

Antimicrobial Properties and Smear Layer Management of Nine Different Root Canal Irrigation Solutions

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by

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DECLARATION

I, Petrus Jacobus van der Vyver, hereby declare that this dissertation, submitted by me in partial fulfillment of the requirements for the degree MSc (Odont) at the University of Pretoria, South Africa, has not been submitted for a degree at any other University.

.....

PJ van der Vyver

Whoever acquires knowledge
and does not practice it
resembles him who ploughs
his land and leaves it unsown.

- Saadi

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SUMMARY

The main objective of root canal therapy is cleaning, shaping and obturating the root canal system in three dimensions, as well as preventing reinfection.

The objective of this *in vitro* study was:

- to establish the antimicrobial efficacy of nine different root canal irrigation solutions,
- to determine the smear layer dissolving capabilities of these irrigating solutions, at various levels in straight root canals, and
- to examine the degree of erosion caused by the irrigation solutions on the root canal walls.

Antimicrobial Effects: In this test the antimicrobial activity of nine irrigation solutions against *E. faecalis* was measured using a disc diffusion test. The antibacterial activity of materials was apparent from circular clear inhibition zones forming around the filtration paper.

Effect on Smear Layer: Root canals were prepared by using Pro Taper nickel titanium rotary files. During preparation, the canals were irrigated with copious amounts of 3.5% sodium hypochlorite for 15 minutes. Thereafter, the teeth were randomly divided into nine groups. One group was kept as control. The other eight groups received a final rinse with one of the following irrigation solutions: 18% EDTA, Sterilox, Top Clear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, Biopure MTAD, Ozonated water and Smear Clear. A total of 25ml of each solution was utilized to irrigate each root canal system, and left undisturbed in the root canal for 2 minutes. Biopure MTAD was left in the root canals for 5 minutes. Finally, the irrigation solutions were removed from the root canals with 10ml sterile distilled water. Each root was sectioned horizontally with a diamond disc in sections corresponding with the coronal, middle and apical levels of the root canal system. Each fragment was fractured laterally and prepared according to standard methods for biological SEM evaluation. The absence or presence of the smear layer as well as the amount of erosion of the peritubular dentine was assessed.

The One-way ANOVA test was used to determine whether there were any statistical significant differences between the different test groups.

No significant inhibition of *E. faecalis* was observed with sterile water (control) and the undiluted solutions of Sterilox, 10% Citric acid and Ozonated water. However, 3.5% NaOCl, 18% EDTA, Top Clear 17% EDTA, 2% Chlorhexidine, Biopure MTAD and Smear Clear showed significant inhibition of *E. faecalis*. For the 1/10 diluted solutions no inhibition of *E. faecalis* was observed with sterile water (control), 3.5% NaOCl, Sterilox, 10% Citric acid and Ozonated water. Biopure MTAD and Smear Clear demonstrated significant inhibition of *E. faecalis* compared to 18% EDTA, Top Clear 17% EDTA and 2% Chlorhexidine. Biopure MTAD was the only solution that showed significant inhibition of *E. faecalis* using a 1/100 diluted solution. No inhibition was observed with the 1/1000 diluted test solutions.

Scanning electron microscope examination revealed that 3.5% NaOCl and Ozonated water had no visible effect on the smear layer. The 10% Citric acid solution slightly modified the smear layer at the coronal and middle levels of the root canals. There was no visible effect on the smear layer at the apical levels. Sterilox and 2% Chlorhexidine removed the smear layer at the coronal levels, modified it slightly in the middle levels and left the smear layer undisturbed in the apical levels of the root canals. 18% EDTA, Top Clear 17% EDTA, Biopure MTAD and Smear Clear removed the smear layer completely at the coronal levels. At the middle and apical levels of the root canals most of the smear layer was removed. However, there were less open dentinal tubules visible at the apical levels compared to the samples examined at the middle levels of the root canals. It was also noted that Top Clear 17% EDTA caused a significantly high percentage of erosion of the peritubular dentine at the coronal levels of the root canals compared to all the other irrigation solutions.

Considering all the results of the present study Biopure MTAD demonstrated the best antimicrobial activity against *E. faecalis*, and removed most of the smear layer at all three levels of the root canal systems without significant erosion of the peritubular dentine.

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CHAPTER 1

Introduction and Literature Review

1.1 History of Endodontics

In October 1910, William Hunter, an English physician and pathologist gave a lecture on focal infection at the University of Montreal. His lecture “The role of Sepsis and Antisepsis in Medicine.” had such an impact that for more than 20 years it blocked research and the teaching of endodontics. Much of his subject matter originated from an earlier relatively unnoticed paper by W.D. Miller, who in 1891 had expounded upon the intimate relationship between the dental and medical professions. Hunter criticized the poor quality of prosthetic dentistry done in the United States. However, it was widely interpreted as an indictment of the pulpless tooth. Consequently, for almost 40 years American dentist continued to extract devitalized teeth. Despite the many hazards restricting their efforts, a number of dentists of that time sought to improve their endodontic skills. Without aseptic techniques and radiographic control a considerable number of endodontic cases ended in failure (1).

Fortunately some pioneer endodontists and others demonstrated successful cases based on sound biological principles and illustrated methods by which strategic teeth could be saved without danger to the patient’s health. – in fact, with improved health. By the late 1940s or early 1950s the theory of focal infection fell and endodontic treatment became an integral part of clinical dentistry (1).

1.2 Principles of Endodontic Treatment

1.2.1 Objective of Endodontics

The objective of endodontic therapy is the restoration of a treated tooth to its proper form and function in the masticatory apparatus. The treated tooth should be in an acceptable state of health (1).

1.2.2 Phases of Therapy

There are three basic phases in endodontic treatment. The first is the diagnostic phase during which the disease to be treated is determined and the treatment plan developed. The second is the preparatory phase during which the contents of the root canal system are removed and the canal cleaned and prepared for the obturation material. The third phase involves the obturation of the canal system with an inert material in order to gain an hermetic seal as close to the cemento-dentinal junction as possible (1).

1.2.3 Importance of Debridement

Endodontic therapy is a debridement procedure that requires removal of the irritants from the canal and periapical tissues if success is to be achieved. The debridement

procedure may be carried out in various ways and may include instrumentation of the root canal, irrigation, placement of medicaments, and electrolysis or surgery. When a root canal is correctly prepared, any acceptable method of root canal obturation will undoubtedly produce a successful result (1).

1.2.4 Respect for Periapical Tissues

Some techniques advocate intentional irrigation of the periapical tissues, many studies have indicated that all enlargement and obturation procedures should be carried out within the confines of the root canal system. Over-instrumentation is the most common cause of postoperative pain. The use of caustic drugs or toxic irrigating solutions should be avoided to ensure protection of periapical tissues (1).

1.2.5 Adequate Restoration of the Treated Tooth

A large number of endodontically treated teeth are lost through fracture due to improper restorations rather than poor endodontics. Adequate restoration of the treated tooth is an integral part of root canal therapy and must be explained to the patient as a part of the treatment plan (1).

1.2.6 Postoperative Observation

Despite a high degree of endodontic success, some root canal treatment failures will occur. It is important that patients are made aware of the necessity for recall in order

to intercept possible failures at an early stage. Re-treatment of some of these cases may be successful but many may require apical surgery or even extraction.

1.3 Microbiological Basis for Endodontic Treatment

It is well established that bacteria are the main etiological factor in the development of dentinal caries and its progression to pulpal and periapical disease (2). Miller (1973) was the first to demonstrate that bacterial invasion of dentinal tubules of both carious and non-carious dentine. He also reported that the tubule microflora in the dentinal tubules consisted of cocci and rods (3). Keyes demonstrated that dentinal caries did not develop in germ-free rats that were fed a range of diets (4), while Kakehashi *et al.* (5), convincingly established the role of bacterial infection in pulpitis and periapical periodontitis. The pulps in germ-free and normal rats were exposed and left open to the oral environment. In gnotobiotic (germ-free) rats, exposed pulps remained healthy, despite the physical damage and food impaction, and initiated repair by way of dentine bridging. Normal rats demonstrated a severe inflammatory response that ultimately resulted in pulpal necrosis, pulpal abscesses and apical periodontitis. This research not only emphasized the important role of bacterial infection in pulpal and periapical disease, but also illustrated the important fact that in the absence of infection, healing and repair of a pulpal wound is predictable (5).

1.3.1 Invasion of Dentinal Tubules by Root Canal Bacteria

Invasion of dentinal tubules by bacteria from supra- and subgingival plaque occurs whenever dentine is exposed in the oral cavity. This can be through carious lesions, restorative or periodontal procedures, tooth wear, enamel and dentine cracks, or dental trauma (6-9). It is important, however, to note that bacteria which are associated with an infected root canal differ from those which are primarily associated with invasion of carious and non-carious dentine. Therefore, although *Streptococcus species* and *Actinomyces species* are major components of dental plaque (10) and may initiate tubule and pulpal infection, obligatory anaerobic bacteria are commonly present in large numbers in infected root canals. The presence of these bacteria may be responsible for continued root canal infection and apical periodontitis (11).

Hundreds of bacterial species are recognized as components of the oral microflora. However, only a of these species appear to have the ability to invade coronal dentine, infect the root canal system, and subsequently invade radicular dentinal tubules (12 - 14). This clearly suggests that many of the oral bacteria species does not have the necessary properties that allow invasion and survival in the intra-tubular environment.

1.3.2 Carious Coronal Dentine

Cariogenic microflora which occur on the surface of fissures, on smooth coronal or root surfaces consist mainly of streptococci, lactobacilli, and *Actinomyces species* (15). *Streptococcus mutans* and *Streptococcus sobrinus* are the main members of the

mutans group of streptococci that are considered etiological agents for induction of coronal dentine and root caries (15-17). Samples taken from the pulpal side of carious dentine lesions of extracted teeth contain larger numbers of Gram-positive anaerobic rods of *Eubacterium*, *Propionibacterium*, and *Bifidobacterium* species, while *Actinomyces* and *Lactobacillus* species comprised the majority of facultative bacteria isolated. Streptococci on the other hand accounted for only a minority of the total isolates (18). Table 1.1 summarizes all the bacterial species identified in carious coronal dentine (19, 20).

1.3.3 Non-carious Dentine

In vivo studies demonstrated that bacteria are able to penetrate the dentinal tubules of non-carious coronal dentine that was exposed to the oral environment (21, 22). Within a week after exposure, inflammatory changes within the pulp can be observed (21). Studies have also demonstrated that microleakage of oral bacteria around restorations (23, 24), as well as through enamel cracks and fractures as a result of trauma(9), may result in pulpal inflammation (24) or periapical disease (25). The composition of the microflora invading exposed non-carious dentine has not been fully elucidated. It is usually dominated by Gram-positive cells (6, 21, 23, 24) and probably resembles the composition of the biofilm infiltrating the tooth-restoration interface (26).

1.3.4 Microflora of the Infected Root Canal

Almost all bacteria recovered from the root canal system are from the oral microflora (13, 26-28) and various factors such as nutrient supply, oxygen tension, and bacterial interactions can influence the development of root canal flora (29, 30). Table 1.2 summarizes those bacterial species that are identified in asymptomatic infected root canals (19, 31).

Intra-radicular infection (32), extra-radicular infection (33), and other pathoses e.g. a true cyst (34, 35) are the main causes of persistent periapical pathology subsequent to root canal treatment. Unlike primary root canal infections, which are typically mixed, consisting of between two and eight bacterial species with obligate anaerobic bacteria dominating the microflora and streptococci making up a significant proportion of the facultative species, the root canal flora from failed endodontic cases are primarily Gram-positive facultative anaerobes and comprise one to two species per canal (36). The most frequently cultivatable microorganisms include bacterial species from *Enterococcus*, *Streptococcus*, *Peptostreptococcus*, and *Actinomyces* species (36, 37, 38). In addition, yeasts, notably *Candida albicans*, have been isolated from cases of endodontic failure (32, 37, 38, 39). *Enterococcus faecalis*, which constitutes a small percentage of the flora in primary root canal infection, is the bacterial species most frequently recovered in root-filled teeth, often as a pure culture (38, 39).

1.3.5 *Enterococcus faecalis*

As previously mentioned, *E. faecalis* is the bacterial species most frequently recovered from root-filled teeth. For bacteria to be involved in the pathogenesis and maintenance of apical periodontitis, they must be able to survive in the inhospitable environment of the obturated root canal where the nutrient supply is limited. Studies have shown that *E. faecalis* is able to withstand a high alkaline environment such as the one generated by calcium hydroxide (11), and appears to be related to a cell proton-pump that is necessary for its survival at high pH (40). Therefore, it is able to form biofilms in calcium hydroxide-medicated root canals (41). In addition, under starved conditions, it shows resistance to sodium hypochlorite (42), heat, hydrogen peroxide, acid and ethanol (43). *E. faecalis* can also survive extended periods of starvation in water (44, 45) within water filled dentinal tubules (46), and in human serum (47). This is most likely to reflect the nutritional supply within non-vital radicular dentinal tubules.

1.3.5.1 Characteristics and strains of *E. faecalis*

Enterococci are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen (48, 49). They are Gram-positive cocci that can occur singly, in pairs, or in short chains. *Enterococcus* species occur in the human intestinal lumen, in the human female genital tract and in lesser numbers the oral cavity (50). They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine and many α -keto acids (48). Enterococci can

survive very harsh environments including extreme alkaline pH (48, 51) and salt concentrations (48, 51). They can also resist bile salts, detergents, heavy metals, ethanol, azide and desiccation (48). They can grow within a range of 10 to 45 °C and can survive a temperature of 60°C for 30 minutes (51).

Since 1970, attention has been drawn to enterococci because they were recognized as major nosocomial pathogens causing bacteremia, endocarditis, bacterial meningitis, urinary tract, and various other infections (52). Studies show that *E. faecalis* is able to translocate from the root canal system to the submandibular lymph nodes of germ-free mice, suggesting that this route of infection may play a role in the pathogenesis of opportunistic infections in patients (53, 54).

1.3.5.2 Prevalence in Secondary Root Canal Infections

E. faecalis is a normal inhabitant of the oral cavity. However, the prevalence of *E. faecalis* is increased in oral rinse samples from patients receiving initial endodontic treatment, as well as from those who are midway through treatment, or patients receiving endodontic re-treatment as compared to those patients with no endodontic history (55).

E. faecalis is associated with different forms of periradicular disease including primary endodontic infections and persistent infections (50). In the category of primary endodontic infections, *E. faecalis* is associated significantly more often with chronic periradicular lesions than with acute periradicular periodontitis or acute periradicular

abscesses. It is found in approximately 4-40% of primary endodontic infections (50). In persistent periradicular lesions the frequency of *E. faecalis* has been shown to be significantly higher. Failed root canal treatment cases for example are nine times more likely to contain *E. faecalis* than are primary endodontic infections (50). Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated prevalence ranging from 24 -77% (37, 39, 56-63). Table 1.3 provides a list of studies that report on the occurrence of *E. faecalis* in root filled teeth with apical periodontitis (36 – 38, 49, 59, 61).

1.3.5.3 Survival and Virulence Rates

E. faecalis possesses some definite virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid (49). It can adhere to host cells, and to express proteins that allow it to compete with other bacterial cells, thereby altering host responses (47, 49). In addition, it can also suppress the action of lymphocytes, potentially contributing to endodontic failure (64).

Because *E. faecalis* is less dependent upon virulence factors, it relies more upon its ability to survive and persist as a pathogen in root canals of teeth (65). *E. faecalis* can ensure survival within the root canal system in several ways:

- It can exhibit widespread genetic polymorphism (55).
- It possesses serine protease, gelatinase and collagen-binding protein, which help it to bind to dentine (66).

- It is small enough to proficiently invade and live within dentinal tubules (47).
- It can endure prolonged periods of starvation until adequate nutritional supply becomes available (67).
- It can resist calcium hydroxide intracanal dressings for more than 10 days (11, 46).
- *E. faecalis* is able to form a biofilm that helps it resist destruction. This enables the bacteria to become 1000 times more resistant to phagocytosis, antibodies and antimicrobials than nonbiofilm-producing organisms (41).

1.3.5.4 Calcium Hydroxide and *E. faecalis*

Calcium hydroxide is a commonly used intracanal medicament applied in root canal treatment. This material has been shown to be ineffective on its own in killing *E. faecalis*, especially when a high pH is not maintained (11, 68, 69). The following reasons have been proposed to explain why *E. faecalis* is able to survive intracanal treatment with calcium hydroxide:

- *E. faecalis* can passively maintain pH homeostasis, as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity (68).
- It contains a proton pump that provides additional means of maintaining pH homeostasis. This is accomplished by pumping protons into the cell to lower the internal pH (69).

- *E. faecalis* is able to survive at a pH of 11.5 or more (69, 70). However, as a result of the buffering capacity of dentine, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques (71). Studies have shown that the presence of dentine has an inhibitory effect on various concentrations of root canal medicaments using the dentine powder model. This was proven in these studies for several intracanal medicaments including calcium hydroxide, sodium hypochlorite, chlorhexidine, and iodine potassium iodide (72, 73).

1.3.5.5 Methods of Eradication of *E. faecalis*

Most of the treatment regimes are aimed at eliminating or preventing *E. faecalis* infection to gain access to the root canal space during treatment, between appointments or even after the treatment has been completed (49).

A protocol which may be considered for eradication is to prepare the apical portion of the root canal to a larger instrument size that will help to eliminate intracanal microorganisms by reaching areas not normally accessible to smaller master apical files (74). The larger sizes also facilitate removal of the innermost dentine. This has the potential of removing intratubular bacteria, and it will open dentinal tubules to allow antimicrobials, irrigating solutions and intracanal medicaments to penetrate more effectively.

Combinations of medicaments to eliminate *E. faecalis* have also been investigated. In one study, a combination of calcium hydroxide with camphorated paramonochlorophenol completely eliminated *E. faecalis* within the dentinal tubules (75). Vitapex, a silicone oil-based hydroxide paste containing 38% iodoform, disinfected dentinal tubules infected with *E. faecalis* more effectively than calcium hydroxide alone (76). Concentrations of 1 to 2% chlorhexidine combined with calcium hydroxide have also demonstrated efficacy at killing *E. faecalis* (75, 77, 78). Chlorhexidine combined with calcium hydroxide will result in greater ability to kill *E. faecalis* than calcium hydroxide mixed with water (77). It is important to note, however, that chlorhexidine alone has been shown to provide as good, or even better, antimicrobial action against *E. faecalis* than calcium hydroxide/chlorhexidine combinations (78, 79).

In another protocol, an attempt was made to produce iodoform-containing gutta-percha points impregnated with chlorhexidine. This method demonstrated little inhibitory action against *E. faecalis* (80, 81).

The antimicrobial activity of various root canal sealers against *E. faecalis* has also been studied. Roth 811 (Roth International Ltd., Chicago, IL), a zinc oxide-eugenol based sealer, has been shown to exhibit the greatest antimicrobial activity against *E. faecalis* when compared to other sealers (82).

The combination of adequate instrumentation, appropriate use of irrigants, medicaments and root canal sealers will optimize the chances of eradicating *E. faecalis* during re-treatment of failed root canal cases.

Additional steps that can be taken to prevent *E. faecalis* from re-entering the root canal space include:

- oral rinse with chlorhexidine before treatment,
- disinfecting the tooth with chlorhexidine or sodium hypochlorite under rubber dam isolation, and /or
- disinfecting gutta-percha points with sodium hypochlorite before insertion into the root canal (83).

Another approach to preventing *E. faecalis* to enter the root canal system is to provide the patient with a well sealed coronal restoration (84).

A new alternative method includes using an obturating system that can provide a more effective seal in the root canal eg. Epiphany (Pentron Corp., Wallingford, CT). This material has been designed to bond to the root canal walls thereby prevent bacterial leakage. A preliminary study showed that this method is better at preventing microleakage of *E. faecalis* than gutta-percha filled root canals (85).

1.4 Effectiveness of Endodontic Procedures

Endodontic procedures are aimed at the management of bacterial infection in the root canal system. It is important to maintain adequate asepsis during the instrumentation procedure and to ensure complete removal of the soft tissue of the pulp. Improper instrumentation may result in tissue remnants adhering to the canal walls (86). If these are concomitantly contaminated, conditions for bacterial growth are enhanced. Current methods available for bacterial reduction in endodontic therapies include mechanical instrumentation to clean and widen the root canal space, and chemical disinfection by irrigation and intracanal medication, known as an antimicrobial dressing (86).

1.4.1 Bacterial Elimination by Mechanical Root Canal

Preparation

Mechanical root canal instrumentation is a primary means of bacterial reduction in endodontics. A study by Byström and Sundqvist (87) demonstrated that mechanical instrumentation can reduce the bacterial counts 100 to 1000-fold in root canals. However, no teeth showed bacteria-free cultures after the first appointment and the residual bacteria in the canals proliferated between appointments.

1.4.2 Elimination of Bacteria by Mechanical Root Canal

Preparation and Chemical Disinfection by Irrigation

The use of irrigants in conjunction with mechanical instrumentation is essential in order to loosen and help remove debris and bacteria. It is also important that the irrigating solution provide antibacterial effects. These antibacterial properties can include the overall killing of bacteria in the root canal system and will also provide disinfection in areas of the canal that are inaccessible to mechanical instrumentation.

The ideal root canal irrigation solution should possess the following properties (88):

1. Digest proteins and dissolve necrotic tissue.
2. Have a low surface tension to reach the apical delta and all areas that cannot be reached by the root canal instruments.
3. Lubricate the canal instruments.
4. Have germicidal and antibacterial properties.
5. Be non-toxic and non-irritating to the periapical tissues.
6. Keep the dentinal debris in suspension.
7. Prevent discoloration of the tooth.
8. Must be readily available and inexpensive.
9. Relatively harmless to the patient and the clinician.

Numerous irrigating solutions have been recommended for clinical use (89). Irrigation with distilled water or saline is effective in eliminating loose debris from the upper and middle third of the root canal, but have little or no effect on the smear layer (90).

1.5 The Dentinal Smear Layer

The aim of root canal instrumentation and irrigation is to prepare a clean, debris-free root canal system prior to obturation. It has been shown with scanning electron microscopy that a layer of superficial debris forms over the surface of the dentinal walls whenever dentine is cut with an instrument (91–93). This layer of debris has been called the smear layer and has been observed by McComb and Smith (92) on the walls of instrumented root canals.

1.5.1 Structure of the Smear Layer

The smear layer consists of ground dentine and predentine, pulpal remnants, odontoblastic processes, remnants of irrigants and bacteria in the case of infected teeth (92-94). According to Cameron (95), the smear layer on the wall of the root canal could have a relatively high organic content in the early stages of instrumentation because of necrotic and/or viable pulp tissue in the root canal. The reported thickness of this layer is 1-5 μ m (93, 96). This thickness may depend on the sharpness and type of the cutting instruments and whether the dentine is cut under dry or wet conditions (97, 98).

Cameron (99) described the smear layer material in two parts:

- First, a superficial smear layer on the surface, and,
- Secondly, smear layer packed into the dentinal tubules. The depth of the dentinal tubule plugs can be between 6 and 40 μm (93). Cameron (99) also concluded that this tubular packing phenomenon of the smear layer is due to the action of burs and endodontic instruments (100). However, Cengiz, Aktener and Piskin (101) proposed that the penetration of the smear material into the dentinal tubules could be caused by capillary action as a result of adhesive forces between the dentinal tubules and the smear material. This hypothesis of capillary action may explain the packing phenomenon observed by Aktener, Cengiz and Piskin (102), who showed that this penetration was increased up to 110 μm by the use of surface-active irrigants during endodontic instrumentation.

1.5.2 Physical Barrier for Bacteria and Disinfectants

The advantages and disadvantages of smear layer and whether it should be removed or not from instrumented root canals before obturation is still controversial in dental literature. Even after chemo-mechanical preparation microorganisms can remain in or migrate into dentine (87,103,104). Some authors propose that the smear layer acts as a barrier to bacterial metabolites, preventing bacterial invasion of the dentinal tubules, rather than being a preferred site for bacterial colonization (105-107). Bacteria, however, not only remain, but also survive and multiply in the smear layer (108-110)

and can also penetrate into the dentinal tubules (111-113). The antimicrobial action of medicaments in the dentinal tubules can also be prevented or delayed by the smear layer (46, 109,110). For this reason it would appear to make smear layer removal advisable.

1.5.3 Smear Layer and Microleakage

Smear layer on root canal walls can act as an intermediate physical barrier and may interfere with adhesion and penetration of root canal sealers into the dentinal tubules. Another advantage of smear layer removal is the ability of root canal sealer to penetrate the dentinal tubules, thereby improving adaptation of obturation material to the root canal walls (107, 114, 115).

Microleakage is defined as the passage of bacteria, fluids and chemical substances between the root structure and any type of filling material. It occurs because there are microscopic gaps at the interface of the filling material and the tooth surface. Smear layer may thus present a passage for substances to leak around or through its particles at the interface between obturation material and the dentine surface. Since the nonhomogenous structure of the smear layer (93), it may slowly disintegrate, dissolving around a leaking filling or obturation material. Pashley and Depew (116) found that microleakage decreased after smear layer removal, but dentine permeability increased. In a study by Saunders and Saunders (117) it was shown that coronal leakage of root canal filling materials was less in smear-free groups than in

those with a smear layer. However, there is a risk of reinfection of dentinal tubules by microleakage if the seal should fail after removal of the smear layer (110).

1.5.4 Removal of the Smear Layer

Electron microscopy has shown that the smear layer contains both organic and inorganic substances (109, 118). It consists mainly of inorganic components as root canal irrigation with sodium hypochlorite has little effect on removal of this layer. Partial or complete removal is only achieved with the aid of acids and chelating agents (109).

1.6 Irrigation Solutions

1.6.1 Sodium Hypochlorite

Sodium hypochlorite (NaOCl) is the most widely used irrigant in endodontics and has aided canal preparation for many years (119). Sodium Hypochlorite is an alkaline solution with a pH of approximately 11 to 12.

Many investigators have demonstrated the germicidal and antibacterial properties of sodium hypochlorite (104, 120, 121, 122). When sodium hypochlorite makes contact with water, NaOCl produces hypochlorous acid and sodium hydroxide. Hypochlorous acid then produces hydrochloric acid and oxygen. The free chlorine has germicidal

properties when it combines with protoplasmatic constituents, such as proteins (123). 5.25% Sodium hypochlorite solutions has been shown to be potently bactericidal against Gram-positive and Gram-negative bacteria, spore-producing microorganisms, and is also effective against viruses. Recent *in vitro* studies have also demonstrated the germicidal effect of 5.25% sodium hypochlorite against some obligate anaerobes commonly found in infected root canals (*Bacteriodes melaninogenicus*, *Bacteriodes fragilis*, *Clostridium perfringes* and *Peptostreptococcus anaerobius*) (124). Siquera et. al., (1997) have demonstrated the killing efficacy of low concentrations of NaOCl against *Enterococcus faecalis* (125).

NaOCl, especially when used in high concentrations is known to be effective in dissolving organic tissue remnants in and disinfecting the root canal system (126). The organic tissue-dissolving activity of NaOCl is well known (127,128) and increases with rising temperatures (129). There is some disagreement in the literature regarding the solvent action of NaOCl on vital pulp tissue. Grey (1970) demonstrated that tissues contained in the lateral canals of vital teeth appeared almost intact and undigested after exposure to NaOCl (130). Using scanning electron microscopy, McComb and Smith arrived at the same conclusion in 1975 (92). Examination of the accessory canals containing vital pulp tissue suggests that some degree of tissue digestion occurs, especially in the portion of the accessory canal adjacent to the principal root canal, but this seems to be due to the digestive action of the NaOCl on that portion of the tissues which has deteriorated in the interval of time between two appointments. The limited activity of NaOCl on tissues still perfused with blood also seems to confirm the clinical impression that during the obturation phase with warm

gutta-percha, it is easy to accomplish filling of the lateral canals of necrotic teeth, whose content has been digested, rather than of vital teeth, which still contain vital pulp tissue (88). Newer studies that have appeared subsequently seem to dispute the above statements (131). Rosenfeld *et al.* (131) demonstrated strong solvent action of full strength Chlorox (NaOCl) on vital uninstrumented teeth. The limited solvent effect in the apical region of the root canals was attributed to the barrier of the apical plug of dentine fillings, narrow lumen, and fibrous nature of the apical pulp tissue. According to these authors, the major obstacle to overcome in the clinical use of NaOCl is the ability to penetrate confined areas. According to Berrutti and Castellucci (88), if a canal is efficiently enlarged in the apical area of the root canal and the irrigant given an adequate period of time, it can exert a digestive effect even on vital, young, healthy human pulp tissue, like that of the accessory canals.

The effectiveness of NaOCl is also time and volume dependent. If a large volume of NaOCl is used to irrigate a canal and the NaOCl stays in contact with the dentinal tubules for a longer period, better results will emerge (132). The solvent effect is also increased by heat. Several studies have shown that warming NaOCl to approximately 60°C, significantly increases the rate and effectiveness of tissue dissolution (133-135).

The concentration of NaOCl used in the clinical environment ranges from 0.5% to 5.25%. The vast majority of authors favour the use of 5.25% NaOCl, since they believe that its toxicity is the same as that of physiologic solution if used as a canal irrigant (110, 121, 128, 136-141). At 5.25% it is much more effective as a solvent of necrotic tissues as compared with more dilute solutions (139), which reduce the

detergent capacity and the ability to remove debris (142, 143) and even reduces the antibacterial properties (93). Other authors favour the use of hypochlorite at low concentrations, between 0.5% and 1%, given its cytotoxicity and irritant effect on the periapex (144). Yet other authors also suggest the use of hypochlorite diluted to 2.5 - 3% (145-151).

There are also some disadvantages however. Various investigations have shown that NaOCl may irritate the periodontal and periapical tissues (152). Many *in vitro* and *in vivo* studies have reported moderate to severe cytotoxicity when NaOCl solution (in clinically recommended concentrations) is extruded through the apex (153,154). Sodium hypochlorite is cytotoxic to all cells, with the exception of highly keratinized epithelium (155). For this reason, the solution should be used with great care in clinical endodontics (156). Clearly because of the potential of toxicity of NaOCl, the investigation of alternative irrigants is important.

1.6.2 Chlorhexidine

Chlorhexidine gluconate has been widely used in periodontics due to its antibacterial activity (157, 158). Its use in endodontics has been proposed both as irrigant and intracanal medicament (159, 160). Chlorhexidine has been studied for its various properties: antimicrobial activity (153, 159, 160, 161); residual antimicrobial activity (161, 162); biocompatibility (153, 163) and action on bacterial lipopolysaccharide (LPS) (164) with the objective of being an alternative to sodium hypochlorite.

Chlorhexidine gluconate is a bisbiguanidine; it is a broad-spectrum antimicrobial agent and therefore it was deduced that it could probably be used as an irrigant. Its disinfective capabilities and adsorption onto dentine contributed to this assumption (165-168).

Chlorhexidine has inhibitory effects on bacteria commonly found in endodontic infections (169), acting against Gram-negative microorganisms (170). One of the mechanisms that explains its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the chlorhexidine molecule to penetrate into the bacteria with toxic effects (158).

Botha and Van der Vyver evaluated the antimicrobial effect of 17% EDTA, TopClear, Sodium Hypochlorite, Sterilox, STEDS, Hydrogen Peroxide (H₂O₂), Ozone Gas, and Chlorhexidine (CHX) on *E. faecalis* in root canals. In this study Chlorhexidine gluconate was effective as an antibacterial root canal disinfectant against *E. faecalis* (171).

Jeansonne and White (160) compared the antimicrobial activity of 2% Chlorhexidine gluconate with that of 5.25% sodium hypochlorite. The number of post-irrigant positive cultures and the number of colony-forming units in positive cultures obtained from Chlorhexidine treated teeth were lower than the numbers obtained from NaOCl treated teeth. When using a combination of 0.2% Chlorhexidine gluconate with 2.5% NaOCl, Kuruvilla and Kamath (166) found that the use of NaOCl and Chlorhexidine

gluconate together produced the highest percentage reduction of post-irrigant positive cultures, this reduction was significant compared to the use of NaOCl alone but not significant compared to the use of Chlorhexidine gluconate alone.

To improve antisepsis in a one-appointment regimen, it has been suggested to rinse the canals with chlorhexidine following irrigation with NaOCl (172, 173). Furthermore, it binds to the surrounding tissues to be released again slowly over extended periods of time, a phenomenon called substantivity (174). It also appears that chlorhexidine can efficiently inhibit the initial adherence and perhaps further accumulation and biofilm formation of yeast and microorganisms (175). A recent clinical study has shown that canals that received a final rinse with 2% chlorhexidine solution were significantly more often free of cultivatable microorganisms than controls irrigated with NaOCl alone (173). However, chlorhexidine preparations have little to no tissue-dissolving capacity (176), and should therefore only be used after thorough irrigation with NaOCl.

1.6.3 Ethylenediamine Tetra-acetic Acid (EDTA)

Ethylenediamine Tetra-acetic acid, was introduced by Nygaard-Ostby in 1957 to facilitate preparation of root canals, particularly in the case of narrow, calcified canals (135). It chelates calcium from both dentine and concretions in the pulp and forms soluble calcium chelates (177, 178). Von der Fehr and Nygaard-Ostby (179) found that EDTA decalcified dentine to a depth of 20 to 30µm in 5 minutes. Fraser (180) stated that the chelating effect was almost negligible in the apical third of root canals.

EDTA is not irritating to pulpal or periapical tissue, is self-limiting, and is not corrosive to endodontic instruments. As an additional benefit, EDTA has been found to inhibit bacterial growth (180). Steward, Kapsimalis and Rappaport (181) found that in combination with urea peroxide, EDTA is very effective to remove debris from a root canal, owing to its bubbling action, and it also improves the cutting capacity of canal instruments.

Chelating agents are used in endodontics for several purposes, like lubrication, emulsification, and flotation (182). They are available in either a viscous suspension or an aqueous solution.

Many authors recommend the use of a chelating agent to remove the smear layer of dentine mud that remains smeared on the internal surface of the canal after instrumentation. This layer which occludes the dentinal tubules and therefore reduces the permeability, is most often constituted of inorganic material and therefore cannot be digested by NaOCl (92, 93, 128). Instead, it can be removed by a chelating agent such as EDTA, used as an irrigating solution together with NaOCl (88).

EDTA solutions have also been combined with a quaternary ammonium bromide (cetrimide) to reduce the surface tension and to increase penetrability of the solution (179). McComb and Smith (92) reported that when this combination (REDTA, Roth International Ltd., Chicago, IL, USA) was used during instrumentation, there was no smear layer except in the apical part of the root canal. Dunavant *et al.* (183)

demonstrated significant antibacterial activity for SmearClear (mixture of 17% EDTA, cetrimide, polyoxyethylene (10) iso-octylcyclohexyl ether) with a 78% decrease in bacterial numbers compared to a 27% decrease in bacterial numbers for an irrigating solution only containing 17% EDTA.

There are no clear-cut recommendations as to the length of time that root canals should be irrigated with aqueous EDTA solutions (184). It must be cautioned that prolonged exposure of root dentine to strong chelators such as EDTA may weaken root dentine (185).

1.6.4 Tetracycline

Tetracycline is a broad spectrum antibiotic, well researched and used in dentistry, especially in periodontology (88). According to Berutti and Castellucci (88) tetracycline is absorbed and then gradually released by the mineralized tissues (dentine and cementum) of the teeth. Furthermore it carries out a chelating action contributing to the removal of the smear layer.

Barkhordar *et al.* (186) compared solutions to remove the smear layer and found that Doxycycline-HCl (100mg/ml) was the most effective in removing the smear layer compared to EDTA and NaOCl. When Haznedaroglu and Hadan (187) compared tetracycline HCl with 50% citric acid, they found that both removed the smear layer. Tetracycline, however did not widen the tubule apertures extensively and did not destroy as much peritubular dentine as did citric acid (187).

1.6.5 Electrochemically Activated Water (ECA)

Various electrode systems have been developed to electrically charge or activate water or watery solutions such as saline. These systems have mainly taken the form of plate reactors and some of these have been developed and commercialized for instance as salt chlorinators for swimming pools. The results of these experiments have been generally disappointing. One of the reasons for these relative failures has been the inefficiency of these electrode systems or reactors to activate chlorine.

Professor Vithold Bhakir of the former Soviet Union began experimenting with a newly configured electrode system. These electrodes consisted of a solid cylinder (the anode) inside a hollow tube (the cathode). A ceramic membrane, creating separate anodic and cathodic chambers, separated these two electrodes from each other. The distance between the two electrodes was very small ($\pm 1,5\text{mm}$), resulting in very large electrical gradients between the two. The electrodes were made from titanium and covered with unique coatings, rendering them impervious to corrosion (188, 189).

ECA is produced from salt solutions of low concentration in a special unit that houses a unique flow-through electrolytic module (FEM). The FEM consists of an anode, a solid titanium cylinder coated with ruthenium-oxide, iridium and platinum, and a cathode, made from titanium, coated with pyro-carbon and glass-carbon. A diaphragm consisting of ultra-filtration, electro-catalytic ceramics on a bed of zirconium, yttrium, aluminium and niobium-oxides separates the anode and cathode (190).

Electrochemical treatment in the anode and cathode chambers of the diaphragm electrolyzer transforms water and dilutes mineral solutions into a metastable state that is characterized by modified values of physical-chemical parameters, notably, the pH and oxidation-reduction potential. The FEM is capable of producing types of solutions that have bactericidal and sporicidal activity, yet are odorless, safe to human tissue and essentially non-corrosive to metal surfaces (191, 192). The ECA devices have been in widespread commercial use in Russia and the commonwealth of Independent States for a number of years, mainly in the areas of hospital disinfection, sterilization, and in agricultural and industrial processes (193).

Electrochemical treatment in the anode and cathode chambers result in the synthesis of two types of solutions. The solution produced in the anodic chamber, termed anolyte, is reputed to demonstrate pronounced microbiocidal effectiveness against bacteria, viruses, fungi and protozoa (193, 194). The anolyte solution has been termed Super-Oxidized Water (191) or Oxidative Potential Water (195). Depending on the type of FEM, the pH of the produced anolyte solution varies; it may be acidic (anolyte), neutral (neutral anolyte) or alkaline (anolyte neutral cathodic). Anolyte has a very high oxidation potential (ORP 700 –1200 mV). The solution produced in the cathodic chamber, termed catholyte, has a very high reduction potential (ORP 700 – 1200 mV) and is thought to be anabolic (196).

Marias (197) compared ECA to 5.25% NaOCl. He found that the cleaning ability of ECA was very similar to that of NaOCl when viewed in an electron microscope.

Marais and Brozel (196) investigated the effect of electro-chemically activated water on biofilm contamination in dental unit water lines. They found that electrochemically activated water was effective in controlling bacterial counts and biofilm in dental unit waterlines.

Marais (198) compared electro-chemically activated water to NaOCl for its cleaning effect on root canal walls. He found that the cleaning efficacy of electro-chemically activated water in root canals was considered to be superior to NaOCl.

Van der Merwe, Marais and Botha (199) compared the antimicrobial efficacy and irrigating potential of different solutions to remove *E. faecalis* from infected canals. ECA gave the best results in removing the smear layer and eliminating *E. faecalis* from the root canals.

Botha and Van der Vyver evaluated the antibacterial effect of ECA as well as the corrosive effect ECA had on the Adec tubing used in the dental unit water lines (200). The two ECA products tested in this study were Sterilox and Radical Waters. The antibacterial activity varied against different bacterial species, Sterilox produced the best antibacterial activity. The corrosive effect on the Adec tubing also varied between the methods used to prepare the solutions.

1.6.6 Citric Acid

Citric acid is highly biocompatible and is commonly used in personal care products (201). It has been used in periodontal procedures as an aid in connective tissue reattachment by exposing collagen fibres on the root surface. Citric acid also exhibits antibacterial properties, as well as bacterial growth inhibition (202). Like EDTA, this demineralizing agent has been recommended as an adjuvant in root canal therapy (203). Citric acid has also been recommended for use as an endodontic irrigant because of its low pH, which causes dentine dissolution and thereby produces similar results to EDTA. Moreover, when used in concentrations of 10%, 25% and 50%, citric acid has been shown to remove the smear layer associated with instrumentation of the canal system (204).

An important aspect related to using EDTA and citric acid as irrigating solutions, is that they can strongly interact with NaOCl (205). Both these agents reduce the available chlorine in solution, rendering the NaOCl ineffective on bacteria and necrotic tissue (206). Therefore, citric acid or EDTA should never be mixed with NaOCl. The same applies to using paste-type EDTA preparations. At a 1:10 ratio; they immediately rid 1% NaOCl solution of all hypochlorite (207). The "bubbling effect" or effervescence used to advocate for such products is proof of this chemical reaction that takes place between hypochlorite on the one hand and EDTA and hydrogen peroxide on the other, resulting in evaporating gas (205). Oxygen evaporates from the aqueous peroxide-hypochlorite mixtures, and chlorine and oxygen gas from corresponding mixtures of NaOCl with EDTA or citric acid (205).

1.6.7 BioPure MTAD

A new root canal irrigation solution called MTAD has recently been proposed (208).

Biopure MTAD irrigation solution contains:

- * Tetracycline (150mg/5ml Doxycycline, Sigma-Aldrich Co., St Louis MO, USA)
- * Acid (Citric acid, Sigma-Aldrich)
- * Detergent (Tween 80, Sigma-Aldrich)

The citric acid and tetracycline removes the smear layer and allows the antibiotic molecule to enter into the dentinal tubules. Thereafter, the detergent has the function of reducing the surface tension and increasing the penetratability of the irrigation solution into the tubules (208).

According to Berutti and Castellucci (2005) BioPure MTAD irrigation solution has the following properties (88):

- Can completely remove the smear layer without significantly altering the dentinal structure (209),
- Solubilizes the organic components of the pulp residues and inorganic components of the dentine (210),
- Efficacious antibacterial solution, even against *E. faecalis* (211-213),
- Is less toxic than most substances commonly used in dentistry (214),
- Does not alter the physical properties of dentine (215),

- Conditions the dentinal surface exposed to the solution. This can prepare dentine surfaces for successive adhesive phases (216),
- Can lead to a reduction in coronal leakage of the teeth obturated with gutta-percha (217).

1.6.8 Ozonated Water

The use of ozonated water for treatment of endodontic infections has been suggested (218, 219). Ozone has also been used in the water industry to eliminate bacteria (220) and its properties could be useful in dentistry (221). Ozone is a blue gas, containing three oxygen atoms, it is an irritant, toxic, unstable and also very reactive (222). The antimicrobial effect from ozone results from oxidation of microbial cellular components. Ozone is generated by passing oxygen through high-voltage (223).

Studies have reported interesting results when ozone-treated water was used in the dental unit (220, 221). Nagayoshi *et al.* (218) observed that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with ultrasonification. They also reported a low level of toxicity against cultured cells. However, Hems *et al.* (219), evaluating the ability of ozone to kill an *E. faecalis* strain verified that its antibacterial efficacy was not comparable to that of NaOCl.

1.7 Objectives of Research Project

The objectives of this *in vitro* study were:

- to establish the antimicrobial efficacy of nine different root canal irrigation solutions,
- to determine the smear layer dissolving capabilities of these irrigating solutions, at various levels in straight root canals, and
- to examine the degree of erosion caused by the irrigation solutions on the root canal walls.

Table 1.1. Bacterial species identified in carious coronal dentine (19, 20).

Bacterial genus or species	Isolation frequency in carious dentine		Bacterial genus or species	Isolation frequency in carious dentine	
	Superficial	Deep		Superficial	Deep
<i>Streptococcus</i>	High	Low-moderate	<i>Propionibacterium</i>	Moderate-high	High
<i>S. mutans</i>			<i>P. acnes</i>		
<i>S. sobrinus</i>			<i>P. avidum</i>		
<i>S. intermedius</i>			<i>P. lymphophilum</i>		
<i>S. morbillorum</i>			<i>P. propionicum</i>	Low	Moderate
<i>S. sanguinis</i>					
<i>Peptostreptococcus</i>	Low	Low	<i>Lactobacillus</i>	High	High
<i>P. anaerobius</i>			<i>L. casei</i>		
<i>P. parvulus</i>			<i>L. plantarum</i>		
<i>P. micros</i>			<i>L. minutus</i>		
<i>Actinomyces</i>	High	Moderate	<i>Fusobacterium nucleatum</i>	Low	Low
<i>A. israelii</i>					
<i>A. naeslundii</i>					
<i>A. odontolyticus</i>					
<i>Eubacterium</i>	High	High	<i>Peptococcus spp.</i>	Low	Low
<i>E. alactolicum</i>					
<i>E. aerofaciens</i>					
<i>E. saburreum</i>					
<i>Veillonella spp.</i>	Moderate	Low	<i>Clostridium spp.</i>	Low	Low
<i>Bifidobacterium spp.</i>	High	High	<i>Porphyromonas spp.</i>	Low	Low
			<i>Prevotella spp.</i>	Low	Low

Table 1.2. Bacterial species commonly found in asymptomatic infected root canals (19, 31).

Gram-positive cocci	Gram-positive rods	Gram-negative cocci	Gram-negative rods
<i>Streptococcus anginosus</i>	<i>Actinomyces Israeli</i>	<i>Capnocytophaga ochracea</i>	<i>Fusobacterium nucleatum</i>
<i>S. sanguinis</i>	<i>A. naeslundii</i>	<i>C. sputigena</i>	<i>Prevotella intermedia</i>
<i>S. mitis</i>			<i>P. melaninogenica</i>
<i>S. mutans</i>	<i>Eubacterium alctolyticum</i>		<i>P. denticola</i>
	<i>E. lentum</i>	<i>Veillonella parvuta</i>	<i>P buccae</i>
<i>Enterococcus faecalis</i>	<i>E. nodatum</i>		<i>P. buccalis</i>
	<i>E. timidum</i>		<i>P. oralis</i>
<i>Peptostreptococcus micros</i>		<i>Campylobacter rectus</i>	
<i>P. anaerobius</i>	<i>Propionibacterium propionicum</i>	<i>C. curpus</i>	<i>Porphyromonas gingivalis</i>
	<i>P. granulosum</i>		<i>P. endodontalis</i>
			<i>Bacteroides gracilis</i>
	<i>Lactobacillus</i>		

Table 1.3. Studies investigating the prevalence of *E. faecalis* in root—filled teeth with an apical periodontitis.

Author/Year	Number of Root-filled Teeth in Study	Number of Root-filled Teeth with Bacterial Growth	Prevalence of <i>E. faecalis</i>	Method of Detection
Molander <i>et al.</i> , 1988 (37)	100	68	32/68 = 47%	Culture
Sundqvist <i>et al.</i> , 1998 (38)	54	24	9/24 = 38%	Culture
Peciuliene <i>et al.</i> , 2000 (59)	25	20	14/20 = 70%	Culture
Pinheiro <i>et al.</i> , 2003 (61)	30	24	11/24 = 46%	Culture
Siquerira & Rôcas 2004 (62)	22	22	17/22 = 77%	PCR
Gomes <i>et al.</i> , 2004 (36)	19	19	6/19 = 32%	Culture
Rôcas <i>et al.</i> , 2004 (49)	30	30	20/30 = 67%	PCR

CHAPTER 2

Materials and Methods

2.1 Irrigation Solutions

The nine different irrigation solutions that were used in this study were as follows:

- **3.5% Sodium Hypochlorite Liquid (NaOCl)** (Rekitt Benckiser South Africa (Pty) Ltd., Elandsfontein, Gauteng, South Africa) (Batch no: 0055366)(Fig. 2.1),
- **EDTA 18% Root Canal Irrigating Solution** (Ultradent Products, Inc., South Jordan, Utah, USA.)(Batch no: B0FVZ)(Fig. 2.2),
- **Sterilox Electrolyte Solution activated in Sterilox Machine** (Optident, International Development Centre, West Yorkshire, UK)(Batch no: MM17604)(Fig. 2.3),
- **TopClear Solution (mixture of 0.2% Cetremide and 17% EDTA)** (Dental Discounts CC, Paulshof, Sandton, South Africa) (Batch no: 10557)(Fig. 2.4),
- **Vista CHX 2% Chlorhexidine Gluconate Solution** (Vista Dental Products, Racine, WI, USA)(Batch no: 090905)(Fig. 2.5),

- **Citric Acid 10% Root Canal Irrigating Solution** (Ultradent Products, Inc., South Jordan, Utah, USA.)(Batch no: B0C3F)(Fig. 2.6),
- **BioPure MTAD Antibacterial Root Canal Cleanser (mixture of 150mg/5ml Doxycycline, Citric Acid and Tween 80)**(Dentsply Tulsa Dental, Johnson City, Tulsa, USA)(Batch no: 040920)(Fig. 2.7),
- **Ozonated Water produced in OH DENT Generator** (Unique Dental, Centurion, South Africa)(Batch no: 0702021)(Fig. 2.8), and
- **SmearClear (mixture of 17% EDTA, cetrimide, polyoxyethylene (10) iso-octylcyclohexyl ether)** (SybronEndo, Glendora CA, USA)(Batch no: 450788)(Fig. 2.9).

2.2 Antimicrobial Effects

In this test the zones of inhibition on plates inoculated with *E. faecalis* were measured. A MacFarland Standard 1 suspension (224) was prepared from an overnight culture of *E. faecalis* (ATCC 49474) and spread onto twenty Casein-peptone-Soymeal-peptone Agar (CASO-Agar) plates (Merck SA (Pty) Ltd., Halfway House, South Africa) with a sterile glass rod (Fig. 2.10).

The prepared agar plates were randomly divided into 10 groups (n=2), two replicates being prepared for each sample solution. The following concentrations of each irrigation solutions were prepared: 100% (Undiluted), 1/10, 1/100 and 1/1000 dilutions. Ten microlitres of each concentration and test solution were dispensed onto four, standardized, sterilized, five millimetre diameter filter paper disks that were placed in each of the divided quadrants of the inoculated Agar plates (Fig. 2.11).

Plates were incubated anaerobically using an Anaerocult A (Merck SA (Pty) Ltd., Halfway House, South Africa) at 37° C for 24 hours, and antibacterial activity evaluated using the conventional agar plate diffusion method. The antibacterial activity of materials was apparent from circular clear inhibition zones forming around the filtration paper. The diameters of these inhibition zones were measured using a micrometer gauge. Measurements were done after incubation at three different positions, for each paper disk. Mean values was calculated for the nine measurements per paper disc on each plate.

2.3 Effect on Smear Layer

The purpose of this part of the study was to compare, at various levels in straight root canals, the smear layer dissolving capability of the nine different irrigation solutions.

2.3.1 Ethical Statement

Teeth collected for this part of the study were extracted for reasons other than for the purpose of this study. Each patient who attended the extraction clinic of the University of Pretoria completed and signed a patient information leaflet and informed consent form (see Addendum A, p 168).

2.3.2 Collection of Material

Forty five single rooted human teeth were collected from the extraction clinic of the University of Pretoria. Immediately after extraction the teeth were rinsed in running water and stored in containers filled with distilled water, at 4°C until needed.

2.3.3 Radiographs

Pre-operative radiographs were taken of each extracted tooth to eliminate teeth with aberrant canal forms, caries, resorption, calcifications, multiple canals, or any other condition, which might negatively influence the cleaning procedure. Teeth with previous root canal treatment were excluded.

2.3.4 Preparation of specimens

The teeth were randomly divided into nine experimental groups of five teeth each. Standardized access cavities were prepared using diamond burs and long shanked round burs (Dentsply Maillefer, Baillaigues, Switzerland). Canals were explored using size 10 K-flexofiles (Dentsply Maillefer, Baillaigues, Switzerland) and the canals were irrigated with distilled water, confirming apical patency and establishing the working length, for each individual tooth. These working lengths were noted for each tooth, at the time of preparation. Each located root canal was prepared by using ProTaper Nickel Titanium Rotary Files (Dentsply Maillefer, Baillaigues, Switzerland)(Fig. 2.12) according to the manufacturer's instructions. During preparation, the canals were irrigated with copious amounts of 3.5% sodium hypochlorite for 15 minutes. The irrigants were delivered with a Probe irrigating needle (Dentsply Tulsa Dental, Johnson City, Tulsa, USA)(Fig. 2.13), which penetrated the prepared root canal within 1-2mm from the established working length. Thereafter, the teeth were randomly divided into nine groups. One group was kept as control. The other eight groups received a final rinse with one of the following irrigation solutions: 18% EDTA, Sterilox, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, BioPure MTAD, Ozonated water and SmearClear. A total of 25ml of each solution was utilized to irrigate each root canal system, and left undisturbed in the root canal for 2 minutes. BioPure MTAD was left in the root canals for 5 minutes. Finally, the irrigation solutions were removed from the root canals by irrigation with 10ml sterile distilled water.

2.3.5 Preparation for Scanning Electron Microscopy (SEM)

- Each root was sectioned horizontally with a diamond disc in sections corresponding with the coronal, middle and apical portions of the root canal system (Fig. 2.14).
- A shallow groove was cut on the external root surface of each root fragment with a diamond disc, taking care not to penetrate the prepared root canal (Fig 2.15). Each fragment was then fractured laterally by placing a small chisel in the groove that was then hit with a hammer so that it split in two (Fig. 2.16).
- The two halves of each sample were prepared according to standard methods for biological SEM evaluation (225, 226) as follows:
- The samples were fixated in 2% gluteraldehyde (Electron Microscopy Sciences, Washington DC, USA) for 1 hour.
- The Gluteraldehyde was sucked off with a pipette and rinsed 3 times in a phosphate buffer solution (PBS - Whittaker MA Bioproducts, Walkersville, USA) for 5 minutes each time.
- Samples were then fixed in 0,25% Osmiumtetroxide (OsO_4) (Merck, Darnstadt, Germany) for 30 minutes and again rinsed 3 times in the phosphate buffer (PBS) for 5 minutes each time.
- Thereafter the samples were rinsed for 5 minutes each time in increasing concentrations of ethanol (Merck, Darnstadt, Germany), namely 30%, 50%, 70% and 3 times in 95%.

- All samples were mounted flat (onto a small aluminum plate using conductive carbon cement) with the fracture side on top in order to be able to view the side with exposed dentinal tubules (Fig. 2.17).
- Samples were then stored in 95% ethanol before drying for about eight hours in a critical point dryer (Polaron, Oxford, England)(Fig. 218).
- All the prepared samples were then coated with gold (Fig. 2.19) in a sputter-coater (Polaron E5200, Whatford, England)(Fig. 2.20) before they were examined in a Scanning Electron Microscope (JEOL JSM 840 Scanning Electron Microscope, Tokyo, Japan).

2.3.6 SEM Specimen Examination

2.3.6.1 Presence of Smear Layer

Two different SEM photomicrographs were taken for each sample. They were coded and examined blind. Two investigators scored the presence or absence of the smear layer on the surface of the root canal or in the dentinal tubules at the coronal, middle and apical portion. For semi-quantative evaluation, the photographs were divided into 10 sub areas by overlaying a grid, which permitted a more precise determination of the ratio of smear free to smeared surfaces. For each of the 10 sub areas, the absence or presence of the smear layer was rated and scored according to the following three appearances:

1. Regularly distributed open dentinal tubule orifices; such surface was rated free of smear layer and scored 10,
2. Scattered open tubule orifices; surface rated partially free of smear layer and scored 5,
3. No visible tubule orifices; surface rated totally smeared and scored 0.

Each SEM photomicrograph was finally scored by adding the scores of the 10 sub areas, thus expressing the result as a percentage of smear layer free surface. The final result for each segment of the root canal was obtained by calculating the mean of all the photomicrographs.

2.3.6.2 Presence of Erosion

The same SEM photomicrographs, utilizing the same principles and techniques were used to examine the amount of erosion of the peritubular dentine:

1. No erosion. All tubules looked normal in appearance and size, and scored 0,
2. Moderate erosion. The peritubular dentine was eroded, and scored 5.
3. Severe Erosion. The intertubular dentine was destroyed, and tubules were connected with each other, and scored 10.

Each SEM photomicrograph was finally scored by adding the scores of the 10 sub areas, thus expressing the result as a percentage of erosion present. The final result for each segment of the root canal was obtained by calculating the mean of all the photomicrographs.

2.4 Statistical Analysis of Data

2.4.1 Antimicrobial Effects

The One-way ANOVA test using Statistix 8 Software (Analytical Software) was used to determine whether there were statistical significant differences between the inhibition zones obtained by the different irrigation solutions.

2.4.2 SEM Specimen Examination

2.4.2.1 Presence of Smear Layer

The One-way ANOVA test using Statistix 8 Software (Analytical Software) was used to determine whether there were significant differences:

- (i) between the nine irrigation regimes, for each different level in the canal; and
- (ii) between the levels, for each different irrigation regime.

2.4.2.2 Presence of Erosion

The One-way ANOVA test using Statistix 8 Software (Analytical Software) was used to determine whether there were significant differences:

- (i) between the nine irrigation regimes, for each different level in the canal; and
- (ii) between the levels, for each different irrigation regime.



Fig. 2.1: 3.5% Sodium Hypochlorite Liquid.



Fig. 2.2: EDTA 18% Root Canal Irrigating Solution.

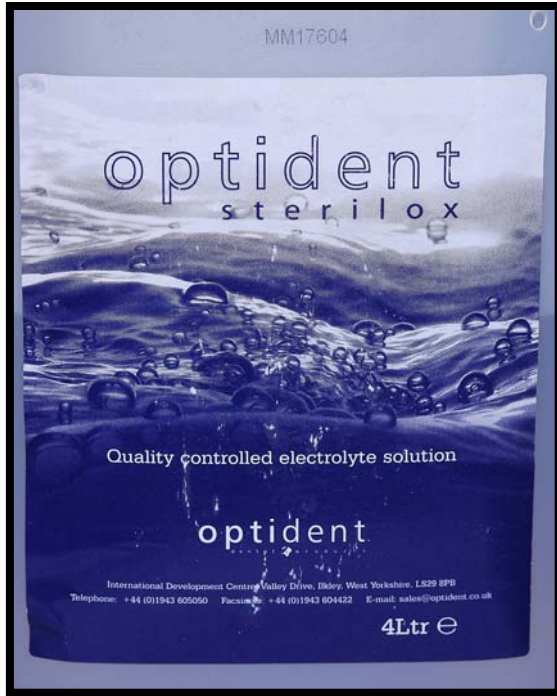


Fig. 2.3: Sterilox Electrolyte Solution and Machine.



Fig. 2.4: TopClear Solution.



Fig. 2.5: Vista CHX 2% Chlorhexidine Gluconate Solution.



Fig. 2.6: Citric Acid 10% Root Canal Irrigating Solution.



Fig. 2.7: BioPure MTAD Antibacterial Root Canal Cleanser.



Fig. 2.8: OH Dent Ozone Generator that was used to produce Ozonated Water.



Fig. 2.9: SmearClear Irrigation Solution.

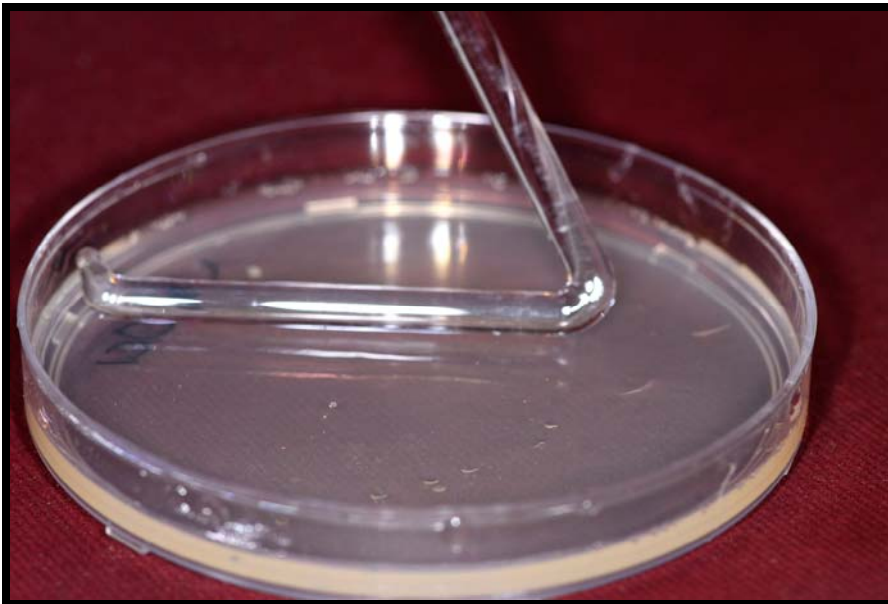


Fig. 2.10: MacFarland Standard 1 suspension of *E. faecalis* spread onto Casein-peptone-Soymeal-peptone Agar (CASO-Agar) plate with a sterile glass rod.



Fig. 2.11: Ten microlitres of each concentration test solution were dispensed onto four, standardized, sterilized, five millimetre diameter filter paper disks.

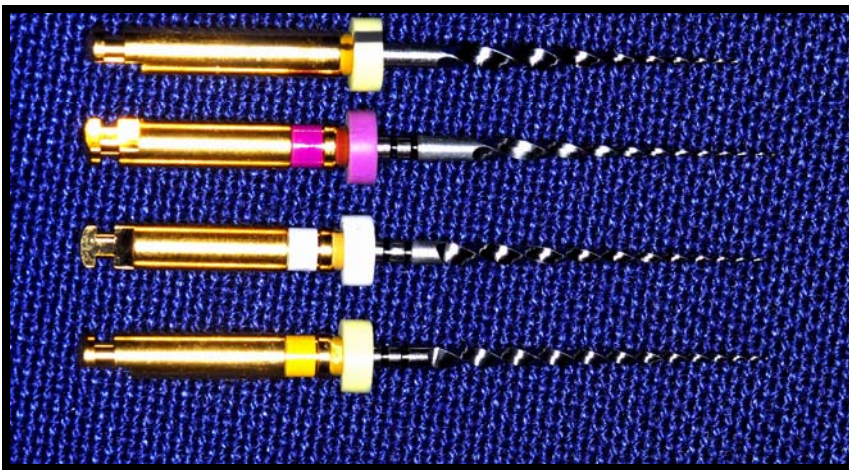


Fig. 2.12: Pro-Taper Nickel Titanium Rotary Files.

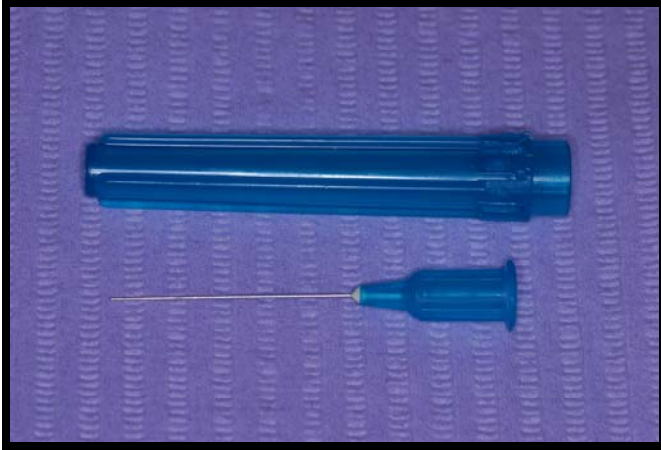


Fig. 2.13: Probe Irrigating Needles.

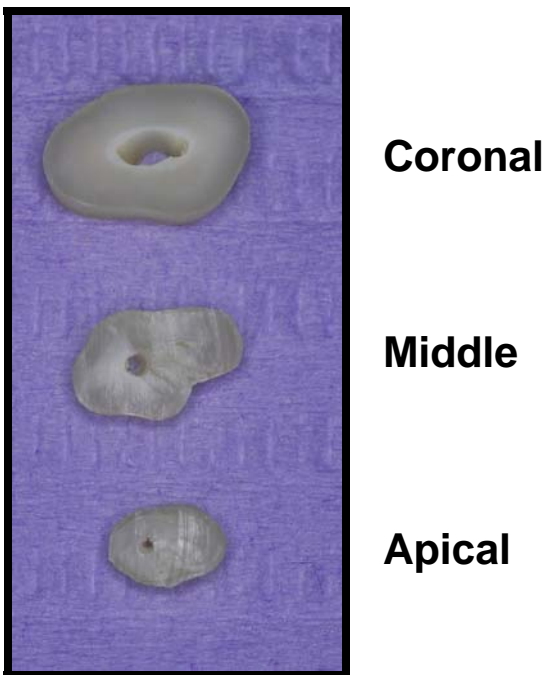


Fig. 2.14: Each root was sectioned horizontally with a diamond disc in sections corresponding with the coronal, middle and apical levels of the root canal system.



Fig. 2.15: A shallow groove was cut on the external root surface of each root fragment with a diamond disc, taking care not to penetrate the prepared root canal.



Fig. 2.16: Each fragment was then fractured laterally by placing a small chisel in the groove that was then hit with a hammer so that it split in two.



Fig. 2.17: Samples were mounted flat onto a small aluminium plate using conductive carbon cement.



Fig. 2.18: Polaron critical point dryer and Sputter-coater.



Fig. 2.19: Samples were coated with gold in the Sputter-coater.



Fig. 2.20: Scanning Electron Microscope (SEM).

CHAPTER 3

Results

3.1 Antimicrobial Results – Inhibition Zones

The means and standard deviations of the zones of inhibition for all the test solutions are presented in Table 3.1 – 3.4. Figures 3.1 - 3.10 shows the inhibition zones obtained for each irrigation solution in the undiluted, 1/10 diluted, 1/100 diluted and 1/1000 diluted solutions.

3.1.1. Undiluted Solutions (100%)

No zones of inhibition were observed adjacent to the filter papers saturated with sterile water (control), Sterilox and Ozonated water. The average zones of inhibition for 3.5% NaOCl, 18% EDTA, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, BioPure MTAD and SmearClear were 9.2mm, 8.3mm, 8.8mm, 6.4mm, 0.7mm, 11.5mm and 10mm respectively. Figure 3.11 shows the comparison of the average areas of inhibition for the undiluted irrigation solutions.

Table 3.5 shows the statistical comparisons between the different inhibition zones for the undiluted irrigation solutions. Statistical analysis using the One-Way ANOVA test showed a statistical significant difference between the

inhibition zones obtained for BioPure MTAD and SmearClear ($p < 0.05$). The zones of inhibition for these two products were significantly larger than those seen around the filter papers saturated with sterile water (control), Sterilox, 10% Citric acid, Ozonated water, 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine. There was no significant difference between the inhibition zones of SmearClear and 3.5% NaOCl ($p > 0.05$), Topclear and 3.5% NaOCl ($p > 0.05$), and 3.5% NaOCl and 18% EDTA ($p > 0.05$).

3.1.2. 1/10 Diluted Solutions

No zones of inhibition were observed adjacent to the filter papers saturated with sterile water (control), 3.5% NaOCl, Sterilox, 10% Citric acid and Ozonated water. The average zones of inhibition for 18% EDTA, TopClear 17% EDTA, 2% chlorhexidine, BioPure MTAD and SmearClear were 0.5mm, 2.2mm, 1.3mm, 9.4mm and 6.3mm respectively. Figure 3.12 depicts the comparison of the average areas of inhibition for the 1/10 diluted irrigation solutions.

Table 3.6 shows the statistical comparisons between the different inhibition zones for the diluted 1/10 irrigation solutions. Statistical analysis using the One-Way ANOVA test showed a statistical significant difference between the inhibition zones obtained for BioPure MTAD and SmearClear ($p < 0.05$). The zones of inhibition for these two products were significantly larger than those seen around the filter papers saturated with sterile water (control), Sterilox,

10% Citric acid, Ozonated water, 3.5% NaOCl NaOCl, 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine. TopClear created a zone of inhibition that was significantly larger in diameter than seen with 2% chlorhexidine and 18% EDTA ($p < 0.05$). There was also a significant difference between the inhibition zones of 2% chlorhexidine and 18% EDTA ($p < 0.05$).

3.1.3. 1/100 Diluted Solutions

No zones of inhibition were observed adjacent to the filter papers saturated with sterile water (control), 3.5% NaOCl, Sterilox, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, Ozonated water and SmearClear. The average zone of inhibition for BioPure MTAD was 2.4mm. Figure 3.13 shows the comparison of the average areas of inhibition for the 1/100 diluted irrigation solutions.

Statistical analysis using the One-Way ANOVA test showed a statistical significant difference between the mean inhibition zones obtained for BioPure MTAD compared to all the other irrigation solutions ($p < 0.05$).

3.1.4. 1/1000 Diluted Solutions

No zones of inhibition were noted adjacent to the filter papers saturated with any of the test solutions (Table 3.4).

3.2. Smear Layer Management and Degree of Erosion

The incidence of smear-free surfaces created by the irrigation solutions are given in Table 3.7 and shown graphically in Figure 3.14. Significant differences were observed between the different irrigating solutions on the coronal, middle and apical levels of the specimens ($p < 0.05$) (Table 3.8 – Table 3.11).

Incidence of erosion of the peritubular and intertubular dentine created by the irrigation solutions are given in Table 3.12 and shown graphically in Figure 3.15. Significant differences were observed between the different irrigating solutions on the coronal, middle and apical levels of the specimens ($p < 0.05$) (Table 3.13 - Table 3.15).

3.2.1. Group A - 3.5% NaOCl Liquid (Control)

Examination of the surface of the root canal walls in teeth irrigated with 3.5% NaOCl showed consistently the presence of heavy smear layer throughout the entire length of the root canals (Fig. 3.16 - Fig. 3.18).

3.2.2. Group B – 18% EDTA

At the coronal level in the root canals, 94 per cent of the observed surface was free of smear layer (Fig. 3.19). At the middle and apical levels, however, the

proportions of smear-free surfaces declined to 63 and 43 per cent respectively. The One-Way ANOVA test demonstrated significant statistical differences between these results ($p < 0.05$) (Table 3.11).

Apparent and regularly distributed open dentinal tubule orifices were visible with a small amount of smear layer on the intertubular dentine surfaces of the samples representing the coronal levels of the root canals (Fig. 3.19). Scattered open tubule orifices with clumps of smear layer were visible on the intertubular and peritubular dentine of most samples at the middle level (Fig. 3.20). A few selected samples demonstrated more regular distributed open dentinal tubule orifices with evidence of smear layer on the intertubular and peritubular dentine surfaces (Fig. 3.21). The samples at the apical level (Fig. 3.22) demonstrated a few partially open tubule orifices and a moderate amount of smear layer covering the dentinal orifices, inter- and peri-tubular dentine.

3.2.3. Group C – Sterilox

This irrigation solution removed most of the smear layer (84%) on the surface of samples examined at the coronal level of root canals (Fig. 3.23). On some selected samples there was evidence of small patches of smear layer on the intertubular dentine.

At the middle and apical levels, however, the proportions of smear-free surfaces declined to 29 and 1 per cent respectively. There were significant

statistical differences between the results at the coronal, middle and apical levels of the root canals treated with Sterilox ($p < 0.05$) (Table 3.11).

Most of samples examined at the middle level of the root canals demonstrated a visible smear layer. A few scattered orifice openings were evident (Fig. 3.24). The samples of the root canals examined at the apical level demonstrated a thick smear layer covering the surface prepared root canal surface (Fig. 3.25).

3.2.4. Group D – TopClear 17% EDTA

The incidence of smear-free surfaces declined from 98 per cent at the coronal level to 60 and 40 per cent respectively at the middle and apical levels. There were significant statistical differences between these results ($p < 0.05$) (Table 3.11).

Most samples at the coronal level demonstrated regular distributed open dentinal tubule orifices with erosion of the peritubular dentine (Fig. 3.26). On two samples there was extensive erosion of the peritubular dentine and in some areas the intertubular dentine was destroyed (Fig. 3.27).

Most of the samples examined in the middle and apical levels of the root canals demonstrated patches of smear layer remnants and regularly distributed open tubule orifices (Fig. 3.28). In contrast, the visible tubule orifices at the apical level were partially exposed (Fig. 3.29).

At the coronal level, 58 per cent of the samples showed extensive erosion of the peritubular dentine. The tubule orifices at the middle level also demonstrated some evidence of erosion of the peritubular dentine (13%), with no evidence of erosion in the apical region of the root canals. The coronal and apical results differed significantly ($p < 0.05$) (Table 3.15). There was also a statistical significant difference between the erosion results obtained for TopClear 17% EDTA at the coronal and middle levels, compared to all the other irrigation solutions examined in the present study ($p < 0.05$) (Table 3.14).

3.2.5. Group E - Chlorhexidine

No smear layer was noted on the surface of most samples (94%) at the coronal level of the root canals in samples irrigated with this solution. Apparent and regularly distributed open dentinal tubule orifices were visible with no evidence of smear layer (Fig. 3.30). The intertubular dentine appeared granular but there was no visible erosion of the peritubular dentine

At the middle level, the proportions of smear-free surfaces declined to 19 per cent. There was a significant statistical difference between the results at the coronal and middle levels of the root canals ($p < 0.05$) (Table 3.11). Most of the surfaces were covered with smear layer. However, a few selected tubule orifices were partially open on most samples of the middle third (Fig. 3.31).

The samples in the apical third demonstrated a thick, irregular smear layer on the surface of all the samples (Fig. 3.32).

3.2.6. Group F - 10% Citric acid

Patches of smear layer with a few partially open tubule orifices was visible on most of the samples at the coronal level of the root canals in this group. (Fig. 3.33).

Very characteristic of the samples at the coronal and middle levels was that it appeared as if the intertubular and peritubular dentine melted together to form a layer covering the dentinal tubule orifices (Fig. 3.33 and Fig. 3.34). Patches of smear layer was visible on most of samples examined at the middle level (Fig. 3.34). The incidence of smear-free surfaces was 19 and 3 per cent for the coronal and middle thirds respectively. There was no visible evidence of erosion of the peritubular dentine at the coronal or middle levels of the root canals.

Almost all the samples that were examined at the apical level of the root canals demonstrated a thick, irregular smear layer (Fig. 3.35).

3.2.7. Group G - BioPure MTAD

The smear layer was completely removed on all the samples examined at the coronal level of the roots. Numerous, regular distributed, large, open dentinal tubules were visible (Fig. 3.36).

At the middle level, most of the smear layer was also removed, and open dentinal tubules were visible on the intertubular dentine (Fig. 3.37).

The samples examined at the apical level (Fig. 3.38) demonstrated a few partially open tubule orifices with patches of smear layer covering the other dentinal orifices, inter- and peri-tubular dentine.

At all levels, the root canals showed more smear-free surfaces than those of the other groups, with the exception of TopClear 17% EDTA. The incidence declined from 100 per cent smear-free surfaces at the coronal level to 97 and 50 per cent respectively at the middle and apical levels. There was a significant statistical difference between the apical and coronal results ($p < 0.05$)(Table 3.11), as well as between the apical and middle level results ($p < 0.05$)(Table 3.11). No significant statistical differences were found between the coronal and middle level results ($p > 0.05$)(Table 3.11).

Despite the high percentage of smear-free surfaces, there was only a small amount of erosion visible of the peritubular dentine at the coronal level.

3.2.8. Group H - Ozonated water

Examination of the surface of the root canal walls in teeth irrigated with Ozonated water showed the presence of smear layer throughout the entire length of the root canals (100 per cent) (Fig 3.39 - Fig. 3.41). However, the smear layer at the coronal level of most samples was thinner and appeared smooth in texture. Smear layer of the samples at the middle level of the root canals appeared thicker and had a mixed texture from smooth to granular. The smear layer at the apical level of the samples was very thick and had an irregular, granular texture.

3.2.9. Group I - SmearClear

At the coronal level of the root canals, 80 per cent of the observed surfaces were free of smear layer. At the middle and apical levels, however, the proportions of smear-free surfaces declined to 33 and 26 per cent respectively. The One-Way ANOVA test demonstrated significant statistical differences between the results obtained at the coronal level compared to those obtained from the middle and apical levels of the root canals ($p < 0.05$) (Table 3.11).

Minimal amounts of smear layer were noted on the surface of most samples at the coronal level (Fig. 3.42). Apparent and regularly distributed open dentinal tubule orifices were visible with a small amount of smear layer visible on the

intertubular dentine surfaces. There was a minimal amount (3 per cent) of erosion visible on the peritubular dentine of some of the samples.

Scattered open tubule orifices with clumps of smear layer were visible on the intertubular and peritubular dentine of most samples at the middle level (Fig. 3.43). A few selected samples demonstrated more regular distributed open dentinal tubule orifices with evidence of smear layer on the intertubular and peritubular dentine surfaces.

The samples at the apical level (Fig. 3.44) demonstrated a few partially open tubule orifices and a moderate amount of smear layer covering the other dentinal orifices, inter- and peri-tubular dentine.



Fig. 3.1: A representative photograph of an *E. faecalis*-seeded CASO-Agar plate incubated for 24 hours after placement of sterile water on the paper discs. Note the absence of inhibition zones.

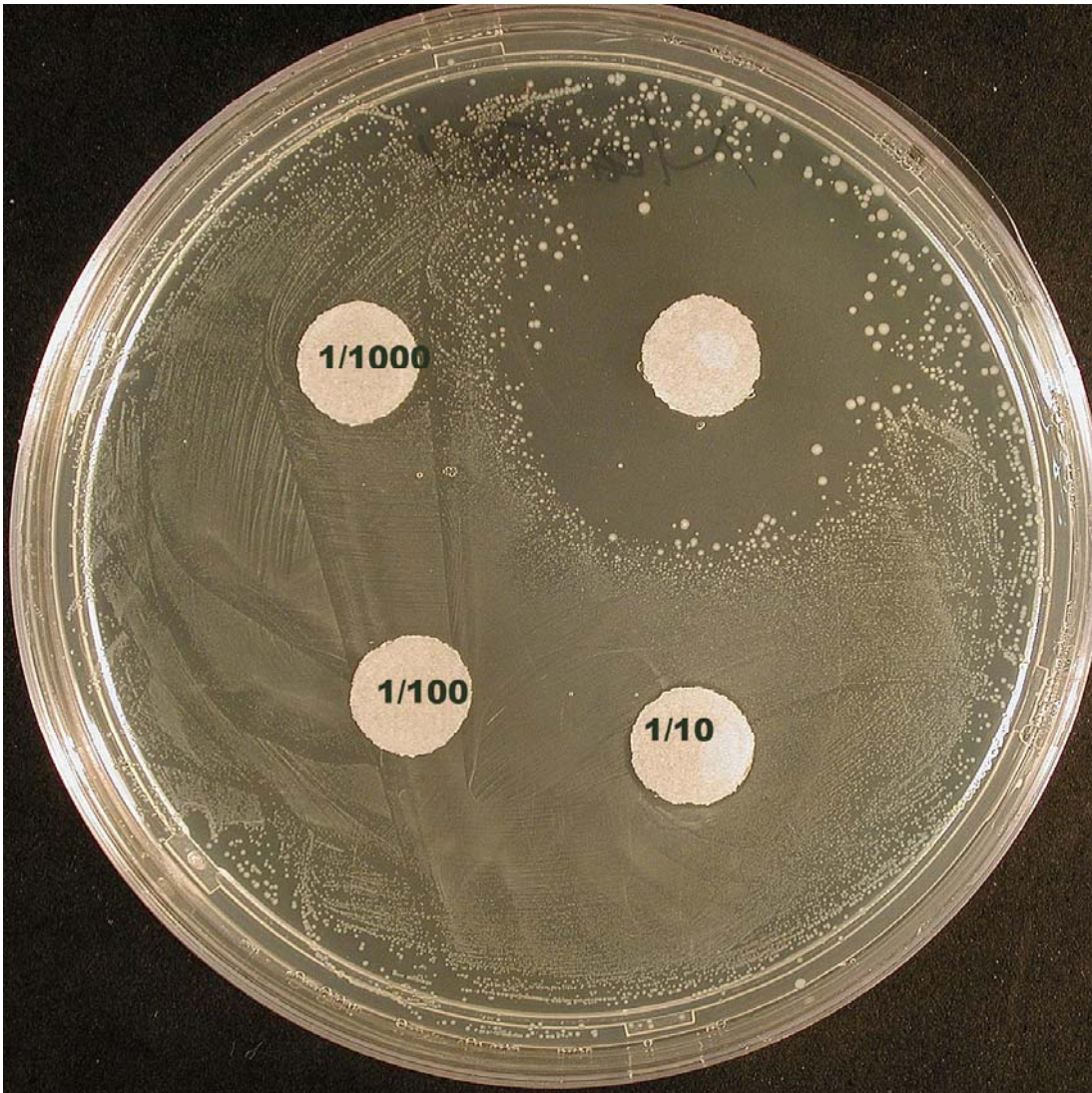


Fig. 3.2: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – 3.5% NaOCl. The average zone of inhibition of the undiluted solution was 9.2mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/10, 1/100 and 1/1000 diluted solutions.

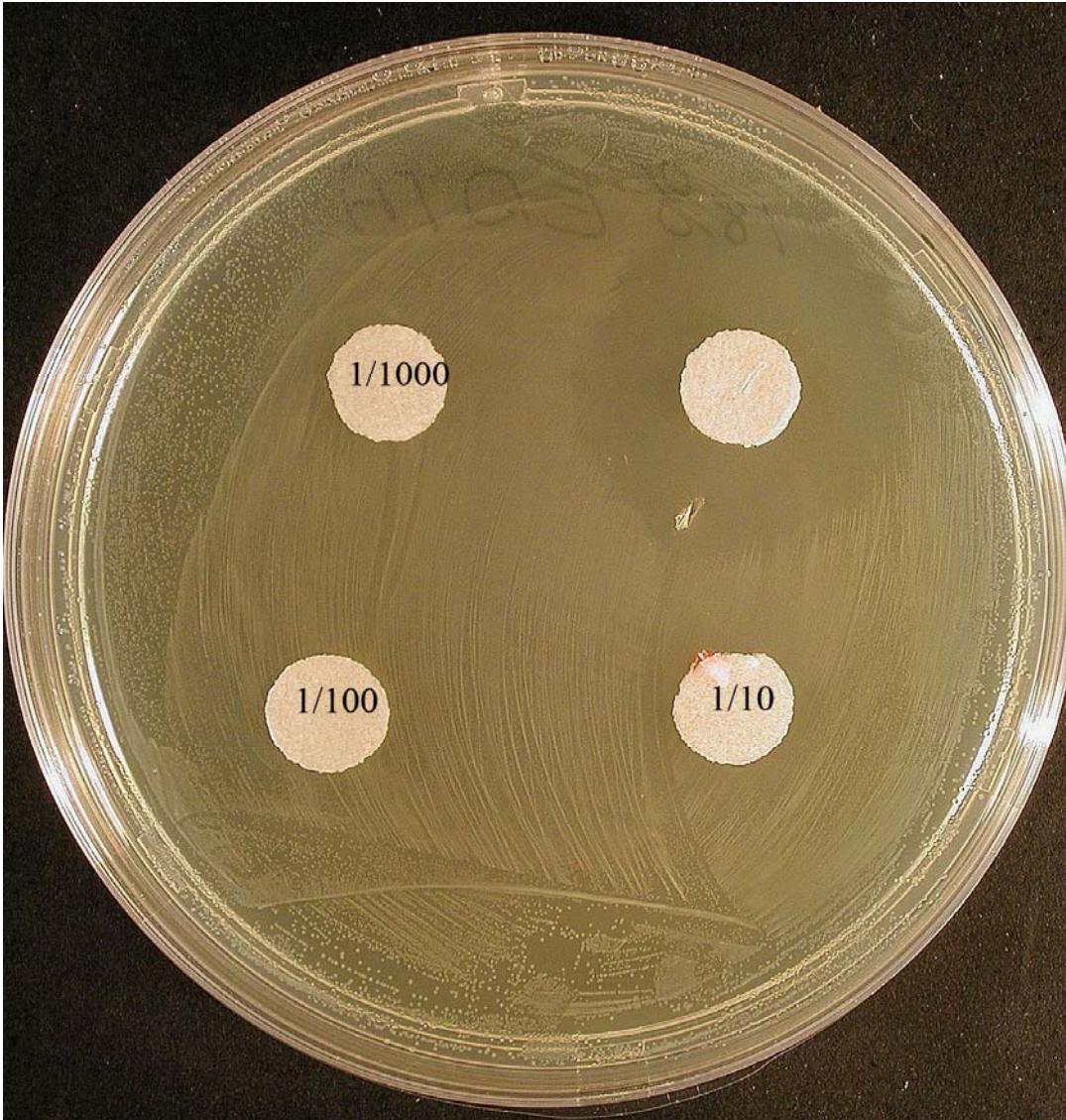


Fig. 3.3: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – EDTA 18% Root Canal Irrigating Solution. The average zone of inhibition of the undiluted solution was 8.3mm. The 1/10 diluted solution created an undefined narrow zone (0.5mm) of inhibition. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/100 and 1/1000 diluted solutions.

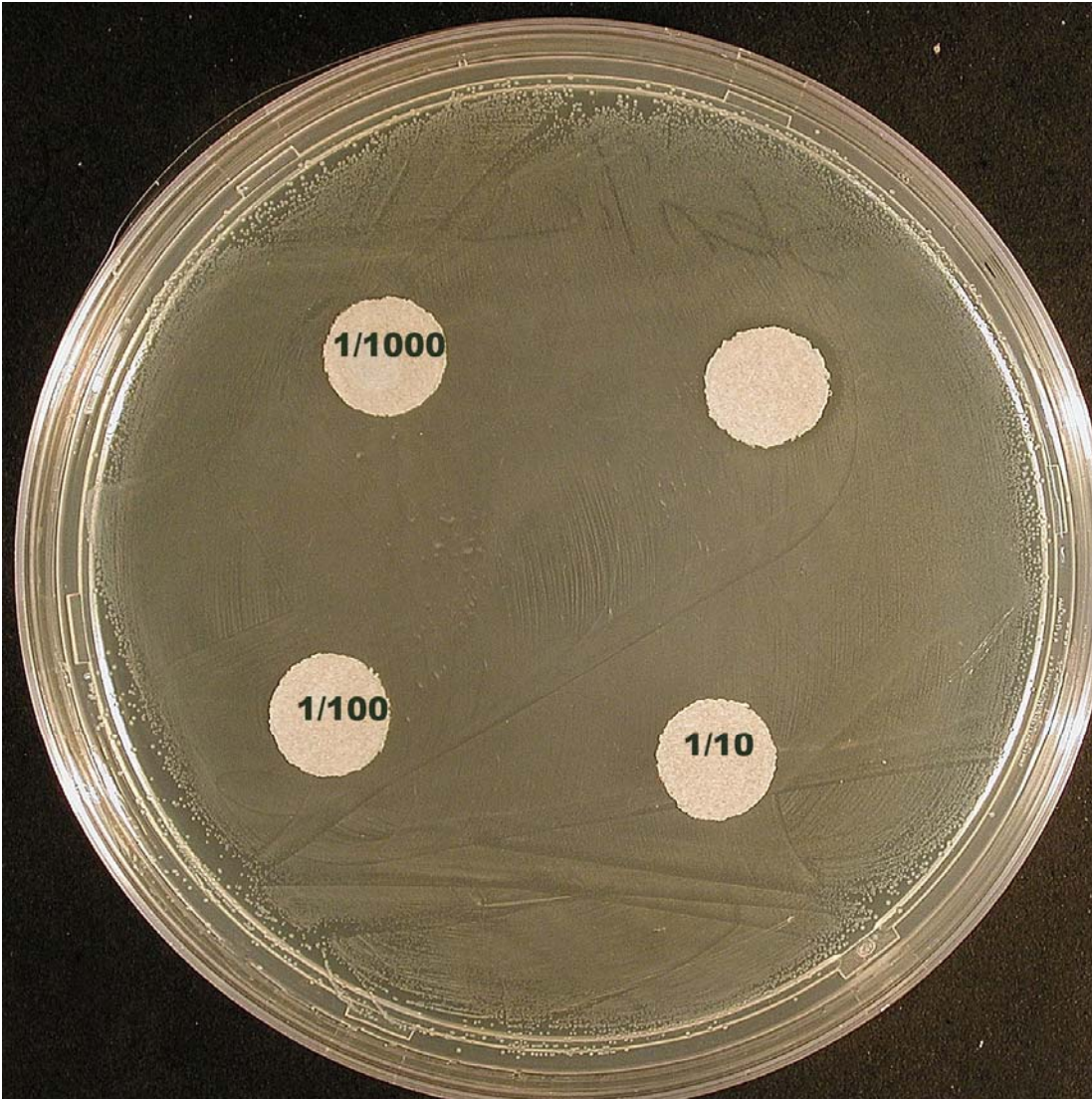


Fig. 3.4: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Sterilox Electrolyte Solution. No zone of inhibition was observed adjacent to the filter papers saturated with the undiluted, 1/10, 1/100 and 1/1000 diluted solutions.

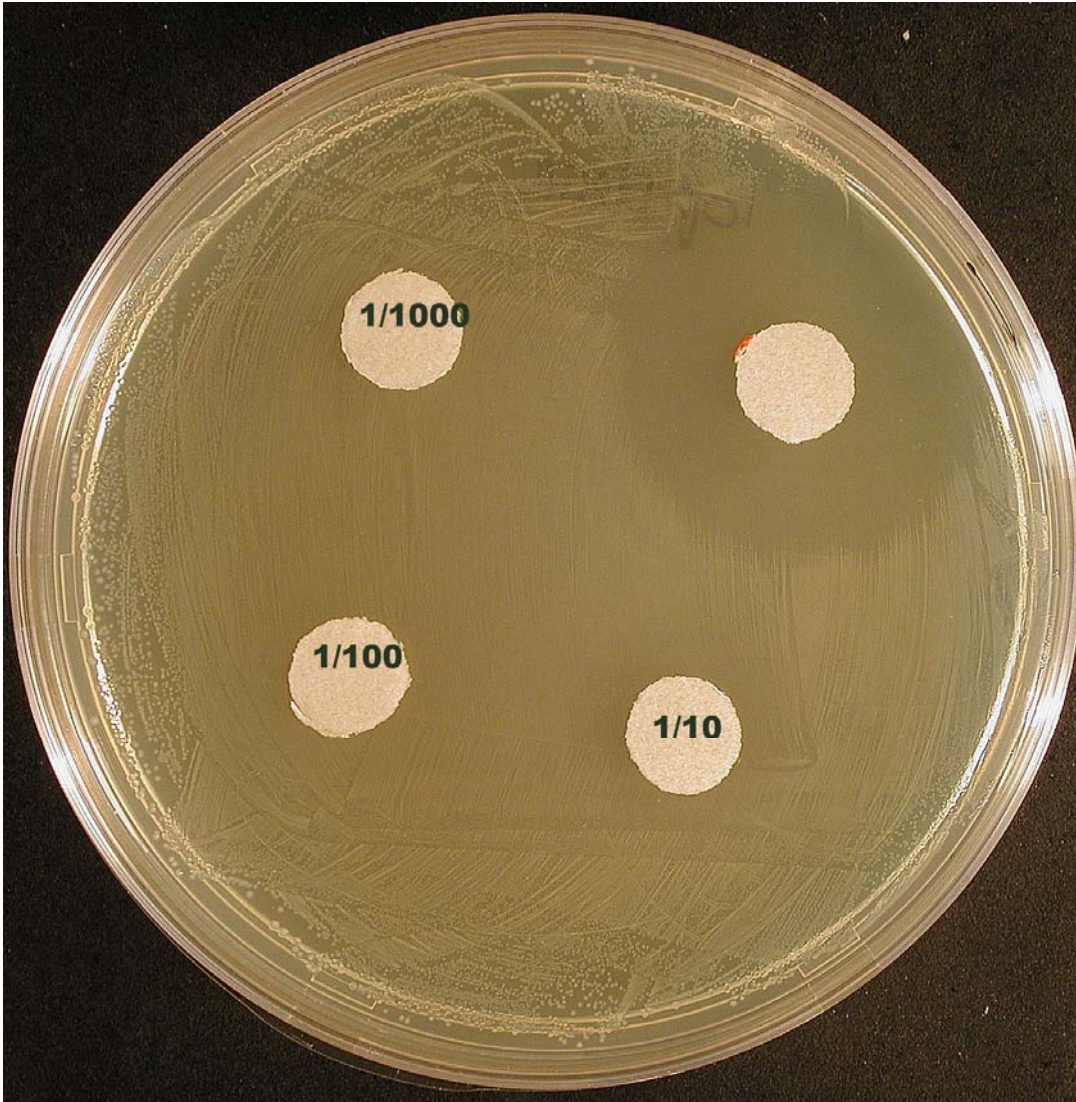


Fig. 3.5: A representative photograph of an *E. faecalis*-seeded CASO-Agar plate incubated for 24 hours – TopClear 17% EDTA solution. The average zone of inhibition of the undiluted solution was 8.8mm. The 1/10 diluted solution created an undefined narrow zone (2.2mm) of inhibition. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/100 and 1/1000 diluted solutions.

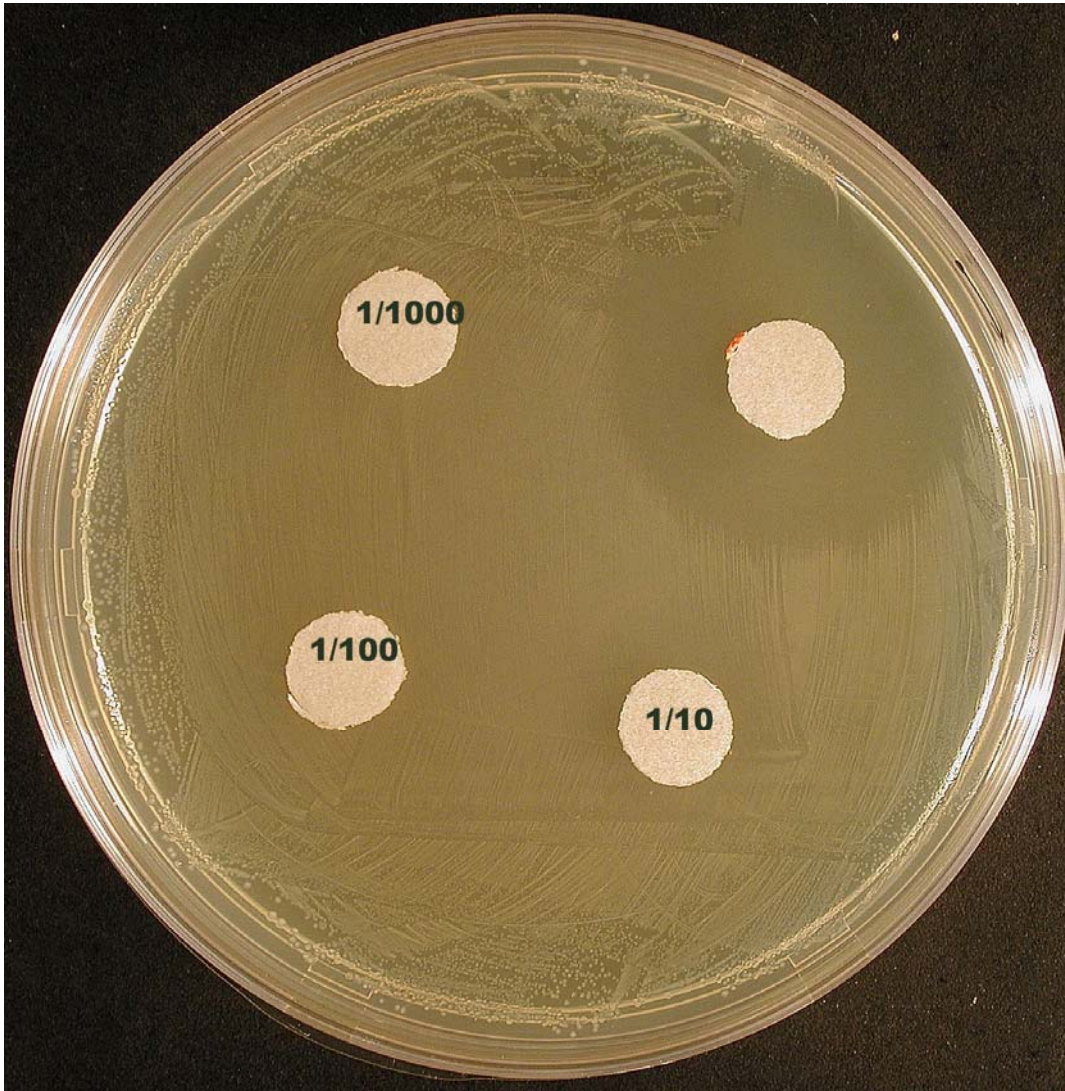


Fig. 3.6: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Vista CHX 2% Chlorhexidine Gluconate Solution. The average zone of inhibition of the undiluted solution was 6.4mm, for the 1/10 diluted solution 1.2mm and for the 1/100 diluted solution 1mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/1000 diluted solutions.

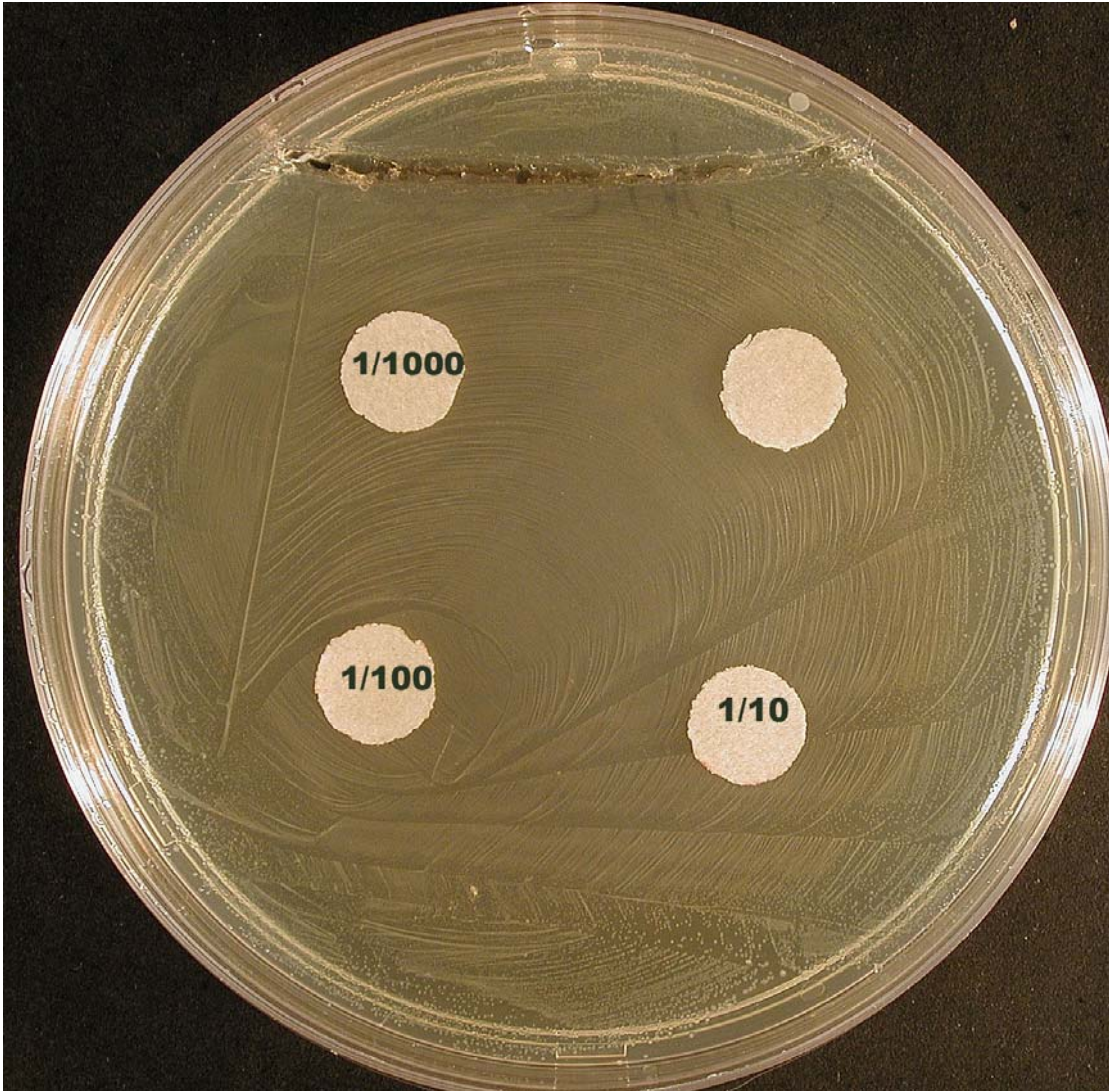


Fig. 3.7: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Citric Acid 10% Root Canal Irrigating Solution. The average zone of inhibition of the undiluted solution was 0.7mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/10, 1/100 and 1/1000 diluted solutions.

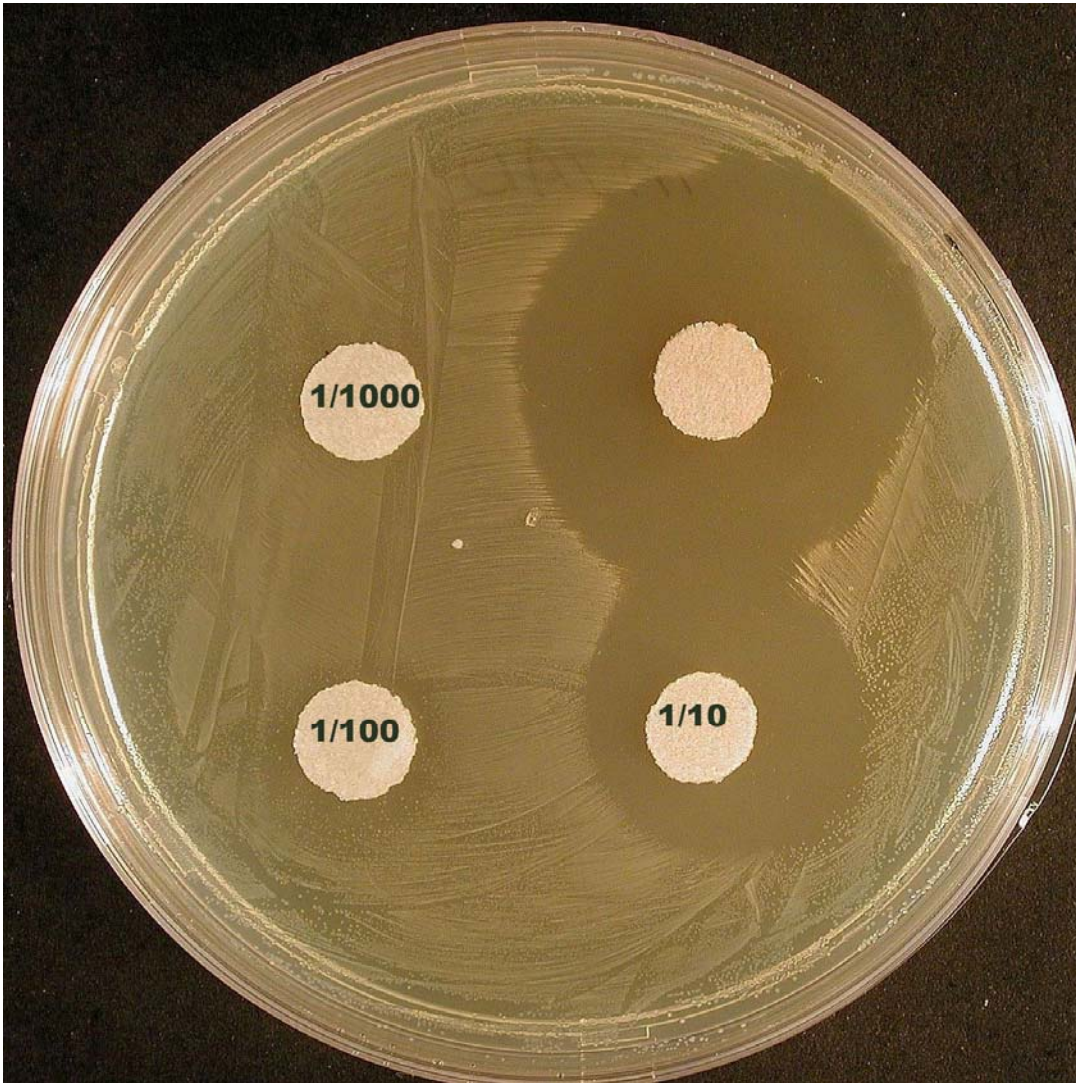


Fig. 3.8: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – BioPure MTAD Antibacterial Root Canal Cleanser. The zone of inhibition of the undiluted solution was 11.5mm, for the 1/10 diluted solution 9.4mm and for the 1/100 diluted solution 2.4mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/1000 diluted solutions.



Fig. 3.9: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Ozonated water. Note the absence of inhibition zones.

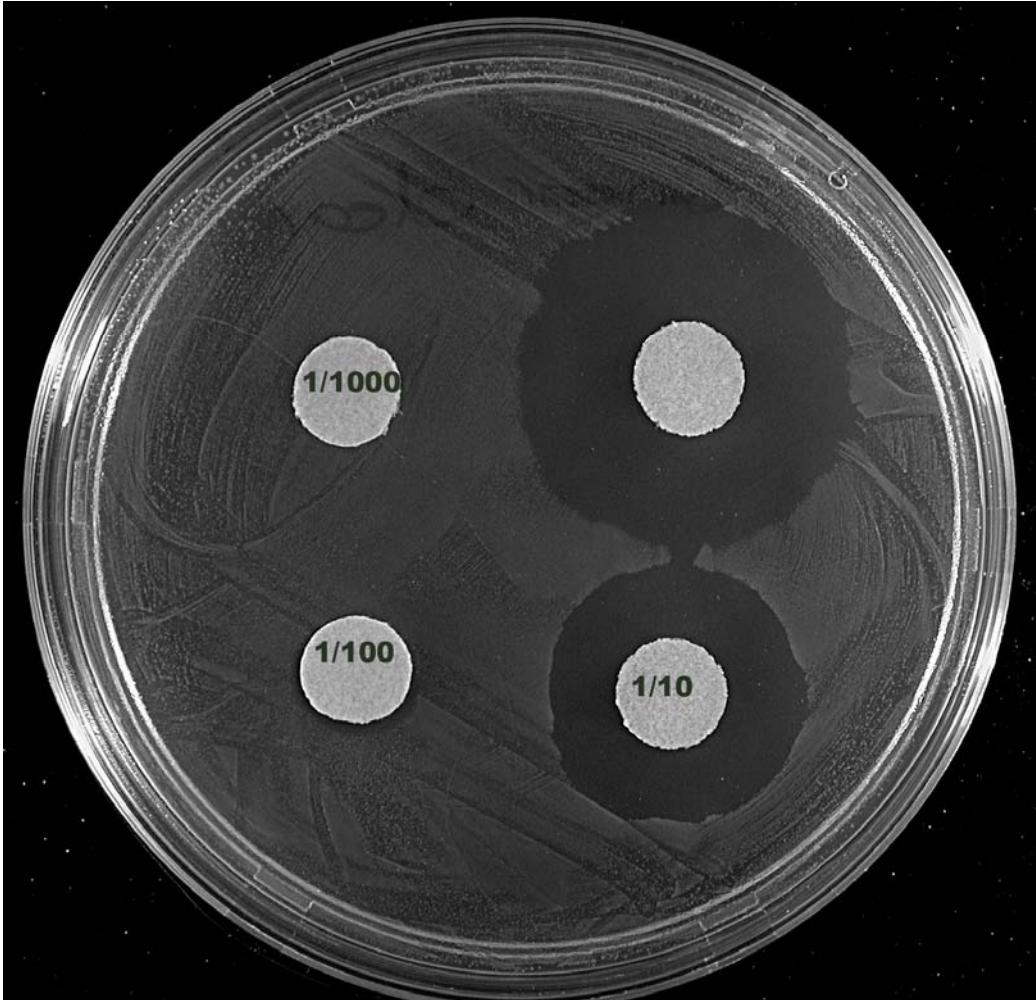


Fig. 3.10: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – SmearClear Irrigating Solution. The average zone of inhibition of the undiluted solution was 10mm. The 1/10 diluted solution created an average zone of inhibition of 6.3mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/100 and 1/1000 diluted solutions.

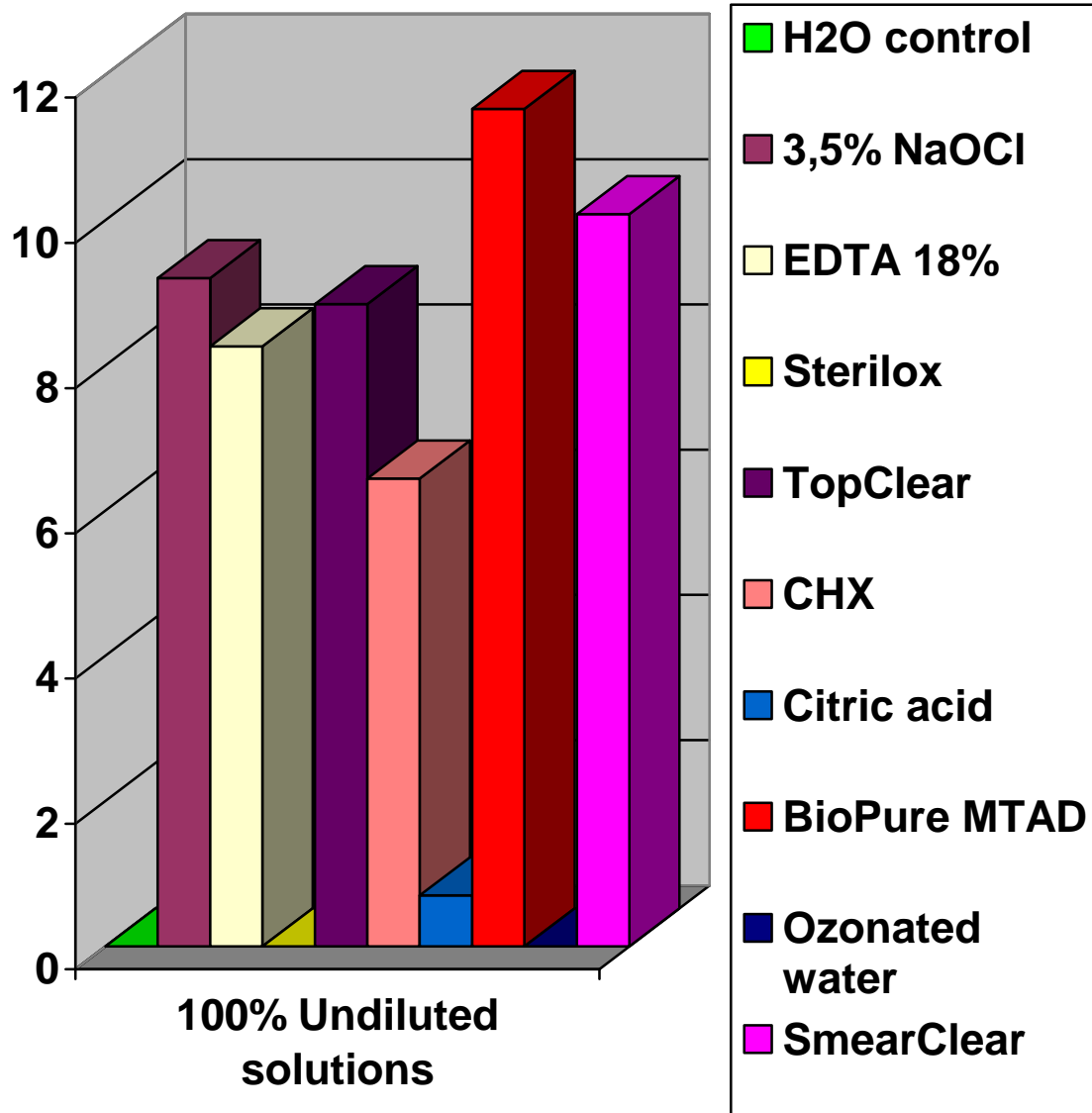


Fig. 3.11: The difference between the average zones of inhibition between the undiluted irrigation solutions.

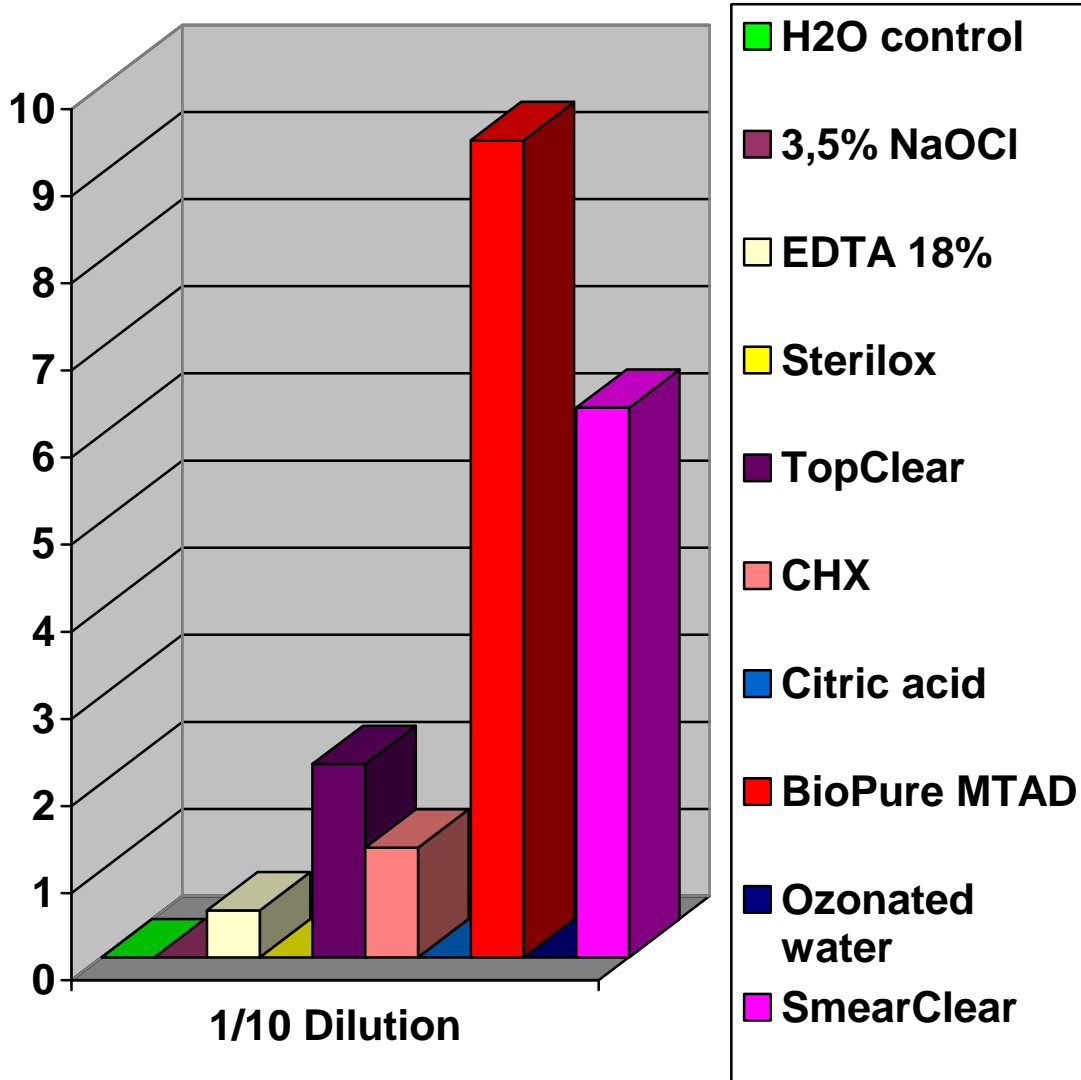


Fig. 3.12: The difference between the average zones of inhibition between the 1/10 diluted irrigation solutions.

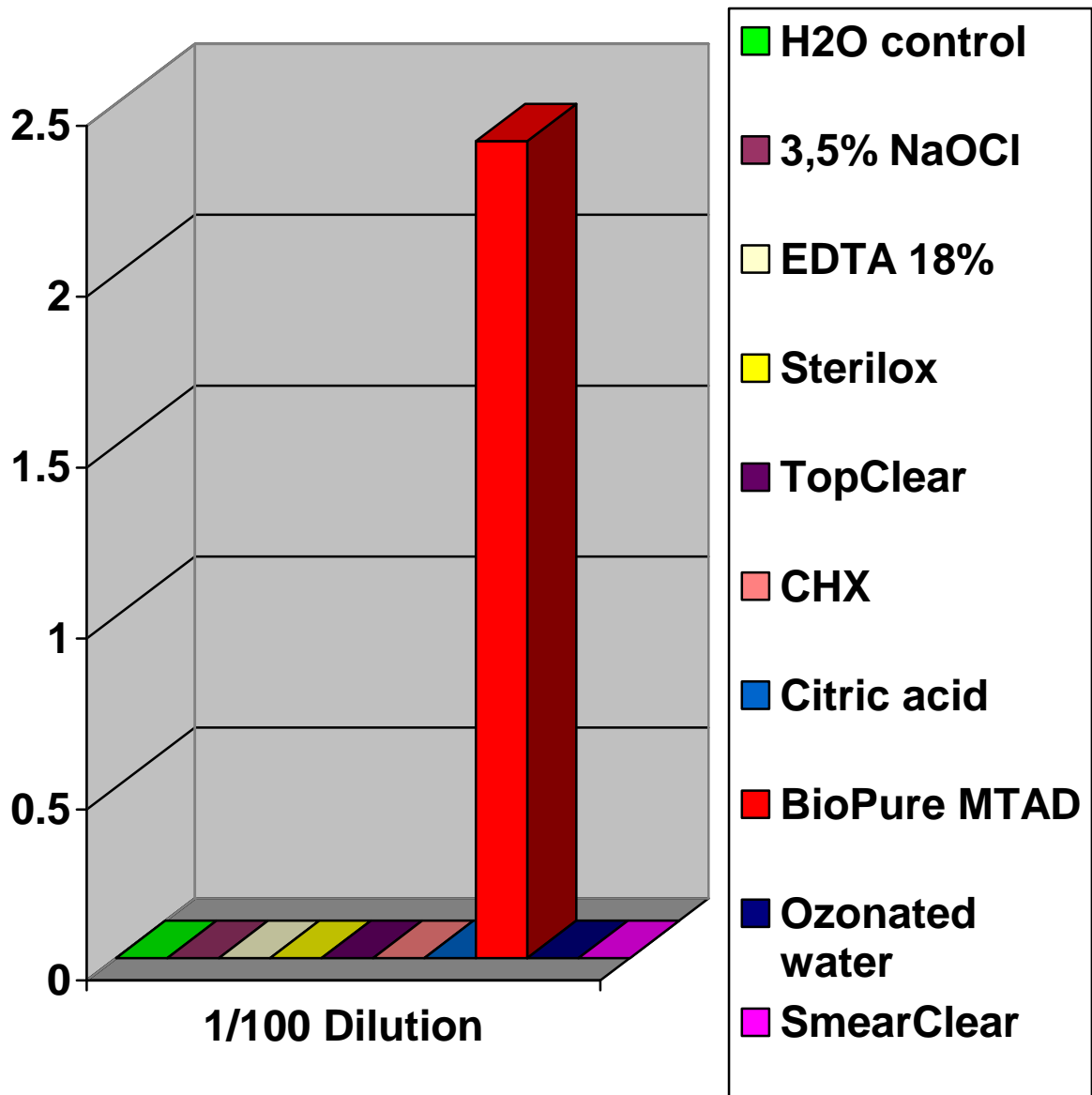
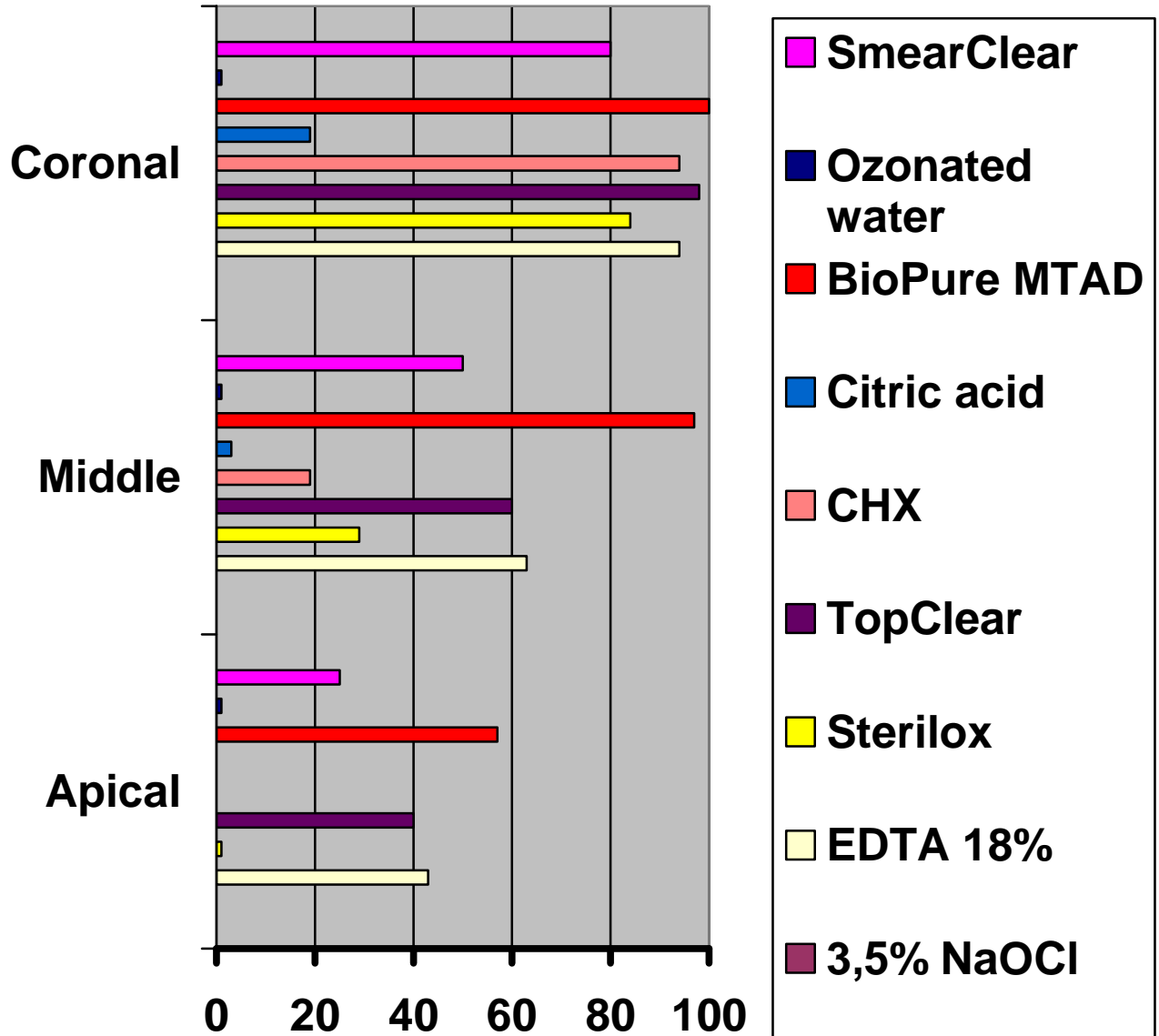


Fig. 3.13: The difference between the average zones of inhibition between the 1/100 diluted irrigation solutions.



Percentage of smear layer-free surfaces

Fig. 3.14: The incidence of smear-free surfaces on the coronal, middle and apical levels of the root canals when using the different irrigation solutions.

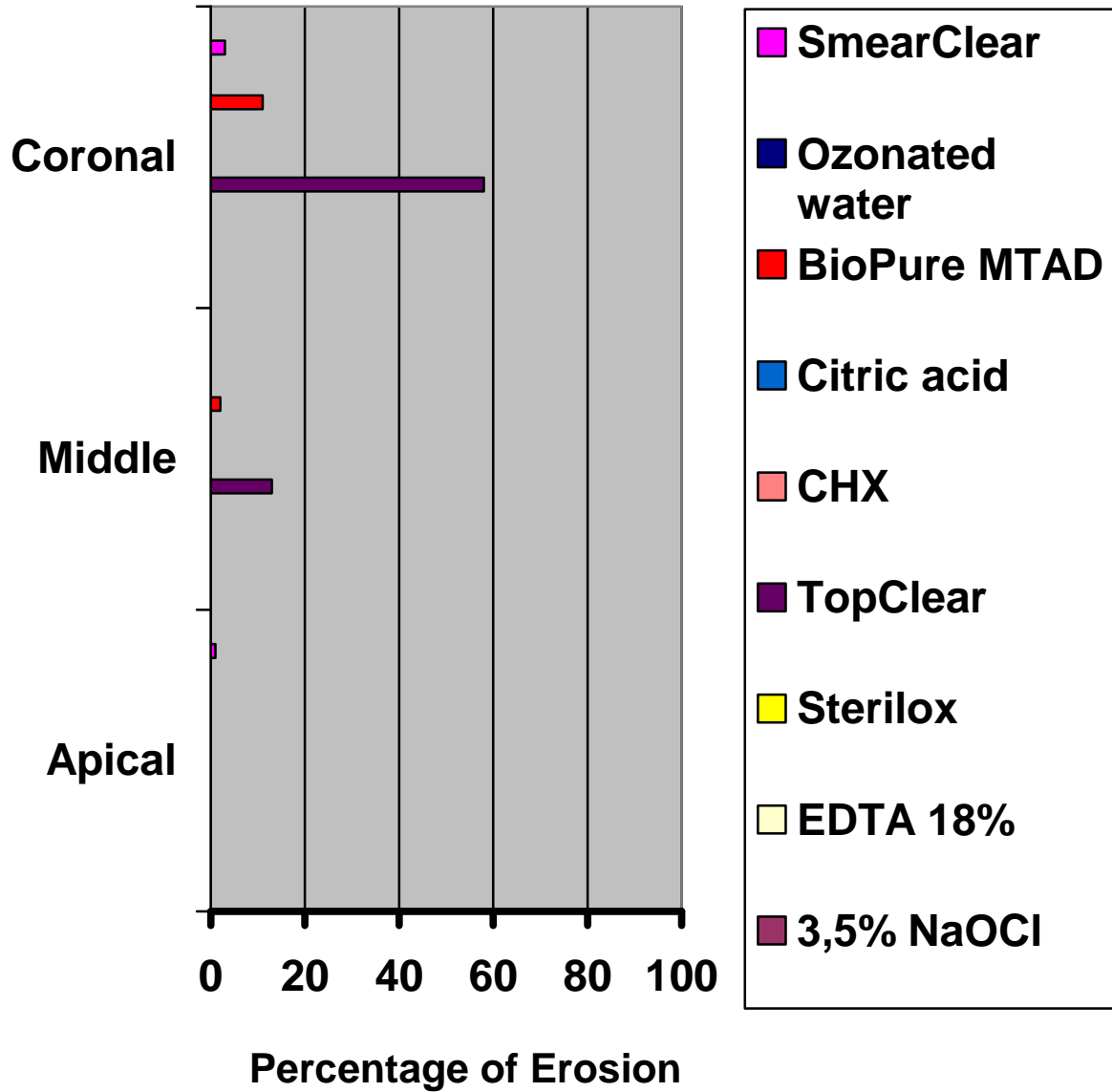


Fig. 3.15: The incidence of erosion on the coronal, middle and apical levels of the root canals when using the different irrigation solutions.

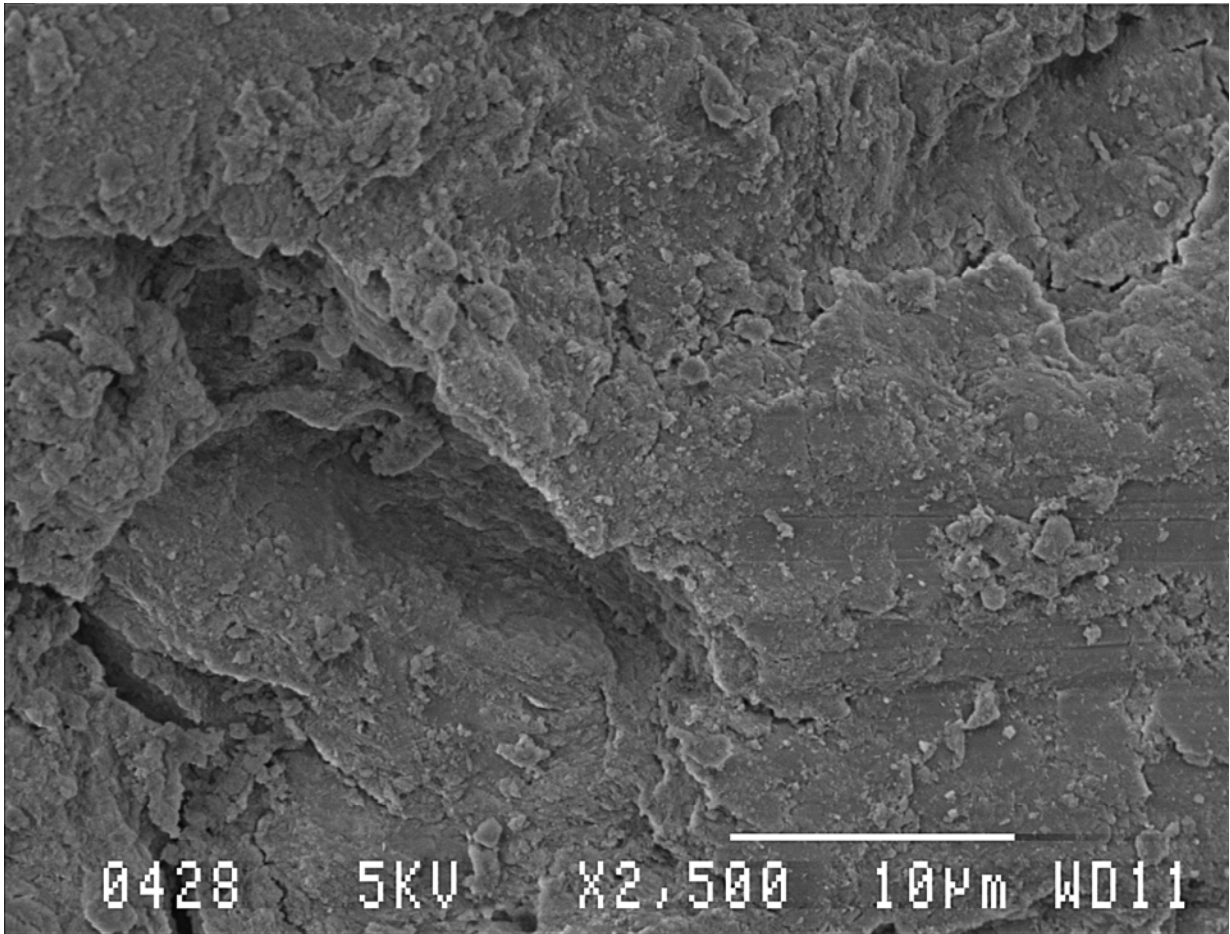


Fig. 3.16: A representative SEM photograph of a prepared surface of a Group A root canal (NaOCl), coronal level, thick smear layer with no visible tubule orifices. The undissolved smear layer appears as a flattened rough, irregular surface. Final magnification 2500X.

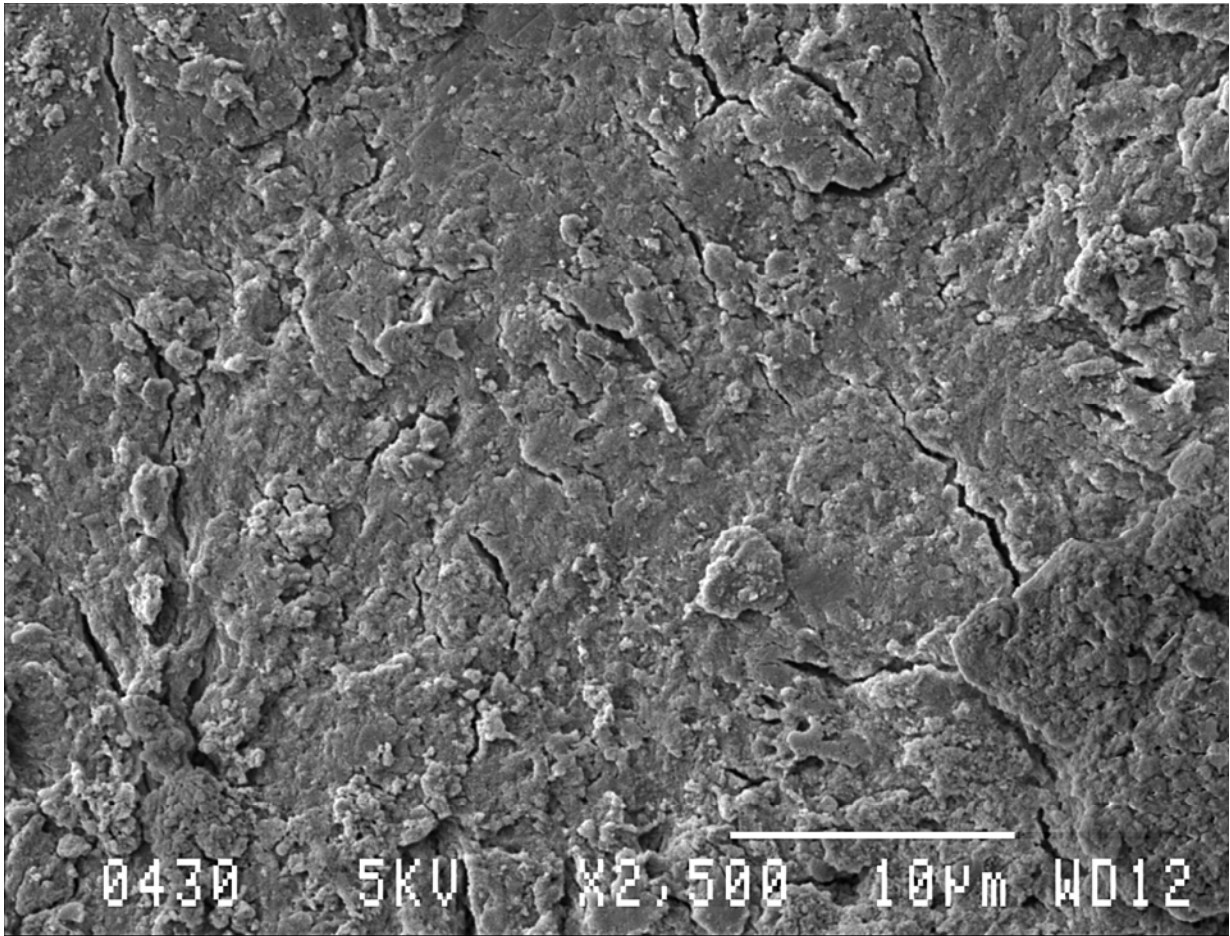


Fig. 3.17: A representative SEM photograph of a prepared surface of a Group A root canal (NaOCl), middle level, thick smear layer with no visible tubule orifices. The undissolved smear layer appears as a flattened rough, irregular surface. Final magnification 2500X.

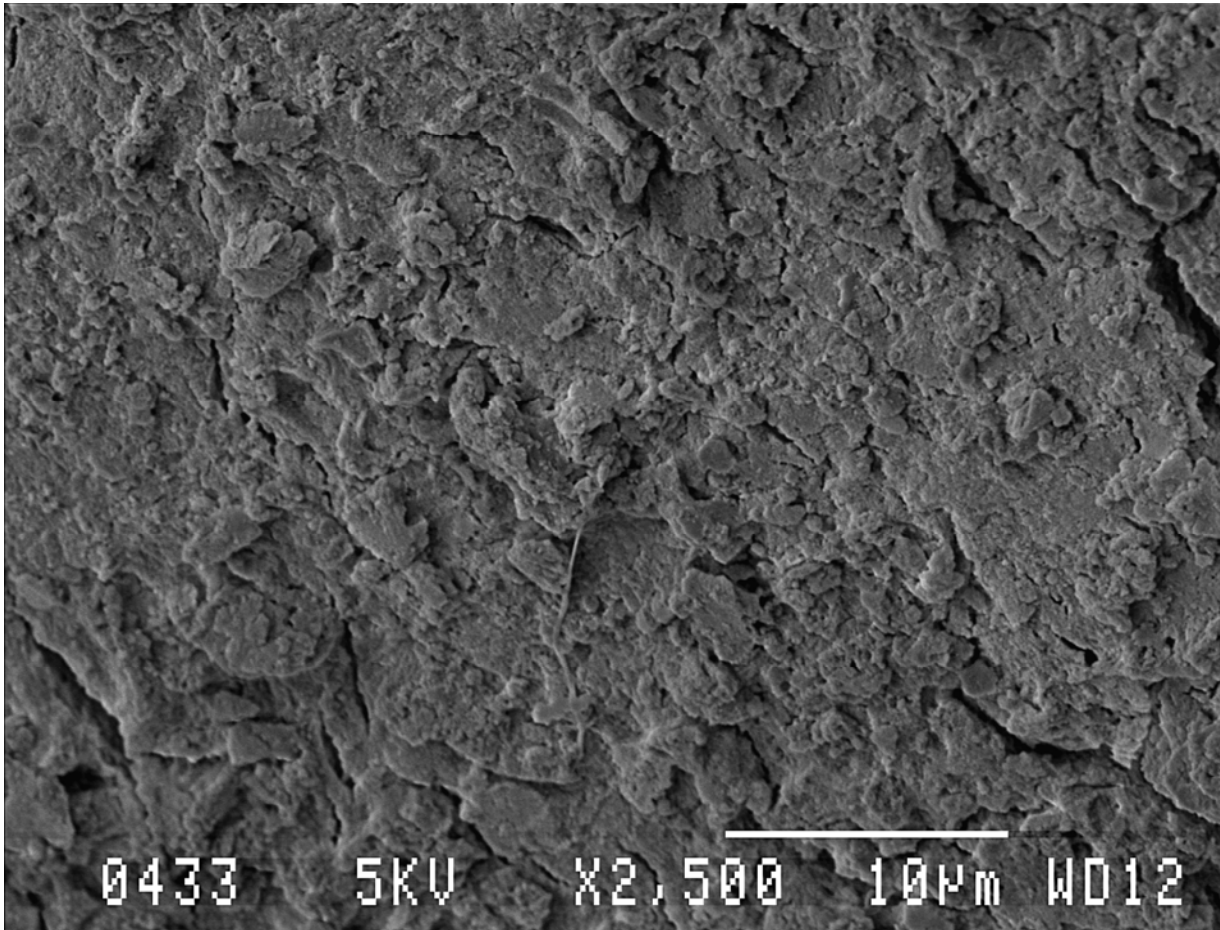


Fig. 3.18: A representative SEM photograph of a prepared surface of a Group A root canal (NaOCl), apical level, thick smear layer with no visible tubule orifices. The undissolved smear layer appears as a flattened rough, irregular surface. Final magnification 2500X.

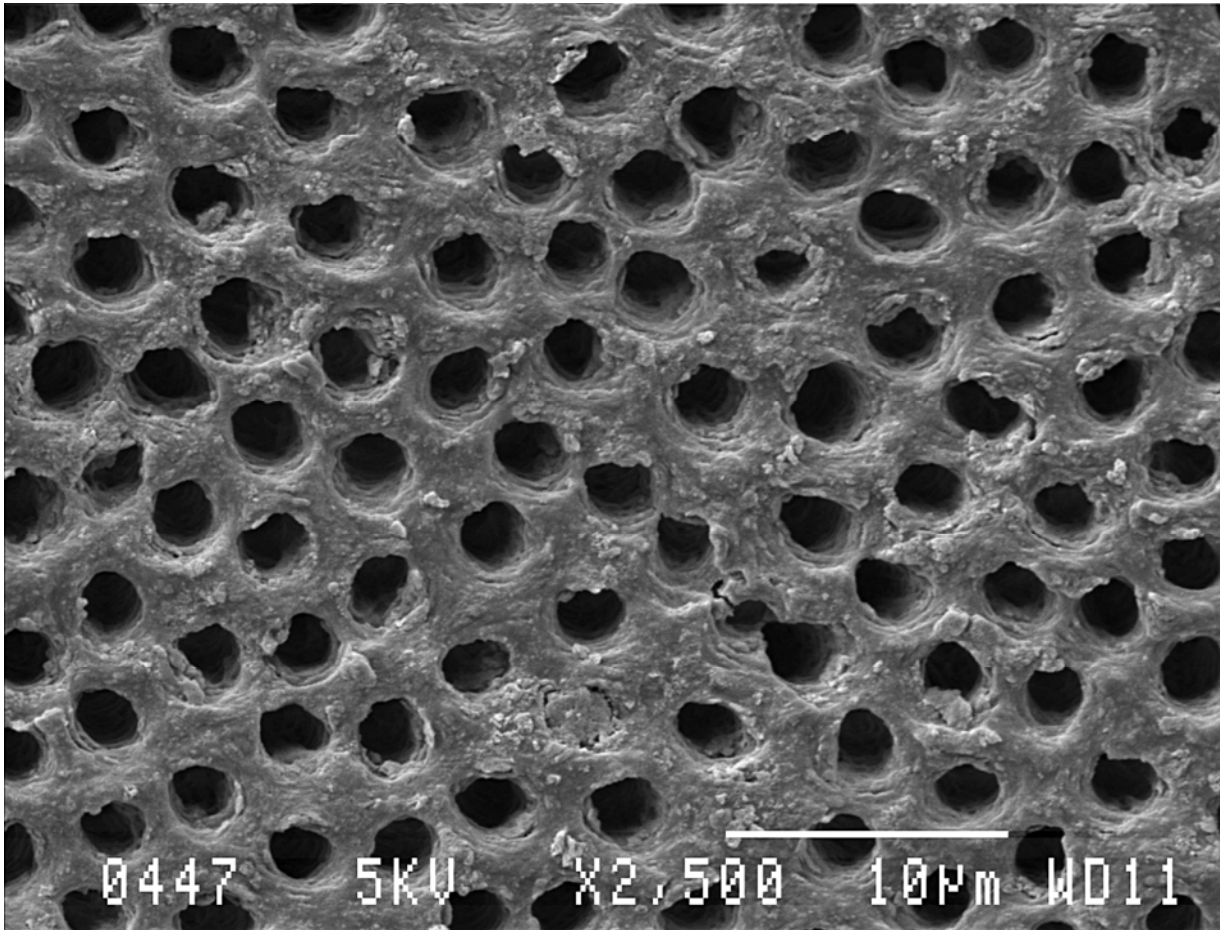


Fig. 3.19: A representative SEM photograph of a prepared surface of a Group B root canal (18% EDTA), coronal level. Apparent and regularly distributed open dentinal tubule orifices are visible. There is a small amount of smear layer left on the intertubular dentine. Final magnification 2500X.

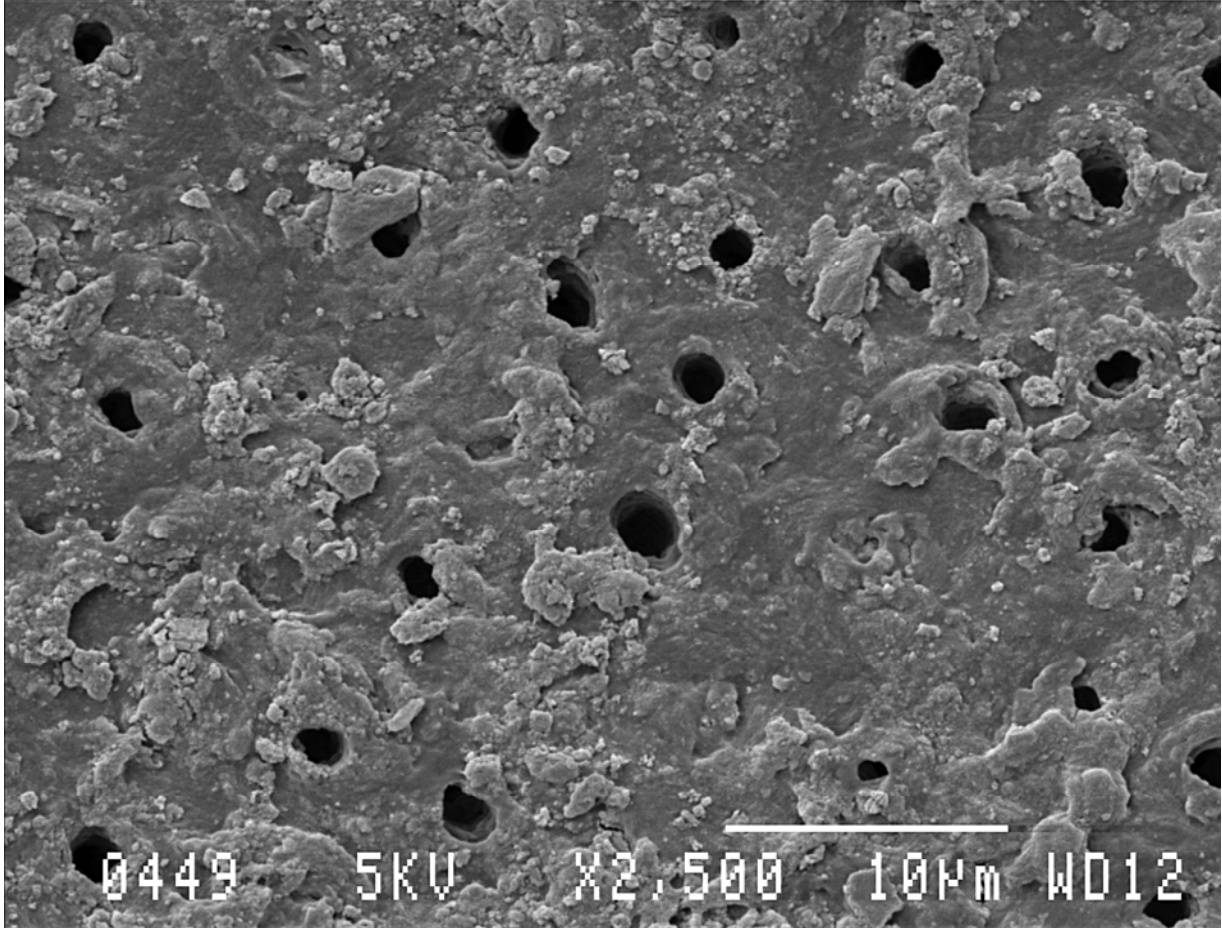


Fig. 3.20: A representative SEM photograph of a prepared surface of a Group B root canal (18% EDTA), middle level. Scattered open tubule orifices with clumps of smear layer visible on the intertubular and peritubular dentine. Final magnification 2500X.

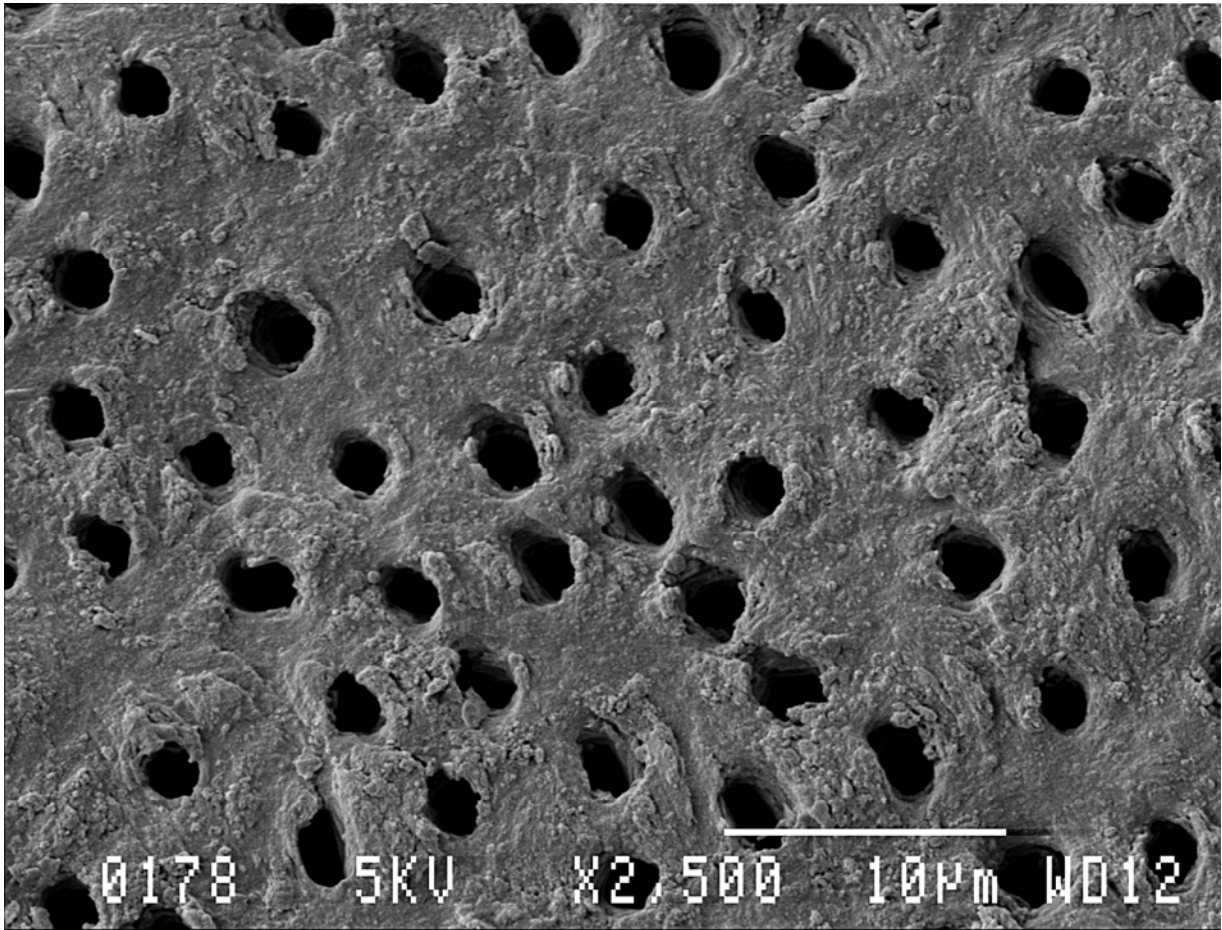


Fig. 3.21: A representative SEM photograph of a prepared surface of a Group B root canal (18% EDTA), middle level. Open tubule orifices with clumps of smear layer visible on the intertubular and peritubular dentine. Final magnification 2500X.

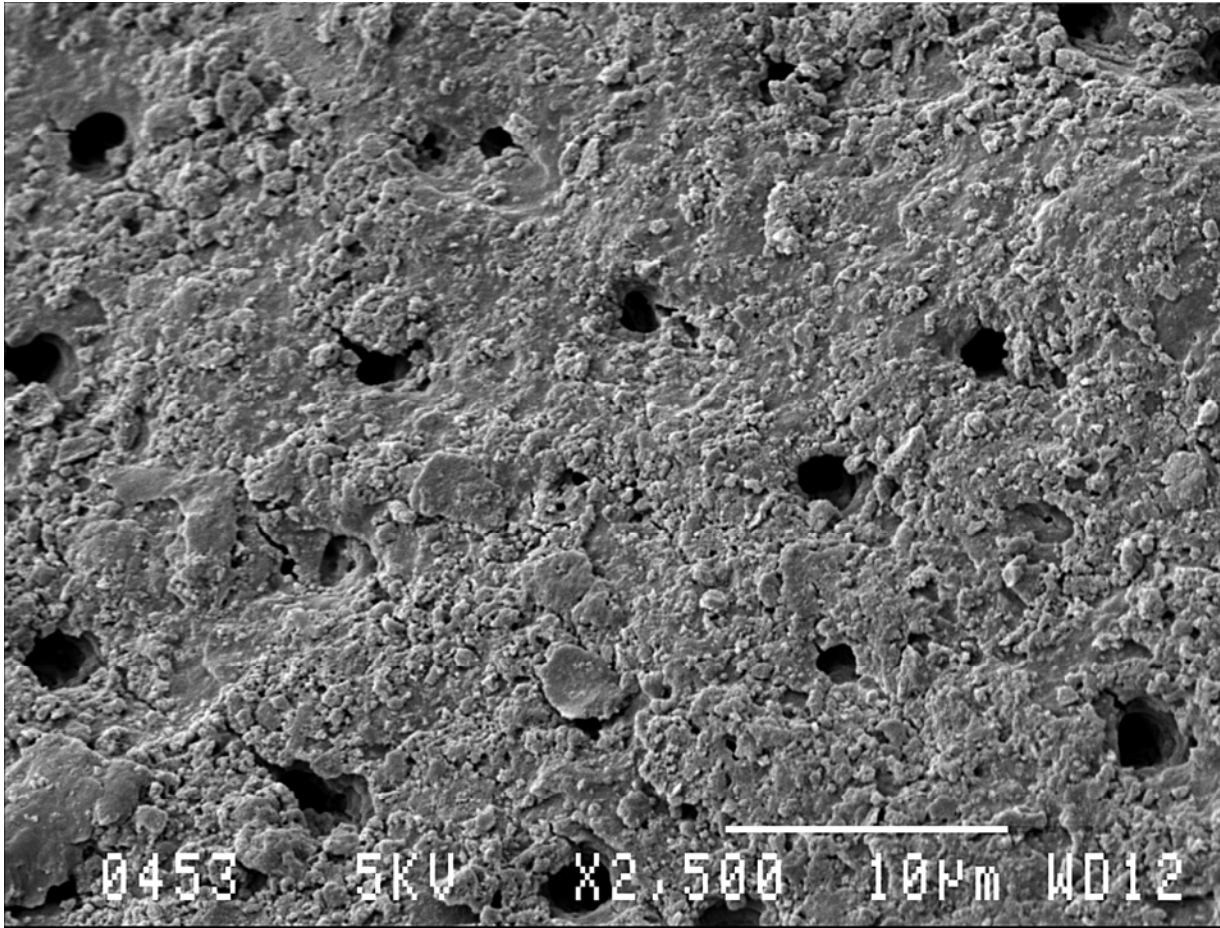


Fig. 3.22: A representative SEM photograph of a prepared surface of a Group B root canal (18% EDTA), apical level. A few partially open tubule orifices is visible with a moderate amount of smear layer covering dentinal orifices, inter and peri-tubular dentine. Final magnification 2500X.

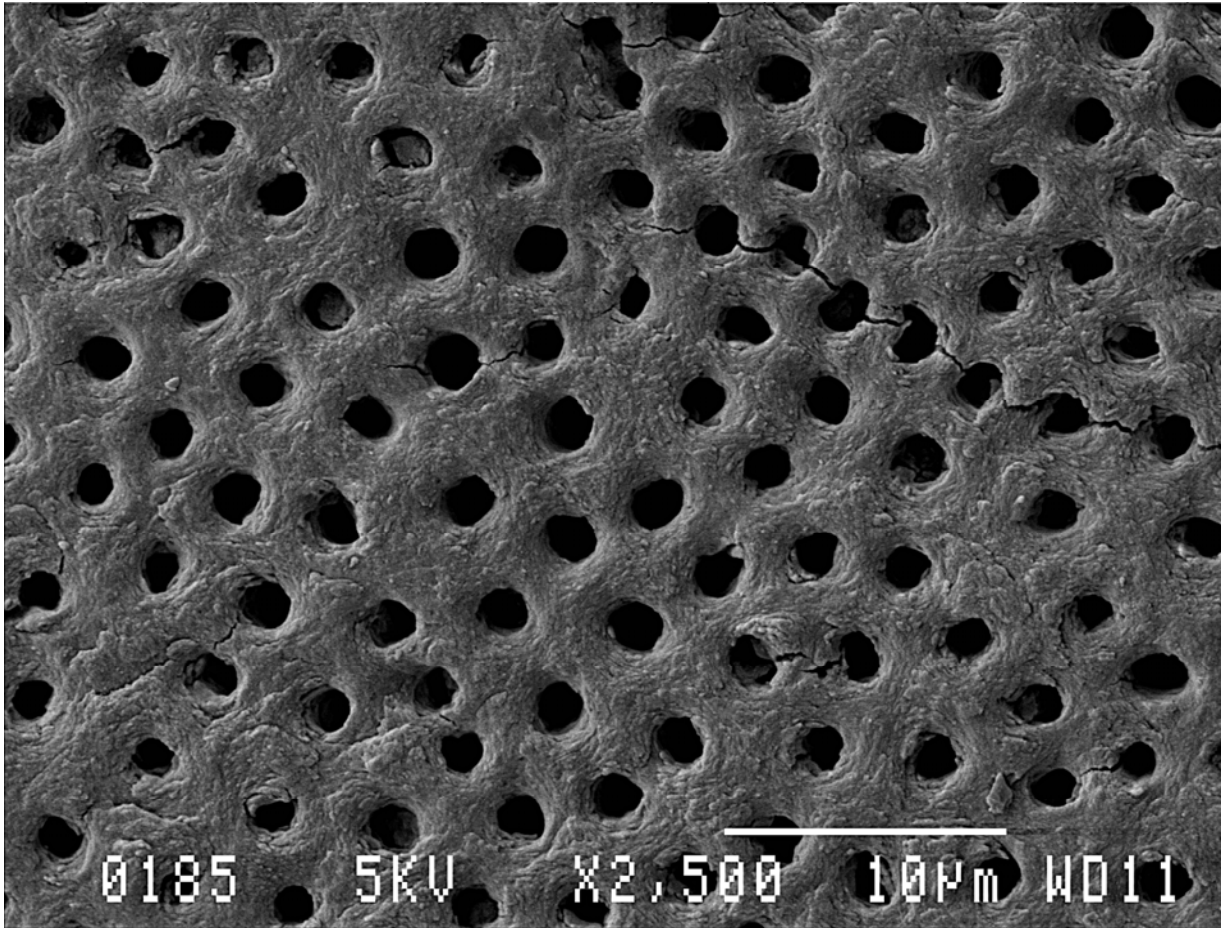


Fig. 3.23: A representative SEM photograph of a prepared surface of a Group C root canal (Sterilox), coronal level. Open dentinal tubule orifices with a small amount of smear layer visible on the intertubular dentine. Final magnification 2500X.

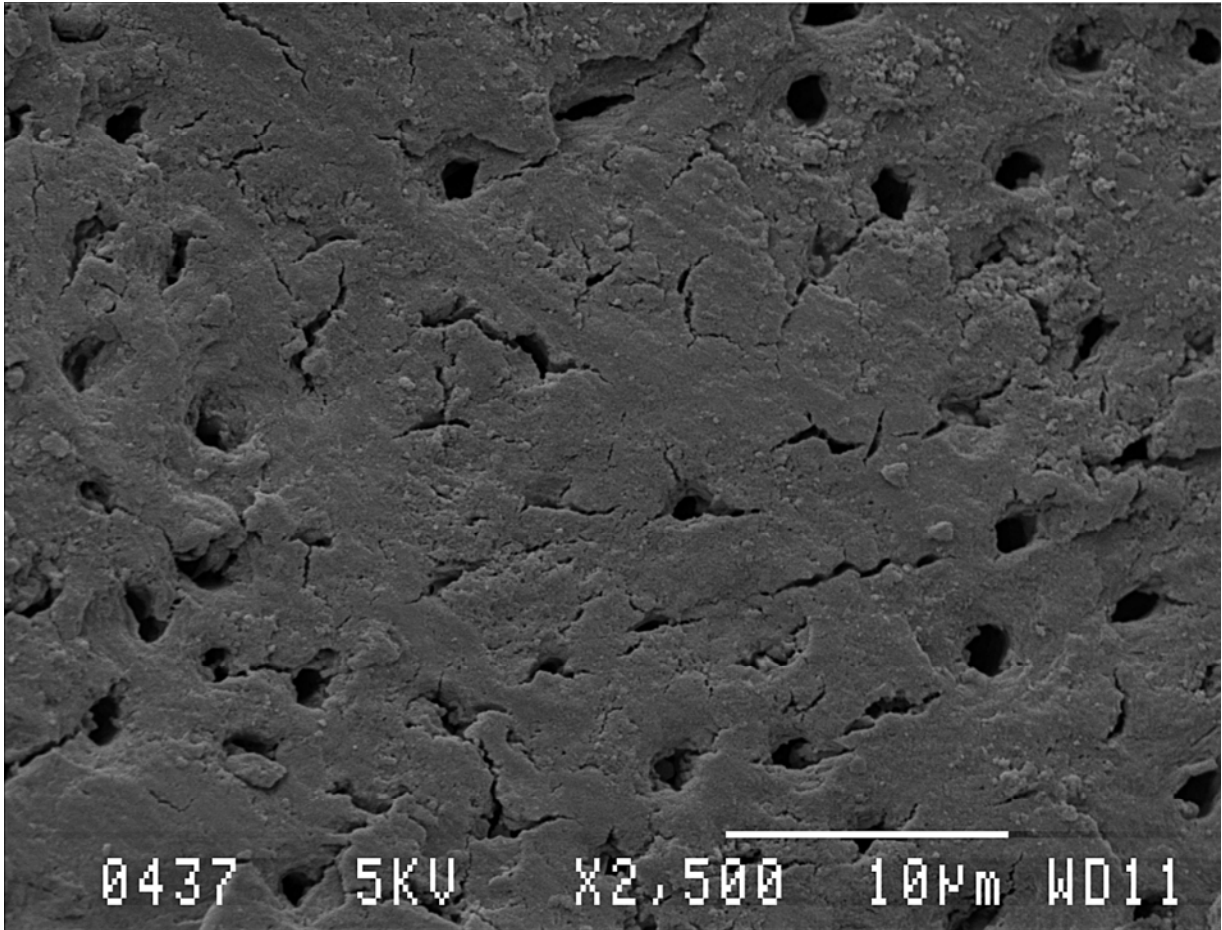


Fig. 3.24: A representative SEM photograph of a prepared surface of a Group C root canal (Sterilox), middle level. A smear layer with some visible tubule orifices is evident. Final magnification 2500X.

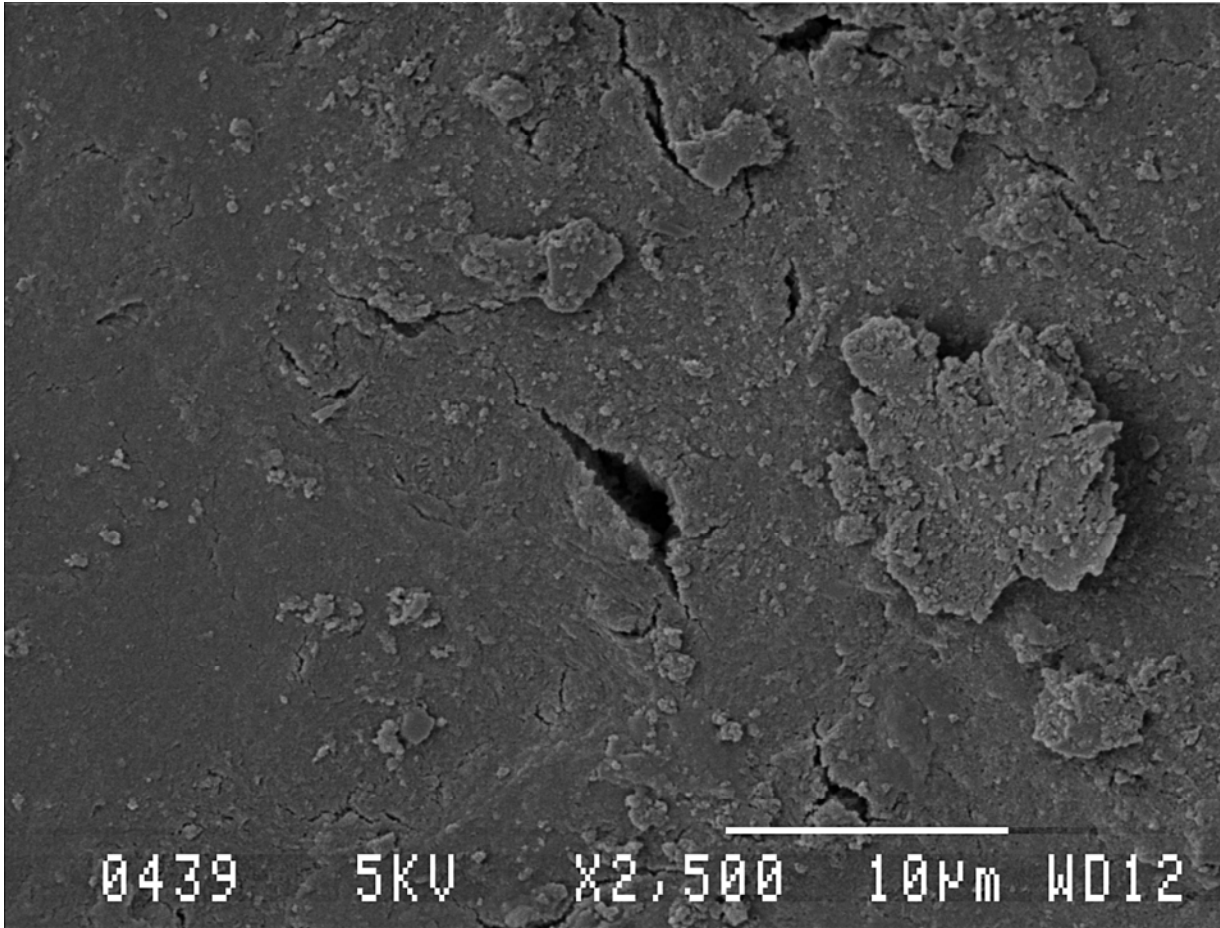


Fig. 3.25: A representative SEM photograph of a prepared surface of a Group C root canal (Sterilox), apical level. A thick smear layer with no visible tubule orifices is evident. Final magnification 2500X.

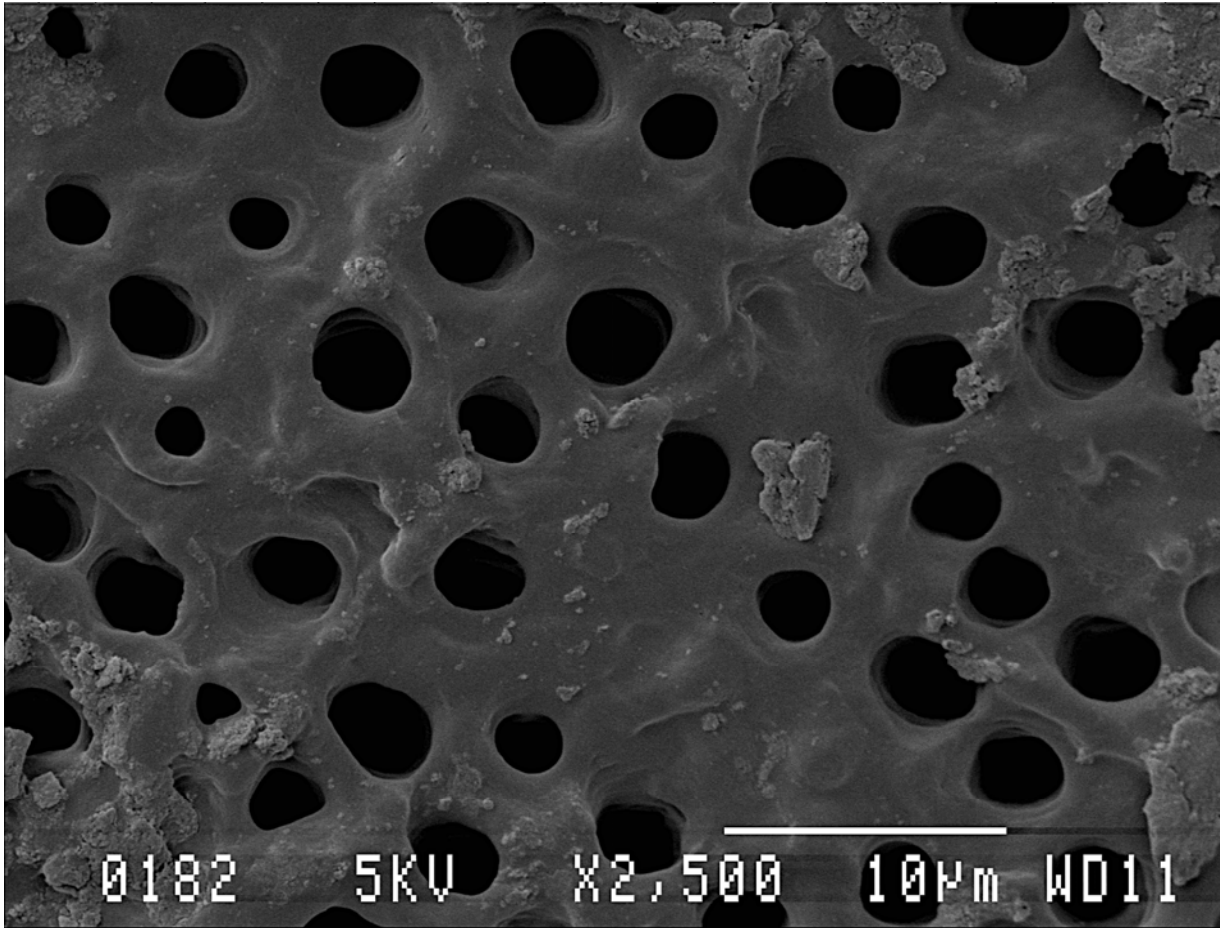


Fig. 3.26: A representative SEM photograph of a prepared surface of a Group D root canal (TopClear 17% EDTA), coronal level. Most of the smear layer is removed. Regular distributed open dentinal tubule orifices with erosion of the peritubular dentine are visible. Final magnification 2500X.

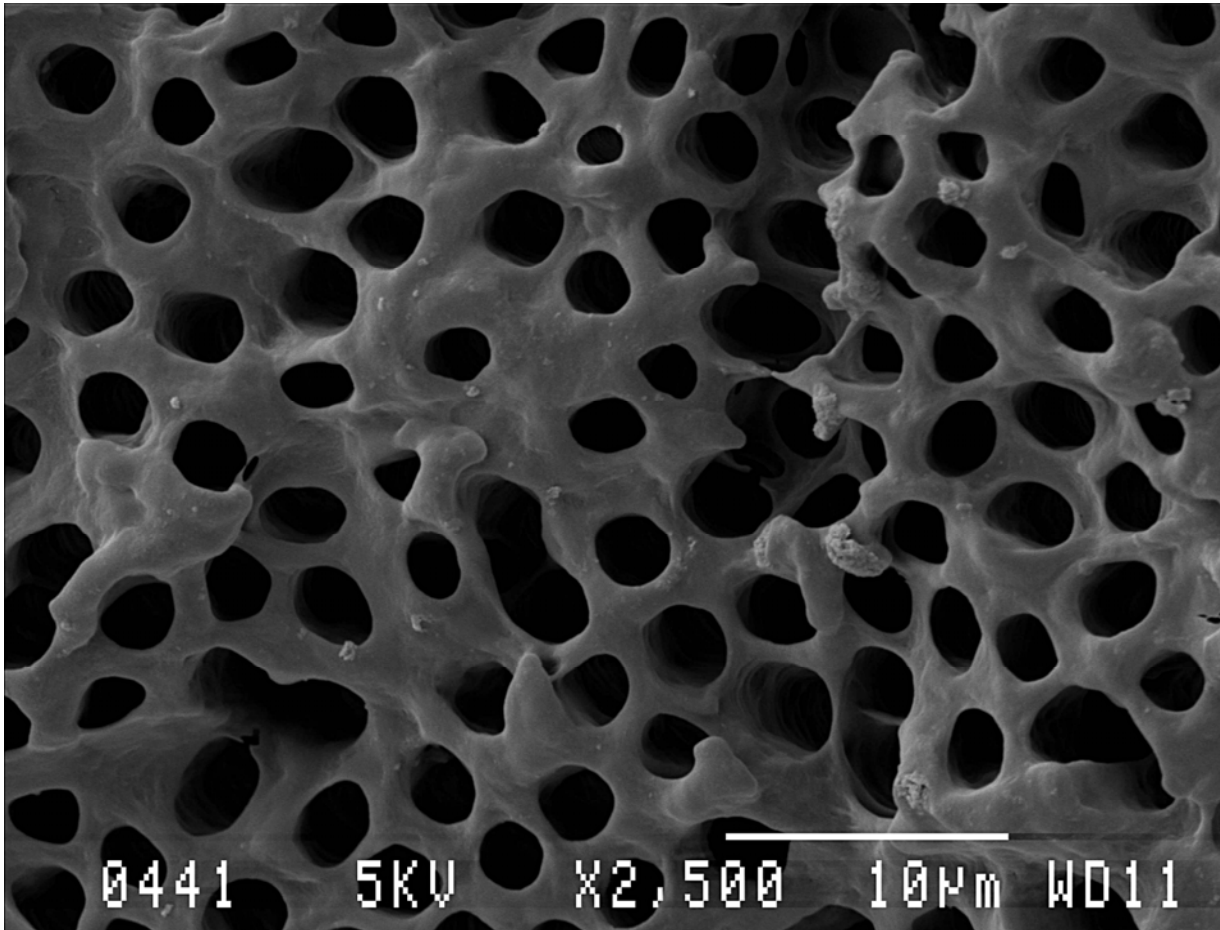


Fig. 3.27: A representative SEM photograph of a prepared surface of a Group D root canal (TopClear 17% EDTA), coronal level. Smear layer is completely removed. Note the extensive erosion of the peritubular dentine and in some areas the intertubular dentine is destroyed. Final magnification 2500X.

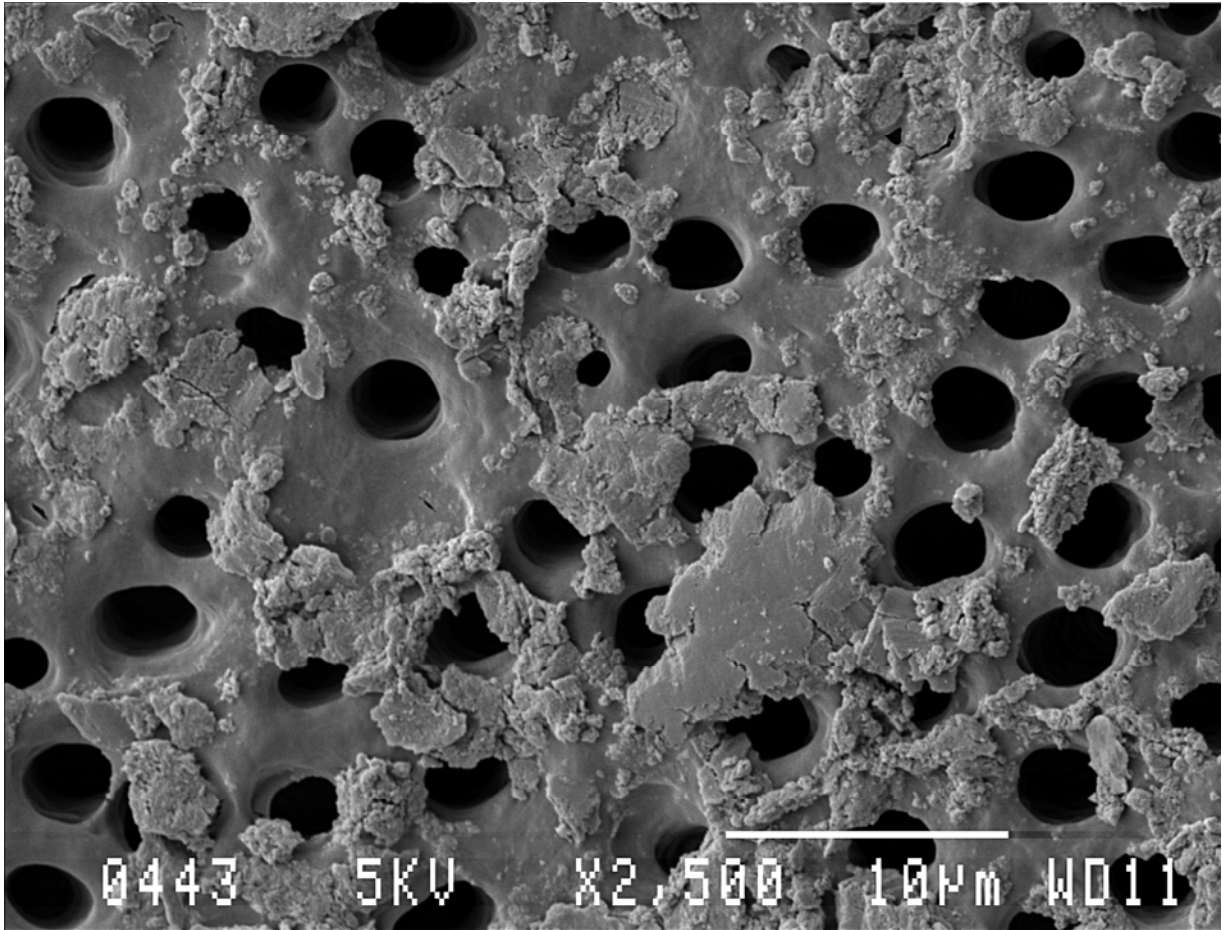


Fig. 3.28: A representative SEM photograph of a prepared surface of a Group D root canal (TopClear 17% EDTA), middle part. Smear layer is partially removed with regularly distributed open dentinal tubule orifices. Note there is some evidence of erosion of the peritubular dentine. Final magnification 2500X.

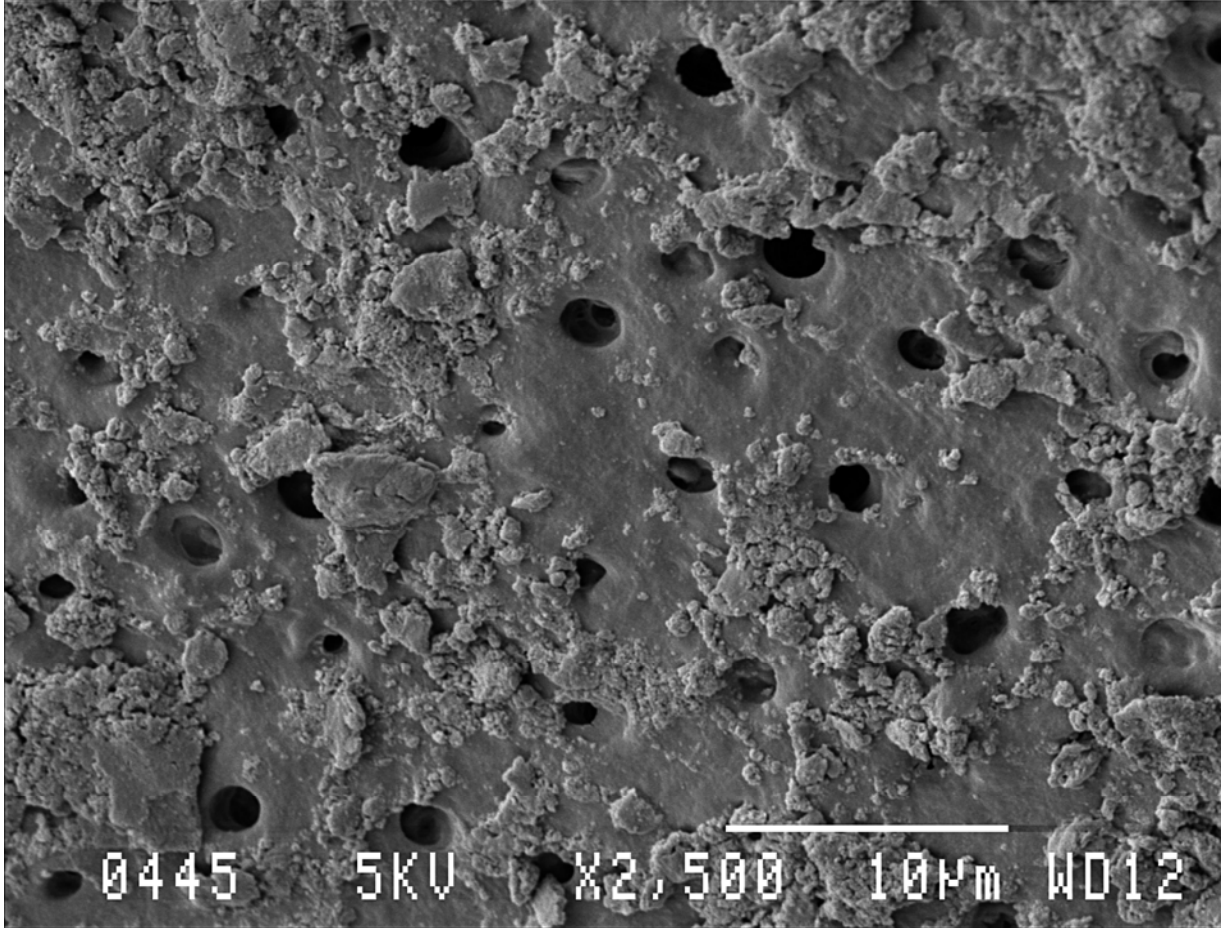


Fig. 3.29: A representative SEM photograph of a prepared surface of a Group D root canal (TopClear 17% EDTA), apical level. Smear layer is partially removed with scattered open dentinal tubule orifices. Final magnification 2500X.

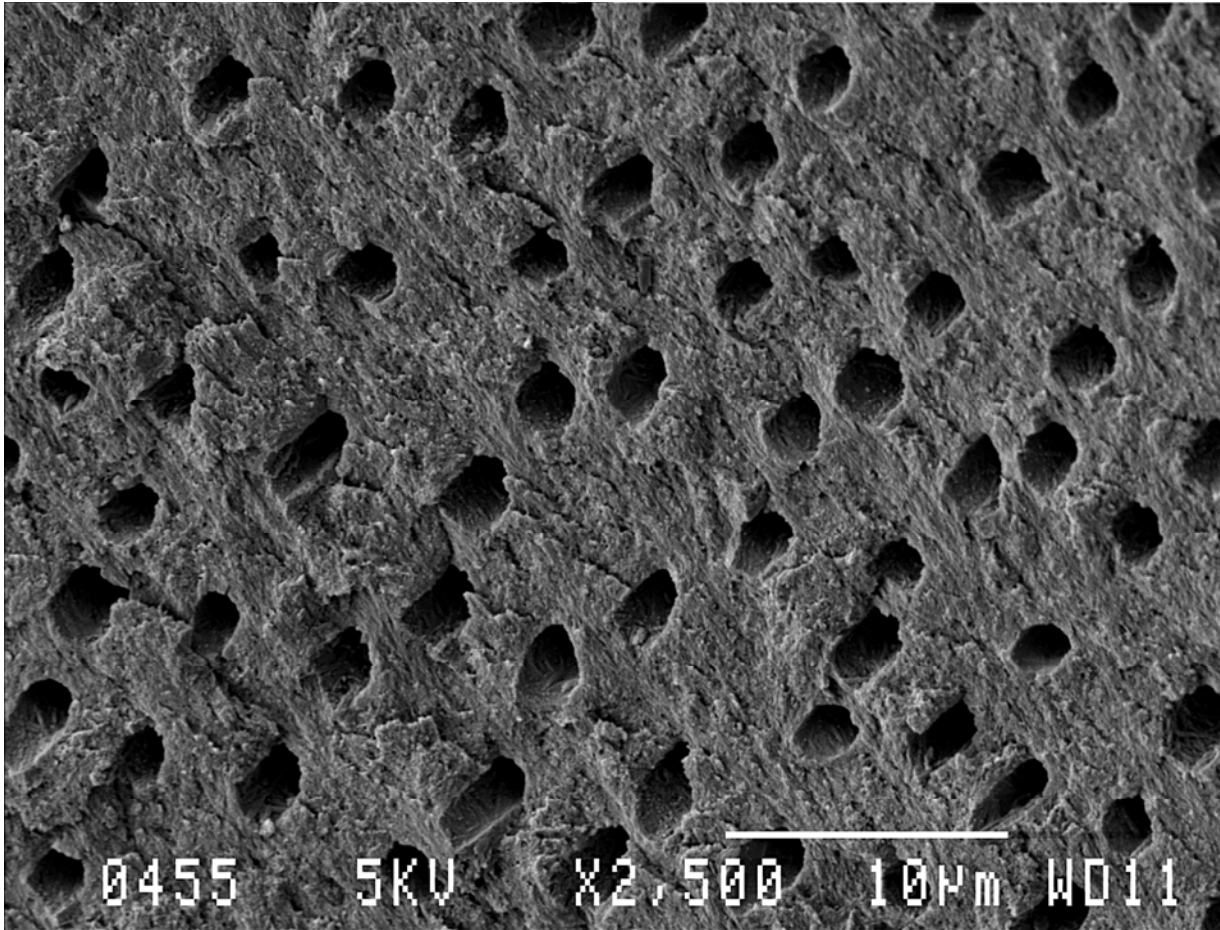


Fig. 3.30: A representative SEM photograph of a prepared surface of a Group E root canal (Chlorhexidine), coronal level. Open dentinal tubule orifices with a granular appearance of the intertubular dentine. Final magnification 2500X.

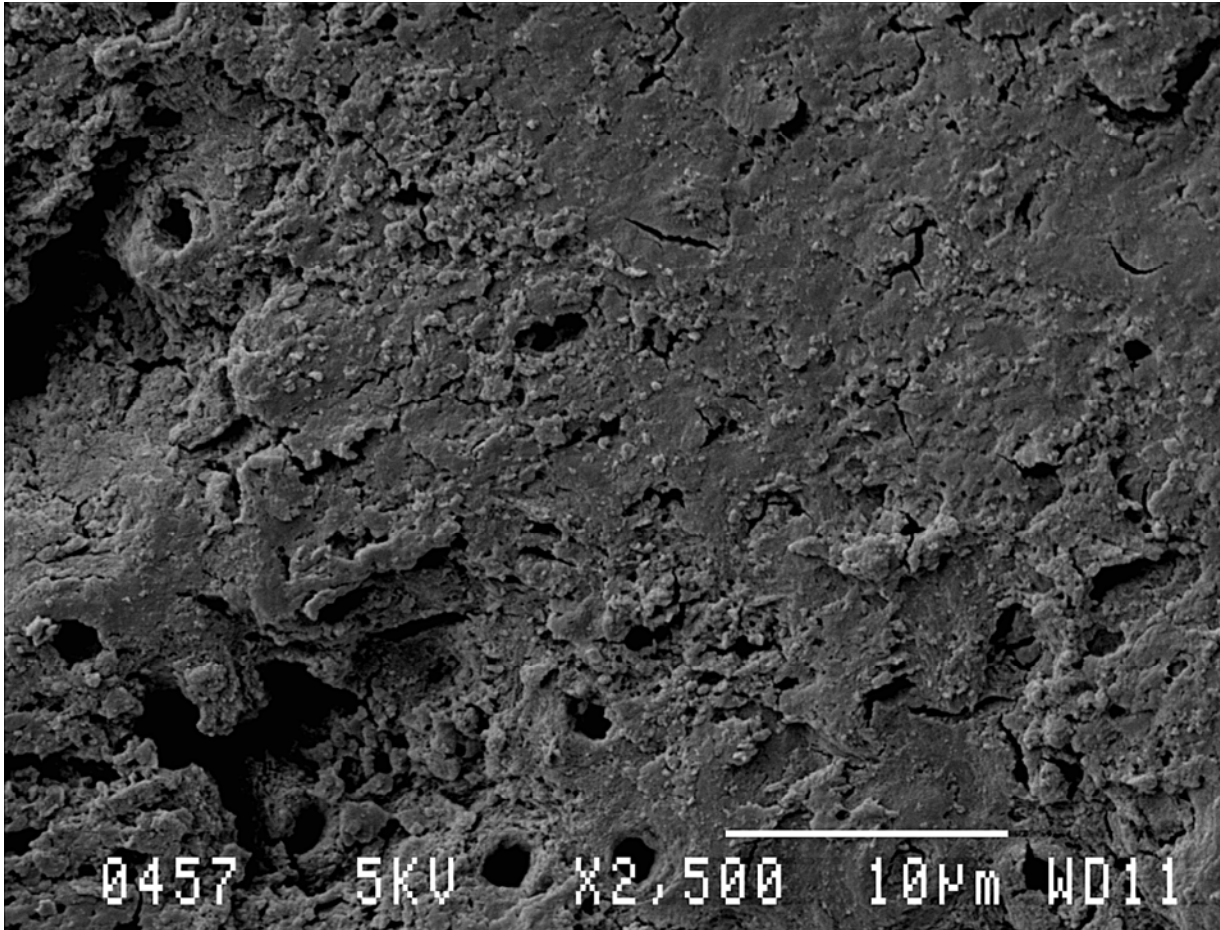


Fig. 3.31: A representative SEM photograph of a prepared surface of a Group E root canal (Chlorhexidine), middle level. A smear layer with a few partially open tubule orifices is evident. Final magnification 2500X.

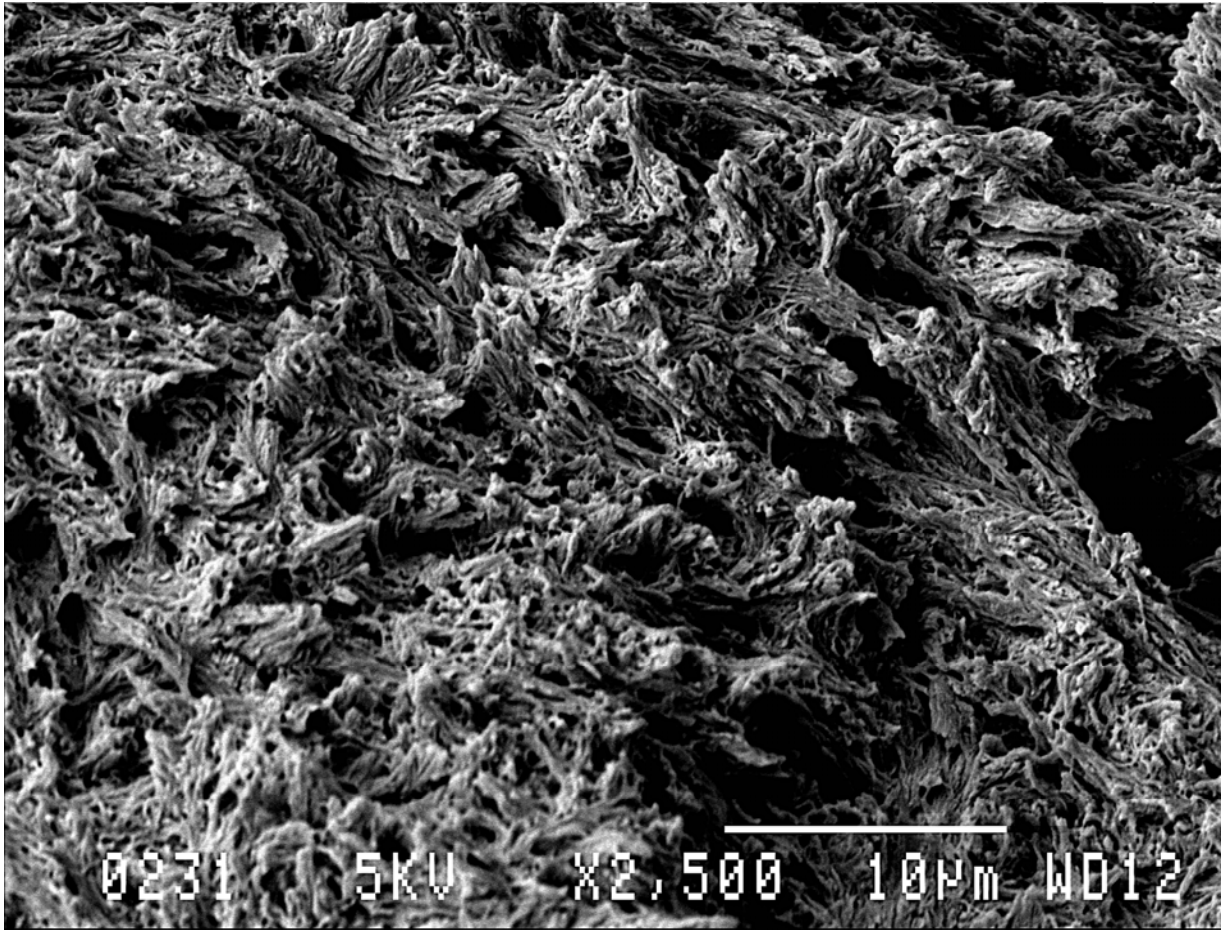


Fig. 3.32: A representative SEM photograph of a prepared surface of a Group E root canal (Chlorhexidine), apical level. A thick, irregular smear layer is visible. Final magnification 2500X.

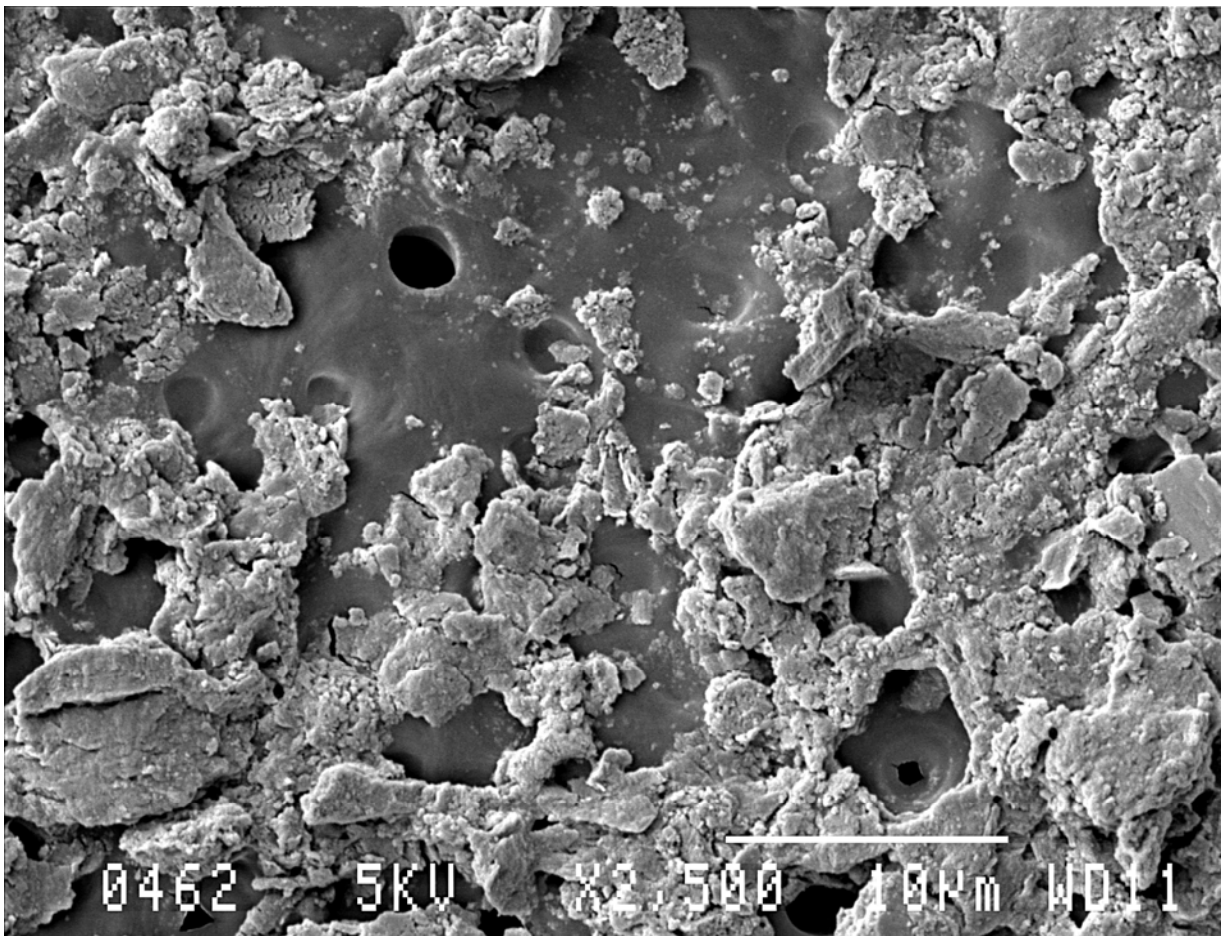


Fig. 3.33: A representative SEM photograph of a prepared surface of a Group F root canal (10% Citric acid), coronal level. Patches of smear layer with one open tubule orifice is visible. Note that it appears as if the intertubular and peritubular dentine melted together to form a layer covering the dentinal tubule orifices. Final magnification 2500X.

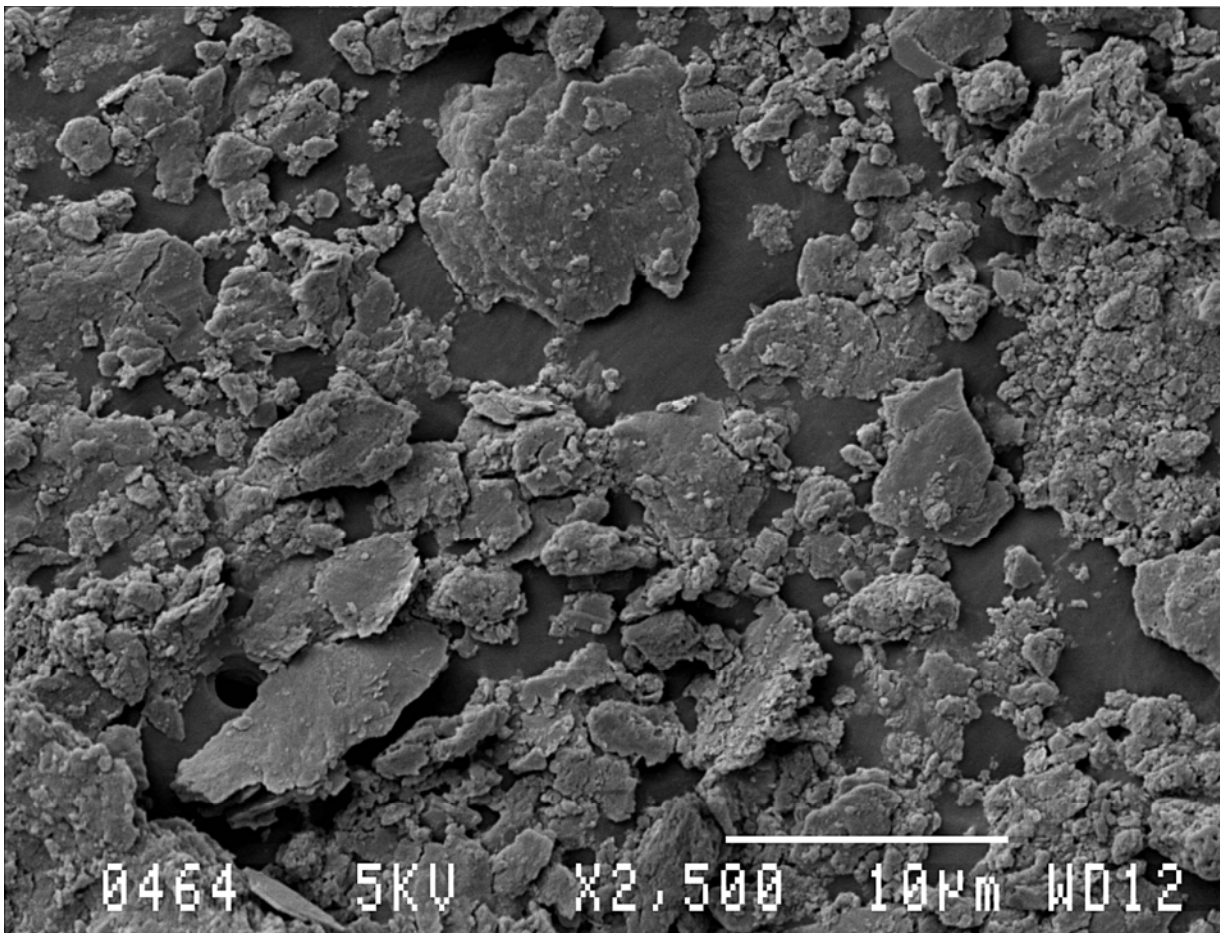


Fig. 3.34: A representative SEM photograph of a prepared surface of a Group F root canal (10% Citric acid), middle level. Patches of smear layer is visible. Note that it appears as if the intertubular and peritubular dentine melted together to form a smooth even layer covering the dentinal tubule orifices. Final magnification 2500X.

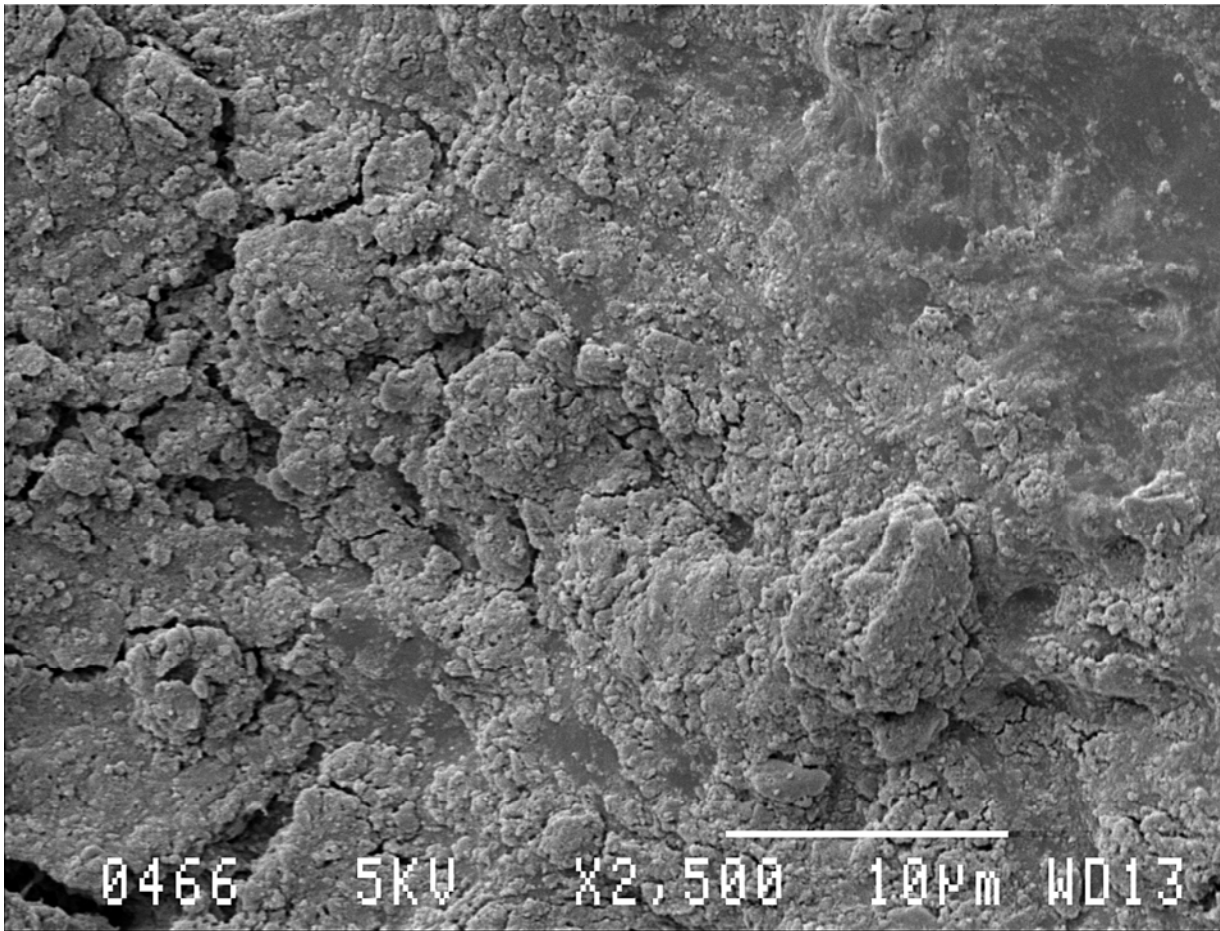


Fig. 3.35: A representative SEM photograph of a prepared surface of a Group F root canal (10% Citric acid), apical level. A thick smear layer with an irregular surface is evident. Final magnification 2500X.

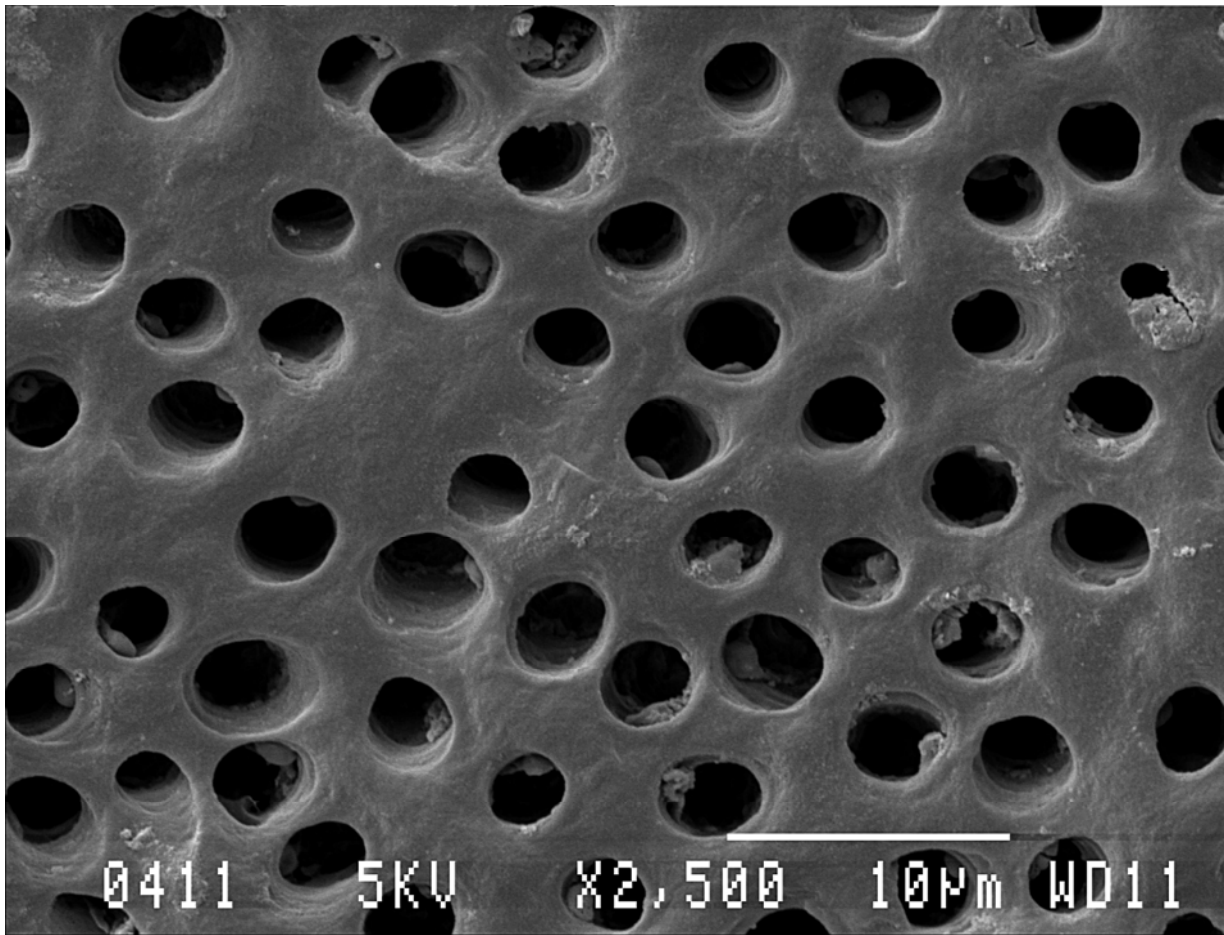


Fig. 3.36: A representative SEM photograph of a prepared surface of a Group G root canal (BioPure MTAD), coronal level. Smear layer is completely removed. Numerous, regular distributed open dentinal tubules are visible. Note there is no erosion visible on the peritubular dentine. Final magnification 2500X.

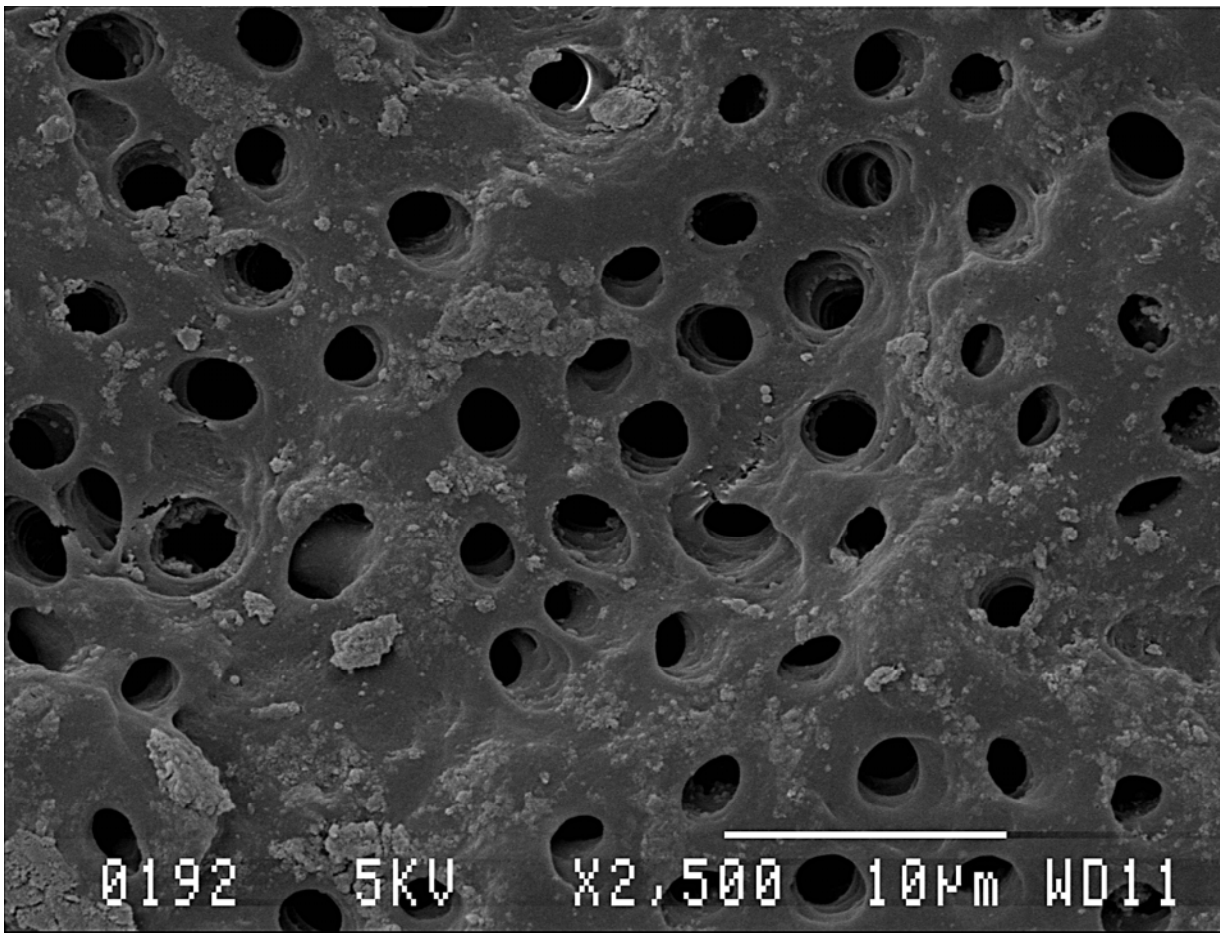


Fig. 3.37: A representative SEM photograph of a prepared surface of a Group G root canal (BioPure MTAD), middle level. Most of the smear layer is removed and open dentinal tubules are visible. Final magnification 2500X.

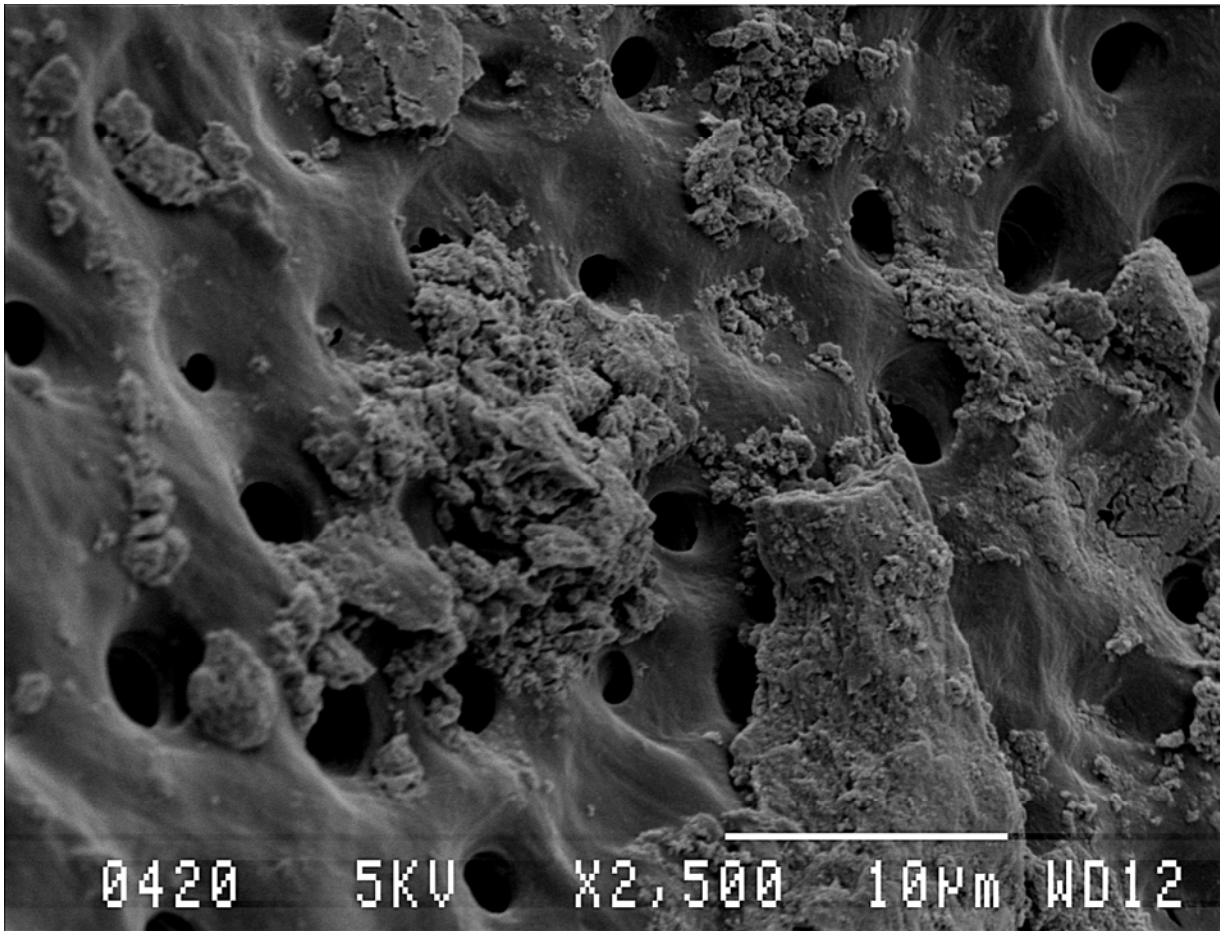


Fig. 3.38: A representative SEM photograph of a prepared surface of a Group G root canal (BioPure MTAD), apical level. Most of the dentinal tubuli is open and visible. There are still patches of smear layer covering some tubules and intertubular dentine. Final magnification 2500X.

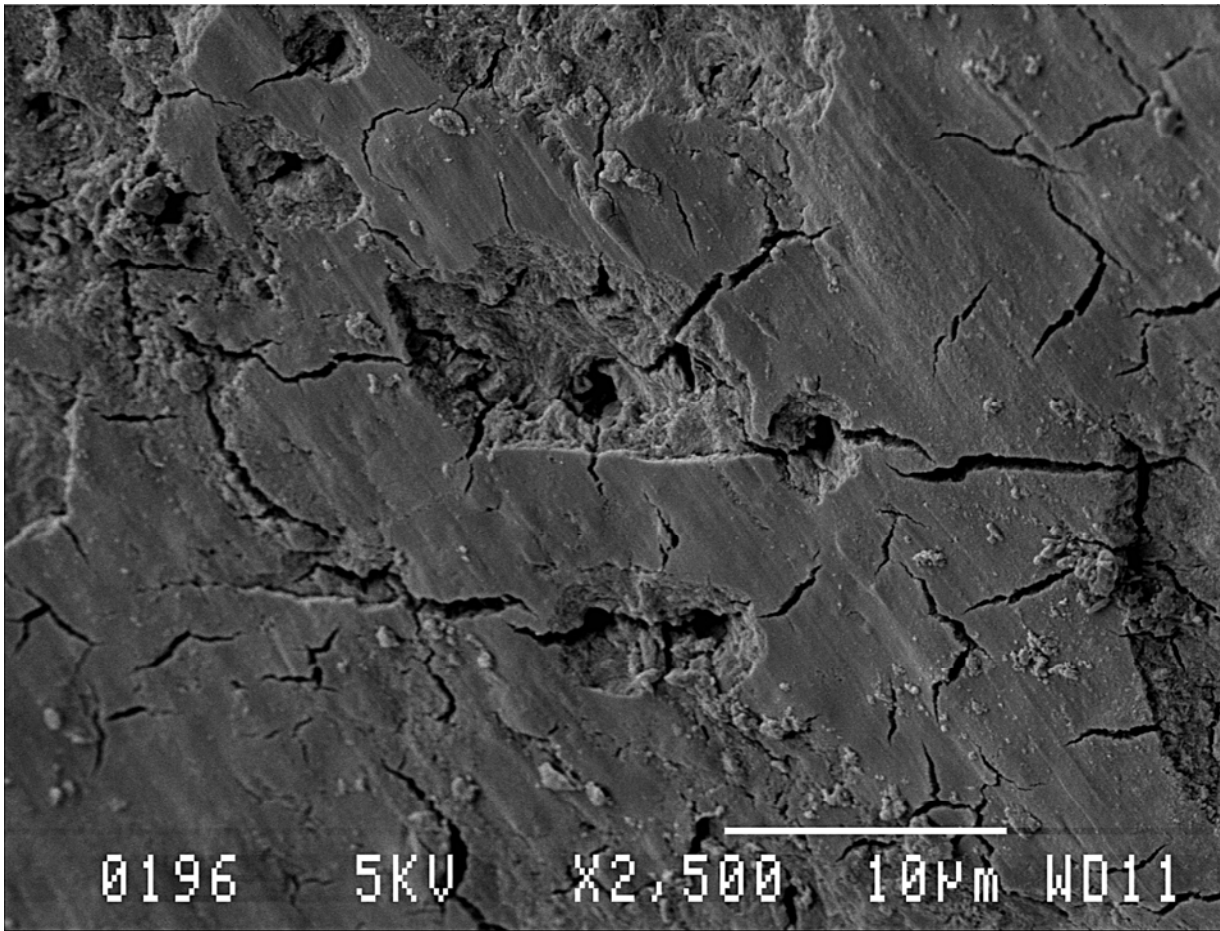


Fig. 3.39: A representative SEM photograph of a prepared surface of a Group H root canal (Ozonated water), coronal level. A thick, smooth smear layer with no visible tubule orifices is evident. Final magnification 2500X.

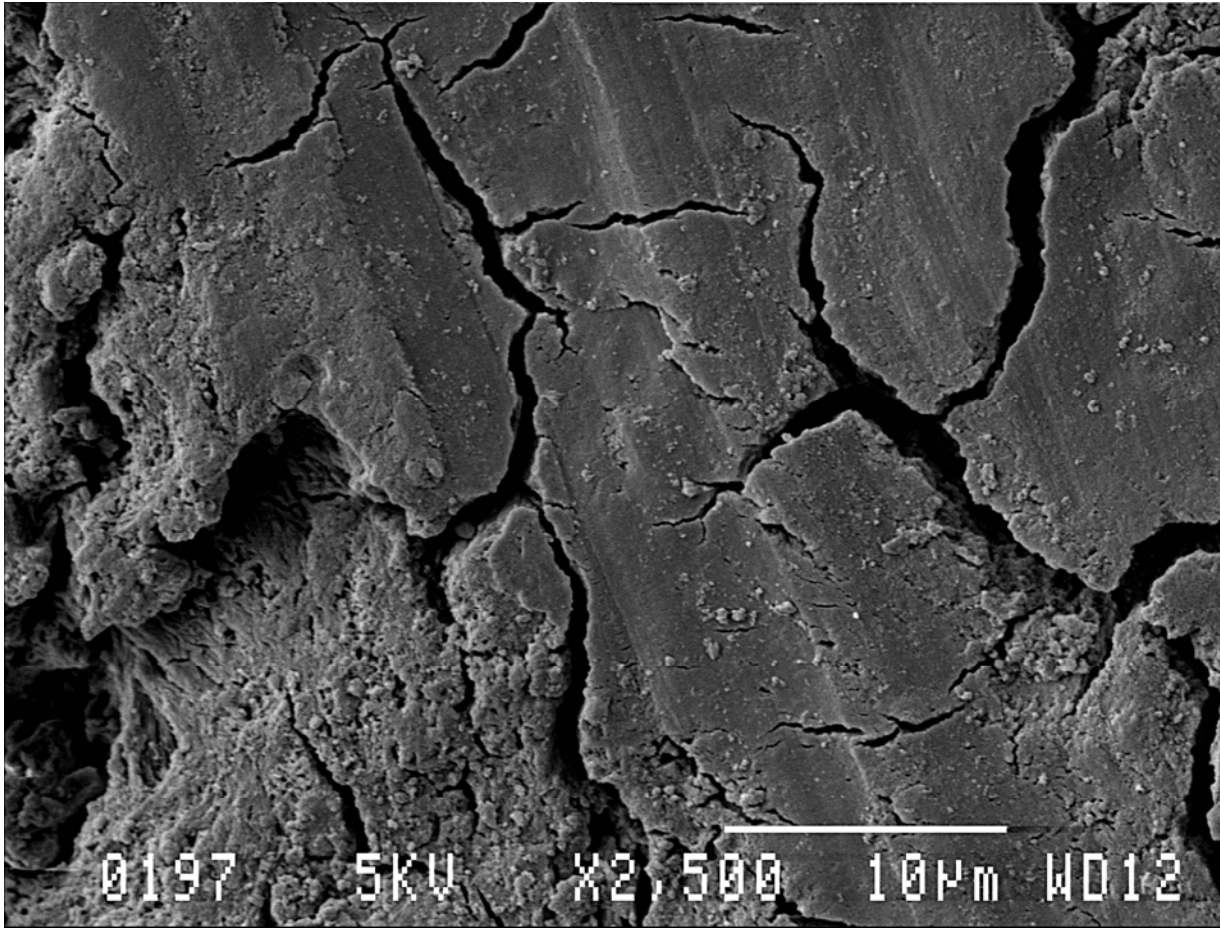


Fig. 3.40: A representative SEM photograph of a prepared surface of a Group H root canal (Ozonated water), middle level. A thick smear layer with a smooth and granular texture is visible. Final magnification 2500X.

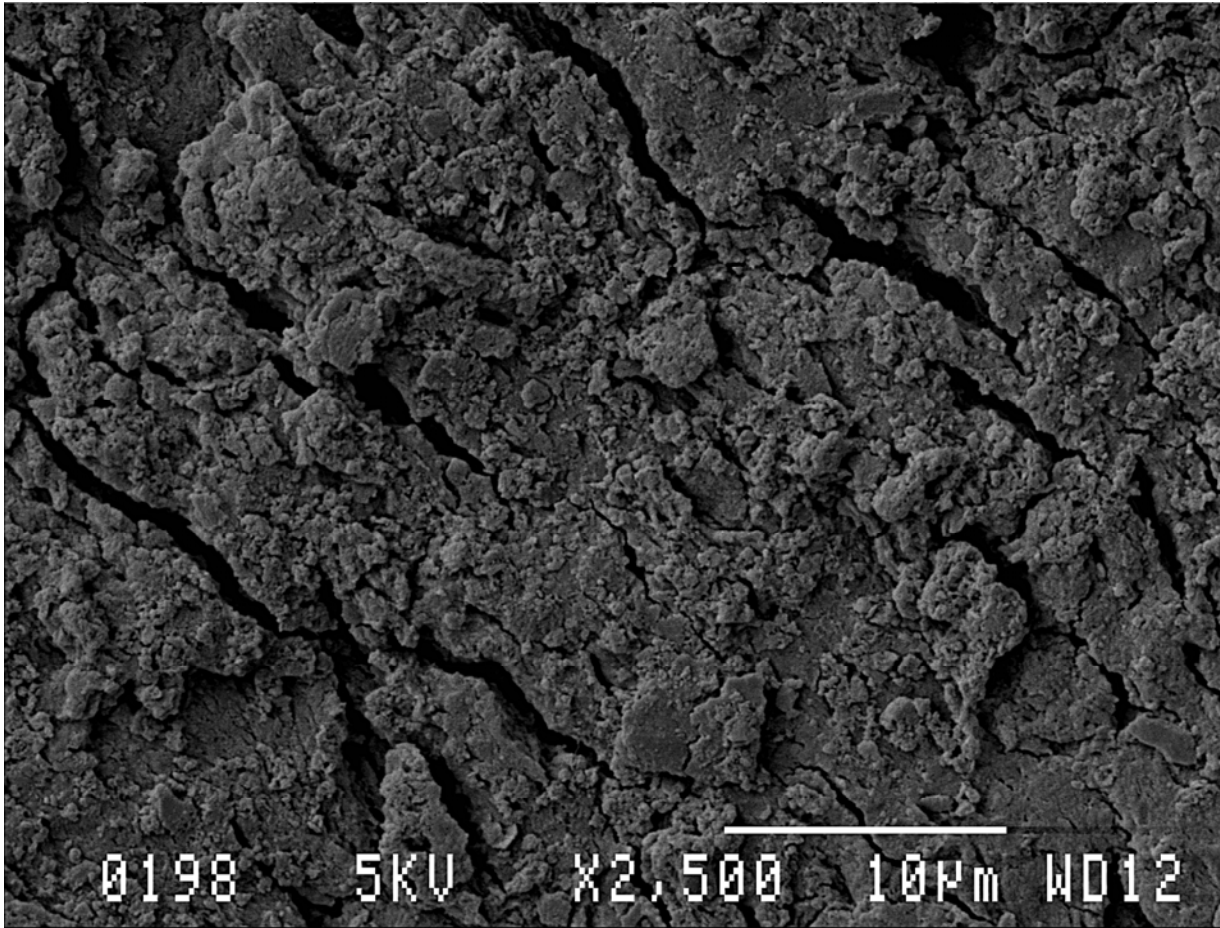


Fig. 3.41: A representative SEM photograph of a prepared surface of a Group H root canal (Ozonated water), apical level. A thick, granular textured smear layer is evident. Final magnification 2500X.

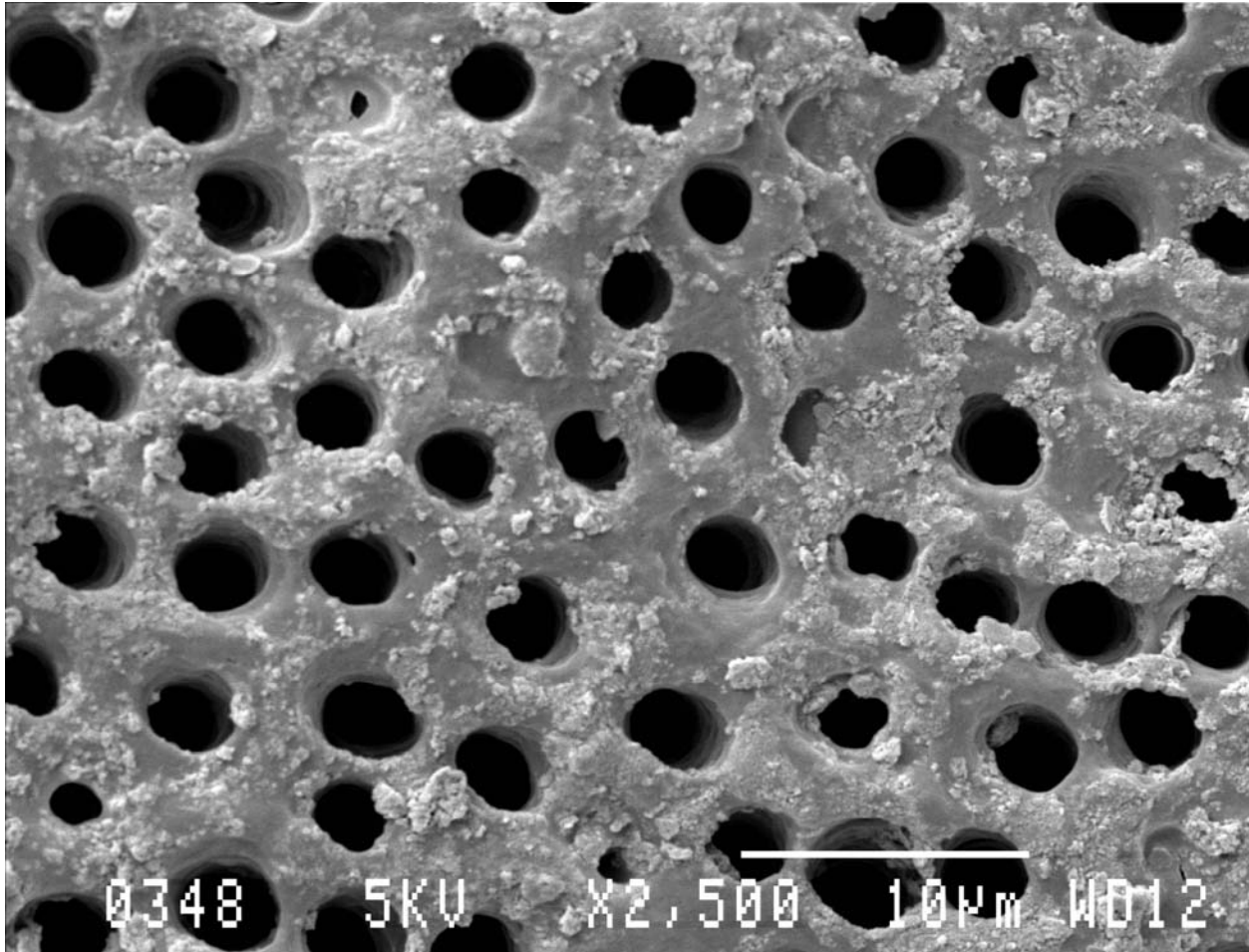


Fig. 3.42: A representative SEM photograph of a prepared surface of a Group I root canal (SmearClear), coronal level. Most of the smear layer is removed. Regular distributed open dentinal tubule orifices visible. Final magnification 2500X.

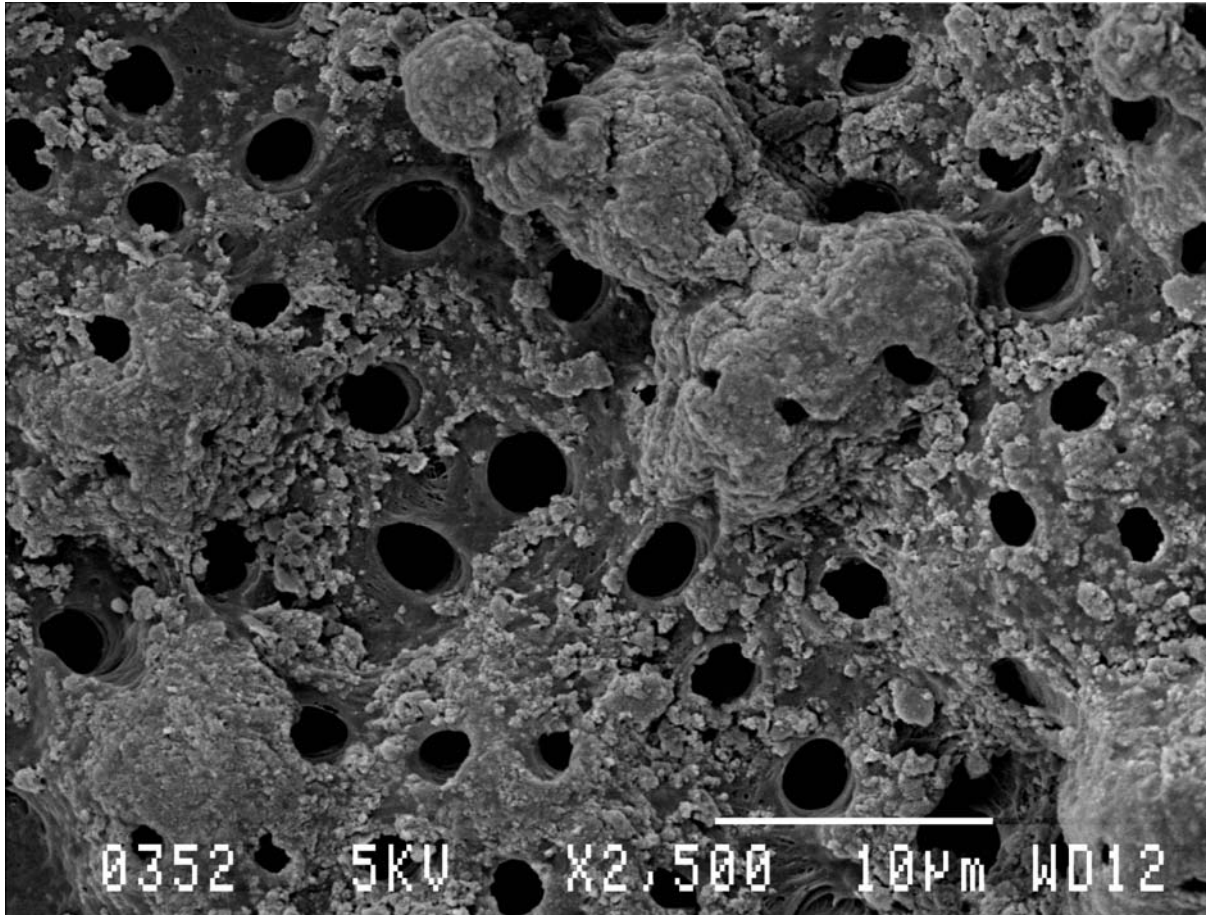


Fig. 3.43: A representative SEM photograph of a prepared surface of a Group I root canal (SmearClear), middle level. Most of the dentinal tubuli is open and visible. There are still patches of smear layer covering some tubules and intertubular dentine. Final magnification 2500X.

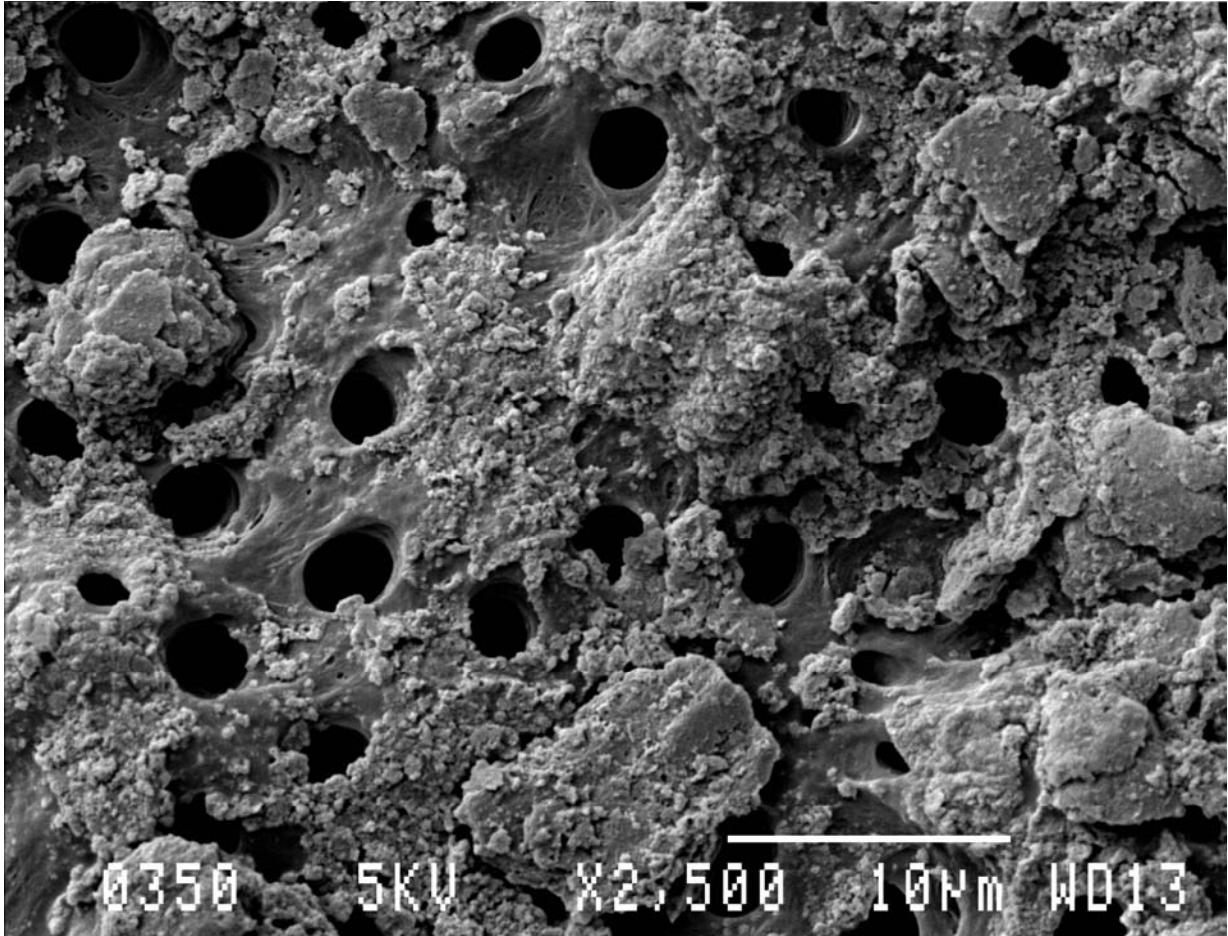


Fig. 3.44: A representative SEM photograph of a prepared surface of a Group I root canal (SmearClear), apical level. Scattered open tubule orifices with clumps of smear layer visible on the intertubular and peritubular dentine. Final magnification 2500X.

Table 3.1. Comparison of *in vitro* antimicrobial activity of the undiluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

100% Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H ₂ O control	0	0	0
3,5% NaOCl	9.20	± 2.51	27.3
EDTA 18%	8.25	± 0.23	2.8
Sterilox	0	0	0
TopClear	8.84	± 0.11	1.2
CHX	6.44	± 0.09	1.4
Citric acid	0.70	± 0.21	30.0
MTAD	11.53	± 0.35	3.0
Ozonated water	0	0	0
SmearClear	10.08	± 0.31	3.1

Table 3.2. Comparison of *in vitro* antimicrobial activity of 1/10 diluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

1/10 Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H ₂ O control	0	0	0
3,5% NaOCl	0	0	0
EDTA 18%	0.54	± 0.02	3.7
Sterilox	0	0	0
TopClear	2.22	± 0.19	8.6
CHX	1.26	± 0.22	17.5
Citric acid	0	0	0
MTAD	9.38	± 0.29	3.1
Ozonated water	0	0	0
SmearClear	6.31	± 0.67	10.6

Table 3.3. Comparison of *in vitro* antimicrobial activity of 1/100 diluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

1/100 Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H ₂ O control	0	0	0
3,5% NaOCl	0	0	0
EDTA 18%	0	0	0
Sterilox	0	0	0
TopClear	0	0	0
CHX	0	0	0
Citric acid	0	0	0
MTAD	2.39	± 0.17	7.1
Ozonated water	0	0	0
SmearClear	0	0	0

Table 3.4. Comparison of *in vitro* antimicrobial activity of 1/1000 diluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

1/1000 Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H ₂ O control	0	0	0
3,5% NaOCl	0	0	0
EDTA 18%	0	0	0
Sterilox	0	0	0
TopClear	0	0	0
CHX	0	0	0
Citric acid	0	0	0
MTAD	0	0	0
Ozonated water	0	0	0
SmearClear	0	0	0

Table 3.5. Significance of difference between the mean values (Table 3.1) of the *in vitro* antimicrobial activity of the undiluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
Sterile water	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
3,5% NaOCI								p<0.05	p<0.05
EDTA 18%	p<0.05			p<0.05			p<0.05	p<0.05	p<0.05
Sterilox	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
TopClear	p>0.05						p<0.05	p<0.05	p<0.05
CHX	p<0.05	p<0.05		p<0.05			p<0.05	p<0.05	p<0.05
Citric acid	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
MTAD	p<0.05							p<0.05	p<0.05
Ozonated water									p<0.05
SmearClear									

Table 3.6. Significance of difference between the mean values (Table 3.2) of the *in vitro* antimicrobial activity of the 1/10 diluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
Sterile water									
3,5% NaOCI									
EDTA 18%	p<0.05			p<0.05			p<0.05		p<0.05
Sterilox									
TopClear							p<0.05		p<0.05
CHX	p<0.05	p<0.05		p<0.05			p<0.05		p<0.05
Citric acid									
MTAD									
Ozonated water									
SmearClear									

Table 3.7: Incidence of smear layer-free surfaces (expressed as a percentage of observed surfaces) (c= coronal level, m= middle level and a= apical level).

Group	A			B			C			D			E		
Irrigation regime	3.5% NaOCL			18% EDTA			Sterilox			TopClear			Chlorhexidine		
Specimen	C	M	A	C	M	A	C	M	A	C	M	A	C	M	A
1	0	0	0	95	45	45	90	30	0	95	60	40	90	20	0
2	0	0	0	90	70	40	80	25	0	100	65	35	100	15	0
3	0	0	0	100	65	40	85	35	0	100	60	40	95	20	0
4	0	0	0	95	60	45	80	25	5	95	55	45	90	15	0
5	0	0	0	90	75	45	85	30	0	100	60	40	95	25	0
Mean	0	0	0	94	63	43	84	29	1	98	60	40	94	19	0
SD	0	0	0	4.2	11.5	2.7	4.2	4.2	2.24	2.7	3.5	3.5	4.2	4.2	0
Group	F			G			H			I					
Irrigation regime	10% Citric			MTAD			Ozonated water			SmearClear					
Specimen	C	M	A	C	M	A	C	M	A	C	M	A			
1	20	5	0	100	95	60	0	0	0	80	25	30			
2	15	0	0	100	100	55	5	0	0	75	20	35			
3	20	0	0	100	100	55	0	0	0	85	30	30			
4	25	5	0	100	95	60	0	0	0	80	25	40			
5	15	5	0	100	95	55	0	0	0	80	30	30			
Mean	19	3	0	100	97	57	1	0	0	80	26	33			
SD (\pm)	4.2	2.7	0	0	2.7	2.74	2.7	0	0	3.5	4.1	4.5			

Table 3.8. Significance of difference between the mean values (Table 3.7) in smear layer scores for the coronal level of the root canals.

	3,5% NaOCl	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
3,5% NaOCl								p>0.05	p<0.05
EDTA 18%	p<0.05			p<0.05			p<0.05	p<0.05	p<0.05
Sterilox	p<0.05	p>0.05		p<0.05	p>0.05		p<0.05	p<0.05	p<0.05
TopClear	p<0.05						p>0.05	p<0.05	p<0.05
CHX	p<0.05	P=0.05		p>0.05			p<0.05	p<0.05	p<0.05
Citric acid	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	p<0.05	p<0.05
MTAD	p<0.05							p<0.05	p<0.05
Ozonated water									p<0.05
SmearClear	p<0.05							p<0.05	

Table 3.9. Significance of difference between the mean values (Table 3.7) in smear layer scores for the middle level of the root canals.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
3,5% NaOCI								p>0.05	p<0.05
EDTA 18%	p<0.05			p>0.05			p<0.05	p<0.05	p<0.05
Sterilox	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05	p<0.05	p<0.05
TopClear	p<0.05						p<0.05	p<0.05	p<0.05
CHX	p<0.05	p<0.05		p<0.05			p<0.05	p<0.05	p<0.05
Citric acid	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	p>0.05	p<0.05
MTAD	p<0.05							p<0.05	p<0.05
Ozonated water									p<0.05
SmearClear									

Table 3.10. Significance of difference between the mean values (Table 3.7) in smear layer scores for the apical level of the root canals.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
3,5% NaOCI								p<0.05	p<0.05
EDTA 18%	p<0.05			p<0.05			p<0.05	p<0.05	p<0.05
Sterilox	p>0.05	p<0.05		p<0.05	p<0.05		p<0.05	p=0.05	p<0.05
TopClear	p<0.05						p<0.05	p<0.05	p<0.05
CHX	p=0.05	p<0.05		p<0.05			p<0.05	p<0.05	p<0.05
Citric acid	p=0.05	p<0.05	p<0.05	p<0.05	p=0.05		p<0.05	P<0.05	p<0.05
MTAD	p<0.05							p<0.05	p<0.05
Ozonated water									p<0.05
SmearClear									

Table 3.11. Significance of difference between the mean values (Table 3.7) in smear layer scores for the apical, middle and apical levels of the root canals.

	Coronal-Middle	Coronal-Apical	Middle-Apical
3,5% NaOCl			
EDTA 18%			p<0.05
Sterilox	p<0.05	p<0.05	p<0.05
TopClear	p<0.05	p<0.05	p<0.05
CHX	p<0.05		
Citric acid			
MTAD		p<0.05	p<0.05
Ozonated water			
SmearClear			p<0.05

Table 3.12. Incidence of erosion of the peritubular dentine (expressed as a percentage of observed surfaces) (c= coronal level, m= middle level and a= apical level).

Group	A			B			C			D			E		
Irrigation regime	3.5% NaOCL			18% EDTA			Sterilox			TopClear			Chlorhexidine		
Specimen	C	M	A	C	M	A	C	M	A	C	M	A	C	M	A
1	0	0	0	0	0	0	0	0	0	50	10	0	0	0	0
2	0	0	0	0	0	0	0	0	0	60	15	0	0	0	0
3	0	0	0	0	0	0	0	0	0	55	10	0	0	0	0
4	0	0	0	0	0	0	0	0	0	55	10	0	0	0	0
5	0	0	0	0	0	0	0	0	0	70	20	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0	58	13	0	0	0	0
SD	0	0	0	0	0	0	0	0	0	7.5	4.4	0	0	0	0
Group	F			G			H			I					
Irrigation regime	10% Citric			MTAD			Ozonated water			SmearClear					
Specimen	C	M	A	C	M	A	C	M	A	C	M	A			
1	0	0	0	10	0	0	0	0	0	0	0	0			
2	0	0	0	15	5	0	0	0	0	5	0	0			
3	0	0	0	5	0	0	0	0	0	0	0	5			
4	0	0	0	10	0	0	0	0	0	5	0	0			
5	0	0	0	15	5	0	0	0	0	5	0	0			
Mean	0	0	0	11	2	0	0	0	0	3	0	1			
SD	0	0	0	4.1	2.7	0	0	0	0	2.7	0	2.2			

Table 3.13. Significance of difference between the mean values (Table 3.11) in erosion scores for the coronal level of the root canals.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
3,5% NaOCI									
EDTA 18%									
Sterilox									
TopClear							p<0.05		p<0.05
CHX									
Citric acid									
MTAD									
Ozonated water									
SmearClear							p<0.05		

Table 3.14. Significance of difference between the mean values (Table 3.11) in erosion scores for the middle level of the root canals.

	3,5% NaOCI	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
3,5% NaOCI									
EDTA 18%									
Sterilox									
TopClear							p<0.05		
CHX									
Citric acid									
MTAD									
Ozonated water									
SmearClear									

Table 3.15. Significance of difference between the mean values (Table 3.7) in erosion scores for the apical, middle and apical levels of the root canals.

	Coronal-Middle	Coronal-Apical	Middle-Apical
3,5% NaOCl			
EDTA 18%			
Sterilox			
TopClear		p<0.05	
CHX			
Citric acid			
MTAD			
Ozonated water			
SmearClear			

CHAPTER 4

Discussion

The intention of this *in vitro* study was to establish the antimicrobial efficacy and smear layer dissolving capability of the nine different irrigation solutions. A scanning electron microscope was used to assess the effectiveness of the various irrigants to remove the smear layer and the amount of erosion caused in the dentinal tubules.

Bacteria play the primary etiological role in the development of necrotic pulps and periapical disease following root canal treatment (227). One of the crucial factors for success of the treatment consists in the eradication of microorganisms and their by-products from the root canal system (36, 228, 229). Among the procedures involved in the control of endodontic infection, instrumentation and irrigation are essential factors in eliminating microorganisms from the root canal system (230, 231). However, mechanical debridement alone does not result in total or permanent reduction of bacteria (104). The use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the numbers of microorganisms (36, 104, 228).

Despite the controversy with regard to the effect of the smear layer on the quality of instrumentation and obturation, the smear layer itself may be infected and may protect bacteria already present in the dentinal tubules (232). Because of these concerns, one may deem it prudent to remove the smear layer in infected root canals and allow penetration of intracanal medicaments into the dentinal tubules of these teeth (213). In order to disinfect the root canal system, including the dentinal tubules, the disinfecting solution must be able to penetrate all components of the root canal system.

E. faecalis was chosen as the test organism in this study because it has been associated with persistent apical inflammation in clinical situations (37, 38).

Love (2001) investigated a possible mechanism that would explain how *E. faecalis* could survive and grow within dentinal tubules and reinfect canals (47). The author postulated that a virulence factor of *E. faecalis* in root-filled teeth with post-treatment disease may be related to the fact that *E. faecalis* cells maintain the capability to invade dentinal tubules and to adhere to collagen in the presence of human serum. It was also chosen because a recent study claimed that Biopure MTAD may not be effective against *E. faecalis* biofilms (183).

An ideal intracanal irrigant or medication should be able to disinfect the dentine and its tubules in one visit. In addition, it should have sustained antimicrobial effects after its use (213).

The most popular irrigating solution is sodium hypochlorite. It is an effective antimicrobial agent (104, 233) and an excellent organic solvent for vital, necrotic and fixated tissues (234). However, it should be noted that it is highly irritating to periapical tissues, especially in high concentrations (235, 236). In the present study the undiluted 3.5% NaOCl demonstrated excellent antimicrobial properties against *E. faecalis*. This is in agreement with findings of Harrison and Hand (1981) who has shown that NaOCl is an effective bacterial agent when it is used undiluted. However, when NaOCl solution was diluted, it was shown to be completely ineffective against *E. faecalis* (237).

The results of the SEM obtained for 3.5% NaOCl confirmed previous reports that NaOCl irrigation during instrumentation leaves the prepared canal wall entirely covered with a smear layer (238).

Some authors recommend the use of a chelating agent as an irrigation solution together with NaOCl (135). In the present study four different chelator solutions was tested : EDTA 18% Root Canal Irrigating Solution, TopClear Solution (mixture of 0.2% cetremide and 17% EDTA), SmearClear (mixture of 17% EDTA, cetrimide,

polyoxyethylene (10) iso-octylcyclohexyl ether) and Citric Acid 10% Root Canal Irrigating Solution.

Three of these solutions (EDTA 18%, TopClear and SmearClear) demonstrated antimicrobial properties against *E. faecalis* and removed most of the smear layer in the coronal part and modified the smear layer in the middle and apical parts of the root canals.

EDTA solutions have also been combined with a quaternary ammonium bromide (cetrimide) to reduce the surface tension and to increase penetrability of the solution (179). McComb and Smith (1975) reported that when this combination (REDTA, Roth International Ltd., Chicago, IL, USA) was used during instrumentation, there was no smear layer except in the apical part of the root canal (92).

TopClear and SmearClear that was used in the present study is a combination solution of EDTA and cetrimide. These solutions demonstrated similar antimicrobial properties against *E. faecalis* compared to the 18% EDTA solution in the undiluted form. It should be noted that SmearClear was the only EDTA containing solution that showed antimicrobial properties to *E. faecalis* after the solutions were diluted to a 1/10 dilution. The SmearClear results of the present study do conform to a recent study done by Dunavant *et al.*, (2006). The latter demonstrated significant antibacterial activity with a 78% decrease in bacterial numbers compared to a 27% decrease in bacterial numbers for an irrigating solution only containing 17% EDTA

(183). They contributed the antimicrobial activity of SmearClear to the addition of the surfactant cetrimide. Cationic surfactants have been reported to have bacteriocidal and fungicidal properties (239).

The 10% Citric acid solution failed to show any antimicrobial properties against *E. faecalis* or to remove the smear layer. The smear layer in some samples representing the coronal aspect of root canals was slightly modified, exposing only one or two dentinal tubules. According to Zehnder et. al., (2005) citric acid appears to be slightly more potent at similar concentrations than EDTA, and both agents show high efficiency in removing the smear layer (240). In addition to their cleansing ability, chelators may detach biofilms adhering to root canal walls (240). It must be noted that in this study only a low concentration of citric acid (10%) as compared to the average concentration of the EDTA -containing solutions was used.

Another group of antiseptic agents that can be added to citric acid irrigants to increase their antimicrobial capacity are tetracycline antibiotics (241). BioPure MTAD is an example of such a product. This endodontic irrigant contains 3% doxycycline hyclate, 4.25% citric acid and 0.5% polysorbate 80 detergent (241). BioPure MTAD represents an innovative approach in simultaneous removal of the smear layer and disinfection of root canals (241, 242). The results of the present study confirmed the antimicrobial properties against *E. faecalis* (undiluted and 1/10 dilution) and the ability to remove the smear layer effectively. This was the only irrigating solution that

removed the smear layer completely in all samples representing the coronal, middle and apical levels of the root canals.

Chlorhexidine is a potent antiseptic and its use in endodontics has been proposed both as irrigant and intracanal medicament (159, 160). The undiluted, 1/10 and 1/100 diluted solutions of 2% chlorhexidine solutions in the present study demonstrated some antimicrobial properties to *E. faecalis*. Despite its use as a root canal irrigant, it cannot be advocated as the main irrigant because chlorhexidine is unable to dissolve necrotic tissue remnants (243) and is also less effective on Gram-negative than on Gram-positive bacteria (244, 245). It must be cautioned here that many *ex vivo* studies use extracted bovine or human teeth uninfected with *E. faecalis*, a Gram-positive facultative species associated with failed root canal treatments (246). However, in primary endodontic infections, which are usually polymicrobial, Gram-negative anaerobes predominate (247). Enterococci are rarely encountered in primary endodontic infections (248).

The chlorhexidine irrigation solution removed the smear layer in the coronal region of the samples examined in this study. However, in the middle regions the smear layer was slightly modified, but in the apical region a thick smear layer was evident in all samples examined. Despite the poor effect on the smear layer of this irrigating solution, it is nevertheless advocated to be the most promising agent to be used as a final irrigant after the smear layer removal with EDTA (240). According to Röllä, Loe and Schoitt (1970) it has an affinity to dental hard tissues, and once bound to a

surface it has prolonged microbial activity (249), a phenomenon called substantivity (250). A final irrigation using chlorhexidine solutions appears advantageous, especially in re-treatment cases, where high proportions of Gram-positive bacteria are to be expected in the root canal system (240).

The undiluted as well the diluted ozonated water that was used in the present study failed to demonstrate any antimicrobial properties against *E. faecalis*. The ozonated water also had no visible effect on the smear layer.

The antimicrobial results of our study are in agreement with Hems *et al.*, (2005) who showed that biofilms incubated for 4 minutes with ozonated water showed no significant reduction in cell viability attributable to ozone alone, whereas no viable cells were detected with NaOCl over the same time period (219). However, the outcome of the present study, differ from the results obtained from a study by Nagayoshi *et al.*, (2004) (218). They observed that ozonated water had almost the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with ultrasonification (215). However, Hems *et al.*, (2005), evaluating the ability of ozone to kill an *E. faecalis* strain verified that its antibacterial efficacy was not comparable to that of NaOCl (219). These differences may be attributed to variations in the irrigant concentration and contact time (218).

Marais (2000) asserted in a preliminary report that the cleansing efficacy of electrochemically activated water (Steds, Radical Waters, Johannesburg, South

Africa), in root canals was considered to be superior to NaOCl (250). He showed that the ECA water removed the bacteria and smear layer in large areas of the root canals (198). However, Marais and Williams (2001) repeated this study and concluded that ECA did not demonstrate any antimicrobial effectiveness against *E. faecalis* (251). In the present study we could not identify any antimicrobial properties for Sterilox against *E. faecalis*. The SEM results for Sterilox were also disappointing, demonstrating a thick smear layer in the coronal, middle and apical levels of the root canals.

Examination of the surfaces of the coronal root canal walls irrigated with TopClear, BioPure MTAD and SmearClear showed erosion of the dentinal tubules. TopClear was very destructive (58% erosion) compared with BioPure MTAD (11% erosion) and SmearClear (3% erosion). Only TopClear (13% erosion) and BioPure MTAD (2% erosion) demonstrated a small amount of erosion in the middle root canal walls. These findings are in agreement with the results of other studies (185, 213) which reported a correlation between the erosive properties of chelating agents and the length of dentine exposure to the solution. Based on the results of these studies, it seems that chelating agents are destructive in the coronal and middle thirds of root canals if in contact with the root dentine for more than one minute. In the present study the root dentine was in contact with the TopClear and SmearClear solutions for two minutes and for five minutes with BioPure MTAD.

Finally, it should be noted that discrepancies in results among the published experiments and the present study are difficult to analyze due to the use of differing experimental bacterial strains, methods and materials.

CHAPTER 5

Conclusions

1. No significant inhibition of *E. faecalis* was observed with sterile water (control) and the undiluted solutions of Sterilox, 10% Citric acid and Ozonated water. However, 3.5% NaOCl, 18% EDTA, TopClear 17% EDTA, 2% Chlorhexidine, BioPure MTAD and SmearClear showed significant inhibition of *E. faecalis*.
2. For the 1/10 diluted solutions no inhibition of *E. faecalis* was observed with sterile water (control), 3.5% NaOCl, Sterilox, 10% Citric acid and Ozonated water. BioPure MTAD and SmearClear demonstrated significant inhibition of *E. faecalis* compared to 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine.
3. No significant inhibition of *E. faecalis* was observed with sterile water (control) and the 1/100 diluted solutions of 3.5% NaOCl, Sterilox, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, Ozonated water and SmearClear. The only solution that showed significant inhibition of *E. faecalis* was BioPure MTAD.
4. No inhibition of *E. faecalis* was observed with the 1/1000 diluted test solutions.

5. BioPure MTAD was the only irrigation solution that inhibited growth of *E. faecalis* in the undiluted, 1/10 diluted and 1/100 diluted solution.
6. SEM examination revealed that 3.5% NaOCl and Ozonated water had no visible effect on the smear layer.
7. SEM examination revealed that 10% Citric acid slightly modified the smear layer at the coronal and middle levels of the root canals. There was no visible effect on the smear layer at the apical levels.
8. SEM examination revealed that Sterilox and 2% Chlorhexidine removed the smear at the coronal levels, modified it slightly in the middle levels and left the smear layer undisturbed in the apical levels of the root canals.
9. SEM examination revealed that 18% EDTA, TopClear 17% EDTA, BioPure MTAD and SmearClear removed the smear layer completely at the coronal levels. At the middle and apical levels of the root canals most of the smear layer was removed. However, there were less open dentinal tubules visible at the apical levels compared to the samples examined at the middle levels of the root canals.
10. SEM examination revealed that TopClear 17% EDTA caused a significantly high percentage of erosion of the peritubular dentine at the coronal levels of the root canals compared to all the other irrigation

solutions. However, BioPure MTAD demonstrated a very low percentage of erosion at the coronal levels of the root canals.

11. Considering all the results of the present study BioPure MTAD demonstrated the best antimicrobial activity against *E. faecalis*, and removed most of the smear layer at all three levels of the root canal systems without significant erosion of the peritubular dentine.

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ADDENDUM A

Pro Forma – Patient information leaflet and informed consent.