parashos and Messer 2007). Thus, perfect apical sealing is desirable to prevent the remaining bacteria and their endotoxins from reaching the root apex (Pommel, Jacquot and Camps 2001). Apical leakage is considered to be a common cause of endodontic therapy failure, and is influenced by many variables such as different filling techniques, the physical and chemical properties of the sealers and the presence or absence of a smear layer (De Almeida *et al* 2000, Pommel, Jacquot and Camps 2001, Sevimay and Kalayci 2005). In the case of coronal leakage, the canal may be re-contaminated in various ways such as contact between the oral bacterial flora and the root canal tubule inlets. However, this most frequently occurs as a result of loss of the temporary filling, or an inadequate endodontic filling or deficient sealing by the crown (Carratu *et al* 2002). It is widely accepted that gutta-percha and sealer fails to achieve an effective seal that can

withstand the challenge of bacterial invasion, thus may result in root canal microleakage (Ray and Trope 1995).

Recently, AH Plus (De Trey, Dentsply, Konstanz, Germany), a sealer based on epoxy resin, was introduced commercially. According to the manufacturer, AH Plus has excellent sealing properties without the release of formaldehyde. AH Plus is generally placed in the root canal without any dentin preparation or dentin adhesive and can be used with any obturating technique (Schwartz 2006).

The purpose of this study was to investigate the effect of the smear layer on the coronal leakage in teeth root-filled with a single gutta-percha cone using AH Plus as the endodontic sealer.

CHAPTER 2

Literature review

2-1- Root canal obturation:

Apical periodontitis is inflammation of periapical tissues which usually occurs due to spread of infection following death of the pulp. Bacterial infection is well documented to be the primary cause of apical periodontitis (Young, Parashos and Messer 2007). Bacteria present may have survived the endodontic procedure, or may have been introduced into the root canal during the course of treatment, or may have appeared after subsequent re-contamination of the root canal system (Torabinejad, Ung and Kettering 1990, Byström and Sundqvist 1985, Kayaoglu *et al* 2005). Our primary aims in endodontic treatment are to prevent or cure apical periodontitis by eliminating bacteria and their by-products from the root canal and to prevent re-contamination of the root canal space. The steps involved include biomechanical preparation which involve cleaning and shaping followed by obturation of the root canal and sound coronal restoration (Ray and Trope 1995, De Almeida *et al* 2000, Sevimay and Kalayci 2005).

An ideal root canal filling should serve three functional objectives: i) to eliminate the surviving bacteria; ii) to prevent the invasion of periapical tissue fluid from reaching such bacteria, if present, in the root canal system, and iii) to prevent re-infection of the root canal space coronally (Sundqvist *et al* 1998).

Two main factors have been implicated to be causes for endodontic failure, residual infection and coronal bacterial leakage

(Torabinejad, Ung and Kettering 1990, Kayaoglu *et al* 2005). Disinfection of the root canal system is essential to control or eliminate the intracanal flora (Oksan *et al* 1993, Kayaoglu *et al* 2005). Three dimensional obturation can be achieved by a proper filling material that adapts completely to dentinal walls and reaches lateral and accessory canals (De Almeida *et al* 2000, Pommel *et al* 2003, Sevimay and Kalayci 2005).

The presence of lateral canals within the root canal system provides communication pathways where necrotic products can pass to periodontal tissues from the furcation or apex (Peters, Wesselink and Moorer 1995). Microleakage can be defined as the passage of periradicular tissue fluids, bacteria and their toxins along the interface between the root canal walls and the obturation material (Hovland and Dumsha 1985).

2-2- Root canal obturation materials:

To achieve a successful root canal treatment it is essential to completely obturate the root canal system with a material that is biocompatible and dimensionally stable (Gutmann 1993, Sevimay and Kalayci 2005). Biocompatibility is necessary because these materials will be in direct contact with periapical tissues for prolonged periods of time. A biocompatible material should not interfere with tissue repair and should stimulate tissue reorganization (Huang *et al* 2002). On the other hand, it has been reported that a complete seal of the root canal system is practically difficult with currently accepted materials and obturation techniques using a combination of gutta-percha and root canal sealer (Gutmann 1993, Hovland and Dumsha 1995, Zmener *et al* 1997). A large variety of root canal sealers are available for use in

combination with different solid or semisolid filling materials. At present, root canal sealers are based on various formulas such as expoxy resin, calcium hydroxide and zinc oxide–eugenol (Huang *et al* 2002).

2-3- Gutta-percha:

The gutta-percha polymer is a trans-1,4- polyisoprene, obtained from the coagulation of latex produced by trees of the Sapotaceae family and mainly derived from Palaquium gutta bail (Friedman *et al* 1975, Marciano, Michailesco and Abadie 1993).

The trans isomer is more linear and crystallized more easily than the cis, thus gutta-perch polymer is harder, more brittle, and less elastic than natural rubber (Friedman *et al* 1975). Gutta-percha is rigid at room temperature, becomes plasticized at 60°C and melts at 100°C. Modern gutta-percha cones are composed of organic (gutta-percha polymer and wax/resin) and inorganic components (zinc oxide and barium sulphate), small percentage of colouring agents and antioxidants could be present (Marciano, Michailesco and Abadie 1993).

2-3-1- Composition of dental gutta-percha cones used for root canal obturation:

The composition of dental gutta-percha has been shown to be approximately 18 to 22% gutta-percha polymer and 37 to 75% zinc oxide (Maniglia-Ferreira *et al 2005)*.

The particular component percentages vary according to the manufacturer. It is evident that since the cones differ in their composition, they may differ in their physical properties, thermal behavior, and even in regard to their biological effect (Tagger and Gold 1988).

Brittleness, stiffness, tensile strength, and radio-opacity have been shown to depend primarily on the proportion of gutta-percha polymer and zinc oxide (Friedman *et al* 1975). A higher zinc oxide content is associated with a lower percentage elongation, reduced ultimate tensile strength, increased brittleness, and thereby a reduced flow and rigidity (Marciano, Michailesco and Abadie 1993). The mechanical properties of gutta-percha are typical of a viscoelastic, partially-crystalline material (Friedman *et al* 1975).

2-4- The role of the root canal sealer in root canal obturation:

Root canal sealer plays an important role in root canal obturation. They have been shown to reduce microleakage and enhance the possible attainment of an impervious seal (De Almeida *et al* 2000, Pommel *et al* 2003, Sevimay and Kalayci 2005). Sealers fill the gaps between individual gutta-percha and between gutta-percha and root canal walls. They also flow to fill accessory and lateral canals. Lubrication is another important function of sealers that facilitates placement of gutta-percha (Hata *et al* 1992, Peters, Wesselink and Moorer 1995, Pommel *et al* 2003). Many sealers have the ability to adhere to dentine and can flow into dentinal tubules in the canals when the smear layer has been removed (Leonard, Gutmann and Guo 1996, Sen, Pïskïn and Baran 1996, Sevimay and Kalayci 2005). The

standard method of obturation of the root canal system is by using a core material in combination with a root canal sealer (Pommel *et al* 2003, Saleh *et al* 2003). With the numerous methods of obturation, the use of a sealer is necessary because the gutta percha does not bond spontaneously to the dentinal walls of the prepared canal (Hata *et al* 1992, Gutmann 1993). The adhesive strength both to the dentin and to the core material is considered an important factor to achieve superior sealing ability (Saleh *et al* 2003).

Both apical and coronal sealing are of equal importance to avoid re-infection and to protect the health of the periapical tissues (De Almeida *et al* 2000, Sevimay and Kalayci 2005).

2-4-1- Root canal sealers:

Many sealers have been introduced into the market and therefore the ability of the sealer to seal the root canal imperviously should be evaluated.

A superior sealer adheres and adapts strongly to the dentin and to the core material (Ahlberg and Tay 1998, Saleh *et al* 2003) and has good cohesive strength for adequate obturation (Saleh *et al* 2003). Setting time and flow ability are important factors to be considered when evaluating a sealer. A slow setting time and flowing as long as possible are desirable features (Kaplan *et al* 2003). The ability to wet the root canal wall and thus good adaptation depends on flow and surface tension of the sealer (Wennberg and Ørstavik 1990). Sealers are usually manufactured of a mixture that hardens through a chemical reaction. It has been suggested that sealers are applied in a

thin layer as they undergo shrinkage after setting, probably caused by approximation between molecules (Wennberg and Ørstavik 1990).

Endodontic sealers can be classified according to their chemical composition. They include those based on zinc oxide and eugenol, epoxy resin, calcium hydroxide and glass ionomer (De Almeida *et al* 2000, Huang et *al* 2002).

For decades, zinc oxide and eugenol sealer (e.g. Grossman's) has been the most widely used endodontic sealer, because it possess strong antimicrobal activity and of its superior physical and chemical properties (Kayaoglu *et al* 2005). Glass ionomer sealers (e.g. Ketac Endo) have the exceptional ability to adhere to dental hard tissue (Saunders and Saunders 1994, Ray and Trope 1995). AH26 is an epoxy based resin sealer that has been shown to release formaldehyde as a by product of its setting reaction. Manufactures found that formaldehyde causes a moderate cytotoxic response. However, AH Plus (De Trey, Dentsply, Konstanz, Germany) also an epoxy based resin sealer introduced later on to the market does not release formaldehyde and has excellent apical sealing (De Almeida *et al* 2000, Sevimay and Kalayci 2005).

2-4-2- AH Plus sealer:

AH Plus is regarded as a new formulation of AH 26 that does not release formaldehyde upon setting. AH 26 sealers were shown to release very small amounts of formaldehyde a result of a chemical setting reaction. However the amount of formaldehyde briefly released was thousands of times lower than the long term release seen with conventional formaldehyde-containing sealers (Pascon and Spångberg

1990). AH plus is available commercially as a paste-paste-mixing system that assures a better mixture. The manufacturers claim that AH Plus sealer has a faster setting time and better radio-opacity when compared to AH 26, and also revealed high dimensional stability, good flow behavior, easy mixing and good tissue tolerance.

2-4-3- Adhesion of AH Plus to dentin:

Root canal filling materials should adhere to the dentinal walls to eliminate any space that allows the penetration of fluids between the filling and the root canal wall (Wennberg and Ørstavik 1990). It was found that AH Plus leaked more than AH 26 (Zmener *et al* 1997) and this may be due to hydrophobic properties of epoxy resin. Several factors affect adhesion; the adherent surfaces should be clean and smooth (Eldeniz, Erdemir and Belli 2005), and surface tension of the adhesive and its ability to wet the surfaces (Saleh *et al* 2002). The different sealer types require different dentin pretreatment for optimal adhesion (Saleh *et al* 2002).

AH 26 and AH Plus are both described as epoxy-based resin sealers that are commonly placed in the canal without any dentin preparation or dentin adhesive and can be used with any obturating technique (Schwartz 2006).

In leakage studies, AH 26 and AH Plus generally performed equal to or better sealing than other sealers tested (De Almeida *et al* 2000, Sevimay and Kalayci 2005, Tay *et al* 2005). A Study done by Pommel and Camps (2001) observed no significant difference in apical leakage of system B compared with other filling techniques. In another study done comparing four different sealers of different chemical compositions the AH plus sealer did not show the best sealing (Cobankara, Adanir and Belli 2004). In general, removal of the smear layer was commonly found to be advantageous for the prevention of leakage (Sevimay and Kalayci 2005, Eldeniz, Erdemir and Belli 2005). Pommel *et al* (2003) recommended moisture control and smear layer removal when using AH 26 as a sealer to take advantage of its excellent adhesive properties.

Sevimay and Kalayci (2005) using the Scanning Electron Microscope found that removal of the smear layer allowed AH Plus sealer to adapt to the dentin and penetrated into the dentinal tubules. The adaptation and penetration of the AH Plus sealer was more prominent in the coronal and middle third of the canal than the apical third, the study also showed better apical sealing and adaptation to dentine than EndoRez sealer.

2-5- Penetration of root canal medicaments and sealers into the dentinal tubules:

Removal of the smear layer has shown better adhesion of obturation materials to the canal walls (White, Goldman and Peck 1984, Sevimay and Kalayci 2005). Chelating agents demineralizing and softening dentin also remove the smear layer from the root canal

wall and potentially allow better dentinal tubule diffusion of the root canal sealers (Wennberg and Qrstavik 1990, *Oksan et al* 2003, Sevimay and Kalayci 2005). An efficient method to remove the organic and inorganic remnants is to irrigate the root canal with Ethylene Diamine Tetra acetic Acid (EDTA) followed by Sodium hypochlorite (NaOCL) (Yamada *et al* 1983, De Almeida *et al* 2000, Sevimay and Kalayci 2005). In a different study Gencoglu, Samani and Günday (1993) assessed the penetration depth of different sealers, including Tubliseal, AH26, Sealapex, Rosin, Roth's 811, and CRCS (Calcium hydroxide Root Canal Sealer), into the dentinal tubules and found the penetration to be 10 to 80 µm deep after removal of the smear layer, whereas no penetration was observed when the smear layer was left intact.

2-6- The smear layer:

The smear layer is the layer that covers the instrumented wall of the root canal as a result of different methods of cleaning and shaping of the root canal (McComb and Smith 1975, Torabinejad *et al* 2002). The smear layer consists of ground dentin and predentin, pulpal remnants, odontoblastic processes, remnants of the irrigant and, bacteria in the case of infected teeth (McComb and Smith 1975, Sen, Wesselink and Turkun 1995). The smear layer on the surface of the canal wall is approximately 1 to 2µm in thickenss (McComb and Smith 1975, Hülsmann, Rümmelin and Schäfers 1997). The components of the smear layer can be forced into the dentinal tubules to varying

distances of up to 40µm (Cengiz, Aktener and Piskin 1990). This can happen as a result of the linear movement and the rotation of instruments and because of the capillary action generated between the dentinal tubules and the smear layer material (Cengiz, Aktener and Piskin 1990).

The clinical significance of smear layer removal remains controversial due to insufficient knowledge of its morphology, composition as well as its physical and biological properties (Clarke-Holke *et al* 2003). In a study investigating the influence of the smear layer on bacterial penetration Clarke-Holke *et al* found 60% of models used with smear layer left intact, leaked and so this study recommends smear layer removal.

Bacteria can remain in or migrate into the dentin in spite of complete chemomechanical preparation (Byström and Sundqvist 1985, O'Connell *et al* 2000). Electron microscopy has shown that the smear layer contains both organic and inorganic substances (Yamada *et al* 1983, Sen Wesselink and Turkun 1995). It seems, however, NaOCL has little outcome on the removal of inorganic components from root canal walls (Yamada *et al* 1983). Complete smear layer removal is attained only with the aid of acids and chelators (Yamada *et al* 1983, Sevimay and Kalayci 2005). Due to smaller particles of the smear layer and a large surface to mass ratio, the smear layer is highly soluble in acids. Several studies have reported that irrigation with a 17% EDTA solution has a superior cleaning outcome on the root canal walls (McComb and Smith 1975, Yamada *et al* 1983, O'Connell *et al* 2000, Sevimay and Kalayci 2005). Following smear layer removal, the root canal walls are clean and the dentinal tubules are clearly

recognizable. The tubule orifices are enlarged because of dissolution of the peritubular dentin (McComb and Smith 1975). Other authors found that the cleaning action is reduced in the direction of the apex and consequently more efficient only in the coronal- and middle-third of the root canal (O'Connell *et al* 2000, Sevimay and Kalayci 2005).

2-7- Dentinal tubules:

In the root, dentinal tubules extend from the pulp-predentin junction to the intermediate dentin just inside the cementum-dentin junction (Majör and Nordahl 1996). Dentinal tubules in the root follow a relatively straight course between the pulp and the periphery in contrast to the typical S- shaped contours of the dentinal tubules in the tooth crown (Majör and Nordahl 1996, Torabinejad *et al* 2002). They range in size from approximately 1 to 3 μm in diameter (Majör and Nordahl 1996). The density or number of the dentinal tubules per square millimeter varies from 4900 to 90,000 (Majör and Nordahl 1996). This density increases in an apical-coronal direction to the root surface and similarly in an external to internal direction from the root surface. At the cementoenamel junction, the number of dentinal tubules has been estimated to be approximately 15,000 per square millimeter (Torabinejad *et al* 2002).

Bacteria and their by-products present in infected root canals may invade the dentinal tubules and remain unaffected during treatment (Ando and Hoshino 1990, Peters *et al* 2001). An Investigator has reported the presence of bacteria in the dentinal tubules of infected teeth at approximately half the distance between

the root canal walls and the cemento-enamel junction (Ando and Hoshino 1990).

Many factors could influence the depth of penetration of bacteria into the dentinal tubules such as the number and the type of bacteria, in addition to the length of exposure and the presence or absence of a smear layer. Due to the difficulties involved in sampling the dentinal tubules, the exact microflora of infected dentinal tubules is unknown (Ørstavik and Haapasalo 1990).

2-8 Instrumentation of root canal

2-8-1 Mechanical preparation and its effects on root canal surface:

The major goals during canal preparation are debridment and cleaning of the root canals and creating radicular access to the complex root canal system for irrigation and placement of root-filling material (Yamada *et al* 1983, Yamashita *et al* 2003, Kayaoglu 2003, Sevimay and Kalayci 2005). However, the complexity of the root canal creates great difficulty for practitioners and even well mechanically prepared canals could contain areas never contacted by endodontic instruments (Evans, Speight, Gulabivala 2001). This has been investigated using high resolution computed tomography where it was found that 35% or more of the canals dentin surface was untouched (Peters *et al* 2001, Hüsbscher, Barbakow and Peters 2003). In one study it was demonstrated that anterior maxillary teeth had significant proportions of their root canal surfaces left uninstrumented, regardless of the access cavity design (Mannan, Smallwood and Gulabivala 2001).

The instrumentation of the root canal with different techniques and devices was investigated and it was found that instrumentation may remove some of the residual tissue and may produce smear layer closely adherent to the canal wall and extending into dentinal tubules (McComb and Smith 1975, Hülsmann, Rümmelin and Schäfers 1997).

Irrigation is an essential adjunct to mechanical preparation (Byström and Sundqvist 1985). It is necessary to suspend and rinse away debris created during instrumentation, to act as a lubricant for instruments, and to remove the smear layer (O'Connell *et al* 2000, Sevimay and Kalayci 2005). The flushing action of the irrigant could be incomplete, and to certain extent tags of tissue may remain bound and could be displaced coronally (Hülsmann, Rümmelin and Schäfers 1997).

If an active irrigant is not used, compacted debri will remain in uninstrumented root canals and leave a space during obturation (Peters *et al* 2001). On the other hand, the combination use of an active irrigant, like sodium hypochlorite (NaOCL) and ethylene-diamine-tetra-acetic acid (EDTA) will help to remove this compacted debris from the non-instrumented anatomy and assist its displacement by virtue of extension of the root filling material into the space that was previously occupied by the debris (Oksan *et al* 1993).

2-8-2- Irrigation of root canal surfaces:

The desirable goal of an active irrigant is to remove the residual tissue and bacterial biofilm from both instrumented and

uninstrumented parts of the root canal system (Evans, Speight and Gulabivala 2001, Peters *et al* 2001). It is evident that penetration of the irrigant or medicament will be dependent upon sufficient apical enlargement and likely canal taper (O'Connell *et al* 2000, Sevimay and Kalayci 2005), in addition to the delivery system and fluid properties of the irrigant (Evans, Speight and Gulabivala 2001, Peters, Boessler and Zehnder 2005,).

2-8-3- Effect of irrigation on instrumented surface and smear layer:

Removal of the smear layer could be achieved by chemical, ultrasonic, and laser treatments. None of these methods have been found to be totally effective (Oksan *et al* 1993, Sevimay and Kalayci 2005). An ideal root canal irrigant should be biologically compatible, have no adverse effect on remaining tooth structure, be antibacterial (Byström and Sundqvist 1985), chemically able to get rid of both organic and inorganic substrate (Yamada *et al* 1983), demonstrate good surface wetting, and be easy to use and effective within clinical limits (Huang *et al* 2001, Hülsmann, Heckendorff and Lennon 2003). The enormous researches done on smear layer removal are predominantly laboratory studies, but unfortunately are difficult to compare because of lack of standardization of methodology (Gulabivala *et al* 2005).

Removal of the smear layer can be achieved by using different concentrations of NaOCL and EDTA (Yamada *et al* 1983, O'Connell *et al* 2000, Sevimay and Kalayci 2005). These are used either as sole irrigants or in conjunction with each other (Yamada *et al* 1983, Cengiz,

Aktener and Piskin 1990, Grawehr *et al* 2003). The smear layer may have a higher organic content because of the presence of pulp tissue in the canal. The use of NaOCL causes progressive dissolution of the organic substrate, and the inorganic component may be removed by EDTA (Yamada *et al* 1983, Cameron 1988). The rotary instrumentation may pack debris into dentinal tubules thus making it more difficult to remove by irrigation, it may be necessary to irrigate with higher final volumes or to allow irrigants to remain in the root canals for longer times to ensure most favorable canal cleanliness (O'Connell *et al* 2000). With nickel-titanium instruments, the chelating gels routinely recommended for use to avoid instrument breakage, this may significantly alter the nature of the smear layer formed (Grandini, Balleri and Ferrari 2002). In a latter study, use of 'Glyde prep' in conjunction with 2.5% NaOCL resulted in a residual smear layer. The flow properties of the agents (fluid vs. gel) may be a causative factor, as the pastes tended to adhere to the grooves in endodontic files, while fluid irrigants tended to flush dentin debri away from instruments (Peters, Boessler and Zehnder 2005).

EDTA is a chelating agent used clinically in a 15-17% saturated solution. It has the ability to demineralize dentine and remove the inorganic component of the smear layer (Young, Parashos and Messer 2007). However, EDTA leaves behind the organic portion of the smear layer (Baumgartner and Mader 1987). Furthermore, organic material inhibits the action of EDTA when used on its own; but when combined with NaOCL, the quantity of inorganic material becomes the limiting factor. The combination of NaOCL and EDTA produces a synergistic effect, resulting in the effective removal of the entire smear layer (Baumgartner and Mader 1987, Grawehr *et al* 2003, Sevimay and

Kalayci 2005). A study found no difference in demineralization properties when comparing different concentrations or types of EDTA (O'Connell *et al* 2000).

An earlier study explored the use of NaOCL in conjunction with hydrogen peroxide but the combined cleaning effect was found to be weakened (McComb and Smith 1975). A comparison of the cleaning effects of 2% chlorhexidine and NaOCL showed the coronal and middle third were cleaner with both agents, while the worst were in the apical third (Yamashita *et al* 2003). Yamada *et al* (1983) evaluated the effectiveness of inorganic and organic acids for smear layer removal and found them to be highly effective, but too aggressive.

To improve the efficacy of smear layer removal without having any deleterious effects on the dentin, various agents such as quaternary ammonium bromide (Cetavlon) with surface wetting and antibacterial properties have been added to EDTA or new agents such as ethylene glycol-b tetraacetic acid (EGTA). However the Liolios, Economides and Parissis-Messimeris (1997) study suggests that the EDTA solution alone is more successful at removal of the smear layer compare to those with other EDTA-based solutions.

2-8-4- Effect of irrigation on uninstrumented surface and biofilm layer:

Using 5.25% of NaOCL on the uninstrumented surface may dissolve organic material and completely remove pulpal remnants and predentin (Baumgartner and Mader 1987). Baumgartner and Mader

(1987) found that combinations of EDTA and NaOCL removed puplal remnants and smear layer leaving a smooth surface in instrumented canals. In uninstrumented surfaces, using the same combination of EDTA and NaOCL alternately, all pulpal remnant and predentin was removed and additionally the diameter of superficial dentinal tubules was enlarged. The study suggested that the combined use of NaOCL and EDTA produced better antimicrobial action than either solution used alone (Baumgartner and Mader 1987). The precise mechanism is unknown but it may be hypothesized that it is because of a combination of EDTA: 1) helping to remove debris obstructing access to the uninstrumented surfaces; and 2) chelating heavy metal ions that help to bind bacterial cells together in the biofilm (Byström and Sundqvist 1985).

2-9- Leakage tests

2-9-1- Types of leakage tests:

In the absence of a universally acceptable protocol, various *in vitro* methods have been developed to evaluate the sealing ability of root canal filling materials. These methods include dye penetration, radioactive isotope tests, bacterial or bacterial metabolite leakage tests, electrochemical techniques and fluid filtration tests (Verissimo and Do Vale 2006). In vitro studies have suggested that assessment of coronal leakage is more clinically relevant than apical leakage (Wu and Wesselink 1993) as microorganisms could penetrate from the coronal to apical aspect easily because of loss of temporary filling, inadequate

endodontic filling or crown sealing (Saunders and Saunders 1994, Carratu *et al* 2002).

2-9-2- Tracer particle test:

Various tracer particles may be used for assessing leakage e.g. dye, radioisotope, and bacterial penetration. The principle is to evaluate the linear penetration of a tracer along the obturated canal of an extracted tooth, which serves as an indicator of the length of the gap between the root filling material and the root canal wall (AliGhamdi and Wennberg 1994). Such tests produce data that can only be regarded as semi-quantitative because these techniques do not provide any information about the volume of tracer in the gaps (Wimonchit, Timpawat and Vongsavan 2002, Camps and Pashley 2003). The results of semi-quantitative tests often lead to the difficulty in drawing firm conclusions as to which filling technique or material was the best in sealing the root canal system. On the other hand, dye penetration is probably the most popular method, because it is simple, sensitive and inexpensive. However, large variations of results make the dye penetration method far from being reproducible and comparable. Linear measurements of dye penetration are made after longitudinal or cross sectioning, or clearing of the specimens (Zakariasen and Stadem 1982).

Dye penetration is the most common method employed in leakage studies. A 0.2% to 2.0% solution of methylene blue dye is the most commonly used dye (Camps and Pashley 2003). Methylene blue dye is soluble in water, can easily diffuse in water-filled gaps

(Wimonchit, Timpawat and Vongsavan 2002), and has a small molecular size (Oliver and Abbott 2001, Camps and Pashley 2003), that is similar to that of bacterial by-products such as butyric acid (Kersten and Moorer 1989). Indian ink, on the other hand, has large carbon particles that are only suspended in water (Wimonchit, Timpawat and Vongsavan 2002), with a particle size comparable to that of bacterial cells (Verissimo and Do Vale 2006). A study which compared the linear extent of leakage after immersion in 5% aqueous solution of methylene blue dye or Indian ink dye showed that the former, which has a lower molecular weight, penetrated more deeply along root canal fillings compared to Indian ink (Ahlberg, Assavanop and Tay 1995). On the other hand, the *in-vitro* penetration of dye should not be considered as equivalent to the *in-vivo* penetration of irritants from an infected canal to cause apical periodontitis. The degree of penetration merely serves as an indicator of the potential for leakage. Such dye penetration tests may be a mean to compare the relative efficacy of two or more techniques, or materials, under the same test conditions (Ahlberg, Assavanop and Tay 1995).

The penetration of dye may be influenced by various factors, such as the presence of entrapped air, and surface tension (Kontakiotis, Georgopoulou and Morfis 2001). Dye can penetrate by capillary action, or by diffusion (Verissimo and Do Vale 2006). Diffusion is the transport of a material in a fluid from a high to a low concentration until equilibrium is reached, whereas capillary action is related to the surface tension of a liquid on the surface of a substrate (dentinal wall) (Kontakiotis, Georgopoulou and Morfis 2001). It has been demonsrtated that methylene blue dye passes faster (by capillary action) in dry gap than (by diffusion) in water-filled gaps along root

fillings (Kontakiotis, Georgopoulou and Morfis 2001). One study emphasized the importance of use of reduced air pressure in dye penetration. The results of the study found that the maximum depth of India ink penetration after using vacuum dye penetration was significantly greater than passive and fluid filtration dye penetrations (Wimonchit, Timpawat and Vongsavan 2002).

Linear assessment of dye penetration along the root-filling interface may be affected by the lack of contrast between the color of the dye, the root filling material, and the tooth substance. In addition, methylene blue dye was found to decolor over time by materials such as calcium hydroxide, mineral trioxide containing zinc oxide eugenol and calcium sulfate. Fuji II glass ionomer cement was the only material tested which did not decolor this dye (Wu, Kontakiotis and Wesselink 1998).

2-9-3- Methodology that uses dyes:

The teeth are sectioned longitudinally, transversely, or cleared and the linear penetration of dye is recorded. Longitudanal sectioning has been found to be more reliable than other sectioning techniques. It has the advantage of allowing the examiner to inspect exposed filling and any dye penetration into the material and at the interface of the dentinal wall on the one side and the obturating material on the other side (Ahlberg, Assavanop and Tay 1995). Ahlberg, Assavanop and Tay (1995) also suggested a variation of this technique; whereby the roots are worn down to visualize the leakage through a thin remaining layer of dentin, thus reducing the dissolution of the dye during the sectioning process. The disadvantages of longitudinal sectioning are

that the axis of cutting is chosen randomly and the probability that the section occurs through the deepest dye penetration is very low giving unreliable results (Camps and Pashley 2003).

According to Ahlberg, Assavanop and Tay (1995), the transverse root sectioning allows one to determine whether or not there is penetration of the dye in each section. The disadvantage of this technique is loss of part of the dentinal tissues and the dye due to the technique itself.

Wimonchit, Timpawat and Vongsavan (2002) used the clearing method which makes viewing of the leakage easier by providing a three-dimensional view of the area. This method allows the maximum depth of dye penetration to be recorded accurately in every direction. It is simple, fast to perform and does not require complex equipment.

CHAPTER 3

AIM AND OBJECTIVES

The **aim** of this study is to compare the sealing ability of AH Plus sealer to the root canal wall in the presence and absence of the smear layer.

This study has the following **objectives:**

1- To assess the adhesion of AH Plus sealer to the root canal wall in the presence of the smear layer.

2- To assess the adhesion of AH Plus sealer to the root canal wall in the absence of the smear layer.

Null Hypothesis

There is no significant difference in dye penetration (leakage) when using AH Plus sealer, in the presence or absence of the smear layer, to bond to the root canal wall.

CHAPTER 4

MATERIALS AND METHODS

4-1- Sample selection

The sample size was forty five extracted human permanent maxillary incisors. The teeth were selected from the teeth extracted in the service rendering clinic at the Oral Health Centers of Mitchells Plain and Tygerberg. Teeth with multiple canals and significant apical curvatures on inspection were excluded from the study. Teeth with open apices and resorptive defects were also excluded. All the teeth were stored in 0.2 % thymol to prevent any bacterial activity during the storage period (Shipper *et al* 2004). The teeth were then immersed in 5% sodium hypochlorite (NaOCl) for approximately 15 minutes to remove any organic material from the root surfaces. All the teeth were carefully cleaned with a sharp knife to remove any calculus or soft tissue debris taken care not to damage the root surface.
Each tooth was sectioned at the amelocemental junction using a water-cooled diamond bur (Horico, Berlin, Germany) and the crown was discarded.

4-2- Instrumentation

All the specimens were instrumented by one operator. The pulp of each tooth was removed with a barbed broach and the root canal was instrumented with a size 15 K-type root canal file (Dentsply, Maillefer, Ballaigues, Switzerland) until the tip of the instrument was

seen just protruding through the apical foramen. The length was noted and the working length was determined as 1mm short of the apex.
The root canals were prepared using the step-back technique and the coronal part of the root canal was flared using gates glidden burs size 070 and 090 (Dentsply, Maillefer, Ballaigues, Switzerland). All the canals were prepared to a size 50 endodontic file to the working length determined for each canal.
During instrumentation, the forty four root canals were irrigated with 3 ml of 3% sodium hypochlorite solution (NaOCl) after each instrument using a 27-gauge needle fitted to a syringe.
Throughout the study, the teeth were kept moist, using sterile gauze soaked in deionized water.

4-3- Obturation:

Prior to obturation, the forty four teeth were randomly divided into two groups of 22 teeth each identified as Group A and Group B. The two groups were irrigated in different ways to either preserve or remove the smear layer, and the remaining tooth was prepared without irrigation and served as the control for the SEM examination.

All the canals in group A received a final flush of 3 ml of buffered 18% ethylene-diamine-tetra-acetic acid (EDTA) (Ultradent Products Inc., South Jordan, UT, USA) **(figure 4.1)**, followed by 3 ml of 3% sodium hypochlorite solution (NaOCl) using a 27-gauge needle to remove any residue from the root canals. The solutions were deliberately forced to extrude through the apical foramen to ascertain patency. The canal was dried with "extra-fine" and "medium" sized paper points (SybronEndo, Orange, CA, USA) at the working length.

Group A was then divided in to two sub-groups A1 and A2. Group A1, consisted of two teeth and group A2, consisted of twenty teeth. The two teeth in group A1, were sectioned and their canals were observed with a scanning electron microscope (Scanning Electron Microanalyser, Hitachi, Japan). In group A2, the AH Plus sealer (Dentsply Detrey, Konstanz, Germany) **(figure 4.2)** was mixed according to the manufacturer's instructions and placed into the root canal with a bladed spiral root filler. A master gutta-percha cone was coated with a thin layer of the sealer and placed in the canal to the working length using a pumping action (Jeffrey, Saunders, and Thomas 1986). The canals were filled adequately with sealer to allow excess material to be extruded onto the coronal surface of the tooth. Immediately after obturation the excess gutta-percha and sealer were cut off flush with the coronal surface of the root canal using a scalpel. No other condensation was performed.

In group B, only 3ml of 3% sodium hypochlorite solution (NaOCL) was used as the irrigant. Group B was also divided in to two subgroups. Group B1, consisted of two teeth and group B2, consisted of twenty teeth. The two teeth in group B1 were sectioning and their canals were observed with a scanning electron microscope.

In group B2 the AH Plus sealer was mixed according to the manufacturer's instructions and placed into the root canal with a bladed spiral root filler. A master gutta-percha cone was coated with a thin layer of the sealer and placed in the canal to the working length using a pumping action (Jeffrey, Saunders and Thomas 1986). The canals were filled adequately with sealer to allow excess material to be extruded onto the coronal surface of the tooth. Immediately after obturation the excess gutta-percha and sealer were cut off flush with

the coronal surface of the root using a scalpel. No other condensation was performed.

Figure 4.1 18% ethylene-diamine-tetra-acetic acid

Figure 4.2 AH Plus sealer

4-4- Preparation of specimens for leakage test:

The obturated roots were stored in distilled water, at 37ºC for 24 hours. The roots were dried and the root apices were sealed with wax (Saunders and Saunders 1994). The teeth were then coated with two layers of nail varnish (Boots No.7, Boots Manufacturing Co., Nottingham, UK), except for 1mm around the opening of the canal **(figure 4.3).**

The specimens were placed in 2% methylene blue dye in a thermo-cycling machine and thermocycled for 500 cycles between 5° C and 55° C with a dwell time of 15 seconds. The roots were then removed from the dye and washed thoroughly under running water. The roots were dried and the nail varnish and sticky wax were removed with a scalpel. The roots were then embedded in a slow setting epoxy resin (Fobroglas, Fowkes Bros, Cape Town, South Africa). The roots were sectioned at the coronal, middle and apical thirds with a water-cooled diamond disk-cutter at slow speed (Minitom, Struers, Denmark **(figure 4.4)**. The smear layer was removed from the specimens using a silicon paper wrapped around two glass slabs under lubrication, so that the extent of dye penetration could be measured under a light microscope.

Figure 4.3 Teeth with apices coated with wax and two layers of nail varnish

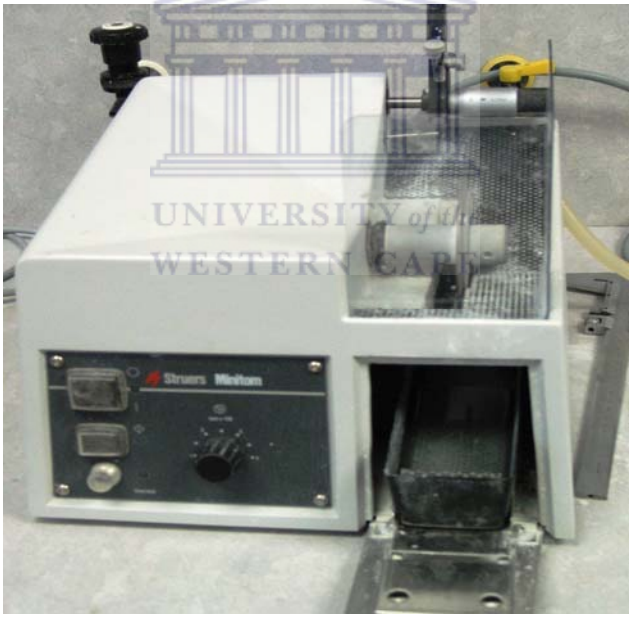

Figure 4.4 Water-cooled diamond disk-cutter

4-5- Preparation of the specimens for scanning electron microscopic examination:

Two teeth from both groups A1 and B1 were selected for scanning electron microscopic examination. The roots were grooved

longitudinally; they were then split into two halves by placing a blade in the groove and applying gentle pressure. Both fractured halves of each root were mounted on an aluminum stub, vacuum-dried, coated with 20 nm of gold and then examined under the scanning electron microscope (SEM) as a control to determine whether the smear layer was removed with this procedure or not **(figure 4.5)**.

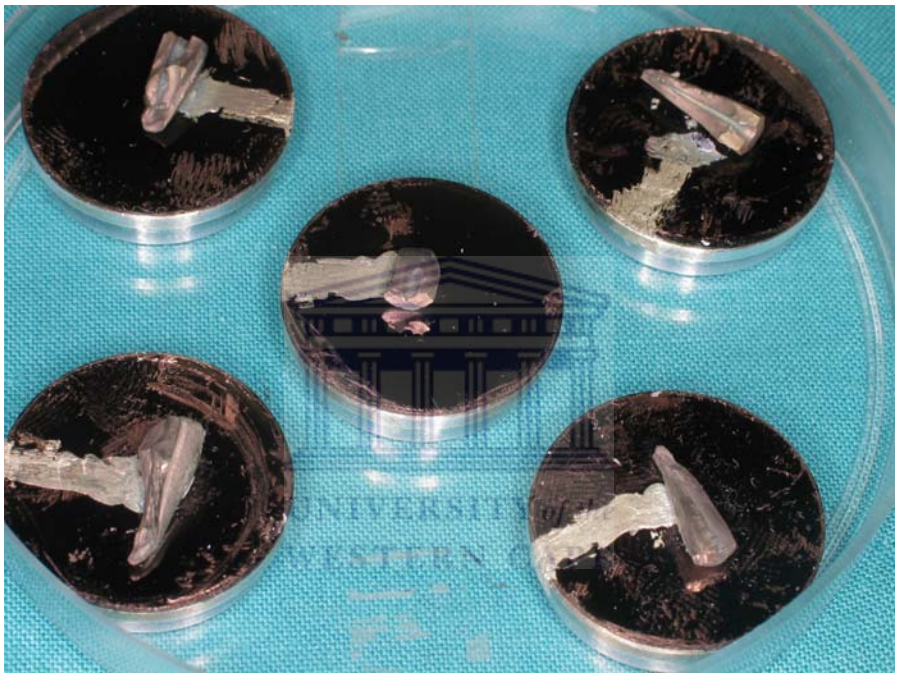

Figure 4.5. Roots mounted on aluminum stub and coated with 20 nm of gold for SEM observation

4-6- Measuring dye penetration and data collection:

The penetration depth of the dye was measured, evaluated and scored according to the criteria in table 4.1

Degree of leakage	Depth of penetration
0	No leakage
1	Up to Coronal third
2	Up to Middle third
3	Apical third

Table 4.1: Criteria for measurement of dye penetration

Coronal dye penetration was measured for each specimen, using a light microscope at a 100 magnification. The dye penetration was measured at each cross-section of the specimen.

CHAPTER 5

RESULTS

5-1- Coronal leakage test:

The raw data of the study for all leakage scores appears in (appendix 1 and 2), and are summarized in table 5.1 and 5.2.

The results of the study showed that of the twenty teeth in the NaOCL+EDTA (group A) seven teeth showed no leakage, while of the twenty teeth in the NaOCL (group B) only five teeth showed no leakage (Table 5.1).

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Level of leakage	NaOCL+ EDTA	NaOCL
Total of specimens	20	20
No leakage	7	5
Leakage	13	15

Table 5.1: Comparison of leakage scores between groups A and B

Of the thirteen teeth in the NaOCL+EDTA (group A) which showed leakage, only one of the specimens leaked to the apical third, and one specimens leaked up to the middle third, while eleven specimens leaked up to the coronal third (table 5.2).

Of the fifteen teeth in the NaOCL (group B) which showed leakage, only two of the teeth leaked up to the apical third, while three teeth

leaked up to the middle third and ten teeth had leakage limited to the coronal third only (table 5.2).

Level of leakage	NaOCL+EDTA	NaOCL
Total specimens that showed leakage	13	15
Coronal third leakage only	11	10
Up to middle third leakage	2	3
Apical third leakage	1	2

Table 5.2: Number of teeth showing level of leakage

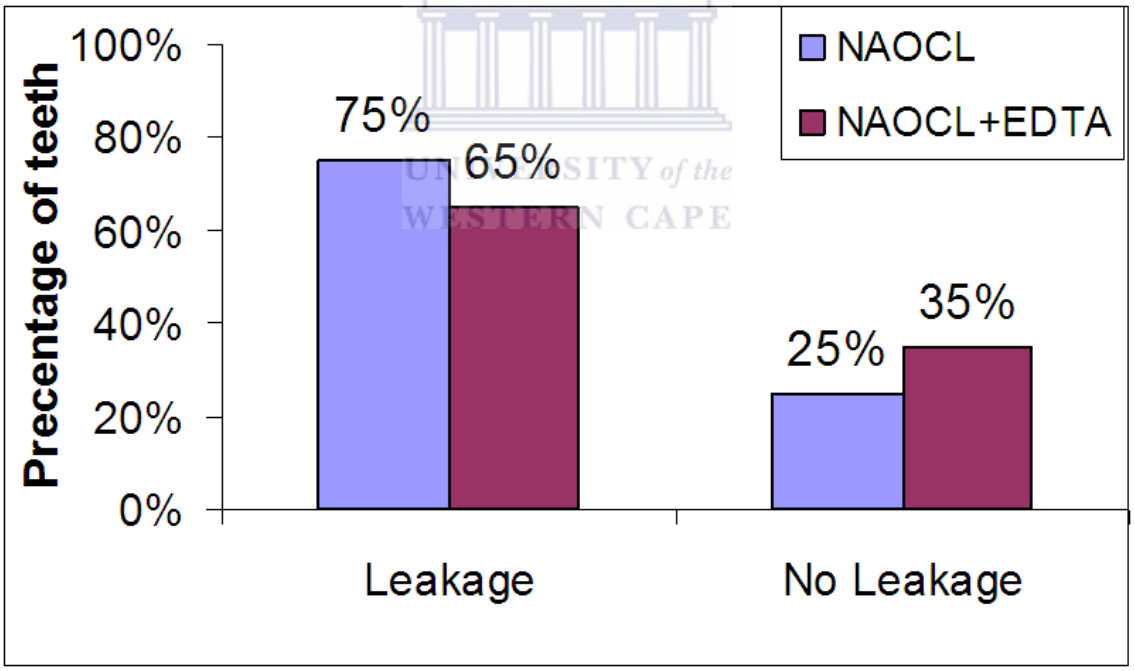

Figure 5.1: Percentage of leakage in each group

As shown in Figure 5.1 the NaOCL+EDTA (group A) had a higher percentage of teeth that showed no leakage (35%) compared to the NaOCL (group B) (25%).

In addition, the NaOCL (group B) had a higher percentage of teeth that showed leakage in both the middle (15%) and apical (10%) thirds compared to the NaOCL+EDTA (group A) which show (5%) in the middle and (5%) in the apical thirds. However in both groups more than 50% of the teeth showed leakage limited to the coronal third of the root. This is graphically illustrated in figure 5.2.

However, comparative analysis of the two groups using a Fisher's Exact test revealed no statistically significant differences in the levels of leakage between the two groups (p = 0.724).

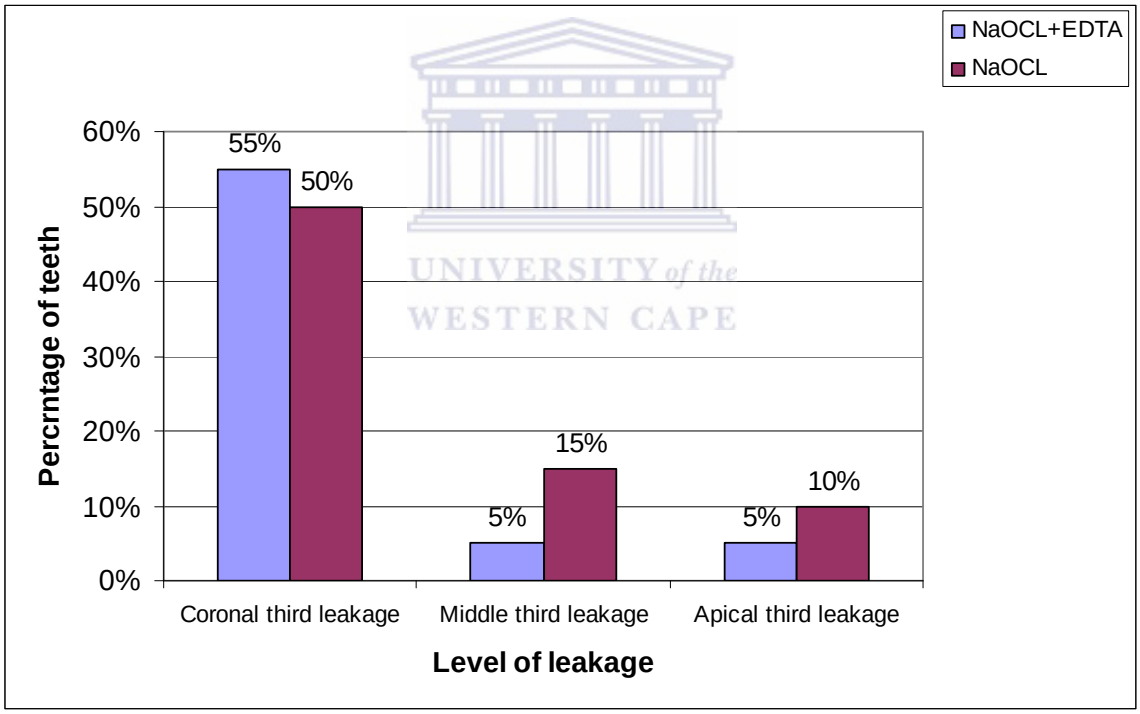

Figure 5.2: Percentage of teeth by level of leakage in each group

5-2- SEM examination:

In the SEM examination, the control group showed a typical smear layer with lots of debri and closed dentinal tubules (figure 5.3). This is consistent with the findings in the literature. The smear layer is a product of the preparation of the root canal and as no flushing was done, the smear layer is largely intact

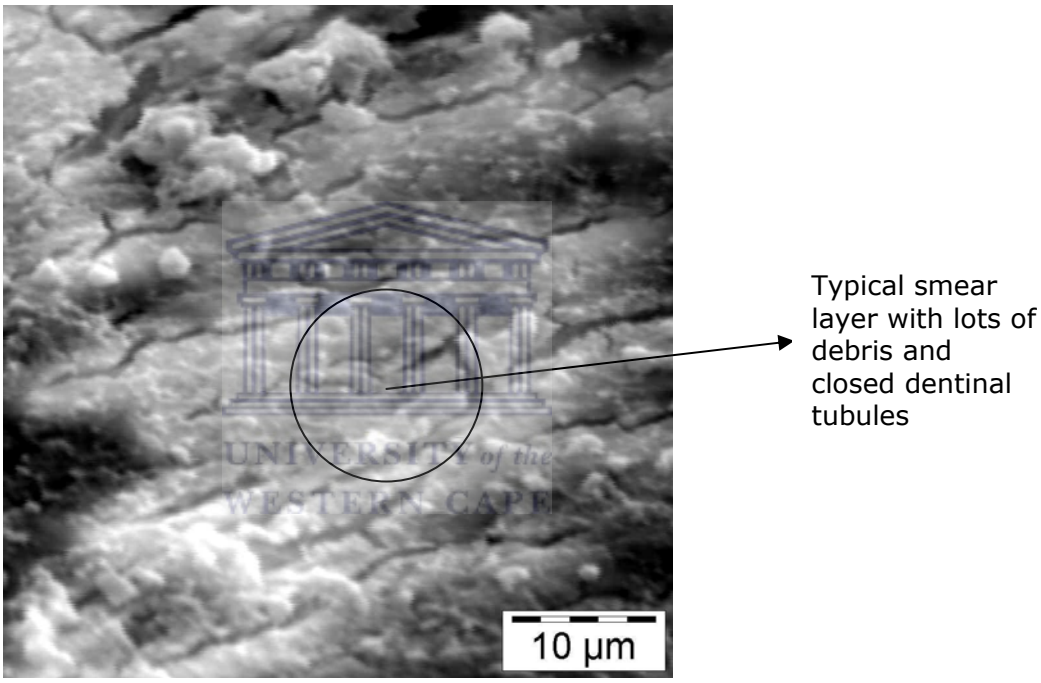

Figure 5.3: Longitudinal section of the control group (x 2200)

The SEM examination of the coronal and middle thirds of teeth in group A (NaOCL + EDTA) revealed well etched dentin and clear openings of the dentinal tubules especially in the coronal (figure 5.4) and the middle thirds of the root canals (figure 5.5). The dentin in the apical third of group A (NaOCL + EDTA) did not appear to be as well etched as the coronal and middle thirds and the dentinal tubules were not clearly opened (figure 5.6).

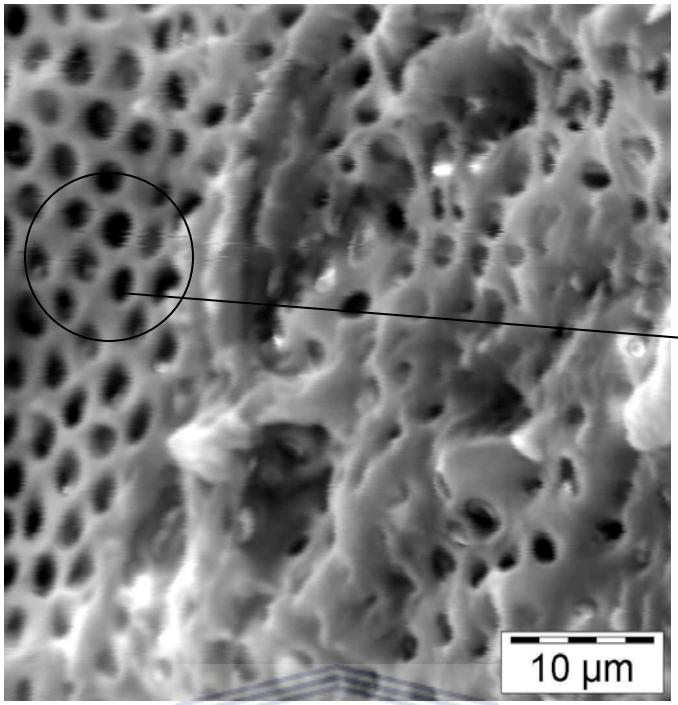

Figure 5.4: Longitudinal section of the coronal third of the NaOCL + EDTA group (x 2200)

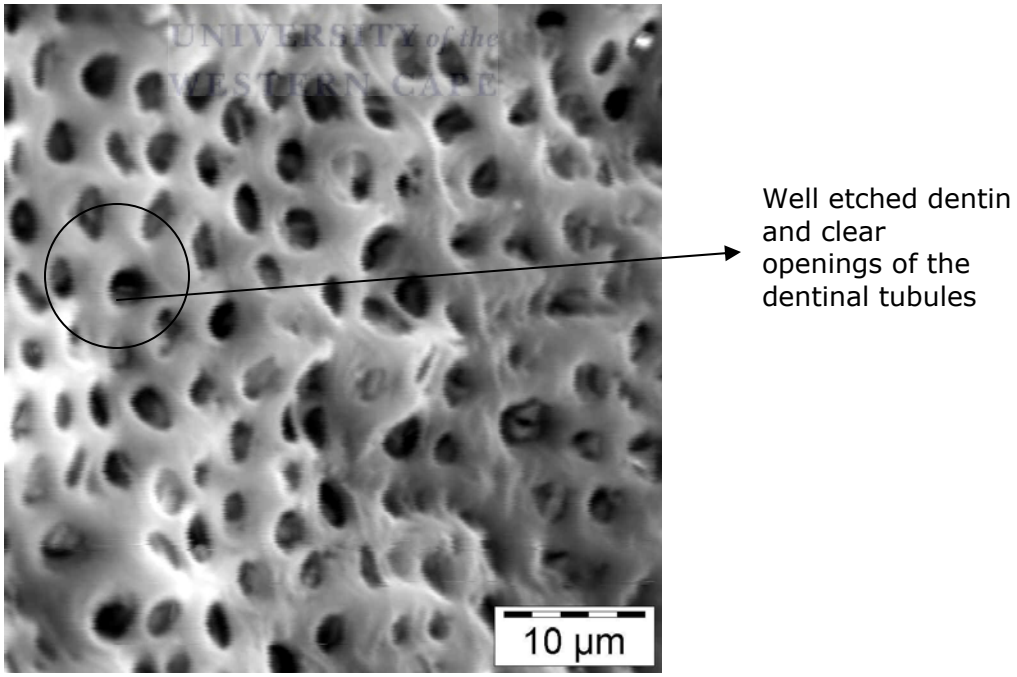

Figure 5.5: Longitudinal section of the middle third of the NaOCL + EDTA group (x 2200).

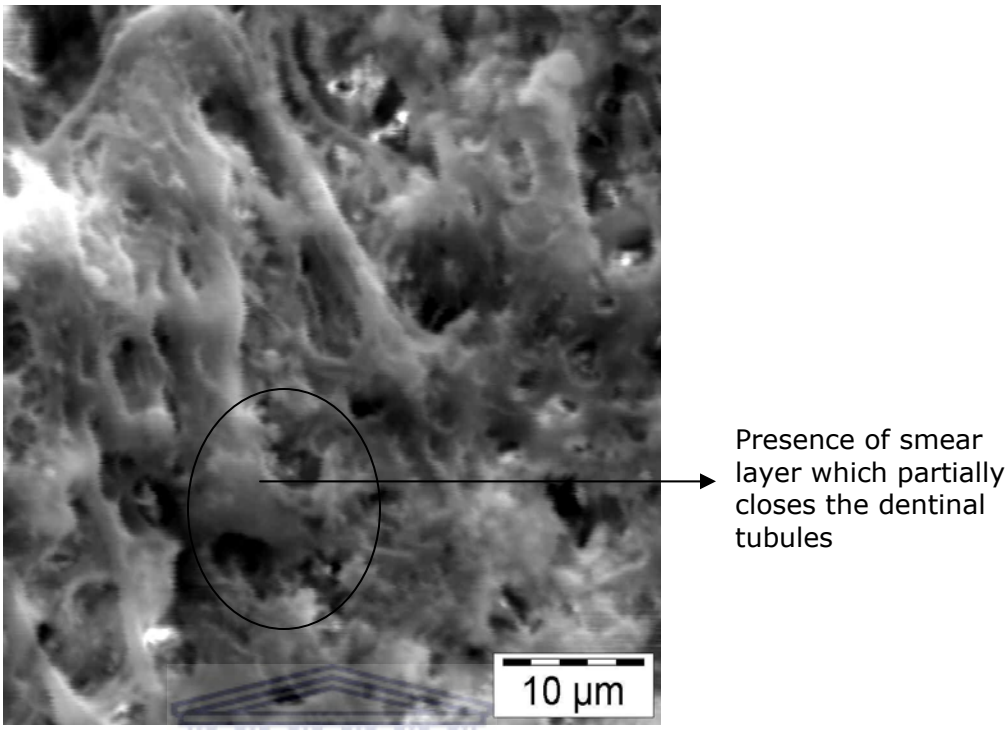

Figure 5.6: Longitudinal section of the apical third of the NaOCL + EDTA group (x 2200).

The coronal and middle thirds of the roots in Group B (NaOCL only) show incomplete opening of the dentinal tubules with some debri covering the opening of the tubules (figure 5.7). The dentin in the apical third of Group B (NaOCL only) also shows more incomplete opening of the dentinal tubules with more debris covering the opening of the dentinal tubules compared to the coronal and middle thirds (figure 5.8).

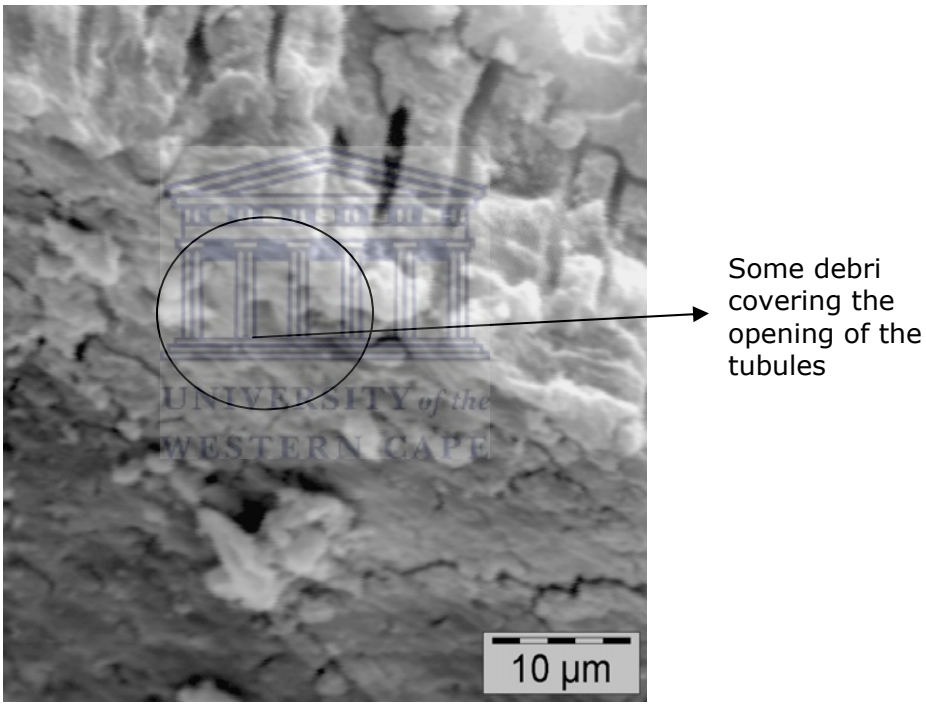

Figure 5.7: Longitudinal section of the Coronal third of the NaOCL group (x 2200)

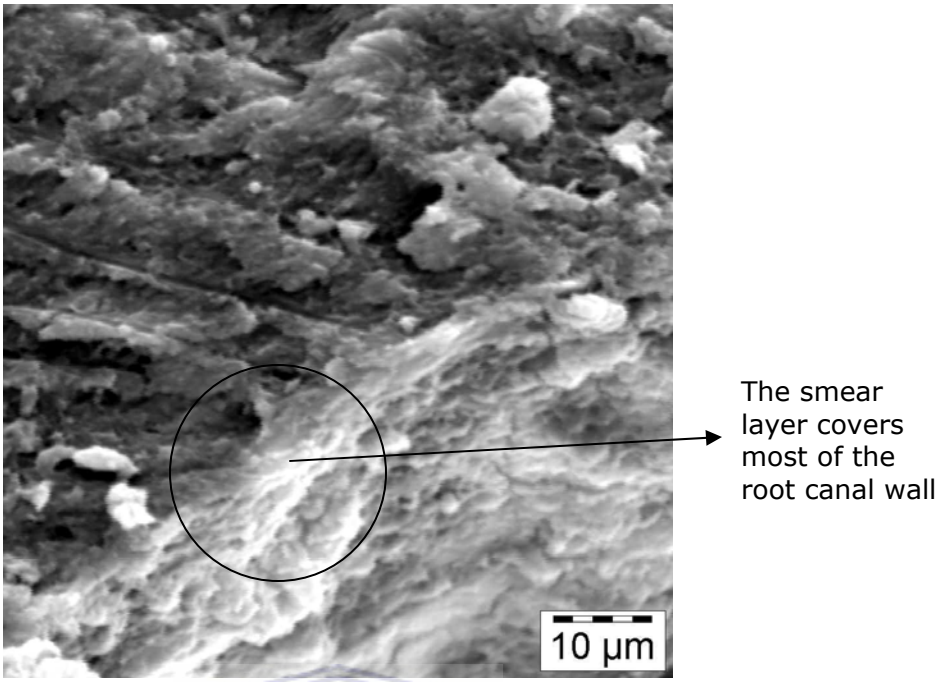

Figure 5.8: Longitudinal section of the apical third of the NaOCL group (x 2200)

The results show that the coronal and middle thirds of group A (NaOCL + EDTA) had the cleanest surface, while the cleaning of the coronal third of group B (NaOCL) was not as efficient when compared to that seen in group A. The worst cleaning occurred in the apical third of both groups and this is also consistent with the findings in the literature.

CHAPTER 6

DISCUSSION

6-1- Methodology of the study:

6-1-1- Tooth sample

When using extracted human teeth for *in vitro* studies, the potential for uncontrollable variation exists. In this study, the specimens were standardized as much as possible, with respect to the tooth type, the taper and dimension of the prepared canal, and were randomly assigned to the two experimental groups. The selected teeth were maxillary central incisors. The coronal portions of all the teeth were removed so that only 10-12 mm of root length remained. This length was considered clinically relevant. The average tooth length for maxillary central incisors is approximately 20 to 23 mm, with the average crown height being 10 mm.

The root canals in the maxillary central incisors are generally straight, and the cross-sectional shape at the mid-root to apical levels often is ovoid or round (Barker *et al* 1973). The root canal curvature and cross-sectional shape may influence the outcome in studies evaluating endodontic obturation materials and techniques. A study comparing the apical seal in straight and curved canals obturated by either lateral compaction or injectable thermoplasticized gutta-percha showed that there was a trend of increased dye leakage in curved canals (Mann and McWalter 1987).

6-1-2- Storage medium:

All the teeth were stored in 0.2 % thymol. Sodium hypochlorite would not have been suitable as storage medium in this study because of its incompatibility with resins (Erdemir *et al* 2004), as AH Plus that was used in this study for obturation purposes is a resin-based sealer.

6-2-Discussion of the results:

6-2-1- Coronal leakage results:

In this study the single cone obturation technique was used because it is recommended in wide and straight canals. However it was not the purpose of this study to compare the single cone obturation technique with other obturation techniques. Molecular size of dyes also affects the degree of penetration. Therefore, methylene blue was chosen in this study because it has a low molecular weight and penetrates more deeply along the root canal filling (Ahlberg, Assavanop and Tay 1995).

Coronal leakage can either occur between gutta-percha-sealer or between sealer- dentin. Most leakage occurs between the root canal and the sealer (Hovland and Dumsha 1985) and this leakage is complicated by the presence of the smear layer.

In the majority of the specimens (70%) of both experimental groups dye penetration occurred between AH Plus sealer and the root canal wall (figure 6.1). It should be noted that leakage occurred only in part and not all around the circumference of the root. It was unknown whether insufficient cleaning or insufficient irrigation or both

caused the leakage. This could be explained by the fact that when the smear layer was removed AH Plus sealer can penetrate better in the dentinal tubules, form a better seal and causes less leakage.

In some specimens (30%), no dye penetration was observed at all for both groups (figure 6.2). The results of this study showed that there was no statistically significant difference in the leakage between those canals with the smear layer intact and those with the smear layer removed. The root canal specimens with intact smear layer had a higher percentage of leakage scores for up to (15%) and beyond (10%) the middle third as compared to those where the smear layer was removed.

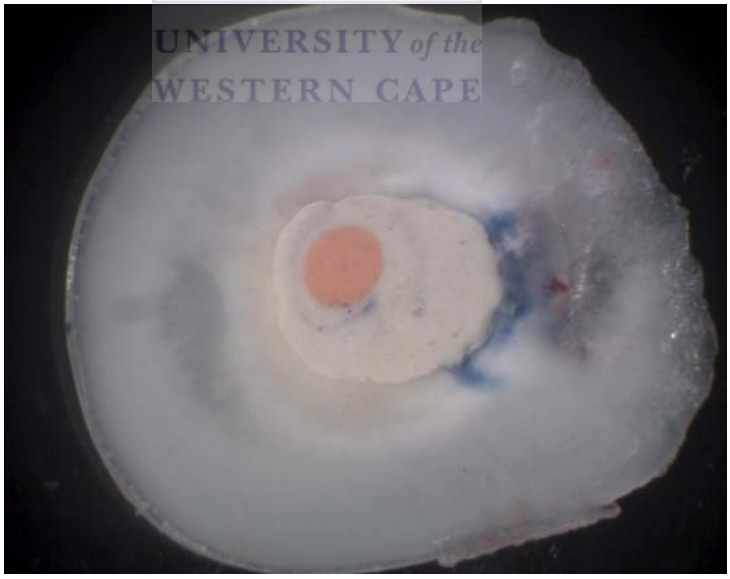

Figure 6.1. Dye penetration occurred at the interface of the AH Plus sealer and root canal wall (x40)

Figure 6.2. Specimen with no dye penetration (x40)

The period of storage of the specimens was 24 hours because longer storage time may result in dissolution of the smear layer giving false readings for leakage. In addition, the influence of salivary, and hence bacterial, contamination and subsequent dissolution of the smear layer *in vivo*, can affect the leakage (Saunders and Saunders 1994).

According to the manufacturer, AH Plus has the advantage of being a paste-paste system that insures rapid and clean mixing, high radio-opacity and faster setting time. However, the fast setting time and shrinkage stress may be the cause of detachment from dentin walls. Silicon oil ingredients in AH Plus sealer can prevent tight sealing to the humid dentinal wall. Formation of voids by the thick sealer with single cone technique may be another cause of dye penetration.

Other variables that were not analyzed and may have contributed to the coronal sealing failure are entrapped air and accessory canals. Therefore, these factors need to be investigated further.

According to the results of the present study, no group showed complete coronal sealing. Significant leakage (65% for NaOCL + EDTA group, and 75% for NaOCL group) was observed for both groups in the coronal third of the canals.

6-2-2- SEM examination results:

Removal of the smear layer allows root canal filling material to penetrate better into the dentinal tubules (Okşan *et al* 1993, Sevimay and Kalayci 2005). 18% EDTA and 3% NaOCL were used as irrigation solution to remove the smear layer in group A. The SEM photograph in figure (6.3) shows a clean dentine surface with clear openings of the dentinal tubules.

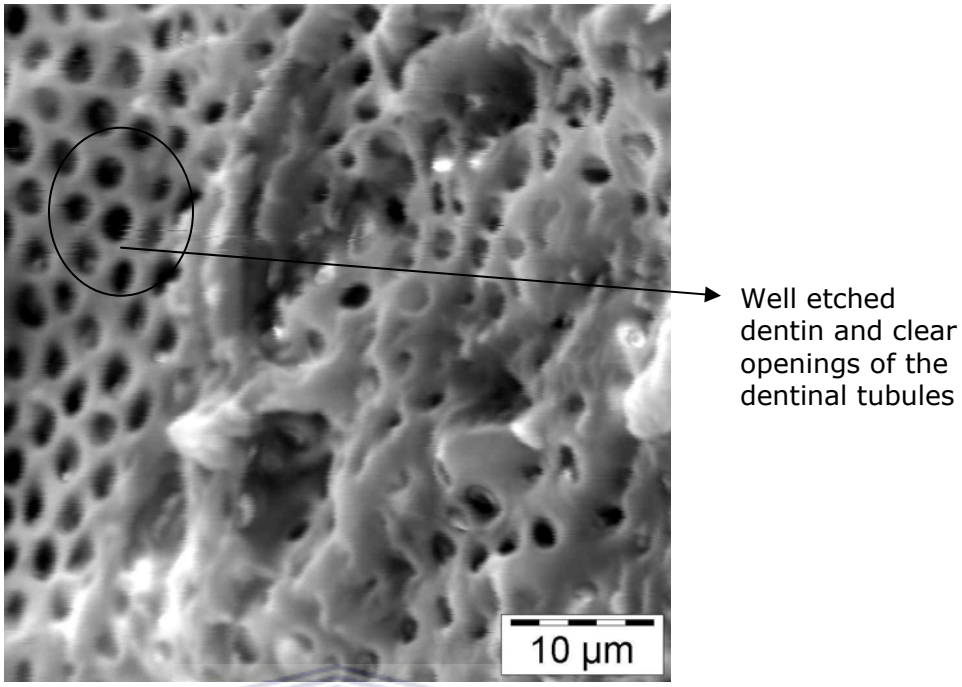

Figure 6.3 Longitudinal section of the coronal third of the NaOCL + EDTA group (x 2200)

The results of the SEM examination demonstrated a more efficient cleaning of the root canal walls in all thirds of group A where the canals were irrigated with 3% NaOCL and 18% EDTA. The canal walls were almost always free of residue and the dentinal tubules were visible. These results are in agreement with other studies who reported that the physio-chemical action of NaOCL is important to remove the organic residue while the EDTA acts mainly on the inorganic residue (Yamada *et al* 1983, Sen, Wesselink and Turkun 1995). Both irrigating solutions showed inefficient cleaning of the apical thirds of the canals and this is in accordance with the results found by Yamada *et al* (1983). The coronal and middle third are obviously wider in diameter, accessible and easily irrigated.

It should be expected that the thickness of the cement lute could have an influence on the coronal leakage as the thicker the material the more likely the inclusion of air voids that may subsequently be a path for leakage. In clinical practice, the use of a single gutta-percha cone is less technique sensitive as compared to the lateral condensation of gutta-percha. However, clinical trials should be conducted to establish the efficacy of this method of root canal obturation specifically using AH Plus as endodontic sealer.

In-vitro studies have been designed to predict clinical performance and to evaluate sealing ability of root canal fillings. Their validity and biological significance have been questioned (Wu and Wesselink 1993). Methodologies reported in the literature are not standardized making them difficult to compare (Verissimo and Do Vale 2006). More studies are needed to closely approximate the clinical situation.

Chapter 7

Conclusion and Recommendation

7.1. Conclusion

The use of EDTA and NaOCL efficiently removes the smear layer and opens the dentinal tubules in the coronal and middle thirds, but less so in the apical third.

The use of NaOCL only did not remove the smear layer from the root canal walls.

There was no statistically significant difference in coronal leakage when using AH-Plus sealer in the presence or absence of the smear layer.

7.2. Limitation

With respect to this study, limitations that could have affected the outcomes of the study are as follows:

The sample size used in the study was relatively small. In general, the greater the sample size, the more reliable are the statistic results.
Another factor that could have limited the outcome of this study is that only one class of sealer had been utilized in the methodology. Other classes of sealers might have different results.

The technique utilized in the preparation for the specimens was a step-back technique. Other modalities of root canal preparation are well documented in the literature. This could be regarded as another limiting factor.

Single-cone obturation technique was used in this study, this could have also limited the outcome of the results.

7.3. Recommendation

With regard to the limitation of the study, the following recommendation can be considered for future research in the field of root canal microleakage.

The sample size could be larger than what was used. This will be in favour of the reliability of the outcome, and increase the predictability of the technique.

Various types of endodontic sealers should be experimented with, this will provide with comparison regarding the optimal sealer that should be used with root canal obturation.

Other preparatory techniques should also be examined. Rotary instrumentation and manual hand instrumentation with the crown down technique are current root canal preparation methods that would be of research interest.

CHAPTER 8

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Appendix 1- Data sheet

Id no	Coronal Third	Middle Third	Apical Third	Total
1	1	0	0	1
2	1	0	1	3
3	0	0	0	0

4	1	0	0	1
5	1	0	0	1
6	0	0	0	0
7	1	0	0	1
8	1	0	0	1
9	0	0	0	0
10	1	0	0	1
11	1	0	0	1
12	0	0	0	0
13	1	0	0	1
14	1	0	0	1
15	0	0	0	0
16	1	1	0	2
17	0	0	0	0
18	1	0	0	1
19	1	0	0	1
20	0	0	0	0

Group A (EDTA+NaOCL)

Appendix 2- Data sheet

Id no	Coronal Third	Middle Third	Apical Third	Total
1	1	0	0	1
2	1	0	0	1

3	0	0	0	0
4	1	1	0	2
5	1	1	1	3
6	1	1	0	2
7	1	1	0	2
8	1	0	0	1
9	1	0	0	1
10	0	0	0	0
11	1	1	1	3
12	1	0	0	1
13	1	0	0	1
14	1	0	0	1
15	0	0	0	0
16	1	0	0	1
17	1	0	0	1
18	0	0	0	0
19	0	0	0	0
20	1	0	0	1

Group B
(NaOCL)

Appendix3

			Level of leakage				Total
			No leakage	Coronal third leakage	Middle third leakage	Apical third leakage	
GroupD	NAOCL	Count	5	10	3	2	20

	% within GroupD	25.0%	50.0%	15.0%	10.0%	100.0%
NAOCL+EDTA	Count	7	11	1	1	20
	% within GroupD	35.0%	55.0%	5.0%	5.0%	100.0%
Total	Count	12	21	4	3	40
	% within GroupD	30.0%	52.5%	10.0%	7.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.714(a)	3	.634	.724		
Likelihood Ratio	1.769	3	.622	.724		
Fisher's Exact Test	1.753			.724		
Linear-by-Linear Association	1.258(b)	1	.262	.355	.178	.081
N of Valid Cases	40					

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is 1.50.

b The standardized statistic is -1.122.