

https://dspace.org/

Smear layer removal ability and antibacterial activity of endodontic irrigants

Bennie, Karen Ruet

2014-02-25
http://hdl.handle.net10539/13950

Downloaded from a DSpace repository. https://dspace.org/

SMEAR LAYER REMOVAL ABILITY AND ANTIBACTERIAL ACTIVITY OF ENDODONTIC IRRIGANTS

Karen Ruet Bennie

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree
of
Master of Science in Dentistry

Johannesburg, 2013

DECLARARATION

I, Karen Ruet Bennie declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

…….Karen Ruet Bennie

…….day of……., 2013

ABSTRACT

The aim of this study was to test various alternating sequences of sodium hypochlorite (NaOCl), anolyte solution, and EDTA for their ability to remove the mineralised portion of the smear layer, and to destroy bacteria.

Forty-eight single canal teeth were collected and randomly divided into six groups, prepared to working length, sterilized and inoculated with *Enterococcus faecalis*. The irrigation protocols were as follows: Group 1 (four roots) 3ml sterile distilled water, Group 2 (four roots) 3ml 6% sodium hypochlorite, Group 3 (ten roots) 3ml 6% sodium hypochlorite followed by 3ml 18% EDTA, Group 4 (ten roots) 3ml 6% sodium hypochlorite followed by 5ml anolyte solution, Group 5 (ten roots) 0.5ml 6% sodium hypochlorite followed by 5ml anolyte solution followed by 3ml 18% EDTA and Group 6 (ten roots) 5ml anolyte solution followed by 3ml 18% EDTA.

Sterile paper points were inserted into the canals after sterilization, inoculation and irrigation. Standard cultivation techniques were used to count the colony forming units of viable bacteria at each phase.

The roots were split longitudinally and prepared for SEM evaluation. Two photomicrographs were randomly taken in the coronal, middle and

apical thirds of each root and the number of patent dentinal tubules counted. The One-way ANOVA was used for statistical evaluation.

The small sample size limited definitive conclusions but the results indicated that the coronal thirds of the roots showed better smear layer removal than the apical thirds, Sodium hypochlorite followed by EDTA showed the best smear layer removal. The various sequences of NaOCl, anolyte solution, and EDTA all had similar antibacterial results.

ACKNOWLEDGEMENTS

I would like to thank the following persons and departments without whose help this research report would not have been possible:

Dr F.S. Botha: Co Supervisor, BSc (Hons) MSc (PU for CHO) PhD (Pret)

Prof. C.P. Owen: Co Supervisor Head of Dept. Prosthodontics, BDS, MScDent, MChD, FICD, FCD(SA)

Dr. S. Tootla: Supervisor, BDS, MSc Dent

Mr. D. van Zyl: Statistician, UNISA

Laboratory for Microscopy and Microanalysis: University of Pretoria

Radical Waters: South Africa

CONTENTS

ABSTRACT ..... **III**

ACKNOWLEDGEMENTS ..... **V**

1. CHAPTER 1 LITERATURE REVIEW **1**

1.1. INTRODUCTION 1

1.2. TOXICITY OF IRRIGANTS 4

1.3. MICROBIOLOGY 6

1.3.1. Caries and Microbial invasion of the Root Canal 7

1.3.2. Microorganisms in Failed Endodontic Treatments 9

1.3.3. Enterococcus faecalis 11

1.3.3.1. Characteristics 11

1.3.3.2. Virulence Factors 12

1.3.3.3. Prevalence in secondary infections 12

1.3.3.4. Eradication of *E. faecalis* 13

1.4. THE SMEAR LAYER CONTROVERSY 14

1.4.1. Removing the Smear Layer 14

1.4.2. Maintaining the Smear Layer 14

1.5. CHEMICAL IRRIGATION 15

1.5.1. Sodium Hypochlorite (NaOCl) 15

1.5.2. EDTA 16

1.5.3. Chlorhexidine 17

1.5.4. Electrochemically Activated Water 19

1.6. CONCLUSION 21

2. CHAPTER 2: AIMS AND OBJECTIVES **23**

2.1. AIM 23

2.2. OBJECTIVES 23

2.3. HYPOTHESIS 24

3. CHAPTER 3: MATERIALS AND METHODS 25

3.1. MATERIALS USED 25

3.2. INCLUSION/EXCLUSION CRITERIA 28

3.3. PREPARATION OF SPECIMENS 29

3.4. LABORATORY PROCEDURES 30

3.4.1. Preparation and sterilisation of teeth 30

3.4.2. Microbiological analysis 30

3.4.3. Irrigation of specimens 32

3.4.4. Scanning Electron Microscopy (SEM) Preparation and Evaluation 33

4. CHAPTER 4: RESULTS 37

4.1. SMEAR LAYER REMOVAL 37

4.1.1. Intra-Group Comparisons 37

4.1.2. Inter-Group Comparisons 51

4.2. ANTIBACTERIAL RESULTS 53

5. CHAPTER 5 DISCUSSION 57

5.1 LIMITATIONS OF THE STUDY 61

5.2 CONCLUSIONS AND RECOMMENDATIONS 64

5.2.1 Summary and Conclusions 64

5.2.2 Recommendations 65

APPENDICES 66

APPENDIX A: ETHICS APPROVAL 66

APPENDIX B: PREPARATION OF ROOTS FOR SEM 67

APPENDIX C: PROTOCOL APPROVAL 68

REFERENCES 69

LIST OF FIGURES

3.1. Inclusion/Exclusion Criteria 28

3.2. Inclusion/Exclusion Criteria 28

3.3. Inclusion/Exclusion Criteria 28

3.4. Inclusion/Exclusion Criteria 28

3.5. SEM photomicrograph 34

4.1. SEM photomicrograph Group 1 coronal 38

4.2. SEM photomicrograph Group 2 middle 39

4.3. SEM photomicrograph Group 3 coronal 41

4.4. SEM photomicrograph Group 3 middle 41

4.5. SEM photomicrograph Group 3 apical 42

4.6. SEM photomicrograph Group 4 coronal 43

4.7. SEM photomicrograph Group 4 apical 44

4.8. SEM photomicrograph Group 5 coronal 45

4.9. SEM photomicrograph Group 5 middle 46

4.10. SEM photomicrograph Group 5 apical 47

4.11. SEM photomicrograph Group 6 coronal 48

4.12. SEM photomicrograph Group 6 middle 49

4.13. SEM photomicrograph Group 6 apical 50

4.14. SEM photomicrograph Group 6 apical 50

4.15. Intra/Inter-group comparison mean no. of patent dentinal tubules 52

4.16. Inter-group comparison of antibacterial results 55

LIST OF TABLES

3.1. Irrigation protocols 32

4.1. Intra-group comparison Group 1 37

4.2. Intra-group comparison Group 2 38

4.3. Intra-group comparison Group 3 40

4.4. Intra-group comparison Group 4 42

4.5. Intra-group comparison Group 5 44

4.6. Intra-group comparison Group 6 47

4.7. Inter-group comparison coronal and middle thirds 51

4.8. Intra-group comparison antibacterial results 54

4.9. Inter-group antibacterial differences 56

1. CHAPTER 1 LITERATURE REVIEW

1.1. Introduction

Endodontic treatment aims at eliminating microorganisms from the infected root canal system and restoring the tooth to its correct form and function. It consists of a diagnostic phase, access preparation, chemomechanical preparation, obturation and restoration (Walton and Torabinejad, 2002).

The diagnostic phase aims at determining the condition of the pulp and periradicular tissues. The diagnosis will determine the treatment options and ultimate treatment plan. To arrive at a proper diagnosis the clinician considers the main complaint, medical and dental history, clinical signs and symptoms and radiographic signs (Baumann and Beer, 2010).

Subsequent to diagnosis the clinician must gain access to the pulpal space. Access preparation aims to remove caries, conserve tooth structure as far as possible, adequately unroof the pulpal chamber, remove the vital and necrotic coronal pulp tissue, locate the canal orifices, gain straight line access to the pulp and establish the margins of the restoration such that marginal leakage will later be minimised (Walton and Torabinejad, 2002; Hargreaves and Cohen, 2011).

Upon completing access preparation the working length of the root canal/s is determined. This includes determining the location of the apex by using radiographs, electronic apex locators or tactile methods. The working length extends from the occlusal or incisal reference point to the apical reference point, the apical constriction which is the narrowest part of the entire canal. The apical constriction is between 0.5mm and 1.5mm from the apical foramen (Kuttler, 1955; Hargreaves and Cohen, 2011).

Once the working length of the canal has been determined the clinician may start the cleaning and shaping procedure. A glide path is made using hand files (West, 2010). The canal space is debrided such that potential irritants are mechanically reduced (Walton and Torabinejad, 2002). Shaping the canal entails tapering it such that it has an even form from the coronal to the apical point of the canal (Schilder, 1974). This facilitates obturation (Young, Parashos and Messer, 2007). Using hand and rotary files, dentine is removed in a uniform layer in all dimensions as far as possible (Walton and Torabinejad, 2002).

This mechanical preparation of the canals leads to the formation of a smear layer that consists of remnants of pulpal tissues, microorganisms and debris (McComb and Smith, 1975; Ballal *et al.*, 2009). The smear layer is acid labile and dissolves in a pH range of 6-6.8 (Pashley, 1990). It is soluble in fluid, fragile and has a poor attachment to dentine

(McComb and Smith, 1975; Mader, Baumgartner and Peters 1984; Clark-Holke *et al.*, 2003). Although mechanical debridement removes a large portion of necrotic or infected tissues and microorganisms within the canal space, chemical irrigation is necessary due to mechanical complexities and the inability of mechanical preparation to disinfect the canal space (Byström and Sundqvist, 1983; Ferraz *et al.*, 2001).

Masterton (1965) stated that chemical irrigation is necessary to aid in removal of residue, tissue remnants and remaining bacteria especially in areas that are otherwise difficult to access by mechanical means (as cited in Baker *et al.*, 1975, Byström and Sunqvist, 1981, Byström and Sundqvist, 1983). Irrigants aid in removing the smear layer (McComb and Smith 1975). Different irrigants possess different properties such as antibacterial, tissue dissolution, lubrication etc. that aid in the chemomechanical preparation of the canal.

Once the canal has been cleaned and shaped it is obturated. The objective of obturation is to create a complete seal along the entire length of the canal from the coronal to the apical extent (Schilder, 1974; Hammad, Qualtrough and Silikas, 2009). Historically obturation has been achieved with the use of gutta percha and an endodontic sealer (Michaud *et al.*, 2008). By creating a fluid-tight seal ingress of bacteria and their toxins can be prevented from reaching the periradicular tissues (Molander *et al.*, 1998; Michaud *et al.*, 2008; Özok *et al.*, 2008). When

there is an area within the canal space that is not adequately filled by the obturating material a space is created where bacteria can proliferate and migrate to the periradicular tissues leading to a secondary pathosis (Molander *et al.*, 1998; Dahlén and Möller, 1992 cited by van der Sluis, Wu and Wesselink, 2005).

Careful restoration of an endodontically treated tooth is essential for longevity of the tooth (Walton and Torabinejad, 2002). Coronal leakage may ultimately lead to failure of the root canal treatment (Saunders and Saunders, 1994). Oral fluids containing bacteria may penetrate an inadequately restored tooth (Swanson and Madison, 1987; Torabinejad *et al.*, 1990). A final restoration needs to be placed soon after the endodontic treatment in order to restore function and aesthetics, provide a coronal seal, prevent microleakage and protect the remaining tooth structure (Barthel *et al.*, 2001; Walton and Torabinejad, 2002).

1.2. Toxicity of Irrigants

The ideal root canal irrigant must be able to decontaminate the dentine and dentinal tubules and dissolve pulpal tissues and remnants. It should provide antibacterial substantivity, that is the ability to exert its antibacterial effect long after its application, (White, Hays and Janer, 1997). It should remove the smear layer, be easy to apply and be

relatively inexpensive. It must be neither toxic nor carcinogenic, should not elicit an allergic reaction, and should not discolour the tooth nor affect the sealing ability of filling materials or restorations (Torabinejad *et al*., 2002; Zehnder, 2006).

The periradicular tissues must be respected during endodontic therapy. Mechanical preparation, chemical irrigants, sealers and obturating materials can potentially cause an inflammatory response in the periodontium. Toxic irrigants and chemicals may gain access to the periodontium through lateral canals, the apex or perforations from procedural errors (Becker, Cohen and Borer, 1974; Gatot *et al*., 1991; Pappen *et al*. 2009; Hülsmann and Hahn, 2000; Grossman, 1978). For example, sodium hypochlorite extruded into the periodontal tissues has been shown lead to a rapid and diffuse haemorrhagic episode (Gatot *et al*., 1991)

Although Ethylenediaminetetraacetic acid (EDTA) and MTAD (mixture of tetracycline, acid and detergent) have been shown to have increased the proinflammatory response of murine macrophages by increasing the release of nitric oxide, there are no reported complications of EDTA extrusion into the periapical tissues. This may be due to the short term use of EDTA in endodontic treatment. (Pappen *et al*., 2009).

Shraer and Legchillo (1989) showed that electrochemically activated water was not toxic to biological tissues (as cited in Solovyeva and Dummer, 2000).

Chlorhexidine digluconate disrupts the cell membranes of neutrophils and crevicular cells at concentrations above 0.005% within five minutes (Agarwal *et al.*, 1997). It has produced mild inflammation in guinea pig subcutaneous tissue within two days. This was followed by a foreign body granuloma after two weeks (Yesilsoy *et al.*, 1995). In human gingival cells the toxicity of chlorhexidine was dependant on the length of exposure and the composition of the medium (Babich et al 1995). Despite all this chlorhexidine has an acceptable biocompatibility in concentrations used clinically. (Mohammadi and Abbott, 2009).

1.3. Microbiology

The major cause of pulpal and periapical infection and inflammation is bacteria (Kakehashi, Stanley and Fitzgerald, 1965; Hahn and Liewehr, 2007). These bacteria can gain access to the pulp in a number of ways including extension of a carious lesion and cavity preparation (Gomes *et al.*, 2004). Those bacteria that are able to colonize and invade the dentinal tubules are influenced by various factors including nutrition, temperature, pH, microbial interactions, host defences and the virulence of the bacteria themselves (Sundqvist, 1992; Gomes, Lilley and

Drucker, 1996). There is a progression of bacterial species in shallow caries, deep caries and periapical pathosis (Duchin and van Houte, 1978; Hoshino, 1985; Milnes and Bowden, 1985). Furthermore a correlation between clinical features and bacterial species has been substantiated (Yoshida *et al.*, 1987).

1.3.1. Caries and Microbial invasion of the Root Canal

The microorganisms involved in a carious lesion are complex and diverse (van Houte, 1994; Becker *et al.*, 2002; Chhour *et al.*, 2004). The oral environment, salivary composition, diet and the age of the carious lesion affect its composition (Johansson *et al.*, 1985; Margolis, Duckworth and Moreno, 1988; Hahn and Liewehr, 2007). A carious lesion may initially contain *Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus* species (Loesche and Syed, 1973; Duchin and van Houte, 1978). These bacteria are acid tolerant (Hahn and Liewehr, 2007) and are able to invade host dentinal tubules, binding to collagen using surface antigen polypeptides (Love, McMillan and Jenkinson, 1997). This invasion causes demineralization and elicits host release of matrix metalloproteinases that promote caries progression (Tjäderhane *et al.*, 1998).

As the lesion progresses there is an evolution from facultative Gram-positive bacteria to lactobacilli and anaerobes which may be due to the oxygen poor environment in deeper dentine (Edwardsson 1974 cited in Hoshino, 1985; Hoshino, 1985). Bacteria such as streptococci are unable

to survive in deep lesions without salivary substrate such as salivary glycoproteins. Proteolytic bacteria dominate over saccharolytic bacteria due to serum-like substrate diffusing from the pulp. (Steinman, Leonora and Singh, 1980; Takahashi *et al.*, 1997; Hahn and Liewehr, 2007)

Due to the fact that *S. mutans* is the predominant bacterium isolated from shallow carious lesions it is thought to be the primary cause of caries (Bowden, 1990; van Houte, 1994; Love and Jenkinson, 2002). Although more *Lactobacillus* species are isolated from deeper carious lesions, there are deep carious lesions with both low and high counts (Hahn, Falkler and Minah, 1991). In low lactobacilli counts there appear to be large variations in the predominant bacterium recovered, varying from Gram-positive non lactobacilli, Gram-positive cocci or *Prevotella intermedia.* (Hahn *et al.*, 1991; Hahn and Liewehr, 2007).

Pulpal inflammation can be elicited from the diffusion of soluble microbial products such as endotoxin or cell walls (Bergenholtz and Lindhe, 1975; Nissan, Segal and Pashley, 1995). Lactic acid is the predominant metabolite recovered from active caries (Hojo *et al.*, 1994).

Carbohydrate fermentation leads to the formation of organic acids within the carious lesion. These acids do not elicit a pain response as the A delta fibres are not stimulated (Panopoulos, Mejare and Edwall, 1983). This may in part be due to the release of calcium and hydrogen

ions from demineralized dentine in the acidic environment (Olgart, Haegerstam and Edwall, 1974; Orchardson, 1978; Hahn and Liewehr, 2007). Carious lesions with high lactobacilli counts are often not sensitive to thermal stimuli (Hahn, Falkler and Minah, 1993).

Pain inducing molecules including ammonia, urea and indole, are produced from protein fermentation (Panopoulos, 1992 cited in Hahn and Liewehr, 2007). Many anaerobic bacteria such as *Fusobacterium nucleatum, Prevotella intermedia* and *Porphyromonas gingivalis* ferment amino acids where the carbohydrate substrate is low (Takahashi *et al.*, 1997). Pain is closely linked to the recovery of these organisms from carious lesions (Massey *et al.*, 1993)

When sucrose is available for fermentation Gram-positive bacteria such as streptococci produce lipoteichoic acid. Lipoteichoic acid is able to diffuse pulpally and elicit an inflammatory response (Rølla *et al.*, 1980, Hahn and Liewehr, 2007).

1.3.2. Microorganisms in Failed Endodontic Treatments

Bacteria responsible for secondary endodontic infections may have remained from the primary infection or gained access to the root canal system after obturation and restoration (Byström *et al.*, 1987; Siqueira and Rôças, 2008).

In primary endodontic infections the predominating bacteria are Gram-negative anaerobic rods. In secondary infection there are few bacterial species, mostly Gram-positive with little predominance of facultatives and anaerobes (Siqueira, 2001).

Enterococcus faecalis is the bacterium commonly associated with persisting endodontic disease and in secondary infections (Sundqvist *et al.*, 1998). However, this bacterium is rarely found in primary (untreated) endodontic infections (Rôças, Siqueira and Santos, 2004). Bacteria that persist in root canals despite chemomechanical treatment must be able to adapt to the different intracanal environment that exists after root canal treatment (Siqueira, 2001). In order to do this they must adhere to the walls of the canal system, penetrate dentinal tubules, accumulate and form biofilms (Ramachandran Nair, 1987; Distel, Hatton and Gillespie, 2002; Nair *et al.*, 2005; Siqueira and Rôças, 2008).

Bacteria remaining in the canals may penetrate the dentinal tubules as deep as 150µm in the apical two thirds of the canal and up to 400µm in the rest of the canal (Haapasalo and Ørstavik, 1987; Sen, Piskin and Demirci, 1995)

In order to survive root canal treatment these bacteria must be able to adapt to an environment low in substrate (Siqueira, 2001). Usually bacteria remaining in dentinal tubules will become entombed in the

tubules if the sealer is able to penetrate the tubules after the smear layer has been removed (Siqueira, 2001; Kokkas *et al.*, 2004). The entombed bacteria die or are prevented from reaching the apical regions (Siqueira, 2001). However, some bacteria have the ability to survive on a low substrate for long periods of time. Bacteria may survive on tissue remnants or fluids that seep through due to coronal leakage (Siqueira, 2001).

1.3.3. Enterococcus faecalis

1.3.3.1. Characteristics

Thiercelin and Jouhaud (1903) showed that *E. faecalis* is a facultative anaerobe, able to survive with or without oxygen, a Gram-positive coccus and can be found singly, paired or in short chains (Schleifer and Kippler-Bälz, 1984). It is a natural inhabitant in the human intestines and female genital tracts where it is present in large quantities. It is also found naturally in the oral cavity but to a lesser extent (Gilmore, 2002; Sedgley, Lennan and Clewell, 2004). It is able to catabolize various sources of energy e.g. carbohydrates, glycerol, lactate, malate, arginine, citrate, agmatine and alpha keto acids (Andrewes and Horder, 1906 cited in Schleifer and Kippler Bälz, 1984). It can survive harsh environments in the presence of antibacterial medicaments (Molander *et al.*, 1999). *E. faecalis* is resistant to bile salts, heavy metals, detergents, ethanol alcohol, azide and dehydration. It can multiply in temperatures

between 10º C and 45º C and can survive at up to 60ºC (Andrewes and Horder, 1906 cited in Schleifer and Kippler-Bälz, 1984; Thiercelin and Jouhaud, 1903 cited in Schleifer and Kippler-Bälz, 1984; Gilmore, 2002).

1.3.3.2. Virulence Factors

E. faecalis is able to survive harsh environments due to its high virulence. Within the root canal system it can bind to dentine as it possesses serine protease, gelatinases and collagen binding protein (Hubble *et al.*, 2003). Penetration of the dentinal tubules is possible due to its small size (Stuart *et al.*, 2006). It can survive long periods with no substrate and recover from starvation using serum from periradicular tissues as a substrate (Love, 2001; Figdor, Davies and Sundqvist, 2003). Once part of a biofilm *E. faecalis* is even more resistant to antimicrobial action (Distel *et al.*, 2002).

1.3.3.3. Prevalence in secondary infections

Endodontically treated teeth with radicular lesions have demonstrated a 24% to 77% prevalence of this bacterium (Engström, 1964 cited in Molander *et al.*, 1998; Molander *et al.*, 1998; Siqueira and Rôças, 2003; Rôças *et al.*, 2004; Siqueira and Rôças, 2004). The wide range of prevalence may be due to culturing as a detection technique (Williams *et al*, 2006). Polymerase chain reaction is more accurate for *E. faecalis* detection, and where PCR has been used to detect *E. faecalis* in

secondary endodontic infections, the prevalence was 67% (Siqueira and Rôças, 2003; Rôças *et al.*, 2004).

1.3.3.4. Eradication of *E. faecalis*

E. faecalis gains access to the root canal space before, during and after endodontic treatment (Rôças *et al.*, 2004).

Better access to the apical portion of the root canal can be gained by preparing this area to a larger master apical file such that irrigants and medicaments can more adequately penetrate the dentinal tubules here. Simultaneously, the infected innermost dentine in the apical portion will be mechanically removed (Card *et al.*, 2002; Stuart *et al.*, 2006). Clegg *et al.*, (2006) found that 6% sodium hypochlorite was the only concentration that was able to remove the biofilm and render the bacteria nonviable. EDTA, although not antibacterial, is important for removal of inorganic matter in the root canal system (Haapasalo *et al.*, 2010). MTAD has shown marked success in the elimination of *E. faecalis* (Torabinejad *et al.*, 2003). Two percent Chlorhexidine used for two minutes can eliminate *E. faecalis* in dentinal tubules up to 100 micrometres (Vahdaty, Pitt Ford and Wilson, 1993).

1.4. The Smear Layer Controversy

1.4.1. Removing the Smear Layer

The smear layer must be removed as it may act as a substrate for remaining bacteria, limit the penetration of obturation materials into dentinal tubules and dissolve due to coronal leakage, opening a pathway for any remaining bacteria to reach the apical regions of the root (White, Goldman and Lin, 1984; Behrend, Cutler and Gutmann, 1996; Vivacqua-Gomes *et al.*, 2002; Clark-Holke *et al.*, 2003). Bacteria can also degrade the smear layer with enzymes that destroy collagen instead of hydroxyapatite (Pashley, 1990). Due to this degradation the smear layer is vulnerable to penetration by bacteria (Behrend *et al.*, 1996; Vivacqua-Gomes *et al.*, 2002).

Smear layer removal improves the seal of root canal filling materials, decreases the coronal leakage, reduces microleakage and improves the mechanical retention of the filling material to dentine (White *et al.*, 1984; Okşan *et al.*, 1993; Behrend *et al.*, 1996). Smear layer removal has also been shown to increase the adaptation of sealer to dentine (Clark-Holke *et al.*, 2003).

1.4.2. Maintaining the Smear Layer

Whilst retaining the smear layer has been shown to block the movement of bacteria (Drake *et al.*, 1994) this blockage is only a delay in bacterial

penetration of the dentinal tubules since the smear layer is not complete (Williams and Goldman, 1985). A bond may form between the smear layer and any sealer instead of the dentine and the sealer (Pashley *et al.*, 1988; Saleh *et al.*, 2003). Since the smear layer has poor adhesion to dentine it could be soluble in fluids and this would increase the chance of microleakage (Clark-Holke *et al.*, 2003). Furthermore it has been shown that where the smear layer remained *Fusobacterium nucleatum, Campylobacter rectus and Peptostreptococcus micros* could be cultivated (Clark-Holke *et al.*, 2003). It would therefore be reasonable to conclude that the smear layer should be removed.

1.5. Chemical Irrigation

1.5.1. Sodium Hypochlorite (NaOCl)

Sodium hypochlorite is a common endodontic irrigant with properties such as the ability to dissolve tissues and exert an antibacterial effect (Shih, Marshall and Rosen, 1970; Moorer and Wesselink, 1982). There appears to be some controversy over the concentration of sodium hypochlorite to be used in endodontic irrigation.

In the presence of dentine and organic matter the effect of sodium hypochlorite is weakened, as tests have shown that it was effective at lower concentrations where there was no organic matter present. However, in the clinical situation lower concentrations are not really as antibacterial as the tests suggest (Haapasalo *et al.*, 2000; Haapasalo *et*

al., 2010). A 0.5% solution of sodium hypochlorite was shown to dissolve necrotic tissues. Higher concentrations dissolve necrotic and vital tissues. A solution of 6% sodium hypochlorite was shown to be more effective than 2% chlorhexidine against microorganisms tested (Zehnder *et al.*, 2002; Carson, Goodell, McClanahan, 2005; Hargreaves and Cohen, 2011).

The addition of surface active agents that decrease the surface tension of sodium hypochlorite leads to an increase in dentinal tubule penetration. These detergents also increase the speed at which sodium hypochlorite is able to dissolve tissues. An example of such a combination product is Chlor-Xtra (Inter-Med. Inc., Racine, WI, USA) (Cameron, 1986; Haapasalo *et al.*, 2010).

The disadvantages of sodium hypochlorite are that it is toxic to tissues; it has been shown to reduce polymerization of resin sealers such as Epiphany (Sybron Endo, Orange, CA, USA) (Nunes *et al.*, 2008); it is unable to remove the smear layer alone; and it is corrosive to endodontic instruments (McComb and Smith, 1975; Neal, Craig and Powers, 1983; Marais and Williams, 2001; Gernhardt *et al.*, 2004).

1.5.2. EDTA

EDTA has a chelating action that assists in creating smear free dentine by dissolving mineralized tissues (Ciucchi. Khettabi and Holz, 1989). In

order to achieve the maximum cleaning effect in canals, alternating the use of a chelating agent such as EDTA with a tissue solvent such as sodium hypochlorite is necessary (Yamada *et al.*, 1983). However, sodium hypochlorite followed by EDTA was not capable of removing the smear layer in all thirds of the canal (Lim *et al.*, 2003). Furthermore prolonged use of sodium hypochlorite followed by EDTA leads to dentinal erosion and a subsequent decrease in flexural strength (Mai *et al.*, 2010).

EDTA used for 1-10 minutes has been shown to remove the smear layer (Calt and Serper, 2000; Scelza *et al.*, 2004). Radicular dentine exposed to 17 % EDTA for more than 1 minute caused erosion of peritubular and intertubular radicular dentine (Calt and Serper, 2002).

1.5.3. Chlorhexidine

Chlorhexidine has been proposed as an alternative irrigant for sodium hypochlorite as it has proven to have the same antimicrobial effect (Jeansonne and White, 1994; Yesilsoy *et al.*, 1995). Furthermore, chlorhexidine used as an endodontic irrigant has the advantage of substantivity (White, Hays and Janer, 1997). Substantivity of chlorhexidine has been seen up to 12 weeks after its application and appears to be related to its concentration and application time (Rosenthal, Spånberg and Safavi, 2004). Chlorhexidine used as an irrigant does not appear to adversely affect the apical seal of

endodontically treated teeth (Ferguson, Marley and Hartwell, 2003). Two percent chlorhexidine is bactericidal resulting in the death of various microorganisms (Gomes *et al*, 2003). Medicaments containing chlorhexidine have eradicated 1-3 day old *E. faecalis* (Lima, Fava and Siqueira, 2001). Two percent chlorhexidine gel seems to have a greater effect against this resilient organism (Ferraz *et al.*, 2001).

Despite these positive attributes, chlorhexidine cannot disrupt the biofilm and unlike sodium hypochlorite, it is unable to dissolve tissues (Clegg *et al.*, 2006, Okino *et al.*, 2004). As a result it has been suggested that chlorhexidine be used as a final irrigant after EDTA and sodium hypochlorite (Kuruvilla and Kamath, 1998).

Sodium hypochlorite in combination with chlorhexidine however, produces a brown precipitate which cannot be removed completely from the canals and reduces the seal of root fillings (Vivacqua-Gomes *et al.*, 2002). Although this precipitate is carcinogenic it is present in insignificant amounts. Chlorhexidine followed by EDTA produces a pink precipitate that is carcinogenic and blocks the dentinal tubules (González-López *et al.*, 2006, Bui, Baumgartner and Mitchell, 2008; van der Bijl, Gelderblom and Thiel, 1984).

1.5.4. Electrochemically Activated Water

Electrochemically activated water has been suggested as a replacement for sodium hypochlorite as an endodontic irrigant (Solovyeva and Dummer, 2000). Electrochemically activated water, also known as super-oxidized water, is produced by passing an electrical current through a saline solution (Selkon, Babb and Moriss, 1999). More than 300 Russian patents exist with this technology although the Western scientific literature is scant (Marais, 2000a; Marais and Williams, 2001). The unit to produce the electrochemically activated water according to the Russian technology consists of a flow-through electrolytic module containing an anode and a cathode separated by a diaphragm (Solovyeva and Dummer 2000). This allows two distinct solutions to be produced, an anolyte and a catholyte. The solutions exist in a metastable state and contain free radicals, various ions and molecules (Solovyeva and Dummer, 2000; Marais and Williams, 2001). The anolyte has a high oxidation potential and can be produced at neutral, alkaline or acidic pH. It is also known as super-oxidized water (Selkon *et al.*, 1999; Solovyeva and Dummer, 2000; Jaju and Jaju, 2011). The anolyte solution has been shown to be antimicrobial (Bakhir *et al.*, 1999 cited in Solovyeva and Dummer 2000; Hotta *et al.*, 1994). The catholyte is an alkaline solution with a high reduction potential that is claimed to have a strong detergent or cleaning effect (Prilutskii and Bakhir 1997 cited in Solovyeva and Dummer, 2000). Both solutions remain in the metastable state for up to 48 hours, thereafter becoming

inactive again (Marais and Williams, 2001). When comparing the root canal cleaning efficacy of electrochemically activated water and sodium hypochlorite it was found that electrochemically activated water removed the smear layer in large areas and even exposed collagen fibres and fibrils more so than sodium hypochlorite. The anolyte solution had a neutral pH and the catholyte a pH of 9.8. Both solutions were produced by STEDS, Radical Waters in Johannesburg according to the Russian technology. The canals were rinsed first with 75ml of anolyte delivered via an ultrasonic unit for 10 seconds between files, then with a final rinse of 75ml of catholyte for a further 30 seconds (Marais, 2000b). Electrochemically activated water leaves a thinner smear layer and results in more open dentinal tubules in the apical and coronal thirds in comparison to sodium hypochlorite (Solovyeva and Dummer, 2000). Electrochemically activated water is not as antibacterial as sodium hypochlorite alone (Rossi-Fedele *et al*, 2010). It has been recommended that canals be cleaned and disinfected by using anolyte in conjunction with a detergent such as catholyte (Marais and Williams, 2001).

A similar irrigant Aquatine EC (Sterilox Puricore, Malvern, PA, USA) showed smear layer removal when used with a final rinse of 17 % EDTA. Aquatine EC is a low concentration saline solution that has been electrochemically charged. This procedure produces hypochlorous acid. Hypochlorous acid is also produced by the body's immune system in the

Oxidative Burst Pathway as a defence against any invading organisms. The FDA approved this product as an endodontic irrigant in 2006 (Garcia *et al.*, 2010).

1.6. Conclusion

Based on the literature sodium hypochlorite has many positive attributes as an endodontic irrigant but its negative characteristics warrant the search for a replacement irrigant (Shih *et al.*, 1970; McComb and Smith 1975)

Sodium hypochlorite followed by EDTA does not produce complete smear layer removal (Lim *et al.*, 2003). Perhaps replacement of sodium hypochlorite with another irrigant will improve the smear layer removal.

Anolyte solution has been suggested as a replacement irrigant for sodium hypochlorite (Solovyeva and Dummer, 2000). It does not produce a smear layer and in fact removes the smear layer and has even exposed collagen fibrils (Marais, 2000b). Anolyte solution, however, is not as antibacterial as sodium hypochlorite (Marais and Williams, 2001).

Chlorhexidine does have the same antibacterial activity as sodium hypochlorite but when followed with sodium hypochlorite and /or EDTA

it produces a precipitate which has been shown to block the dentinal tubules and is carcinogenic (van der Bijl *et al.*, 1984; Vivacqua-Gomes *et al.*, 2002; González-López *et al.*, 2006; Bui *et al.*, 2008).

From the literature it would thus seem reasonable to test further combinations of irrigants for their effect in removing the smear layer of endodontically treated teeth, and in particular, sodium hypochlorite, anolyte solution and EDTA.

2. CHAPTER 2: AIMS AND OBJECTIVES

2.1. Aim

To test various alternating sequences of sodium hypochlorite, anolyte solution, and EDTA for their ability to remove the mineralised portion of the smear layer, and to destroy bacteria.

2.2. Objectives

1. To test six groups of irrigation protocols for their ability to remove the smear layer of the coronal, middle and apical thirds of the canal as described by scanning electron microscopic evaluation. Group 1 (sterile distilled water) is the negative control and Group 3 (sodium hypochlorite followed by EDTA) is the positive control.

Group 1: Sterile distilled water

Group 2: 6% sodium hypochlorite

Group 3: 6% sodium hypochlorite followed by 18% EDTA

Group 4: 6% sodium hypochlorite followed by anolyte solution

Group 5: 6% sodium hypochlorite followed by anolyte solution followed by 18% EDTA

Group 6: anolyte solution followed by 18% EDTA

2. To compare the antimicrobial efficacy of these irrigant groups against *E. faecalis*, a virulent microorganism associated with endodontic infections. Group 1 (sterile distilled water) is the negative control and Group 2 (sodium hypochlorite) is the positive control.

2.3. Hypothesis

The null hypothesis is that there will be no difference between any of the irrigation protocols in their ability to remove the smear layer and to display antimicrobial activity against *E. faecalis*.

3. CHAPTER 3: MATERIALS AND METHODS

The teeth used for this study were collected from the stores at the Dental Research Institute at University of the Witwatersrand. These teeth were previously collected by Prof. E. S. Grossman for the purpose of dental research. An ethical waiver was issued by the Human Research Ethics Committee of the University of the Witwatersrand for the use of these specimens for this study as well as for the use of *E. faecalis* (Clearance number M050760) (Appendix A).

Sixty single canal human teeth were used. The teeth had been stored in saturated thymol. After collection they were stored in sterile distilled water at room temperature.

3.1. Materials Used

The laboratory materials used were as follows:

- Sterile Ringer's solution (Merck SA (Pty) Ltd., Halfway House, South Africa)
- Casein-peptone Soymeal-peptone Broth (CASO Broth) (Merck SA (Pty) Ltd., Halfway House, South Africa)

- Anaerocult A® (Merck SA (Pty) Ltd., Halfway House, South Africa)

The Scanning Electron Microscope materials were as follows:

- 2% gluteraldehyde (Electron Microscopy Sciences, Washington DC, USA)
- Phosphate buffer (PBS – Whittaker MA Bioproducts, Walkersville, USA)
- 0.25% Osmium tetroxide (OsO_4) (Merck, Darmstadt, Germany)
- Ethanol (Merck, Darmstadt, Germany)
- Critical Point Dryer (Polaron, Oxford, England)
- Gold Sputter Coater (Polaron E5200, Watford, England)
- Scanning Electron Microscope- SEM (JEOL JSM 840 Scanning Electron Microscope, Tokyo, Japan)

The irrigation solutions used were as follows:

- Sterile distilled water (Aqua Purification B.P, Reitzer Pharmaceuticals, Plettenburg Bay, South Africa)
- EDTA 18% Root Canal Irrigating Solution (Ultradent Products, Inc., South Jordan, Utah, USA.)(Batch no: B5SQW)
- Chlor-XTRA 6 % Sodium Hypochlorite (Inter-Med, Inc., Racine, WI, USA) (Batch no: 2011-0611)

- Electrochemically Activated Water, Anolyte Solution (Radical Waters, Kyalami, JHB, South Africa)

The endodontic instruments and dental materials used were:

- 10, 15, 20, 25 K-hand files (Mani, Inc., Utsunomi Ya, Tochigi, Japan)
- ProTaper nickel titanium rotary files (Dentsply, Maillefer, Baiillaigues, Switzerland)
- Syringe (Ultradent Inc.,USA)
- 27 gauge Endo-EZE 1” Irrigator Tip (Ultradent, Inc., South Jordan, Utah, USA)
- Sterile absorbent paper points (KentDental, Tremblay-en-France, France) (Lot 010508)
- GC Fuji I (GC Corporation, Tokyo, Japan) (Lot 1012141)
- EcoTemp (Ivoclar Vivadent, New York, USA) (Lot J15944)

The software used:

- Excel © (Microsoft)
- Image J Version 1.45 (U.S. National Institutes of Health, Bethesda, Maryland, USA)
- SPSS Version 20 (IBM).

3.2. Inclusion/Exclusion Criteria

Pre-operative radiographs were taken of each tooth specimen. Teeth observed to have root fractures, multiple canals, complicated canal forms and/or pulp stones or calcifications were eliminated from the study. Forty eight teeth were selected from those deemed appropriate after radiographs were taken. Some examples of roots used and excluded are shown in figures 3.1 to 3.4.



Fig 3.1
Lower root included
Upper excluded



Fig 3.2
Both roots included



Fig 3.3
Both roots included



Fig 3.4
Excluded

3.3. Preparation of specimens

The teeth were decoronated at the level of the cementoenamel junction. Thereafter the roots were scaled and cleaned of any deposits using curettes. The canals of each root were explored using a 10 K hand file. The working length was established and recorded by piercing the apex of the canal with the 10 K hand file until it was just visible at the canal apex, and 0.5 mm was subtracted from this length. A glide file path was established using the 10 K hand file and a 15 K hand file. Thereafter the tooth was prepared using ProTaper nickel titanium rotary files in an endodontic hand piece according to the manufacturer's instructions. The canals were prepared using S1 and S2 files, followed by a 20 K hand file, F1 rotary file, 25 K hand file and finally the F2 rotary file. The canals were prepared up to length with S1, S2, F1 and where possible with F2 rotary files. Between each hand and rotary file and as often as necessary the canals were rinsed with sterile distilled water. The apices of the roots were sealed with GC Fuji I (GC Corporation, Tokyo, Japan) and the orifice sealed with EcoTemp (Ivoclar Vivadent, New York, USA) (Lot J15944) material in order to keep the internal environment isolated. The teeth were stored in sterile distilled water at room temperature.

3.4. Laboratory procedures

3.4.1. Preparation and sterilisation of teeth

The teeth were randomly divided into six groups and placed in sterile Ringer's solution for 72 hours. Four groups contained 10 roots each and two groups contained four roots each. The Ringer's solutions were replaced at 24 hour intervals. The roots were sonified three times and were then sterilized at 121 °C for 15 minutes in an autoclave.

In order to maintain sterile conditions the study was conducted in a positive sterile airflow laboratory, working in a laminar flow cabinet, using sterile gloves, masks and instruments.

3.4.2. Microbiological analysis

The 48 roots were placed in sterile bottles containing Casein-peptone Soymeal-peptone Broth (CASO Broth) and anaerobically incubated using Anaerocult A® at 37°C for a period of three days.

Sterile paper points were inserted into the canals then placed onto CASO Agar plates and incubated anaerobically using Anaerocult A® at 37 °C for 72 hours. Negative cultures confirmed that the roots were sterile and did not contain any anaerobic bacteria before the inoculation procedure.

A MacFarland Standard-I suspension (MacFarland, 1907) (8 x 10^8 colony-forming units [CFU]) of *E. faecalis* (ATCC49474) was prepared from cultures on CASO Agar and incubated for 24 hours. A 1% suspension was added to the Broth and incubated anaerobically using Anaerocult A® at 37˚C for three days.

The roots were sampled by inserting sterile paper points in the canals to soak up the inoculated broth from the canals. Each paper point was placed into a vial containing 0.9 ml sterile Ringers solution. This served as a 1:10 dilution in sterile Ringers solution from which serial dilutions were made. One hundred micro litres of each suspension was spread onto CASO- Agar by means of the standardised glass spreading technique to quantify colony forming units of bacteria (Gerhardt *et al.*, 1981).

The following method as described by Bauman (2011) illustrates the plate count method:

a) Serial dilutions: a series of 10-fold dilutions is made
b) Planting: a 0.1ml sample from each dilution is poured onto a plate and spread with a sterile rod.
c) Counting: plates are examined after incubation. Some plates may contain so many colonies that they are too numerous to count (TNTC). The number of CFUs is multiplied by the reciprocal of

the dilution to estimate the concentration of bacteria in the original culture.

3.4.3. Irrigation of specimens

The roots were then irrigated for one minute for each irrigant according to the following protocol:

Table 3.1: Table of irrigation protocols

GROUP	STERILE DISTILLED WATER	6% SODIUM HYPOCHLORITE	ANOLYTE SOLUTION	18% EDTA
1 (4 roots: control)	3ml			
2 (4 roots: control)		3ml		
3 (10 roots: test)		3ml followed by		3ml
4 (10 roots: test)		3ml followed by	5ml	
5 (10 roots: test)		0.5ml followed by	5ml followed by	3ml
6 (10 roots: test)			5ml followed by	3ml

Thereafter the irrigants were rinsed out of the canals with 10 ml of sterile distilled water. The irrigants were all delivered using a syringe (Ultradent Inc.,USA) and 27 gauge Endo-EZE 1” Irrigator Tip (Ultradent, Inc., South Jordan, Utah, USA) within 2 mm of the canal apex.

Sterile paper points were inserted into the canal of a tooth in each group and suspended in 0.9 ml sterile water and serial dilutions were made and plated onto CASO Agar plates in triplicate for one root per group to quantify the CFU of bacteria that survived the irrigation process.

A table was made of the the CFU/ml for each group at each dilution before and after irrigation. The percentage difference was calculated between the CFU before and after irrigation for each group. The percentage differences were compared using the t-test on SPSS Version 20 (IBM). A p-value ≤ 0.05 indicated a significant statistical difference at a 95% confidence interval. The inter-group percentage differences were compared using the One-way ANOVA test. The Tukey HSD or Tamhane test was used for multiple comparisons to establish between which which groups, any differences lay. To determine whether the Tukey HSD or Tamhane test was used the Test of Homogeneity of Variances was completed. If this test revealed a difference > 0.05, the Tukey HSD test was used for multiple comparisons. If the Test of Homogeneity of Variances revealed a difference ≤ 0.05, the Tamhane test was used. Thereafter the roots were stored in sterile distilled water until preparation for Scanning Electron Microscopy.

3.4.4. Scanning Electron Microscopy (SEM) Preparation and Evaluation

The roots were prepared for SEM according to the standard biological methods (see Appendix B).

SEM photomicrographs were randomly taken in the coronal, middle and apical thirds of each section. Due to financial constraints the number of

photomicrographs (n) taken was limited to the minimum necessary to establish statistical results i.e. reject the null hypotheses and agree with results of similar studies. As such n is not always equal within or between groups. Using a random number table the roots that were photomicrographed were selected. Two SEM photomicrographs were taken per third per group. This was done by superimposing a numbered grid over a section and selecting random numbers from a statistical random number table. The selected block was magnified to 2500x. Using Image J software (U.S. National Institutes of Health, Bethesda, Maryland, USA) the open tubules in each photomicrograph were counted and read into the software by two previously calibrated expert individuals independently. Open, partially open and closed tubules were defined before counting and it was decided that partially open tubules and closed tubules were not counted (figure 3.5).

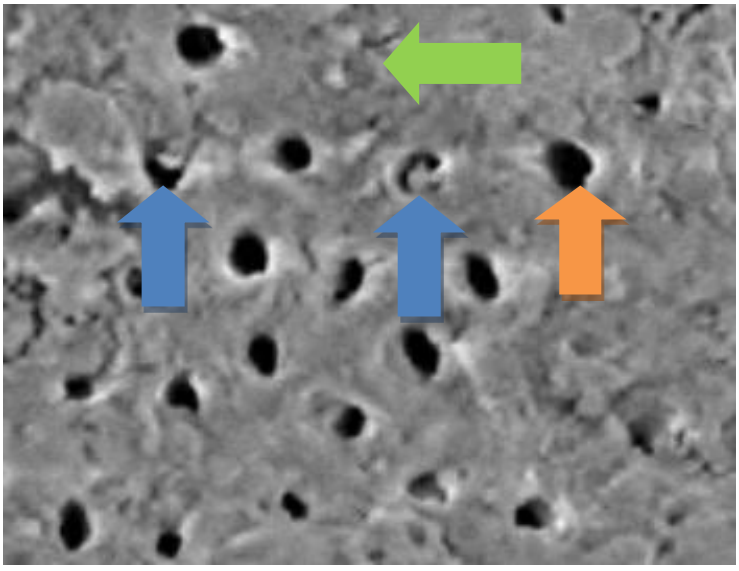

Figure 3.5: Partially occluded tubule (blue arrows), fully patent tubule (orange arrow), and closed tubule (green arrow).

Open tubules were defined as round, with no smear layer or matter overlying the tubule opening. The peritubular dentine must be visualised. Bacteria may be present inside the tubule such that a sealer will entomb it when penetrating the canal. The bacteria may not be covering the opening of the canal. An Excel © (Microsoft) spread sheet was created listing the number of open tubules for each photomicrograph of each third of each root by both expert examiners and compared. A percentage difference was calculated from the number of cells that differed from the total number of cells. After establishing the initial percentage difference the expert examiners re-counted the open tubules in those photomicrographs to determine if the difference was due to a capturing error. Where the expert examiners ultimately differed, the definition of an open tubule was re-emphasised and a consensus was reached after discussion. Further statistical analysis was completed using SPSS Version 20 (IBM). The One-way ANOVA test was used to establish if there was an intra-group and inter-group significant statistical difference. A p-value ≤ 0.05 indicated a significant statistical difference at a 95% confidence interval. The Tukey HSD or Tamhane test was used for multiple comparisons to establish between which thirds or between which groups, any differences lay. To determine whether the Tukey HSD or Tamhane test was used the Test of Homogeneity of Variances was completed. If this test revealed a difference > 0.05, the Tukey HSD test was used for

multiple comparisons. If the Test of Homogeneity of Variances revealed a difference ≤ 0.05, the Tamhane test was used.

4. CHAPTER 4: RESULTS

4.1. Smear Layer Removal

4.1.1. Intra-Group Comparisons

4.1.1.1. Group 1: Sterile Distilled Water

Table 4.1: Results for Group 1(sterile distilled water)

	Mean no. patent tubules	Std. deviation	Std. error	[95% CI] Lower bound-Upper bound	Minimum-Maximum no. of Patent Tubules
Coronal (n=11)	2.82	4.834	1.457	-0.43-6.07	0-14
Middle (n=10)	2.90	4.122	1.303	-0.05-5.85	0-12
Apical (n=12)	0.25	0.622	0.179	-0.14-0.64	0-2
Total	1.91	3.720	0.647	0.59-3.23	0-14

The One-way ANOVA test revealed no significant statistical differences ($p>0.05$).

Examination of the walls of the canals irrigated with sterile distilled water revealed a thick smear layer along the entire length of the canal. Bacteria adhering to the smear layer and dentine could clearly be seen (figure 4.1).

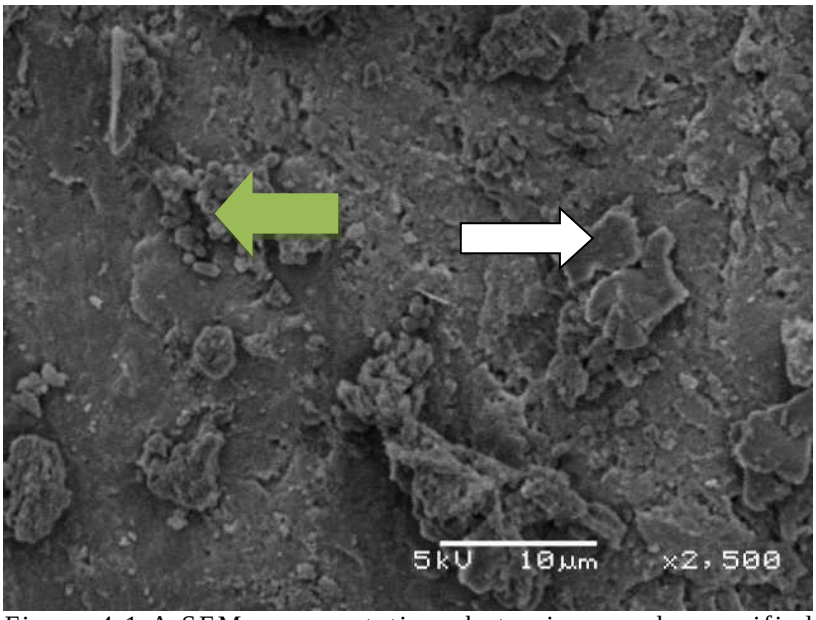

Figure 4.1 A SEM representative photomicrograph magnified to 2500x of the coronal third of a Group 1 root after irrigation with sterile distilled water. An intact smear layer (white arrow) and remaining bacteria (green arrow) can be easily seen.

4.1.1.2. Group 2: 6% sodium hypochlorite

Table 4.2 Results for Group 2 (sodium hypochlorite)

	Mean no. Patent tubules	**Std. deviation**	**Std. error**	**[95% CI] Lower bound-Upper bound**	**Minimum-Maximum no. of Patent Tubules**
Coronal (n=10)	6.60	12.782	4.042	-2.54-15.74	0-42
Middle (n=10)	3.20	3.120	0.987	0.97-5.43	0-8
Apical (n=10)	1.80	4.686	1.482	01.55-05.15	0-15
Total	3.87	8.046	1.469	0.86-6.87	0-42

The One-way ANOVA test revealed no significant statistical differences ($p>0.05$).

Examination of the photomicrographs of the walls of the canals revealed a thick smear layer along the entire length of the canal. The smear layer appeared rough and irregular. Few or no bacteria could be visualized. Few to no dentinal tubules were open in all thirds of the canal (figures 4.2).

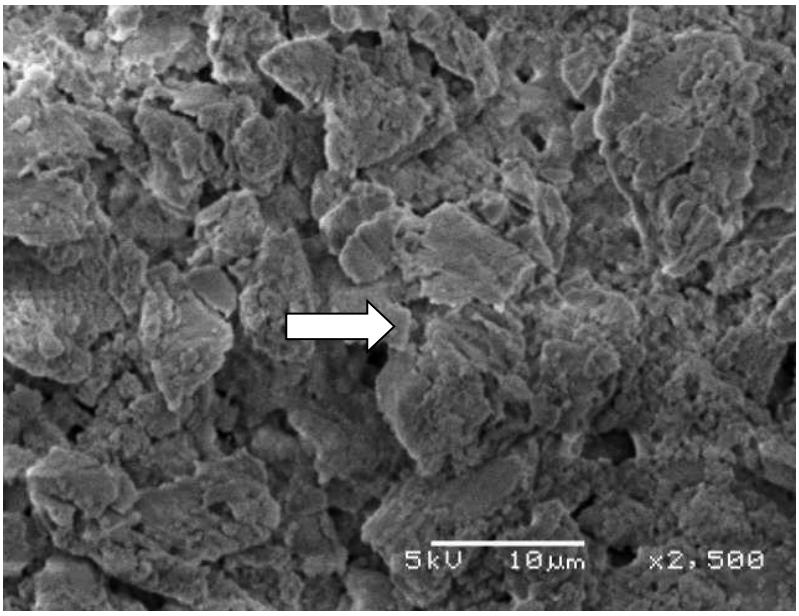

Figure 4.2 A SEM representative photomicrograph magnified to 2500x of the middle third of a Group 2 root after irrigation with 6% sodium hypochlorite. A thick irregular smear layer can be seen.

4.1.1.3. Group 3: 6% sodium hypochlorite followed by 18% EDTA

Table 4.3 Results of Group 3 (sodium hypochlorite followed by EDTA)

	Mean no. Patent tubules	**Std. deviation**	**Std. error**	**[95% CI] Lower bound-Upper bound**	**Minimum-Maximum no. of Patent Tubules**
Coronal (n=12)	9.92	6.829	1.971	5.58-14.26	3-22
Middle (n=12)	9.92	7.103	2.050	5.40-5.86	2-26
Apical (n=12)	2.83	4.764	1.375	-0.19-5.86	0-12
Total	7.56	7.008	1.168	5.18-9.93	0-26

The One-way ANOVA test revealed a statistically significant difference between these results ($p<0.05$). The Tukey HSD test revealed significant differences between the coronal and apical thirds (<0.05) and between the middle and apical levels (<0.05).

The photomicrographs of the walls of the coronal and middle thirds of the canals showed a moderate number dentinal tubules completely open. Most tubules were partially open and thus were not counted. A thin irregular smear layer could still be seen (figures 4.3 and 4.4).

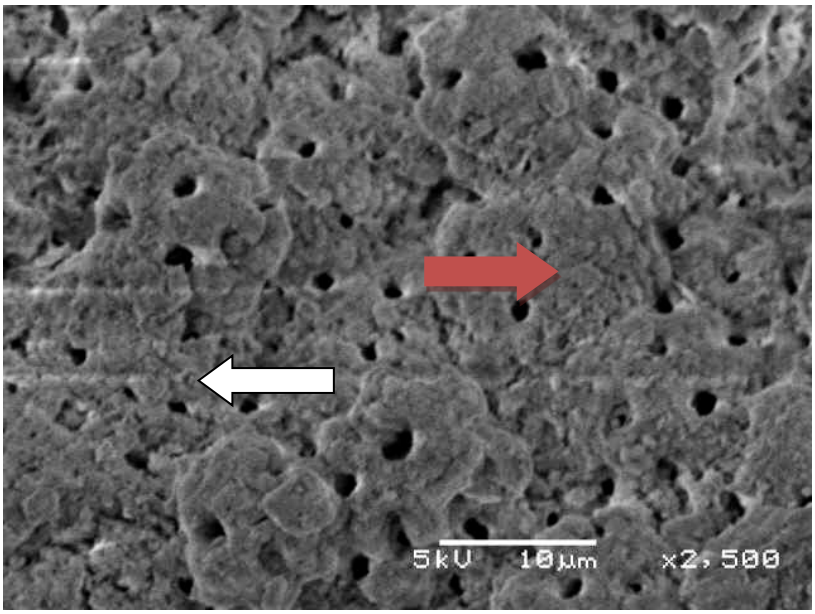

Figure 4.3 A SEM representative photomicrograph magnified to 2500x of the coronal third of a Group 3 root after irrigation with 6% sodium hypochlorite followed by 18% EDTA. Most of the dentinal tubules are partially open (white arrow). There is an irregular smear layer partially or totally covering the dentinal tubules and the intertubular dentin (red arrow).

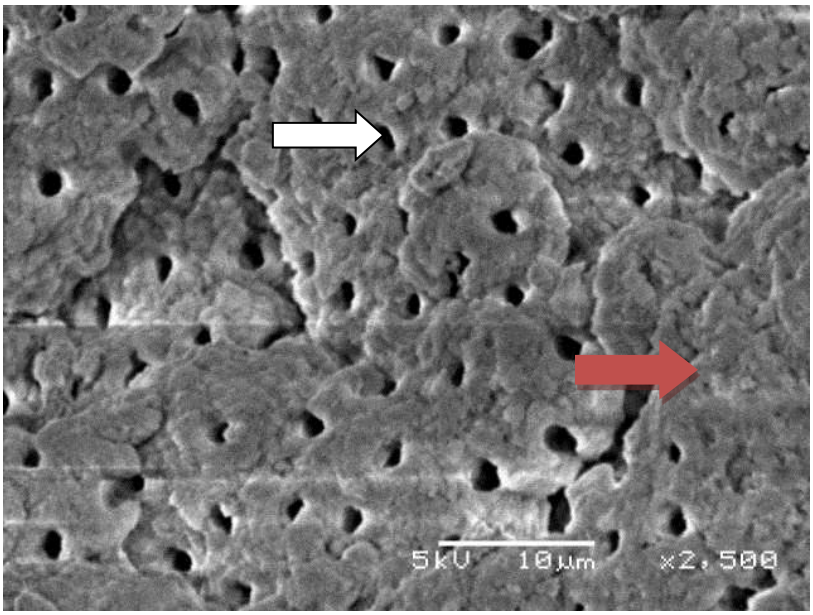

Figure 4.4 A SEM representative photomicrograph magnified to 2500x of the middle third of a Group 3 root after irrigation with 6% sodium hypochlorite followed by 18%EDTA. A regular distribution of open and partially open dentinal tubules can be seen (white arrow). Patches of flat smear layer is seen over a few tubules and on the intertubular dentin (red arrow).

The apical third had a thicker irregular smear layer with very few to no open dentinal tubules (figure 4.5).

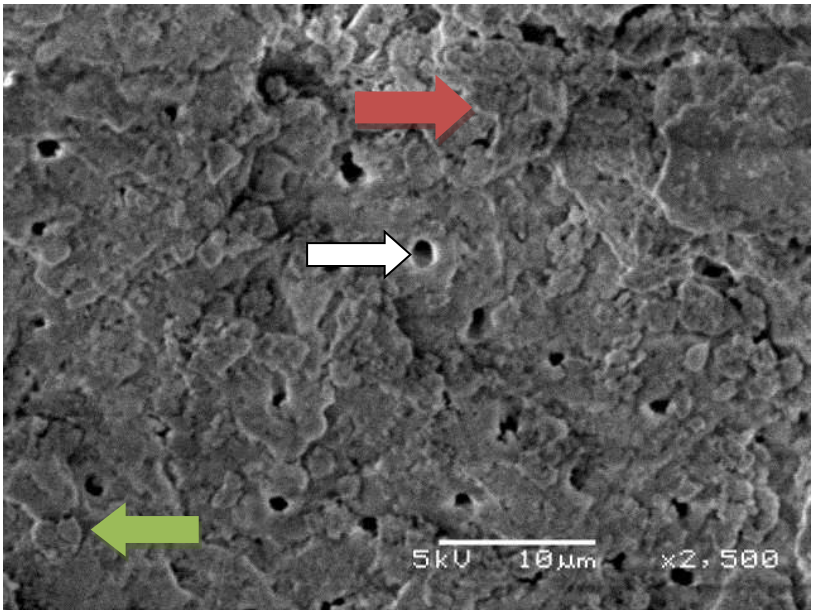

Figure 4.5 A SEM representative photomicrograph magnified to 2500x of the apical third of a Group 3 root after irrigation with 6% sodium hypochlorite followed by 18% EDTA. A few dentinal tubules are completely patent (white arrow). Most are totally covered by an irregular smear layer (red arrow) and bacteria (green arrow).

4.1.1.4. Group 4: 6% sodium hypochlorite followed by anolyte solution

Table 4.4 Results of Group 4 (sodium hypochlorite followed by anolyte solution)

	Mean no. Patent tubules	**Std. deviation**	**Std. error**	**[95% CI] Lower bound-Upper bound**	**Minimum-Maximum no. of Patent Tubules**
Coronal (n=12)	13.75	16.912	4.882	3.00-24.50	0-45
Middle (n=12)	2.83	5.271	1.522	-0.52-6.18	0-18
Apical (n=12)	3.17	4.509	1.302	0.30-6.03	0-14
Total	6.58	11.465	1.911	2.70-10.43	0-45

The One-way ANOVA test revealed a statistical significant difference between these results ($p<0.05$). The Tamhane test failed to reveal where the statistical difference was on multiple comparisons. However, the expert examiners pointed out that there was a marked visual difference observed between the coronal and apical thirds.

Examination of the photomicrographs of the coronal third of the roots in Group 4 revealed areas where there were thick clusters of smear layer covering the dentinal tubules and intertubular dentine, interspersed with areas where the smear layer was completely removed and the dentinal tubules were open (figure 4.6). In the middle third there were less completely open dentinal tubules with greater areas that had a thick smear layer.

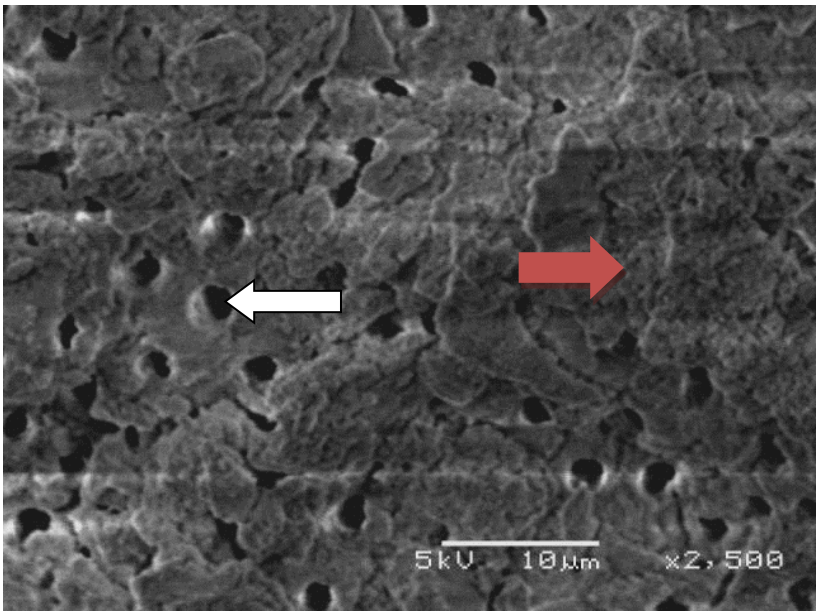

Figure 4.6 A SEM representative photomicrograph magnified to 2500x of the coronal third of a Group 4 root after irrigation with 6% sodium hypochlorite followed by anolyte solution. A moderate number of patent (white arrow) and partially occluded dentinal tubules can be viewed. Most of the intertubular dentin and other dentinal tubules are covered in a thick irregular smear layer (red arrow).

In the apical third there were few to no open dentinal tubules and the smear layer appeared flat and irregular (figure 4.7).

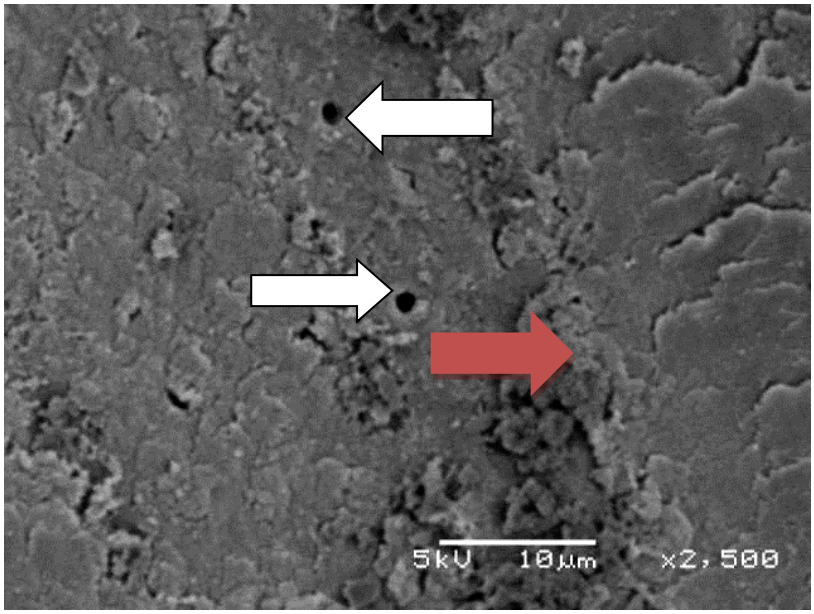

Figure 4.7 A SEM representative photomicrograph magnified to 2500x of the apical third of a Group 4 root after irrigation with 6% sodium hypochlorite followed by anolyte solution. Only two dentinal tubules can be seen (white arrows). The rest of the dentinal tubules and intertubular dentine is covered in a thick flat smear layer (red arrow).

4.1.1.5. Group 5: 6% sodium hypochlorite followed by anolyte Solution followed by 18% EDTA

Table 4.5 Results of Group 5 (sodium hypochlorite followed by anolyte solution followed by EDTA)

	Mean no. Patent tubules	**Std. deviation**	**Std. error**	**[95% CI] Lower bound-Upper bound**	**Minimum-Maximum no. of Patent Tubules**
Coronal (n=18)	18.61	16.881	3.979	10.22-27.01	1-51
Middle (n=16)	12.44	15.718	3.930	4.06-20.81	0-62
Apical (n=16)	6.00	9.223	2.306	1.09-10.91	0-31
Total	12.60	15.101	2.136	8.31-16.89	0-62

The One-way ANOVA test revealed a statistically significant difference between these results ($p<0.05$). The Tamhane test revealed a significant difference between the coronal and apical levels ($p=0.032$).

Examination of the coronal photomicrographs showed regularly distributed open and partially open dentinal tubules. Small clusters of smear layers could be visualised over some tubules and intertubular dentine (figure 4.8).

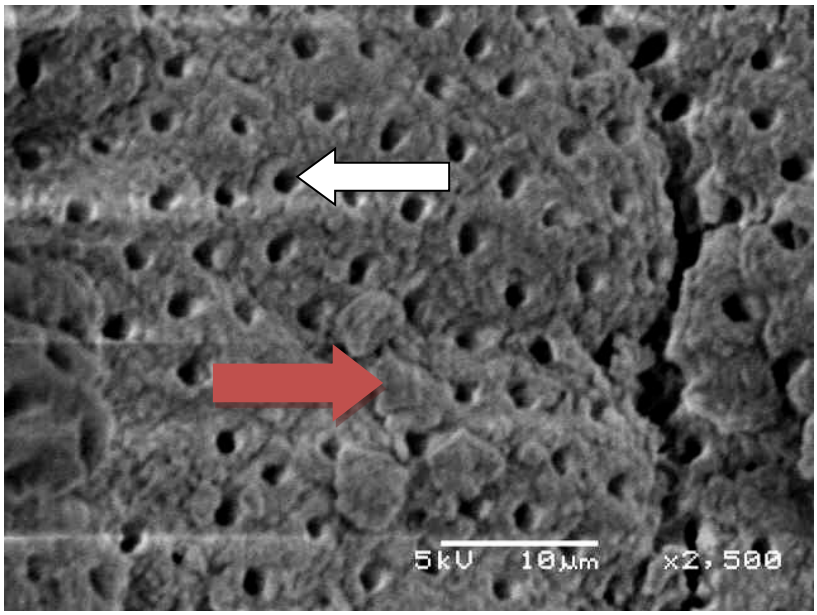

Figure 4.8 A SEM representative photomicrograph magnified to 2500x of the coronal third of a Group 5 root after irrigation with 6% sodium hypochlorite followed by anolyte solution followed by 18% EDTA. The photomicrograph appears to have been taken at an angle to the long axes of the dentinal tubules. Most of the tubules are open (white arrow). A small amount of smear layer can be seen scantily scattered on parts of the intertubular dentine (red arrow).

The middle third showed larger areas covered by large clusters of smear layer interspersed with sections where the dentinal tubules were patent (figure 4.9).

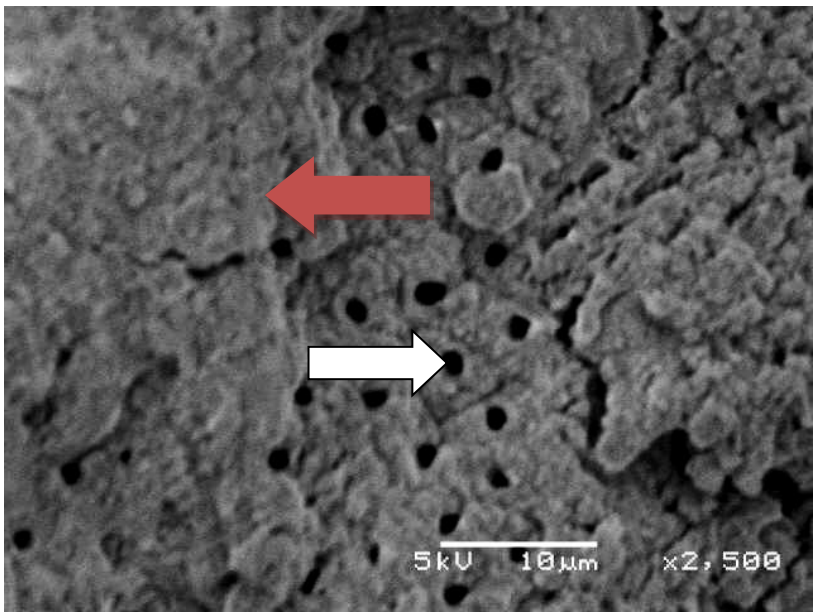

Figure 4.9 A SEM representative photomicrograph magnified to 2500x of the middle third of a Group 5 root after irrigation with 6% sodium hypochlorite followed by anolyte solution followed by 18% EDTA. A low to moderate number of dentinal tubules are open (white arrow). A thick irregular smear layer can be seen covering most of the intertubular dentine and remaining dentinal tubules (red arrow).

The apical third showed few open dentinal tubules with most of the tubules and intertubular dentine covered by a thick irregular smear layer (figure 4.10).

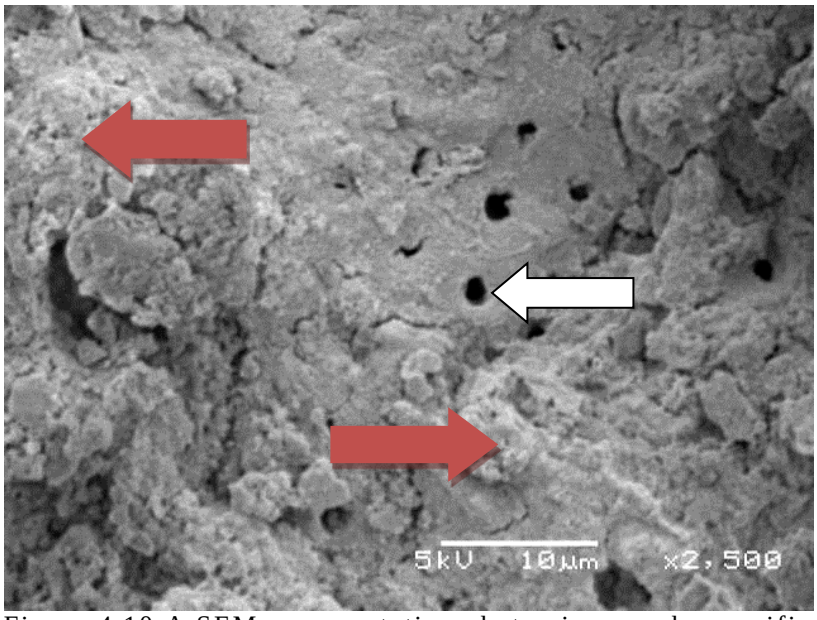

Figure 4.10 A SEM representative photomicrograph magnified to 2500x of the apical third of a Group 5 root after irrigation with 6% sodium hypochlorite followed by anolyte solution followed by 18% EDTA. Very few dentinal tubules can be seen (white arrow). A thick irregular smear layer is covering most of the intertubular dentine and dentinal tubules (red arrows).

4.1.1.6. Group 6: anolyte Solution followed by 18% EDTA

Table 4.6 Results of Group 6 (anolyte solution followed by EDTA)

	Mean no. Patent tubules	**Std. deviation**	**Std. error**	**[95% CI] Lower bound-Upper bound**	**Minimum-Maximum no. of Patent Tubules**
Coronal (n=10)	52.10	11.883	3.758	43.60-60.60	35-71
Middle (n=12)	43.17	30.120	8.695	24.03-62.30	2-82
Apical (n=12)	24.17	26.974	7.787	7.03-41.31	1-86
Total	39.09	26.866	4.607	29.71-48.46	1-86

The One-way ANOVA test revealed a statistical significant difference between these results ($p<0.05$). The Tukey HSD test revealed a significant difference between the coronal and apical levels ($p<0.05$).

Examination of the photomicrographs of the coronal and middle thirds showed regularly distributed open dentinal tubules. The intertubular dentine was for the most part smear free. The lateral dentinal tubules or ramifications could be seen. Peritubular dentin could be seen. Some remaining bacteria could be visualised within the canals or on the intertubular dentine (figures 4.11 and 4.12).

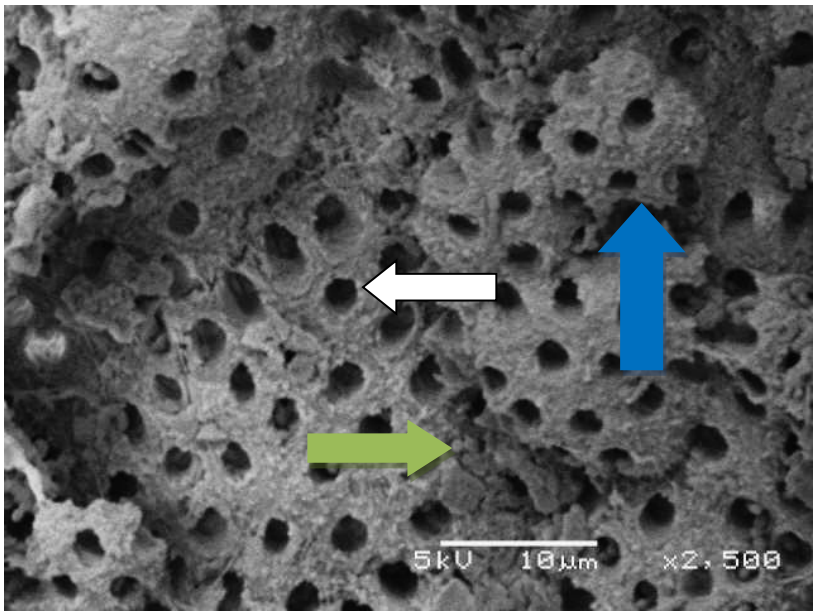

Figure 4.11 A SEM representative photomicrograph magnified to 2500x of the coronal third of a Group 6 root after irrigation with anolyte solution followed by 18% EDTA. The dentinal tubules are open (white arrow) but some bacteria can be seen within and surrounding the tubules (green arrow). Part of the dentine appears fractured off from its original position (blue arrow). This may be due to the process of splitting the root longitudinally for SEM preparation and examination.

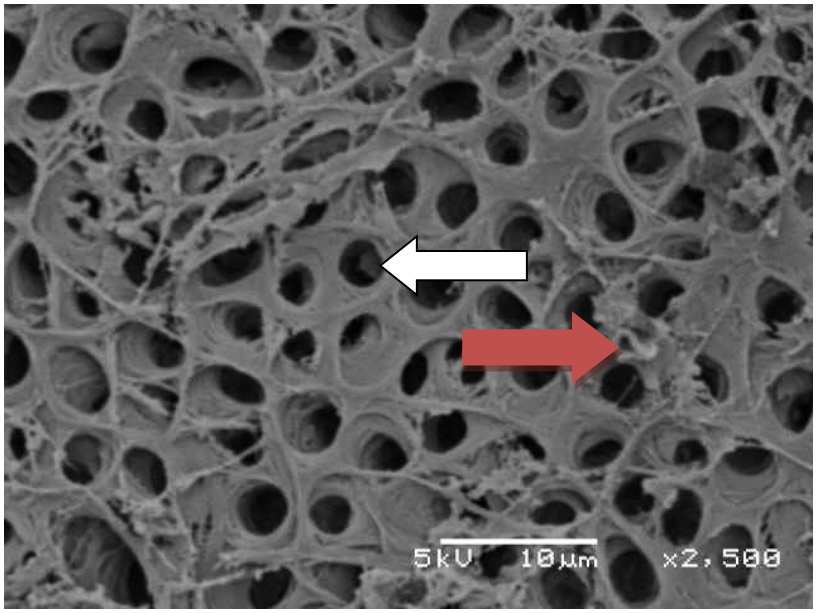

Figure 4.12 A SEM representative photomicrograph magnified to 2500x of the middle third of a Group 6 root irrigated with anolyte solution followed by 18% EDTA. Regularly distributed open dentinal tubules can be seen (white arrow). A thin smear layer is loosely present over some of the intertubular dentine and a few dentinal tubules (red arrow).

The photomicrographs of the apical thirds showed a low to moderate number of open dentinal tubules. There was still a fine smear layer on the intertubular dentine. Remaining bacteria could be seen on the intertubular dentine or within the tubules (figures 4.13 and 4.14).

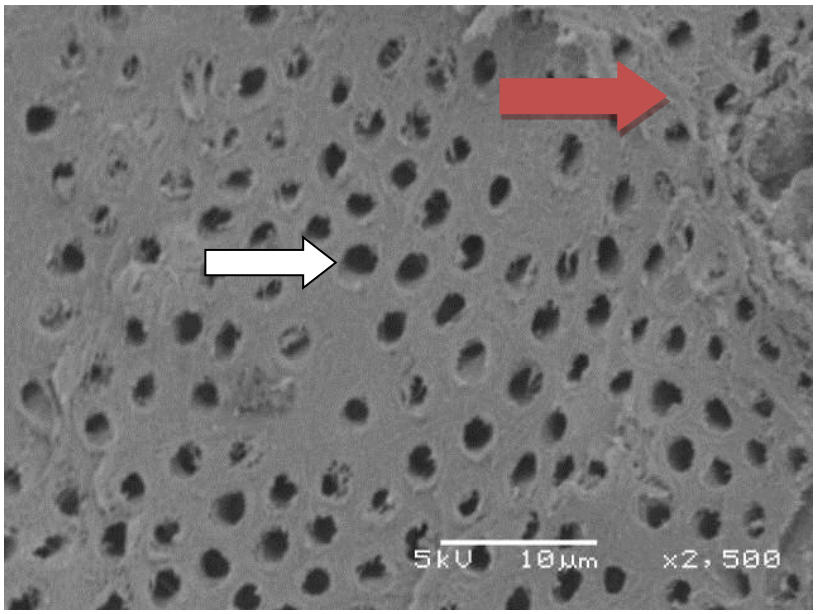

Figure 4.13 A SEM representative photomicrograph magnified to 2500x of the apical third of a Group 6 root irrigated with anolyte solution followed by 18% EDTA. A moderate number of tubules are open (white arrow). There is a fine fibrous-like smear layer covering the intertubular dentine and part of the dentinal tubules (red arrow).

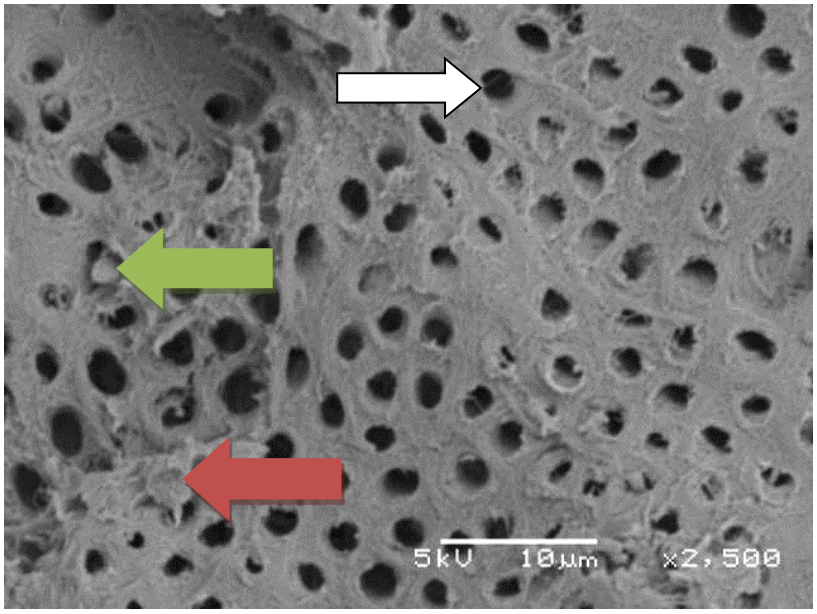

Figure 4.14 A SEM representative photomicrograph magnified to 2500 x of the apical third of a Group 6 root after irrigation with anolyte solution followed by 18% EDTA. Most of the dentinal tubules are open (white arrow). A few bacteria are present within the tubules (green arrow). A thin smear layer is present over the intertubular dentine and tubules (red arrow).

4.1.2. Inter-Group Comparisons

Using One-way ANOVA the results for the coronal, middle and apical thirds of all 6 groups were compared. Significantly statistical differences were revealed ($p<0.05$) (table 4.7).

Table 4.7 Inter-group significant differences after multiple comparisons for the coronal and middle thirds.

Groups		Significance p-value
Coronal		
6	1	<0.05
	2	
	3	
	4	
	5	
5	1	<0.05
Middle		
6	1	<0.05
	2	<0.05
	3	<0.05
	4	<0.05

As the Test of Homogeneity of Variances showed a significance of ≤ 0.05 for the coronal thirds, the Tamhane test was used for multiple comparisons and showed statistically significant differences between Group 6 and all the other groups. There was also a significant statistical difference between group 5 and group 1. These results are represented graphically in figure 4.15. The Tamhane test was similarly used for multiple comparisons of the middle third of all groups tested and showed statistically significant differences between Group 6 and Groups 1-4 (table 4.7).

.

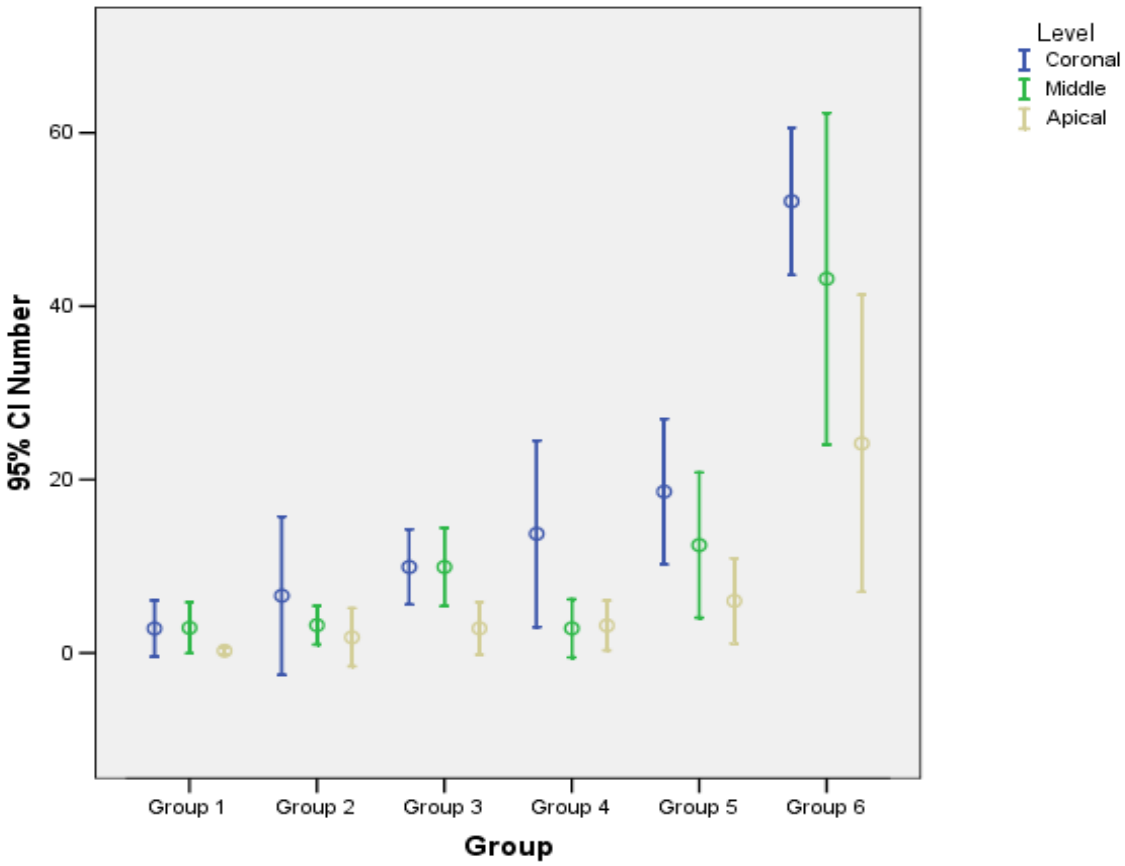

Figure 4.15 Graphical representation of the comparison of the mean number of patent dentinal tubules for the coronal, middle and apical thirds of all 6 groups at a 95% Confidence Interval.

In the comparison of the apical thirds a statistical anomaly occurred. The One-way ANOVA showed a statistical difference. The Test of Homogeneity of Variances was less than 0.05 but the Tamhane test failed to show significant statistical differences on multiple comparisons. The expert examiners pointed out that there was a marked visual difference observed between Group 6 and all other groups

4.2. Antibacterial Results

The intra-group comparisons showed statistically significant difference in the CFUs before and after irrigation for all groups (Table 4.8).

Group	Average CFU/ml (Range of CFU/ml)	Std. deviation	Std. error	[95% CI]		Percentage Difference	Significance p-value
				Lower Bound	Upper Bound		
1 Before	$6.6x10^7$ ($5.8x10^7$-$7.2x10^7$)	$5.9x10^6$	$2.4x10^6$	$6.0x10^7$	$7.2x10^7$	92.4563±1.6181	<0.05
1 After	$5.0X10^6$ ($3.0x10^6$-$6.2x10^6$)	$1.3x10^6$	$5.2x10^5$	$3.7x10^6$	$6.3x10^6$		
2 Before	$7.1x10^7$ ($6.0x10^7$-$7.7x10^7$)	$6x10^6$	$2.4x10^6$	$6.5x10^7$	$7.8x10^7$	100.0000±0.00	<0.05
2 After	.0000 (0.0-0.0)	0.0	0.0	0.0	0.0		
3 Before	$2.8x10^7$ ($1.6x10^7$-$3.6x10^7$)	$8.2x10^6$	$3.4X10^6$	$2.0x10^7$	$3.7x10^7$	100.0000±0.00	<0.05
3 After	0.000 (0.0-0.0)	0.0	0.0	0.0	0.0		
4 Before	$1.0x10^8$ ($9.5x10^7$-$1.2x10^8$)	$1.3x10^7$	$5.2X10^6$	$8.9x10^7$	$1.2x10^8$	100.0000±0.00	<0.05
4 After	0.000 (0.0-0.0)	0.0	0.0	0.0	0.0		
5 Before	$8.4x10^7$ ($8.3x10^7$-$1.0x10^8$)	$1.1x10^7$	$4.3x10^6$	$7.2x10^7$	$9.5x10^7$	99.9998±.0003	<0.05
5 After	$2x10^2$ (0.0-$5x10^2$)	$2.3x10^2$	$9.3x10^1$	0.0	439.3081		
6 Before	$5.0x10^7$ ($3.8x10^7$-$6.7x10^7$)	$9.8x1^6$	$4.0x10^6$	$3.9x10^7$	$6.0x10^7$	99.9998±.0002	<0.05
6 After	$1x10^2$ (0.0-$3x10^2$)	$1.3x10^2$	$5.2x10^1$	0.0	$2.3x10^2$		

Table 4.8 Intra-group antibacterial results.

The inter-group comparisons showed statistically significant results (p=0.000). Since the Test of Homogeneity of Variance showed a significance ≤ 0.05, the Tamhane test was used to reveal a statistically significant difference between Group 1 and all other groups for a 95% confidence interval (figure 4.16 and table 4.9).

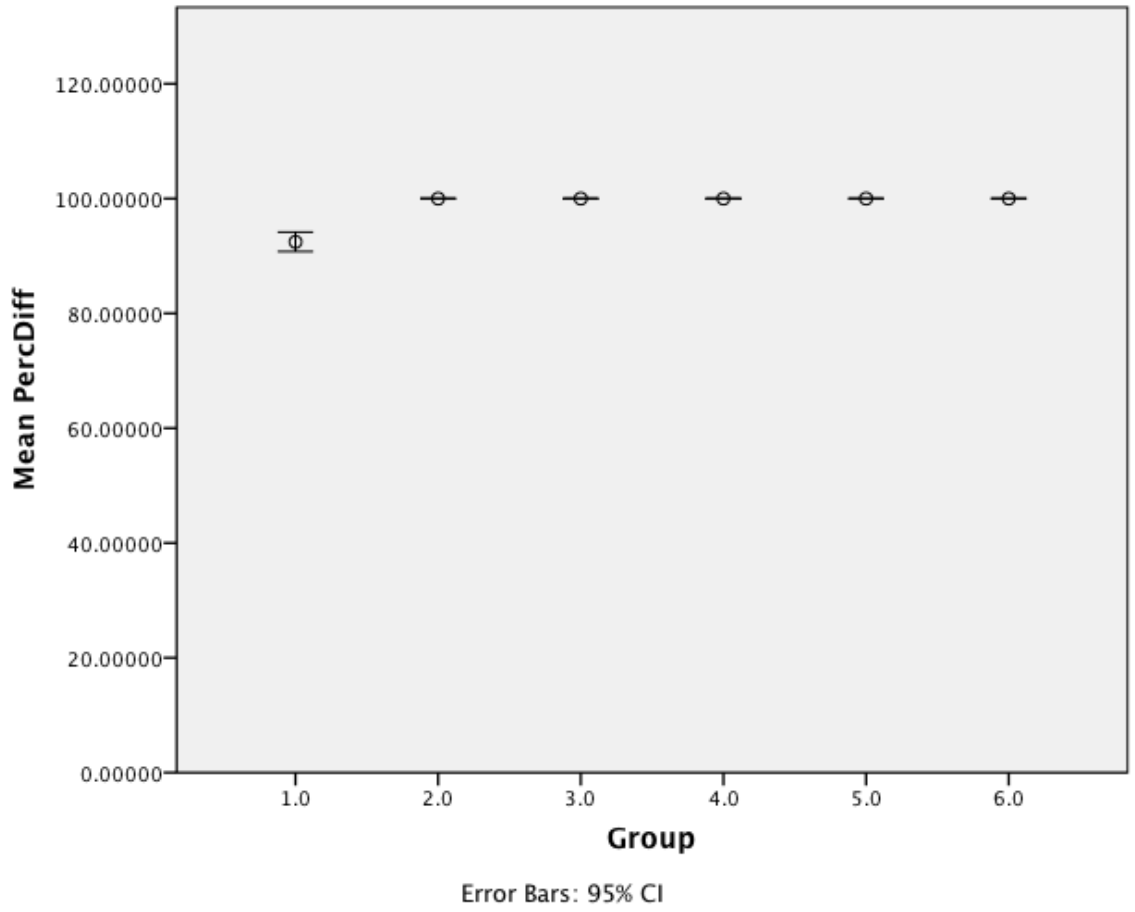

Figure 4.16 Inter-group antibacterial results

Table 4.9 Inter-group significant statistical antibacterial differences.

Significant Differences between Groups		p-value
1	2	<0.05
	3	<0.05
	4	<0.05
	5	<0.05
	6	<0.05

On examination of the SEM photomicrographs there are more bacteria remaining in the canals after irrigation in groups 1, 5 and 6. These may or may not be viable.

5. CHAPTER 5 DISCUSSION

Endodontic treatment aims at eliminating microorganisms from the infected root canal system and restoring the tooth to its correct form and function. To clean the canals and eradicate bacteria the canal must be prepared by mechanical and chemical methods (Walton and Torabinejad, 2002). Mechanical methods alone are not effective in removing all the debris and destroying bacteria. Irrigation solutions with antibacterial and detergent properties are thus used as an adjunct to mechanical preparation (Byström and Sundqvist, 1981; Ferraz *et al.*, 2001).

Mechanical preparation of the canals also leads to the formation of a smear layer. This smear layer is an amorphous layer of unpredictable diameter and volume that consists of remnants of pulpal tissues, microorganisms and debris from canal preparation (McComb and Smith, 1975; Ballal *et al.*, 2009). The smear layer should be removed as it may act as a substrate for any remaining bacteria. Smear layer removal improves the seal of the root canal filling materials, decreases the coronal leakage, reduces microleakage and improves the mechanical retention of the filling material to dentine (White *et al.*, 1984; Okşan *et al.*, 1993; Behrend *et al.*, 1996; Vivacqua-Gomes *et al.*, 2002; Clark-Holke *et al.*, 2003).

Dentinal tubule structure is different between different individuals, between different teeth in the same individual, and between different parts in the same tooth (Ferrari *et al.*, 2000). For this reason it is difficult to make a true comparison of the effect of smear layer removal or of the antibacterial activity of different irrigants. A sample with a poor dentine matrix will be more adversely affected by a detergent than a hypermineralised sample. Furthermore, dentinal tubule diameter changes with age and trauma (Morse, 1991; Mjör, 2001). Teeth with smaller diameter or less tubule density may give the impression of poorer smear layer removal when counting patent dentinal tubules within a given area and comparing this result to a tooth that has higher tubule density.

Tubules may also differ in size, diameter, shape and number depending on their position in the tooth. The tubule diameter is larger near the pulp than the dentino-enamel junction (DEJ). Furthermore the average density of dentinal tubules appears to be reduced in radicular dentine compared with cervical dentine. Higher tubule density is seen on the lingual and buccal pulpal walls than on the mesial and distal pulpal walls (Garberoglio and Brännström, 1976; Marshall 1993; Ferrari *et al.*, 2000)

Smear layer removal

Where sodium hypochlorite was the sole irrigant there was a thick irregular smear layer that had a different structure to that remaining after sterile distilled water was used. This research is in agreement with other research that has shown sodium hypochlorite cannot remove the inorganic portion of the smear layer (McComb and Smith, 1975; Marais and Williams, 2001).

For all other irrigant sequences there was better smear layer removal in the coronal third. This may be explained by the fact that the irrigation solution did not reach the apical and possibly the middle third due to an operator error, anatomical difficulties or the needle diameter being too large to reach the apical third. The irrigants may also have removed more smear layer had they been in contact with the smear layer and dentine for a longer time or been replaced more frequently (Byström and Sundqvist, 1983; Yamada *et al.*, 1983; Calt and Serper, 2000; Ferraz *et al.*, 2001; Zaccaro Scelza *et al.*, 2004).

In this study, alternating the use of a tissue solvent (sodium hypochlorite) and a chelating agent (EDTA) improved smear layer removal. Alternating sodium hypochlorite with anolyte solution removed the smear layer clinically more than sodium hypochlorite alone. This indicates that anolyte solution may be responsible for the

increased smear layer removal (Byström and Sundqvist, 1983; Yamada *et al.*, 1983; Ferraz *et al.*, 2001; Garcia *et al.*, 2010).

No one group was able to completely remove the smear layer in all thirds of the canal, but where anolyte solution was followed by 18% EDTA there was improved smear layer removal in all thirds compared with the other groups. Thus, anolyte solution followed by EDTA may be a promising irrigation protocol and with further research to establish the ideal volume, contact time and irrigation method, it may be a replacement for sodium hypochlorite.

Antibacterial activity

Where 3 ml of the sodium hypochlorite was used (Groups 2, 3 and 4), the CFU count after irrigation was always zero. Thus 3 ml of 6% sodium hypochlorite with surfactant molecules used for one minute was effective against *E. faecalis* under the conditions of this study.

In Groups 5 (sodium hypochlorite followed by anolyte solution followed by EDTA) and 6 (anolyte solution followed by EDTA) the CFU count after irrigation was so close to zero that the percentage difference before and after irrigation was deemed statistically insignificant compared to the groups that had 3 ml of 6% sodium hypochlorite as one of the irrigants (Groups 2, 3 and 4). Sterile distilled water (Group 1)

had the highest CFUs after irrigation and the percentage difference was deemed statistically significant compared to all other groups. This indicates that although chemical irrigation does significantly reduce the intracanal CFU count, an antibacterial irrigant is more effective. Statistically the CFU after irrigation in Groups 5 and 6 compared to Groups 2, 3 and 4 may be deemed insignificant but clinically the remaining microorganisms in Groups 5 and 6 cannot be discounted. Some authors have suggested that any remaining bacteria that are not entombed in the dentinal tubules during obturation may potentially multiply and migrate apically leading to failure of the endodontic treatment (Byström *et al.*, 1987; Sjögren *et al.*, 1997). *E. faecalis* is particularly virulent and may survive for long periods with little or no substrate (Molander *et al.*, 1999; Love, 2001; Figdor *et al.*, 2003), so it is important that irrigants have antibacterial properties.

5.1 Limitations of the study

Due to financial constraints the sample size was kept to the absolute minimum that was statistically determined. Subsequently some anomalies arose during the statistical evaluation which may have been avoided with a larger sample size. In this study the number of open dentinal tubules was counted for each photomicrograph of each root in a group at the various levels. This allowed for quantification of the smear layer removal to compare the irrigation protocols. Similarly, the

antibacterial results are derived from a small sample size and as such are only indicative of the antibacterial activity of all irrigants tested.

In similar studies of smear layer removal the evaluation was semi-quantitative i.e. the photomicrographs were given a score out of ten based on the overall appearance of the smear layer removal. For example the score would be 0 for an intact smear layer, 5 for partial smear layer removal and 10 for complete smear layer removal. The counts were added for all the thirds within a group and a percentage calculated. These percentages were then compared (van der Vyver, 2007).

In either case there is room for error. In this study the independent evaluator must be calibrated so that a tubule that is partially open will not be included in the number of completely open tubules. This method does not make provision for those tubules that are partially open but so lightly covered by smear layer that an endodontic sealer may in fact penetrate such a tubule. Perhaps similar counts can be done for partially open dentinal tubules.

The categorical results are based on an impression of the overall cleanliness of the canal. This is not a mathematical quantity and relies on the examiner's discretion. This evaluation method does however include partially open tubules.

The teeth used formed part of a collection of teeth that were stored in saturated thymol for an undetermined period of time. The effects of different storage media over various periods of time is not well established in the literature (Titley *et al.*, 1998; Santana *et al.*, 2007). It may be preferable to use freshly extracted teeth that have been stored in saline or sterile distilled water.

Traditional cultivation techniques were used in this study and since standard PCR is more sensitive to microbial species the cultivation method may not be an accurate representation of whether *E. faecalis* remained in the canals (Williams *et al.*, 2006). This may indicate that a zero CFU count after irrigation, as seen with 3 ml 6% sodium hypochlorite, is in fact not zero. Since both methods have their faults and merits perhaps future research should use both methods to determine the antibacterial activity of these irrigation protocols.

The manufacturers of 18% EDTA suggest in the instructions not to use the product for longer than 1 minute in total. When examining the smear layer removal in similar studies it was found that increasing the contact time of EDTA did improve the smear layer removal. These studies have shown better smear layer removal using 17% EDTA for 2 to 10 minutes (Calt and Serper, 2000; Scelza *et al.*, 2004). One, three and ten

millilitres of 17% EDTA applied for one minute has shown equal debris removal (Crumpton, Goodell and McClanahan, 2005)

All the irrigation solutions and especially anolyte solution may be more effective in larger volumes, longer contact times and with activation.

5.2 Conclusions and Recommendations

5.2.1 Summary and Conclusions

Due to the small sample size and other limitations of this study, definitive conclusions cannot be made, but the results indicate the following:

- For any irrigant group the coronal third showed better smear layer removal than the apical third.
- 5ml anolyte solution followed by 3 ml 18% EDTA for one minute showed the best smear layer removal results for all thirds.
- Chemical irrigation significantly decreases the intracanal *E. faecalis* CFUs.
- Sterile distilled water is not as effective in decreasing the intracanal CFUs as other irrigants tested and is not considered antibacterial.
- All other irrigant protocols are equally antibacterial.

5.2.2 Recommendations

Future research must be conducted to determine the exact volume, contact time and irrigation method of anolyte solution followed by EDTA to improve the smear layer removal and antibacterial results.

Furthermore one could include MTAD as an alternative to EDTA. The study may also be conducted with a lower concentration of sodium hypochlorite, longer contact times and activation of irrigants.

With regards to the antibacterial studies future research should involve using PCR methods and cultivation to assess the antibacterial results.

APPENDICES

APPENDIX A: Ethics Approval

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10086, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5758
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-100806 -1- update

15/08/2011

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigators: **Dr Karen Bennie (Student No. 521707)**

Project title: **(New title) Smear layer removal ability and antibacterial activity of endodontic irrigants**
(Previous title) **NaOCL and.CHX interact to form a precipitate that prevents sealer penetration. Can the benefits of both irrigants be maintained and the precipitate formation prevented with an intervening rinse of distilled water?**

Reason: This is a wholly laboratory study using extracted teeth stored in the Division of Experimental Odontology collected by Prof E S Grossman for previous research (Clearance M050760). Prof Grossman has given permission for the teeth to be used. The study will include use of a stock culture of *Enerococcus faecalis* There are no humans involved.

UNIVERSITY OF THE WITWATERSRAND
2011 -08- 15
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Research Office, Senate House, Wits

cal Waiver

APPENDIX B: Preparation of roots for SEM

A shallow longitudinal groove was cut on the external buccal and lingual surfaces of each root fragment without penetrating the canal. A chisel was placed in the groove and using a hammer the root was split in half longitudinally. The two halves were prepared for SEM according to the standard methods for biological SEM examination.

The halves were fixated with 2% gluteraldehyde for 1 hour. Using a pipette the Gluteraldehyde was sucked off and the sections rinsed with phosphate buffer solution for 5 minutes 3 times each. The sections were then fixed in 0.25% OsO_4 for half an hour and again rinsed with phosphate buffer for 5 minutes 3 times each. The sections were rinsed with 30%, 50% and 70% ethanol for 5 minutes each time. Thereafter the sections were rinsed with 95% ethanol 3 times for 5 minute each time and stored in 95% ethanol. The sections were removed from the 95% ethanol and dried in a critical point dryer for 8 hours. The sections were mounted on an aluminium plate with conductive carbon cement with the split side (canal surface) showing in order to view the dentin. The samples were sputter coated with gold and then examined under a Scanning Electron Microscope- SEM (JEOL JSM 840 Scanning Electron Microscope, Tokyo, Japan).

APPENDIX C: Protocol Approval

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2076

Reference: Ms Salamina Segole
E-mail: salamina.segole@wits.ac.za
06 December 2011
Person No: 521707
PAG

Dr KR Bennie
261 Olivier Street
Brooklyn
0181
Pretoria, South Africa

Dear Dr Bennie

Master of Science in Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled "*Smear layer removal ability and antibacterial activity of endodontic irrigants*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

References

Agarwal S, Piesco NP, Peterson DE, Charon J, Suzuki JB, Godowski KC, Southard GL (1997). Effects of sanguinarium, chlorhexidine and tetracycline on neutrophil viability and functions in vitro. *J Periodont Res* 32:335-344.

Andrewes FW, Horder TJ (1906). A study of the streptococci pathogenic for man. *Lancet* 11:708-13, 775-82, 852-5. Cited by Schleifer and Kilpper-Bälz (1984). Not read in the original.

Babich H, Wurzburger BJ, Rubin YL, Sinensky MC, Blau L (1995). *Cell Biol Toxicol* 11:79-88.

Baker NA, Eleazer PD, Averbach RE, Seltzer S (1975). Scanning electron microscopic study of the efficacy of various irrigating solutions. *J Endod* 1:127-135.

Bakhir VM, Grishin VP, Panicheva SA, Toloknov VI (1999). Assessment of the effectiveness of medical instruments sterilized by electrochemically activated solutions and computer modeling of the dynamics of hospital infections. *Medizinskaia Tekhnika* 2:14-6. Cited by Solovyeva and Dummer (2000). Not read in the original.

Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S (2009). Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: a scanning electron microscopic study. *J Endod* 35:1573-6

Barthel CR, Zimmer S, Wussogk R, Roulet JF (2001). Long-term bacterial leakage along obturated roots restored with temporary and adhesive fillings. *J Endod* 27:560-2

Bauman RW (2011). *Microbiology alternate edition with diseases by body system*. 3rd ed. UK: Pearson Education, Inc.

Baumann MA, Beer R (2010). *Endodontology*. 2nd ed. Stuttgart: Thieme

Becker GL, Cohen S, Borer R (1974). The sequelae of accidentally injecting sodium hypochlorite beyond the root apex. Report of a case. *Oral Surg* 38:633-8

Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, *et al*. (2002). Molecular analysis of bacterial species associated with childhood caries. *J Clin Microbiol* 40:1001-9.

Behrend GD, Cutler CW, Gutmann JL (1996). An *in-vitro* study of smear layer removal and microbial leakage along root-canal fillings. *Int Endod J* 29:99-107.

Bergenholtz G, Lindhe J (1975). Effect of soluble plaque factors on inflammatory reactions in the dental pulp. *Scand J Dent Res* 83:153-8.

Bowden GHW (1990). Microbiology of root surface caries in humans. *J Dent Res* 69:1205-10.

Bui TB, Baumgartner JC, Mitchell JC (2008). Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. *J Endod* 34:181-5

Byström A, Happonen RP, Sjögren U, Sundqvist G (1987). Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. *Endod Dent Traumatol* 3:58-63

Byström A, Sundqvist G (1981). Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 89:321-8.

Byström A, Sundqvist G (1983). Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg* 55:307-12

Calt S, Serper A (2000). Smear layer removal by EGTA. *J Endod* 26:459-61.

Calt S, Serper A (2002). Time-dependant effects of EDTA on dentin structures. *J Endod* 28:17-19

Cameron JA (1986). The effect of a fluorocarbon surfactant on the surface tension of the endodontic irrigant, sodium hypochlorite. A preliminary report. *Aust Dent J* 31:364-368.

Card SJ, Sigurdsson A, Ørstavik D, Trope M (2002). The effectiveness of increased apical enlargement in reducing intracanal bacteria. *J Endod* 28:779-83.

Carson KR, Goodell GG, McClanahan SB (2005). Comparison of the antimicrobial activity of six irrigants on primary endodontic pathogens. *J Endod* 31:471-3

Chhour K, Nadkarni MA, Byun R, Martin EF, Jacques NA, Hunter N (2004). Molecular analysis of microbial diversity in advanced caries. *J Clin Microbiol* 43:843-9

Ciucchi B, Khettabi M, Holz J (1989). The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: a scanning electron microscopic study. *Int Endod J* 22:21-8

Clark-Holke D, Drake D, Walton R, Rivera E, Guthmiller JM (2003). Bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer. *J Dent* 31:275-81.

Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR (2006). The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. *J Endod* 32:434-7

Crumpton BJ, Goodell GG, McClanahan SB (2005). Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. *J Endod* 31:536-8.

Dahlén G, Möller AJR (1992). Microbiology of endodontic infections. In: Contemporary oral microbiology and immunology. Slots J, Taubman MA editors. St Louis:Mosby-Year book:444-75. Cited in van der Sluis *et al.*, (2005). Not read in the original.

Distel JW, Hatton JF, Gillespie MJ (2002). Biofilm formation in medicated root canals. *J Endod* 28:689-93.

Drake DR, Wiemann AH, Rivera EM, Walton RE (1994). Bacterial retention in canal walls in vitro: effect of smear layer. *J Endod* 20:78-82

Duchin S, van Hout J (1978). Relationship of *Streptococcus mutans* and lactobacilli to incipient smooth surface dental caries in man. *Arch Oral Biol* 23:779-86.

Edwardsson S (1974). Bacteriological studies on deep areas of carious dentine. *Odontol Revy Suppl* 32:1-143. Cited in Hoshino (1985). Not read in the original.

Engström B (1964). The significance of enterococci in root canal treatment. *Odontol Revy* 15:87-106. Cited in Molander *et al.*, (1998). Not read in original.

Ferguson DB, Marley JT, Hartwell GR (2003). The effect of chlorhexidine gluconate as an endodontic irrigant on the apical seal: long-term results. *J Endod* 29:91-4.

Ferrari M, Mannocci F, Vichi A, Cagidiaco MC, Mjör IA (2000). Bonding to root canal: structural characteristics of the substrate. *Am J Dent* 13:255-60.

Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, de Souza-Filho FJ (2001). In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. *J Endod* 27:452-5.

Fidgor D, Davies JK, Sundqvist G (2003). Starvation survival, growth and recovery of *Enterococcus faecalis* in human serum. *Oral Microbiol Immunol* 18:234-9.

Garberoglio R, Brännström M (1976). Scanning electron microscopic investigation of human dentinal tubules. *Arch Oral Biol* 21(6):355-62.

Garcia F, Murray PE, Garcia-Godoy F, Namerow KN (2010). Effect of aquatine endodontic cleanser on smear layer removal in the root canals of ex vivo human teeth. *J Appl Oral Sci* 18:403-8.

Gatot A, Arbelle J, Leiberman A, Yanai-Inbar I (1991). Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. J Endod 17:573-4.

Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieq NR, Phillips GB, editors (1981*). Manual of methods for general bacteriology*. 2nd printing. Washington: American Society for Microbiology, pp 179-207.

Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M (2004). Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. *Int Endod J* 37:272-80

Gilmore MS (2002*). The Enterococci: pathogenesis, molecular biology, and antibiotic resistance*. Washington: AMS Press.

Gomes BP, Lilley JD, Drucker DB (1996). Clinical significance of dental root canal microflora. *J Dent* 24:47-55.

Gomes BP, Pinheiro ET, Gadê-Neto CR, Sousa EL, Ferraz CC, Zaia AA *et al.* (2004). Microbiological examination of infected dental root canals. Oral Microbiol Immunol 19:71-6.

Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L *et al.* (2003). Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *Int Endod J* 36:267-75.

González-López S, Camejo-Aguilar D, Sanchez-Sanchez P, Bolanos-Carmona V (2006). Effect of CHX on the decalcifying effect of 10% citric acid, 20% citric acid, or 17% EDTA. *J Endod* 32:781-4.

Grossman LI (1978). Paresthesia from N2 or N2 substitute. Report of a case. *Oral Surg Oral Med Oral Pathol* 45:114-5

Haapasalo M, Ørstavik D (1987). In vitro infection and disinfection of dentinal tubules. *J Dent Res* 66:1375-9.

Haapasalo M, Shen Y, Qian W, Gao Y (2010). Irrigation in endodontics. *Dent Clin North Am* 54:291-312.

Haapasalo HK, Sirén EK, Waltimo MT, Ørstavik D, Haapasalo MP (2000). Inactivation of local root canal medicaments by dentine: an in vitro study. *Int Endod J* 33:126-31.

Hahn CL, Falkler WA Jr, Minah GE (1991). Microbiological studies of carious dentine from human teeth with irreversible pulpitis. *Arch Oral Biol* 36:147-153.

Hahn CL, Falkler WA Jr, Minah GE (1993). Correlation between thermal sensitivity and microorganisms isolated from deep carious dentin. *J Endod* 19:26-30.

Hahn CL, Liewehr FR (2007). Relationships between caries bacteria, host responses, and clinical signs and symptoms of pulpitis. *J Endod* 33:213-9

Hammad M, Qualtrough A, Silikas N (2009). Evaluation of root canal obturation: a three-dimensional in vitro study. *J Endod* 35:541-4

Hargreaves KM, Cohen S (2011). Cohen's pathways of the pulp. 10th ed. St Louis: Mosby Elsevier.

Hojo S, Komatsu M, Okuda R, Takahashi N, Yamada T (1994). Acid profiles and pH of carious dentin in active and arrested lesions. *J Dent Res* 73:1853-7.

Hoshino E (1985). Predominant obligate anaerobes in human carious dentin. *J Dent Res* 64:1195-8.

Hotta K, Kawaguchi K, Saitoh F, Saito N, Suzuki K, Ochi K, Nakayama T (1994). Antimicrobial activity of electrolyzed NaCl solutions: effect on the growth of *Streptomyces* spp. *Actinomycetologica* 8:51-6.

Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ (2003). Influence of *Enterococcus faecalis* proteases and the collagen-binding protein, Ace, on adhesion to dentin. Oral Microbiol Immunol 18:121-6.

Hülsmann M, Hahn W (2000). Complications during root canal irrigation-literature review and case reports. *Int Endod J* 33:186-93.

Jaju S, Jaju PP (2011). Newer root canal irrigants in horizon: A review. *Int J Dent* 2011. http://www.hindawi.com/journals/ijd/2011/851359/

Jeansonne MJ, White RR (1994). A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod* 20:276-8.

Johansson I, Ericson T, Bowen W, Cole M (1985). The effect of malnutrition on caries development and saliva composition in the rat. *J Dent Res* 64:37-43.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965). The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 20:340-9

Kokkas AB, Boutsioukis AC, Vassiliadis LP, Stavrianos CK (2004). The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. *J Endod* 30:100-2.

Kuruvilla JR, Kamath MP (1998). Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. *J Endod* 24: 472-6.

Kuttler Y. Microscopic investigation of root apexes (1955). *J Am Dent Assoc* 50:544-52.

Lim TS, Wee TY, Choi MY, Koh WC, Sae-Lim V (2003). Light and scanning electron microscopic evaluation of Glyde File Prep in smear layer removal. *Int Endod J* 36:336-43.

Lima KC, Fava LR, Siqueira JF Jr (2001). Susceptibilities of *Enterococcus faecalis* biofilms to some antimicrobial medications. *J Endod* 27:616-9.

Loesche WJ, Syed SA (1973). The predominant cultivable flora of carious plaque and carious dentine. *Caries Res* 7:201-16.

Love RM (2001). *Enterococcus faecalis*-a mechanism for its role in endodontic failure. *Int Endod* J 34:399-405.

Love RM, Jenkinson HF (2002). Invasion of dentinal tubules by oral bacteria. *Crit Rev Oral Biol* Med 13:171-83.

Love RM, McMillan MD, Jenkinson HF (1997). Invasion of dentinal tubules by oral streptococci is associated with collagen recognition mediated by the antigen I/II family of polypeptides. *Infect Immun* 65:5157-64.

MacFarland J (1907). The nephelometer: an instrument for estimating the numbers of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J Am Med Assoc* 49:1176-8.

Mader CL, Baumgartner JC, Peters DD (1984). Scanning electron microscopic investigation of the smeared layer on root canal walls. *J Endod* 10:477-83.

Mai S, Kim YK, Arola DD, Gu LS, Kim JR, Pashley DH *et al*. (2010). Differential aggressiveness of ethylenediamine tetraacetic acid in causing canal wall erosion in the presence of sodium hypochlorite. J Dent 38:201-6.

Marais JT (2000a). Investigations into the application of electrochemically activated water in dentistry. *SADJ* 55(Suppl 7):381-5.

Marais JT (2000b). Cleaning efficacy of a new root canal irrigation solution: a preliminary evaluation. *Int Endod J* 33:320-25.

Marais JT, Williams WP (2001). Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution. *Int Endod J* 34:237-43.

Margolis HC, Duckworth JH, Moreno EC (1988). Composition of pooled resting plaque fluid from caries-free and caries-susceptible individuals. *J Dent Res* 67:1468-75.

Marshall GW (1993). Dentin: microstructure and characterization. *Quintessence Int* 24:606-617.

Massey WL, Romberg DM, Hunter N, Hume WR (1993). The association of carious dentin microflora with tissue changes in human pulpitis. Oral Microbiol Immunol 8:30-5.

Masterton JB (1965). Chemical debridement in the treatment of infected pulpless teeth and chronic periapical abscesses. Dent Pract (Bristol) 15:162. Cited in Baker et al. (1975). Not read in the original.

McComb D, Smith DC (1975). A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod* 1:238-42.

Michaud RA, Burgess J, Barfield RD, Cakir D, McNeal SF, Eleazer PD (2008). Volumetric expansion of gutta-percha in contact with eugenol. *J Endod* 34:1528-32.

Milnes AR, Bowden GH (1985). The microflora associated with the developing lesions of nursing caries. *Caries Res* 19:289-97.

Mjör IA (2001). Pulp-dentin biology in restorative dentistry. Part 5: clinical management and tissue changes associated with wear and trauma. *Quintessence Int* 32:771-788.

Mohammadi Z, Abbott PV (2009). The properties and applications of chlorhexidine in endodontics. *Int Endod J* 42:288-302.

Molander A, Reit C, Dahlén G, Kvist T (1998). Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J* 31:1-7.

Molander A, Reit C, Dahlén G (1999). The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. *Endod Dent Traumatol* 15:205-9.

Moorer WR, Wesselink PR (1982). Factors promoting the tissue dissolving capability of sodium hypochlorite. *Int Endod J* 15:187-96.

Morse DR (1991). Age related changes of the dental pulp complex and their relationship to systemic aging. *Oral Surg Oral Med Oral Pathol* 72:721-745.

Nair PN, Henry S, Cano V, Vera J (2005). Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 99:231-52.

Neal RG, Craig RG, Powers JM (1983). Effect of sterilization and irrigants on the cutting ability of stainless steel files. J Endod 9:93-6.

Nissan R, Segal H, Pashley D, Stevens R, Trowbridge H (1995). Ability of bacterial endotoxin to diffuse through human dentin. *J Endod* 21: 62-4.

Nunes VH, Silva RG, Alfredo E, Sousa-Neto MD, Silva-Souso YT (2008). Adhesion of Epiphany and AH Plus sealers to human root dentin treated with different solutions. *Braz Dent J* 19:46-50.

Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JA (2004). Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. *Int Endod J* 37:38-41.

Okşan T, Aktener BO, Sen BH, Tezel H (1993). The penetration of root canal sealers into dentinal tubules. A scanning electron microscopic study. *Int Endod J* 26:301-5.

Olgart L, Haegerstam G, Edwall L (1974). The effect of extracellular calcium on thermal excitability of the sensory units in the tooth of the cat. *Acta Physiol Scand* 91:116-22.

Orchardson R (1978). The generation of nerve impulses in mammalian axons by changing the concentrations of the normal constituents of extracellular fluid. *J Physiol* 275:177-89.

Özok AR, van der Sluis LW, Wu MK, Wesselink PR (2008). Sealing ability of a new polydimethylsiloxane-based root canal filling material. *J Endod* 34:204-7.

Panopoulos P (1992). Factors influencing the occurrence of pain in carious teeth. *Proc Finn Dent Soc* 88:155-60. Cited in Hahn and Liewehr (2007). Not read in the original.

Panopoulos P, Mejàre B, Edwall L (1983). Effects of ammonia and organic acids on intradental sensory nerve activity. *Acta Odontol Scand* 41:209-15.

Pappen FG, Souza EM, Giardino L, Carlos IZ, Leonardo MR, Leonardo RT (2009). Endodontic chelators induce nitric oxide expression by murine-cultured macrophages. *J Endod* 35:824-8.

Pashley DH, Tao L, Boyd L, King GE, Homer JA (1988). Scanning electron microscopy of the substructure of smear layers in human dentine. *Arch Oral Biol* 33:265-70.

Pashley DH (1990). Clinical considerations of microleakage. *J Endod* 16:70-7.

Prilutskii VI, Bakhir VM (1997). Electrochemically activated water: anomalous properties, mechanism of biological action. M.,VNIIIMT. Cited in Solovyeva and Dummer (2000). Not read in the original.

Ramachandran Nair PN (1987). Light and electron microscopic studies of root canal flora and periapical lesions. *J Endod* 13:29-39.

Rôças IN, Siqueira JF Jr, Santos KR (2004). Association of *Enterococcus faecalis* with different forms of periradicular disease. *J Endod* 30:315-20.

Rølla G, Oppermann RV, Bowen WH, Ciardi JE, Knox KW (1980). High amounts of lipoteichoic acid in sucrose-induced plaque in vivo. *Caries Res* 14:235-8.

Rosenthal S, Spånberg L, Safavi K (2004). Chlorhexidine substantivity in root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98:488-92.

Rossi-Fedele G, Figueiredo JA, Steier L, Canullo L, Steier G, Roberts AP (2010). Evaluation of the antimicrobial effect of super-oxidized water (Sterilox®) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model. *J Appl Oral Sci* 18:498-502.

Saleh IM, Ruyter IE, Haapasalo MP, Ørstavik D (2003). Adhesion of endodontic sealers: scanning electron microscopy and energy dispersive spectroscopy. *J Endod* 29:595-601

Santana FR, Pereira JC, Pereira CA, Neto AJ, Soares CJ (2007). Influence of method and period of storage on the microtensile bond strength of indirect composite resin restorations to dentine. *Braz Oral Res* 22:352-7.

Saunders WP, Saunders EM (1994). Coronal leakage as a cause of failure in root-canal therapy: a review. *Endod Dent Traumatol* 10:105-8.

Scelza MF, Pierro V, Scelza P, Pereira M (2004). Effect of three different time periods of irrigation with EDTA-T, EDTA, and citric acid on smear layer removal. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98:499-503.

Schilder H (1974). Cleaning and shaping the root canal. *Dent Clin North Am* 18:269-96.

Schleifer KH, Kilpper-Bälz R (1984). Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Int J Syst Bacteriol* 34:31-4.

Sedgley CM, Lennan SL, Clewell DB (2004). Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiol Immunol* 19:95-101.

Selkon JB, Babb JR, Moriss R (1999). Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox, for the disinfection of endoscopes. *J Hosp Infect* 41:59-70.

Sen BH, Piskin B, Demirci T (1995). Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. *Endod Dent Traumatol* 11:6-9.

Shih M, Marshall JF, Rosen S (1970). The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 29:613-9.

Shraer TI, Legchilo AN (1989). Use of electrochemically activated 0.8% potassium chloride solutions in the treatment of empyema and parietal abscesses of the lung. *Grudn Khir* Jul-Aug:64-8.

Siqueira JF Jr (2001). Aetiology of root canal treatment failure: why well-treated teeth can fail. *Int Endod J* 34:1-10

Siqueira JF Jr, Rôças IN (2003). PCR methodology as a valuable tool for identification of endodontic pathogens. *J Dent* 31:333-9.

Siqueira JF Jr, Rôças IN (2004). Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 97:85-94.

Siqueira JF Jr, Rôças IN (2008). Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod* 34:1291-1301.

Sjögren U, Figdor D, Persson S, Sundqvist G (1997). Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* 20:297-306.

Solovyeva AM, Dummer PM (2000). Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *Int Endod J* 33:494-504.

Steinman RR, Leonora J, Singh RJ (1980). The effect of desalivation upon pulpal function and dental caries in rats. *J Dent Res* 59:176-85.

Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006). *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 32:93-8.

Sundqvist G (1992). Ecology of the root canal flora. *J Endod* 18:427-30.

Sundqvist G, Figdor D, Persson S, Sjögren U (1998). Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85:86-93.

Swanson K, Madison S (1987). An evaluation of coronal microleakage in endodontically treated teeth. Part I. Time periods. *J Endod* 13:56-9.

Takahashi N, Saito K, Schachtele CF, Yamada T (1997). Acid tolerance and acid-neutralizing activity of *Porphyromonas gingivalis, Prevotella intermedia* and *Fusobacterium nucleatum*. *Oral Microbiol Immunol* 12:323-8.

Thiercelin E, Jouhaud L (1903). Reproductionde l'enterocoque; taches centrales; granulations peripheriques et microblasts. *C. R. Seances Soc Biol* 55:686-88. Cited in Schleifer and Kilpper-Bälz (1984). Not read in the original.

Titley KC, Chernecky R, Roussouw PE, Kulkarni GV (1998). The effect of various storage methods and media on shear-bond strengths of dental composite resin to bovine dentin. *Arch Oral Biol* 43:305-11.

Tjäderhane L, Larjavo H, Sorsa T, Uitto VJ, Larmas M, Salo T (1998). The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res; 77(8):1622-29

Torabinejad M, Handysides R, Khademi AA, Bakland LK (2002). Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94:658-66.

Torabinejad M, Shabahang S, Aprecio RM, Kettering JD (2003). The antimicrobial effect of MTAD: an in vitro investigation. *J Endod* 29:400-3.

Torabinejad M, Ung B, Kettering JD (1990). In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endod* 16:566-9.

Vahdaty A, Pitt Ford TR, Wilson RF (1993).Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. *Endod Dent Traumatol* 9:243-8

van der Bijl P, Gelderblom WC, Thiel PG (1984). On the mutagenicity of parachloroaniline, a breakdown product of chlorhexidine. *J Dent Assoc S Afr* 39:535-7.

van der Sluis LW, Wu MK, Wesselink PR (2005). An evaluation of the quality of root fillings in mandibular incisors and maxillary and mandibular canines using different methodologies. *J Dent* 33:683-8.

van der Vyver PJ (2007). Antimicrobial properties and smear layer management of nine different root canal irrigation solutions [dissertation]. Pretoria: University of Pretoria.

van Houte J (1994). Role of micro-organisms in caries etiology. *J Dent Res* 73:672-81.

Vivacqua-Gomes N, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ (2002). Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. *Int Endod J* 35:791-5.

Walton RE, Torabinejad M (2002*). Principles and practice of endodontics*. 3rd ed. Philadelphia: Saunders.

West JD (2010). The endodontic Glidepath: "Secret to rotary safety". *Dentistry Today September*. http://www.dentistrytoday.com/endodontics/3478-endodontic-glidepath-secret-to-rotary-safety. [Accessed 31.1.2012].

White RR, Goldman M, Lin PS (1984). The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. *J Endod* 10:558-62.

White RR, Hays GL, Janer LR (1997). Residual antimicrobial activity after canal irrigation with chlorhexidine. *J Endod* 23:229-31.

Williams S, Goldman M (1985). Penetrability of the smeared layer by a strain of *Proteus vulgaris*. *J Endod* 11:385-88.

Williams JM, Trope M, Caplan DJ, Shugars DC (2006). Detection and quantitation of *E. faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. *J Endod* 32:715-21.

Yamada RS, Armas A, Goldman M, Lin PS (1983). A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions:Part 3. *J Endod* 9:137-42.

Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M (1995). Antimicrobial and toxic effects of established and potential root canal irrigants. *J Endod* 21:513-5.

Yoshida M, Fukushima H, Yamamoto K, Ogawa K, Toda T, Sagawa H (1987). Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis. *J Endod* 13:24-8.

Young GR, Parashos P, Messer HH (2007). The principles of techniques for cleaning root canals. *Aust Dent J* 52(Suppl):S52-63.

Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T (2002). Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94:756-62.

Zehnder M (2006). Root canal irrigants. *J Endod* 32:389-98.