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Ciclo XXIII

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Maximizing disinfection procedures in endodontics

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Introduction

We are living in the age of evidence-based medicine. Any new concept and technique to be used on patients should ideally be assessed in randomized controlled clinical trials against their respective gold standards. This, however, poses a major problem particularly in endodontic research. A favourable outcome of root canal treatment is defined as the reduction of a radiographic lesion and the absence of clinical symptoms of the affected tooth after a minimal observation period of 1 yr. (1). Alternatively, the so called surrogate outcome (dependent) variables yielding quicker results, such as the microbial load remaining in the root canal system after different treatment protocols, can be defined. However, these do not necessarily correlate with the "true" treatment outcome (2). Endodontic success is dependent on multiple factors (3), and a faulty treatment step can thus be compensated. For instance if cultivable microbiota remain after improper canal disinfection, they can theoretically be entombed in the canal system by a perfect root canal filling (4), and clinical success may still be achieved (5). On the other hand, in a methodologically sound clinical trial, single treatment steps have to be randomized and related to outcome. Otherwise, the results do not allow any conclusions and no causative relationships may be revealed (6).

The above issues may be viewed as the reason (or as an excuse) for the fact that no randomized controlled clinical trials exist on the effect of irrigating solutions on treatment outcome in the endodontic literature. As of yet, we largely depend on data from in vitro studies and clinical trials with microbial recovery after treatment as the surrogate outcome. Clinical recommendations based on such findings are merely deductive and need to be interpreted with care.

Chapter 1

1.1Root Canal Infection

A traditional concept that explains infectious processes occurring in humans suggests that diseases are produced as the result of the aggressive invasion of harmful microorganisms, which battle with the human host's defences, triggering mechanisms that release antibodies and immune cells. The impact of such an approach generates a predisposition to search for those "most dangerous" microorganisms that can cause/ trigger the most severe damage to the host. In line with this view, infectious processes of the oral cavity were proposed to be caused by a relatively small number of organisms from the diverse collection of species found in the human mouth (7). In caries, for example, the frequent isolation of *Streptococcus mutans* from carious lesions (8–12) generated a considerable number of studies to explore the ex vivo features of this bacterium. Research findings showing the significant acid-tolerant capabilities of *S. mutans* defined this organism as "the" agent responsible for initial enamel and dentine demineralization. Similarly, in periodontal disease, the frequent recovery of proteolytic microorganisms from deep periodontal pockets, such as *Porphyromonas gingivalis*, increased the attention of periodontists to these bacteria because they were considered key etiological agents of the disease (11,12). The main disadvantage with this traditional view of the infectious process, especially in oral infections, is that the determination of true cause-and-effect relationships is not always possible. Consequently, the predominance of certain microorganisms at a given site may be the result of the disease itself rather than that of the initiating agent (13). Recently, the "ecological plaque hypothesis" (14–19) has improved on these classic infectious concepts to explain the aetiology of caries and periodontal disease. This hypothesis suggests that the organisms associated with the disease may also be present at sound sites, but at levels too low to represent a clinical threat. In other words, disease is produced as the result of changes in the local environmental conditions that will shift the balance of the resident flora.

Root canal infections have a different nature than that of caries or periodontitis because they become established in originally sterile compartments of the oral cavity. In many cases, this led to the concept that the aetiology of root canal infections involves only a single pathogen. For example, the predominance of certain proteolytic black-pigmented anaerobic organisms in cultures from infected root canals associated with acute symptoms suggested that these organisms are foremost etiological agents in such cases (20,21). Recently, the frequent recovery of Enterococcus faecalis in root canals associated with persistent infections brought

about an intense research interest in this bacterium. E. faecalis has become the ideal organism to test different irrigants, medicaments, and antiseptic solutions used in endodontics ex vivo, with findings that revealed its innate resistance capacity (22–24). This extensive interest in E. faecalis, perhaps driven by its ability to grow under almost any laboratory condition (25), resulted in the concept that the organism is the sole etiological agent for chronic endodontic infections. Consequently, the focus on E. faecalis resulted in much less information on the existence of other organisms in such infections that may possess similar tolerating characteristics to E. faecalis and that would shed light on the existence of a polymicrobial persisting community. Thus, it is not surprising that ecological parameters in root canal infections are not often discussed.

From an ecological perspective, the root canal can be considered a highly controlled environment with a limited number of niches. Although niches are composed by a variety of environmental factors that limit the growth of one species relative to others (26), the main limiting factors in root canal niches that influence bacterial colonization are, for instance, oxygen and nutrient availability (27). After root canal treatment, other limiting factors become involved, such as pH and the short/long-term effects of the antibacterial medicaments applied. Bacterial survival in such controlled environments, especially after root canal treatment, is based on the capacity of organisms to adapt to the existing conditions.

Although traditional views suggest that the organisms surviving root canal treatment are a selected group of the “most robust” organisms, the application of ecological parameters indicates that bacterial survival after root canal treatment will depend not on the robustness of the organisms, but on how good an adaptor the organism is to the new limiting factors in their corresponding niches. Furthermore, as in every natural microenvironment, the adaptive capabilities of individual organisms are exponentially augmented when growing in biofilm communities. The foundation for this ecological approach to endodontic infections suggests that the most dangerous “pathogen” is not an individual species, but a polymicrobial entity that undergoes physiological and genetic changes triggered by changes in the root canal environment.

Currently, there is no substantial evidence indicating that certain microorganisms of the microbial flora in root canal infections are more virulent than others. With this in mind, Sundqvist and Figdor (28) stated that a proper definition for endodontic pathogens should include every organism capable of inducing the tissue destruction in apical periodontitis. In reality, however, the majority of endodontic-microbiology studies refer to the endodontic

pathogen as the bacterium isolated from a symptom-associated root canal that grows in the laboratory in a specific media. By this approach, the most frequently recovered species will assume the role of major endodontic pathogen. In persistent root canal infections, for example, the frequent occurrence of monocultures of E. faecalis has raised suspicion that this bacterium may be the sole organism persisting in the root canals. Considering that mono-infections rarely if ever occur in nature, it is possible that the apparent pure cultures of E. faecalis could be the result of sampling and culturing techniques that favour it over other organisms at the site that were either in low numbers or were physiologically inactive or dormant. For instance, in a commonly cited study (29), from the total 100 root- filled teeth with apical periodontitis sampled E. faecalis was reported as the most frequently recovered organism (32%), although in 32% of the cases with persistent lesion no microbe could be isolated. In yet nine root-filled teeth without periapical lesion that showed bacterial growth, the organism was found in one case. In a similar study, 25 root-filled teeth requiring retreatment were sampled and E. faecalis was found in 14 of those 20 teeth with bacterial growth (30). However, it would seem that this study was focused primarily in proving the occurrence of E. faecalis in root-filled teeth rather than in exploring the microbial flora in persisting infections. Similarly, in a recent study using a sophisticated nested PCR technique, the target bacterium E. faecalis was found in 41 of 50 (82%) untreated root canals and in 38 of 50 (76%) treatment failure associated root canals (31). As in other related works (32– 35), PCR methodology seems to be exclusively directed to find only E. faecalis, ignoring the rest of the flora present that may be as important as E. faecalis in provoking the treatment failures.

On the other hand, recent investigations have confirmed the polymicrobial nature of root canal infections (36, 57). In a study with monkeys (36), different combinations of bacteria were experimentally inoculated in root canals and periapical lesions were induced. The teeth were treated endodontically and followed-up radiographically and histologically for 2 to 2.5 years. In the root canals with bacteria present when the root filling was removed, 30 of the 31 canals had persisting periapical lesions. Importantly, more of these non-healed lesions were associated with various combinations of bacterial strains, that is, mixed infections, than single strains. Previously, the same research group (38) also found that when an “eight-strain collection” of species, derived from one infected root canal, was re-inoculated in equal proportions into other monkey teeth, species such as Bacteroides oralis (now Prevotella oralis) dominated in mixed infections and showed a more potent capacity for tissue destruction. Furthermore, B. oralis could not be reisolated from inoculated root canals after the experimental period when inoculated as a pure culture. In another study using the tissue

cage model implanted subcutaneously in the backs of rabbits, the same col- lection of eight bacterial strains from monkey root canals were inoculated in different combinations and individual species. The combination of B. oralis, Fusobacterium necrophorum, Peptostreptococcus anaerobius, and Streptococcus milleri was the most predominant and induced higher titers of circulating antibodies than that obtained with individual inoculations, such as E. faecalis (39).

Even if we accept the polymicrobial nature of root canal infections, one of the major problems in understanding endodontic infections is that we still extrapolate between individual organisms growing in liquid (planktonic) cultures and the in vivo situation. A significant literature now exists demonstrating that the physiology of a bacterium in planktonic culture is profoundly different from that of the same organism growing on a surface in a biofilm [see review by Costerton et al. (40)]. For instance, planktonic bacteria are more sensitive to antimicrobial agents because of their ease of diffusion within the bulk fluid, whereas biofilm bacteria are notably resistant to these agents (41–45). In this context, the study of biofilms in root canal infections has included biofilms formed by mixed cultures of anaerobic bacteria in extracted teeth (46, 47) or by pure cultures of E. faecalis (48, 49). Biofilms of five root canal isolates have also been used to test the antimicrobial efficacy of endodontic irrigants, such as sodium hypochlorite (NaOCl) (2.25%), 0.2% chlorhexidine, 10% povidone iodine, and 5 ppm colloidal silver, with NaOCl shown to be the most effective agent of this group (50). In addition, Chavez et al. tested the alkaline tolerance of species isolated from chronically infected root canals and found that E. faecalis and other Gram-positive organisms, such as Lactobacillus paracasei, Olsenella uli, or Streptococcus gordonii, shared similarly high alkaline-tolerant capabilities when growing in planktonic conditions. S. anginosus, S. oralis, and F. nucleatum, on the other hand, were greatly affected by the alkaline stress (see Fig. 1) (51). Of importance, however, was the observation that this difference in alkaline tolerance was not apparent when the strains were tested in biofilms because all seven strains showed a similar high tolerance to alkaline pH (Fig. 1). These findings not only show the capacity of root canal bacteria other than E. faecalis to adapt to alkaline stress, but also provide further evidence that bacteria in surface-adhered biofilm consortia are more resistant to environmental stress than when grown in liquid culture.

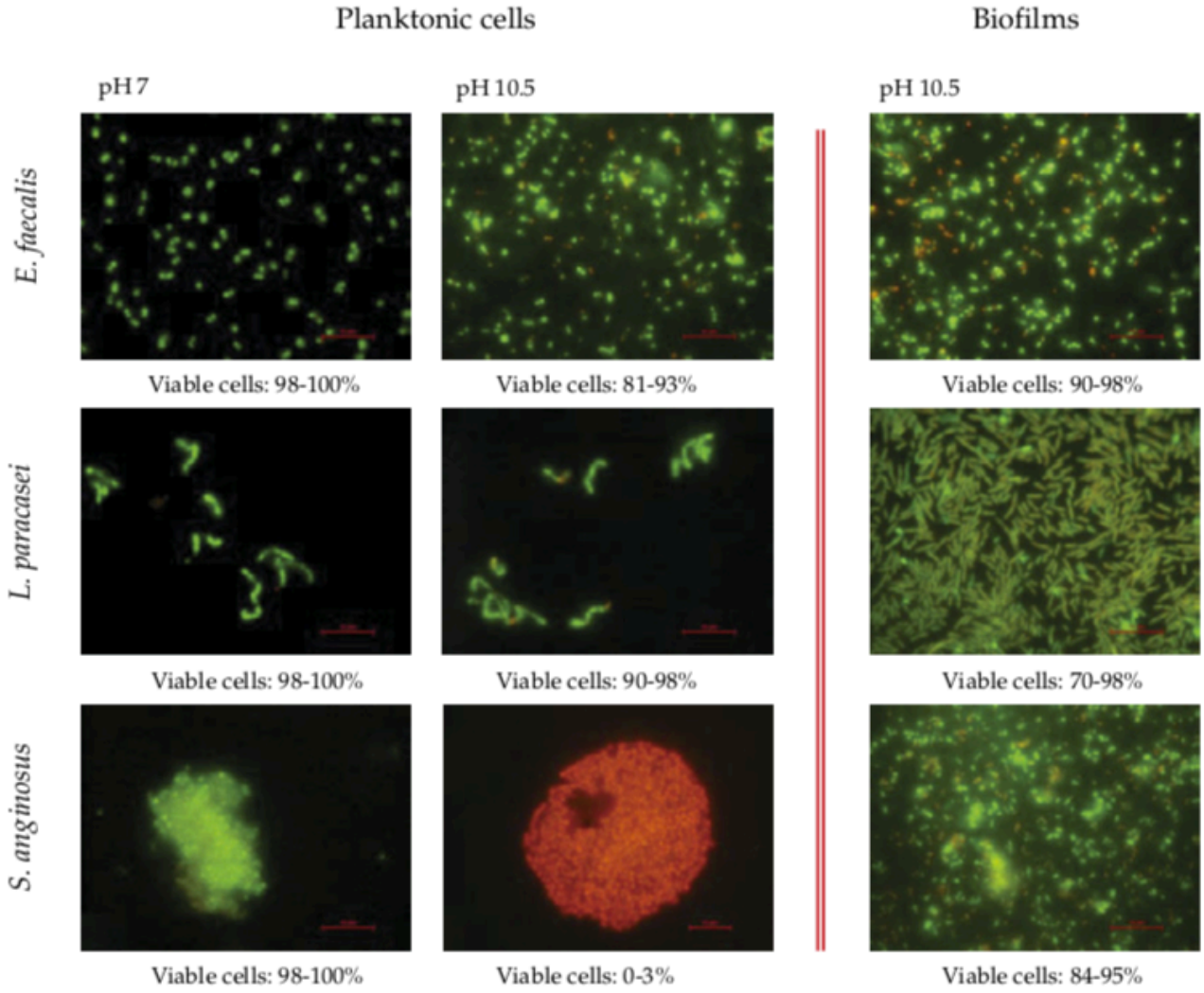

Figure 1. Fluorescence micrographs using Live/Dead fluorescence staining for bacterial viability. Cells stained fluorescent green represent viable cells, whereas cells stained fluorescent red are nonviable or damaged. In the first column, images show planktonic cells of three root canal strains at neutral media (pH 7). The middle column shows planktonic cells after exposure to pH 10.5 for 4 hours, and the right column shows biofilm cells exposed to alkaline challenge (pH 10.5) for 4 hours. Bars, 2 m. Images are published with permission of Blackwell Publishing. International Endodontic Journal, Chávez de Paz et al. (65)

As the host defense loses its access to the necrotic pulp space, opportunistic microorganisms selected by harsh ecological conditions and the low-oxygen environment aggregate in the root canal system (52). These microbial communities may survive on organic pulp tissue remnants and exudate from the periodontium (53, 54). Consequently, clusters of microorganisms in

necrotic teeth and teeth with failed root canal treatments are typically found in the apical root canal area, where they have access to tissue fluid (52). In long-standing infections, root canal bacteria can invade the adjacent dentin via open dentinal tubules (55, 56).

Primary root canal infections are polymicrobial, typically domi- nated by obligately anaerobic bacteria (53). The most frequently isolated microorganisms before root canal treatment include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, Lactobacillus species and Gram-positive facultative Streptococcus species (53). The obligate anaerobes are rather easily eradicated during root canal treatment. On the other hand, facultative bacteria such as nonmutans Streptococci, Enterococci, and Lactobacilli, once established, are more likely to survive chemomechanical instrumentation and root canal medication (57). In particular Enterococcus faecalis has gained attention in the endodontic literature, as it can frequently be isolated from root canals in cases of failed root canal treatments (58, 59). In addition, yeasts may also be found in root canals associated with therapy-resistant apical periodontitis (60).

It is likely that all of the microorganisms able to colonize the necrotic root canal system cause periapical inflammatory lesions. Enterococci can survive in monoculture, but cause only minor lesions (38). Certain Gram-negative taxa appear to be more virulent (53). The outer membrane of Gram-negative bacteria contains endotoxin, which is present in all necrotic teeth with periapical lesions (61), and is able to trigger an inflammatory response even in the absence of viable bacteria (62). Furthermore, the levels of endotoxin in necrotic root canals are positively correlated to clinical symptoms such as spontaneous pain and tenderness to percussion (63). Virulent Gram-negative anaerobic rods depend on the presence of other bacteria in their environment to survive and establish their full pathogenic potential (38). Such aggregations of microorganisms in an extracellular polysaccharide matrix associated with a surface (in our case the inner root canal wall) are called biofilms (64). There is convincing evidence that microorganisms organized in this manner are far less susceptible to antimicrobial agents than their planktonic counterparts, which have traditionally been used to test the antimicrobial efficacy of substances in vitro (65, 66). If a bacterially inoculated broth is confronted with an antimicrobial fluid, the efficacy of that agent can appear to be very convincing, similar as with agar-diffusion tests. However, in the root canal system biofilms and infected dentinal tubules make disinfection much more difficult and thus study models such as standardized infected bovine dentin blocks (67) or in vivo models appear to be more valid than the above mentioned study designs. Furthermore, it has been shown that organic

and inorganic dentin components, which are suspended in the irrigant during chemomechanical instrumentation, inhibit most antimicrobial agents (68, 69).

In conclusion, the biofilm concept and the specific conditions in the pulpless root canal microniche cannot be overestimated when considering the actions of different irrigating solutions.

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1.2 ROOT CANAL INSTRUMENTATION

The objectives of mechanical preparation are two-fold:

1. To facilitate irrigation Conventional radiography does not enlighten the clinician about the true complexity of the root canal system. Lateral canals, fins, anastomoses and ramifications are invariably present, with some canals being joined by narrow isthmi. The main canal is rarely round, but often oval, ribbon- like or even 'C'-shaped, depending upon the tooth. One seminal study has demonstrated up to 53% of the canal will remain unreached by instrumentation following preparation (1). Therefore, mechanical preparation facilitates penetration of irrigants into these complex anatomical spaces. Although some dentine-containing micro-organisms will be removed during mechanical preparation, research suggests that a considerable amount of the canal will not be contacted by a file, therefore irrigants play a crucial role in destroying micro-organisms, neutralizing endotoxin and removing organic tissue components (2).

2. To facilitate obturation as cleaning and shaping does not remove all micro-organisms from the canal, obturation aims to entomb any residual pathogens and limit recolonization by preventing the passage of nutrients from both coronal and apical aspects. Mechanical preparation facilitates obturation. Schilder's principles of canal preparation still hold true today (3). The idea of creating a continuously tapering preparation, free from mechanical errors, allows the best chance of a well-condensed obturation, with the absence of voids.

1.2.1 The crown down approach

The majority of micro-organisms are in the coronal portion of the canal and pulp chamber (4). Thus, whatever instruments are used, a crown down approach and only initial scouting of the canal prior to working length determination is sensible. This technique involves shaping the canal from the coronal aspect first and progressively working more apically with smaller diameter instruments (5) (Figure 2).

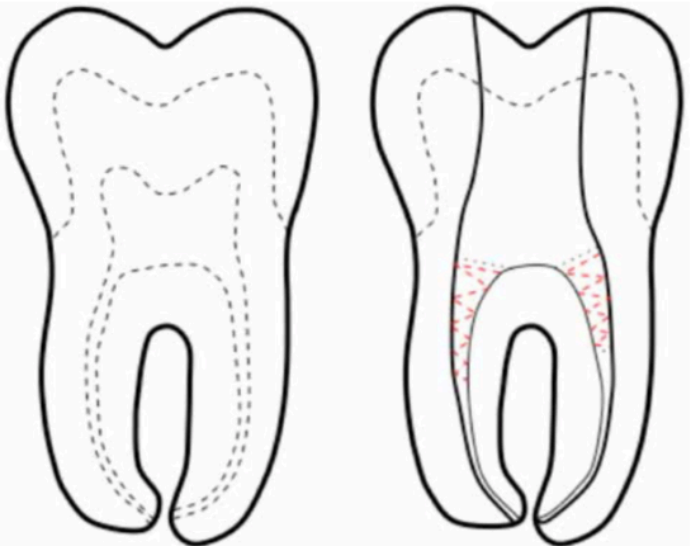

Figure 2. The crown down approach: the coronal third of the canal system is enlarged using GG or orifice-shaping files. The enlargement is directed away from the furcation and has the simultaneous benefit of removing dentine overhanging the orifices to allow optimal straight-line access.

Such an approach:

- Minimizes the transportation of pathogens further into the canal system;
- Allows a greater amount of irrigant to be held in the canal, facilitating debris removal and disinfection;
- Removes coronal curvatures and facilitates straight-line access;
- Improves accuracy of working length determination as reduction of curvature after working length determination may alter the working length and result in a tendency to transport the canal and over-enlarge the apical foramen;
- Reduces file binding in the coronal portion of the canal, facilitating working length assessment and further reducing the risk of instrument separation through torsional failure.

Traditionally, Gates Glidden (GG) instruments would be used for the crown down procedure but many rotary filling systems now have orifice shapers to begin the preparation. If clinicians elect to use GGs it is wise to remember a Size 6 GG has an apical diameter of 1.5 mm (ISO 150), with sizes stepping down in 0.2 mm increments to a Size 1 GG at 0.50 mm (ISO 50). As such, even the smallest of GGs can be very destructive if used carelessly. Avoid using sizes above GG 3 (0.90 mm: ISO 90). Whatever instruments are used, caution must be taken with regard to the furcation region, the instruments being used away from the furcation (anti-curvature

filing) (6). Despite the aforementioned advantages, it is easier to create blockages and ledges with an aggressive or careless crown down approach, thus highlighting the importance of recapitulation.

1.2.2 Working length determination

The apical extent of preparation should be kept within the canal system: over extension can reduce success up to 62% and, for every mm short of the apex, underextension reduces success by 12% (7). Methods used to estimate the maximum working length for instrumentation include apical gauging by tactile sensation, instrumentation without local anaesthetic, using pre-operative radiographs alone, the paper point technique, working length radiographs (WLRs) with files *in situ* and, most recently, the use of electronic apex locators (EALs). Historically, the most widely accepted method is by placing a file to the estimated length, then taking a confirmatory radiograph, but the radiographic apex rarely corresponds with the anatomical apex (8) It follows that WLRs can only give an estimation of the correct termination of preparation.

Modern impedance-based multifrequency EALs are reliable and accurate >90% of the time (9) These devices are only accurate at a ZERO reading. Any reading given other than ZERO should not be used as a marker of apical extent. The ZERO reading is reached when the file contacts the periodontal ligament. Thus, by definition this is over extended and, to calculate the working length, one must subtract 0.5 mm from the ZERO reading length (10). For more information readers are referred to other papers on the subject of EALs (11,12).

The 2013 Faculty of General Dental Practitioners Selection Criteria for Dental Radiography states '*Unless there is confidence about working length(s) derived from an electronic apex locator, at least one good-quality radiograph is necessary to confirm working length(s)*' (13). From this one could extrapolate that WLRs are no longer always necessary. We recommend that a combination of techniques is used.

1.2.3 Size of apical preparation

There is equivocal evidence regarding the effect of the size of apical preparation on the success of endodontic treatment (14,15) Smaller apical preparation has the advantage of minimizing the risk of transportation and extrusion of debris and irrigant. Conversely, a more aggressive apical preparation will remove more infected dentine and allow greater access to irrigants but may increase the risk of perforation and extrusion of debris and irrigants. Traditional teaching advocated using a master apical file which was three sizes larger than the first file to bind (16) Subsequent work has shown this method to be inaccurate (17). In addition, most apical foramina are not round but ovoid in shape and it is questionable whether infected dentine needs to be removed as appropriate irrigation penetrates dentine and kills micro-organisms (18). A modern approach to apical enlargement focuses on irrigation. Irrigant must reach the apical 1 mm of the canal (19). Evidence suggests that irrigants do not flow greater than 1–2 mm past the syringe tip. Ideally, the irrigating syringe tip must be placed within 1–2 mm of the apex (20). A conventional 30 gauge needle corresponds to the tip of an ISO 30 file, therefore an apical preparation smaller than this may result in the inability to place the needle tip within the apical 2 mm and thus there may be inadequate irrigation in this area. We suggest that an apical preparation of 0.25–0.30 mm (ISO 25–30) should be considered a good target. In addition, it has been demonstrated that larger taper preparations enhance cleaning and irrigation and subsequently reduce bacterial load (21). One study has shown only modest increases in irrigation with taper increases beyond 0.04 (22). The clinician must therefore be aware that increasing taper carelessly may also increase the risk of excessive tooth structure removal and perforation without added benefit. If canals are sclerosed or very curved such large preparation may not be possible.

1.2.4 Preparation techniques

New endodontic instrumentation systems are being continually introduced on to the market, allowing clinicians to complete endodontic treatment with simpler protocols, faster. Accordingly, there has been a paradigm shift towards nickel titanium rotary file systems. Nonetheless the clinician must understand the importance of hand filing: the clinician that cannot hand file is handicapped in the ‘art of endodontics’.

1.2.5 Hand file instrumentation

Hand files afford the clinician greater tactile feedback than rotary instruments and are often invaluable in determining the direction and magnitude of curvatures and canal configurations. There are two main types of files: Hedstrom and K files. The former are machined stainless steel cylinders that cut aggressively. The latter are twisted stainless steel that are more flexible and less aggressive. The cross-section varies depending on the type of file. All have 16 mm fluted portions and follow ISO dimensions. New instruments are available in nickel titanium. These instruments are flexible and potentially safer but cannot be pre-curved and negate some of the benefit of hand filing in the early stages, especially in curved canals.

Shaping the canal with hand files can be undertaken in numerous ways, depending upon the canal anatomy. Techniques for total canal preparation with hand files includes 'step-back', 'crown-down', 'double flare' and 'anticurvature filing' (5,6,23,24). Techniques for manipulation of the files during preparation include circumferential filing, 'balanced force' (25), watchwinding and push-pull. Thus the former describe the strategy and the latter describes the method of achieving that. 'Step-back' and 'double flare' techniques both involve determining the working length and choosing a master apical file size, then using progressively larger files at shorter lengths in order to create a continuous taper. Stainless steel hand files are all standard 2% ISO taper. The operator can choose the degree of taper created by adjusting the lengths to which progressively larger files are inserted. Traditional step back, using increments of 1 mm creates a canal with a 5% taper. If the clinician wishes to develop a larger taper, then reducing the increments to 0.5 mm will result in a canal with a 10% taper. One common pitfall with both these techniques is under preparation of the middle third of the canal. This poses problems when obturating using cold lateral compaction techniques, as accessory points cannot penetrate past the coronal third, resulting in an obturation which resembles an 'inverted wine bottle'.

The 'balanced force' technique involves turning the file clockwise up to 90° followed by an anti-clockwise movement of 180° or more whilst maintaining apical pressure (25). The first movement engages the dentine, whilst the second movement releases and cuts the canal wall. This permits predictable, centred dentine removal. Though 'balanced force' may be used in all canals, it is an especially effective and safe technique for hand filing curved canals. Circumferential and push-pull filing techniques are more suitable for straight, wide canals, C-shaped or ovoid canals: the walls of the canal are reamed with an oscillating apico-coronal

movement. As a rule, the use of stainless steel endodontic instruments should be avoided in rotary hand-pieces as they can be aggressive and are prone to breakage.
Stainless steel files may be pre-curved to the estimated shape of the canal, preferably with a designated instrument to avoid contamination. It is useful to indicate the direction of the curve by marking it with the pointer on the rubber stop. After using each successive file, always irrigate and recapitulate with a fine file, such as #10, to disrupt and to agitate the plug of 'dentine mud' which builds up apically which can result in loss of working length.

1.2.6 Patency filing

Patency filing is the process of placing an ISO 10 file (or smaller) 0.5 mm passively beyond the apex (26). It is imperative that the file is not excessively rotated, as this can enlarge the apical foramen. This removes dentine plugs that can be compacted in the apical region. These can harbour bacteria and may result in deviation of the instrument tip if not cleared. Ensuring patency of canals improves the success of RCT7.

1.2.7 The era of nickel titanium

The most notable development in endodontics in the last 25 years is the introduction of nickel titanium (NiTi) instruments (27) This alloy, composed of 55% nickel and 45% titanium has several properties which are desirable for endodontics; most notably, NiTi has super elasticity and shape memory. This helps to keep the file centred in the canal and reduces the risk of procedural errors. Although NiTi instruments are commonly associated with rotary techniques, many manufacturers also produce hand file versions of their rotary systems, which are designed to be used in the same sequence. The super elasticity of nickel titanium does, however, prevent these files being pre-curved. Recent advances in material technology now afford greater flexibility and cyclic fatigue resistance (28) These include *M-wire* (Dentsply, Tulsa) and *HyFlex CM* or *Controlled Memory* (Coltene/Whaledent, Germany). *M-wire* is now used in the production of single file systems (see below). *HyFlex CM* instruments can also be pre-bent, reducing the risk of ledging, transportation or perforation. This may potentially revolutionize nickel- titanium technology.

1.2.8 Rotary file systems

Since the introduction of nickel titanium it has been possible to prepare root canals using a motor safely and predictably. Rotary instrumentation increases cutting efficiency. Although speed reducing motor hand-pieces can be coupled to existing units, the use of dedicated electric endodontic motors is recommended. The torque and speed can be adjusted to match the instrument manufacturers' specifications precisely and many have auto reverse to prevent files binding in the canal and exceeding the torque limit. Rotary files usually create preparations of greater taper than the conventional ISO 2%, with some systems exhibiting variable taper throughout the length of the file.

Although most practitioners will be familiar with the manufacturers' protocol for such instruments, Table 1 offers a list of guidelines relevant to all using rotary instrumentation (29).

■ Create straight line access to the coronal or middle third of the root before using a hand or rotary instrument
■ Create a glide path up to a #20 hand file to the apex before using hand or rotary instruments in that part of the canal
■ Fine files frequently: after 3–4 pecks of a rotary instrument, remove, irrigate and recapitulate with fine files
■ Thoroughly clean the flutes of the instrument after removal from the canal
■ Maintain patency throughout by taking a small (ISO 8 or 10) file 0.5 mm beyond the working length
■ Never force a rotary instrument. If resistance is met; stop, increase the amount of coronal flaring, irrigate and recapitulate
■ If challenging anatomy is present, always prepare the canal with hand files before introducing rotary instruments
■ Don't try to bypass ledges with rotary instruments; always use hand files for this
■ Prepare sufficient coronal flaring to ensure that the minimum amount of the file is contacting the canal walls
■ Always introduce the file into the canal whilst it is rotating. Do not stop and start the motor once the file is in the canal
■ Make sure that you have an accurate working length before using rotary files in the apical area. Aggressive enlargement and transportation can occur if a rotary file goes beyond the apex
■ Any time saved in preparation should be used in irrigation

Table 1. Tips for using rotary NiTi file systems modified from the AAEs Guidelines.

Most manufacturers would recommend the use of a 'glide path' to ensure safe and efficient passage of the instruments to full working length. By taking an ISO 20 hand file to the length to which a NiTi instrument is to go will significantly reduce the risk of instrument fracture, as covered below. There are ranges of NiTi instruments that are advocated for developing a glide path (eg *Pathfile* (Dentsply, Tulsa, USA). The manufacturers indicate these for use in sclerosed or difficult to negotiate canals. These should be used at slow speeds and with caution. It remains sensible to create a glide path with hand instruments first. The finer details of file design and shape will not be covered in this paper but the clinician should be aware that

many of the properties of an instrument are not simply governed by the material but the shape of the instrument. It is important to know the cutting efficiency, the taper size, and the instrument diameters at the tip.

- Although rotary NiTi file systems can be advantageous for preserving the original canal anatomy, they have limitations: When straight files are placed into curved roots the instrument can straighten the canal, resulting in a 'zip' apically where the apex is expanded. This is virtually impossible to fill. Rotary instruments should not be left rotating for more than 3–4 pecks of the apex to prevent such zipping and the ensuing difficulties this presents for obturation.
- Rotary preparations are circular, thus they are less useful in ribbon and 'C'-shaped canals, which are better prepared with hand files using circumferential techniques.
- Rotary files have a propensity to separate by two mechanisms (30) First, torsional failure can occur by the file continuing to rotate whilst one part of it is bound against the canal. Secondly, continuous rotation of the file in a curved canal can result in cyclical failure. The move to single use instruments reduces the risk of instrument separation but this will never mitigate the risks of poor technique. Always inspect the tips of instruments during use: if the threads are unwinding there is a risk of separation, so discard them. Nonetheless, NiTi rotary instrumentation is safe and effective if care is taken and manufacturer's instructions are followed (31).

1.2.9 Reciprocating systems

Reciprocation involves the file rotating in both anti-clockwise and clockwise directions: essentially a form of mechanized 'balanced force'. The anti-clockwise movement engages dentine following which the clockwise turn releases the file from the canal before re-engaging the canal wall, shearing dentine and creating the preparation. The reciprocating motion and single file system has several important benefits:

- Decreased risk of cyclical failure as the files are rotating at a lower RPM;
- Decreased risk of torsional failure as the filing motion repeatedly disengages the dentine, thus preventing binding and instrument fracture;
- More cost-effective endodontic treatment as the current reciprocating systems are 'single file'.
- A simplified protocol with only three choices of instrument for small, regular or large

canals.

Currently, there are two systems on the market, *Wave One* (Dentsply- Maillefer, Ballaigues, Switzerland) and *Reciproc* (VDW, Munich, Germany). *Wave One* utilizes an 170°:50° anti-clockwise: clockwise movement and *Reciproc* 150°:30°. This means that it will take three reciprocating movements for both file systems to rotate 360°. Although marketed as a single file system, the ecommended protocol for *Wave One* still involves the initial use of hand files (32). The manufacturers of *Reciproc* advocate that production of a glide path with hand files is not required in most cases (33). It remains good practice to establish a glide path with 0.20 ISO files before any NiTi instrument is used to working length. These instruments surpass conventional rotary instruments in resisting cyclical and torsion fatigue and, although similar in concept, *Wave One* has greater resistance to torsional fatigue than *Reciproc* and *Reciproc* has greater resistance to cyclical fatigue than *Wave One* (34). This means that *Reciproc* is more suited to curved canals and *Wave One* to narrow or sclerosed canals.

1.2.10 Which system is best?

The method of instrumentation used (hand or rotary) does not appear to influence success rates (7), although one study found better success rates with rotary instruments amongst general practitioners (39). Although manufacturers are becoming more aware of the importance of robust supporting evidence, clinicians must not be duped by the marketing and should research the systems independently, if possible. We recommend practitioners remain open-minded about using differing systems using extracted teeth to trial new filing systems. Finally, always remember the mantra ‘files shape and irrigants clean’: no system of instrumentation renders the canal bacteria free (40,41) Irrigation is the key to success in endodontics and will be discussed in the next chapter.

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Chapter 2

IRRIGATION

During endodontic treatment mechanical debridement alone will not rid the root canals of bacteria (1) regardless of whether this is done by hand files or rotary instruments (2). First, instruments do not access the complex shape of the root canal system (3-6). Secondly, within these inaccessible regions complex biofilms can develop that are not easily disrupted. Thirdly, instrumentation creates a smear layer that further prevents decontamination of the canal surface dentine and prevents a good adaptation of the obturation material to the canal wall. A sound irrigation regimen can help to deliver antimicrobials to these inaccessible areas of the root canal system, penetrate and remove biofilm and smear layer and even penetrate the dentine.

2.1 Type of irrigant

A recent Cochrane Systematic Review showed no difference between different endodontic irrigants (7). However, these results should be interpretedwith caution. A 'no difference' result is a reflection of the paucity of well-conducted clinical studies rather than taking as fact that no difference exists. The irrigant has several primary goals: dissolution of organic tissue and pulpal remnants, be they vital or necrotic, dissolution of select inorganic components, killing of micro-organisms and neutralization of endotoxin.

Many different irrigants and combinations of irrigants have been used in RCT to achieve these goals. These include:

- Sodium hypochlorite;
- Chlorhexidine;
- Sterilox;
- EDTA;
- Iodine potassium iodide;
- Hydrogen peroxide;
- Local anaesthetic, saline and/or water;
- Mixtures of irrigants (QMIX®).

See Table 1 for a summary of their differing properties (8). When used alone, very few irrigants offer a complete spectrum of ideal properties.

	TYPE	ACTION ON FLORA	TISSUE DISSOLUTION	ENDOTOXIN DEACTIVATION	INORGANIC SMEAR LAYER	SUBSTANTIVITY	TOXIC?	ALLERGENIC?	COST
SODIUM HYPOCHLORITE >1%	HALOGEN IONS	✓✓	✓✓✓	✓	X	X	X	-VE	£
CHLORHEXIDINE 0.2%	BISGUANIDE	✓✓	X	✓	X	✓	?	+VE	££
HYPOCHLOROUS ACID		✓✓✓	X	?	X	X	✓	?	££
EDTA	CHELATING AGENT	✓	X	X	✓✓✓	X	✓	-VE	££
IODINE POTASSIUM IODIDE	HALOGEN IONS	✓✓	X	X	X	X	✓	+VE	££
HYDROGEN PEROXIDE	PEROXIDE	✓	X	X	X	X	X	-VE	£
SALINE/WATER/ LOCAL ANAESTHETIC	PLACEBO!	X	X	X	X	X	✓	-VE	£

Whenever dentine is cut using hand or rotary instruments, the mineralized tissues are not shredded or cleaved but shattered to produce considerable quantities of debris. Much of this, made up of very small particles of mineralized collagen matrix, is spread over the surface to form what is called the smear layer. Identification of the smear layer was made possible using the electron microprobe with scanning electron microscope (SEM) attachment, and first reported by Eick et al. (1970). These workers showed that the smear layer was made of particles ranging in size from less than 0.5–15 lm. Scanning electron microscope studies of cavity preparations by Bra¨nnstrÖm & Johnson (1974) demonstrated a thin layer of grinding debris. They estimated it to be 2–5 lm thick, extending a few micrometres into the dentinal tubules.

The smear layer in a cavity and in the root canal may not be directly comparable. Not only are the tools for dentine preparation different in coronal cavities, but in the root canal the dentinal tubule numbers show greater variation and there are likely to be more soft tissue remnants present. The first researchers to describe the smear layer on the surface of instrumented root canals were McComb & Smith (1975). They suggested that the smear layer consisted not only of dentine as in the coronal smear layer, but also the remnants of odontoblastic processes, pulp tissue and bacteria. Lester & Boyde (1977) described the smear layer as ‘organic matter trapped within translocated inorganic dentine’. As it was not removed by sodium hypochlorite irrigation, they concluded that it was primarily composed of inorganic dentine. Goldman et al. (1981) estimated the smear thickness at 1 lm and agreed with previous investigators that it was largely inorganic in composition. They noted its

presence along instrumented canal surfaces. Mader et al. (1984) reported that the smear layer thickness was generally 1–2 lm. Cameron (1983) and Mader et al. (1984) discussed the smear material in two parts: first, superficial smear layer and second, the material packed into the dentinal tubules. Packing of smear debris was present in the tubules to a depth of 40 lm. Bra¨nnstro¨m & Johnson (1974) and Mader et al. (1984) concluded that the tubular packing phenomenon was due to the action of burs and instruments. Components of the smear layer can be forced into the dentinal tubules to varying distances (Moodnik et al. 1976, Bra¨ nnstro¨ m et al. 1980, Cengiz et al. 1990) to form smear plugs (Fig. 2). However, Cengiz et al. (1990) proposed that the penetration of smear material into dentinal tubules could also be caused by capillary action as a result of adhesive forces between the dentinal tubules and the material. This hypothesis of capillary action may explain the packing phenomenon observed by Aktener et al. (1989), who showed that the penetration could increase up to 110 lm when using surface-active reagents in the canal during endodontic instrumentation. The thickness may also depend on the type and sharpness of the cutting instruments and whether the dentine is dry or wet when cut (Barnes 1974, Gilboe et al. 1980, Cameron 1988). In the early stages of instrumentation, the smear layer on the walls of canals can have a relatively high organic content because of necrotic and/or viable pulp tissue in the root canal (Cameron 1988). Increased centrifugal forces resulting from the movement and the proximity of the instrument to the dentine wall formed a thicker layer which was more resistant to removal with chelating agents (Jodaikin & Austin 1981). The amount produced during motorized preparation, as with Gates- Glidden or post drills, has been reported as greater in volume than that produced by hand filing (Czonstkow- sky et al. 1990). However, McComb & Smith (1975) observed under SEM that instrumentation with K-reamers, K-files and Giromatic reciprocating files created similar surfaces. Additional work has shown that the smear layer contains organic and inorganic substances that include fragments of odontoblastic processes, microorganisms and necrotic materials (Pashley 1992). The generation of a smear layer is almost inevitable during root canal instrumentation. Whilst a noninstrumentation technique has been described for canal preparation without smear formation, efforts rather focus on methods for its removal, such as chemical means and methods such as ultrasound and hydrodynamic disinfection for its disruption. Root canal preparation without the creation of a smear layer may be possible. A noninstrumental hydrodynamic technique may have future potential (Lussi et al. 1993), and sonically driven polymer instruments with tips of variable diameter are reported to disrupt the smear layer in a technique called hydrodynamic disinfection (Ruddle 2007).

When viewed under the SEM, the smear layer often has an amorphous irregular and granular appearance (Bra¨ nnstro¨ m et al. 1980, Yamada et al. 1983, Pashley et al. 1988) (Fig. 3). The appearance is thought to be formed by translocating and burnishing the superficial components of the dentine walls during treatment (Baumgartner & Mader 1987).

3.1 The significance of the smear layer

Root canal treatment usually involves the chemomechanical removal of bacteria and infected dentine from within the root canals. The process is often followed by an intracanal dressing and a root filling. Amongst important factors affecting the prognosis of root canal treatment is the seal created by the filling against the walls of the canal. Considerable effort has been made to understand the effect of the smear layer on the apical and coronal seal (Madison & Krell 1984, Goldberg et al. 1985, 1995, Evans & Simon 1986, Kennedy et al. 1986, Cergneux et al. 1987, Saunders & Saunders 1992, 1994, Gencog˘lu et al. 1993a, Karago¨z-Ku¨c¸u¨kay & Bayirli 1994, Tidswell et al. 1994, Lloyd et al. 1995, Behrend et al. 1996, Chailertvanitkul et al. 1996, Vassiliadis et al. 1996, Taylor et al. 1997, Timpawat & Sripanaratanakul 1998, Economides et al. 1999, 2004, von Fraunhofer et al. 2000, Froe´s et al. 2000, Goya et al. 2000, Timpawat et al. 2001, Clark-Holke et al. 2003, Cobankara et al. 2004, Park et al. 2004).

Workers have reached different conclusions, with current knowledge of interactions between the smear layer and factors such as filling technique and sealer type being limited. In addition, the methodology of studies, the type and site of leakage tests, and the sample size should be taken into account and consideration given to these variables before conclusions are reached (Shahravan et al. 2007).

Some authors suggest that maintaining the smear layer may block the dentinal tubules and limit bacterial or toxin penetration by altering dentinal permeability (Michelich et al. 1980, Pashley et al. 1981, Safavi et al. 1990). Others believe that the smear layer, being a loosely adherent structure, should be completely removed from the surface of the root canal wall because it can harbour bacteria and provide an avenue for leakage (Mader et al. 1984, Cameron 1987a, Meryon & Brook 1990). It may also limit the effective disinfection of dentinal tubules by preventing sodium hypochlorite, calcium hydroxide and other intracanal medicaments from penetrating the dentinal tubules.

3.2 Should the smear layer be removed?

The question of keeping or removing the smear layer remains controversial (Drake et al. 1994, Shahravan et al. 2007). Some investigations have focussed on its removal (Garberoglio & Bra¨ nnstro¨ m 1976, Outhwaite et al. 1976, Pashley 1985), whilst others have consid- ered its effects on apical and coronal microleakage (Madison & Krell 1984, Goldberg et al. 1995, Chailertvanitkul et al. 1996), bacterial penetration of the tubules (Pashley 1984, Williams & Goldman 1985, Meryon & Brook 1990) and the adaptation of root canal materials (White et al. 1987, Gencog˘lu et al. 1993a, Gutmann 1993). In support of its removal are:

1. It has an unpredictable thickness and volume, because a great portion of it consists of water (Cergneux et al. 1987).

2. It contains bacteria, their by-products and necrotic tissue (McComb & Smith 1975, Goldberg & Abramovich 1977, Wayman et al. 1979, Cunningham & Martin 1982, Yamada et al. 1983). Bacteria may survive and multiply (Bra¨nnstro¨m & Nyborg 1973) and can proliferate into the dentinal tubules (Olgart et al. 1974, Akpata & Blechman 1982, Williams & Goldman 1985, Meryon et al. 1986, Meryon & Brook 1990), which may serve as a reservoir of microbial irritants (Pashley 1984).

3. It may act as a substrate for bacteria, allowing their deeper penetration in the dentinal tubules (George et al. 2005).

4. It may limit the optimum penetration of disinfecting agents (McComb & Smith 1975, Outhwaite et al. 1976, Goldberg & Abramovich 1977, Wayman et al. 1979, Yamada et al. 1983). Bacteria may be found deep within dentinal tubules (Bystro¨m & Sundqvist 1981, 1983, 1985) and smear layer may block the effects of disinfectants in them (Goldberg & Abramovich 1977, Wayman et al. 1979, Yamada et al. 1983, Baumgartner & Mader 1987). Haapasalo & Ørstavik (1987) found that in the absence of smear layer, liquid camphorated monochlorophenol disinfected the den- tinal tubules rapidly and completely, but calcium hydroxide failed to eliminate Enterococcus faecalis even after 7 days of incubation. A subsequent study con- cluded that the smear layer delayed but did not abolish the action of the disinfectant (Ørstavik & Haapasalo 1990). Bra¨nnstro¨m (1984) had previously stated that following the removal of the smear layer, bacteria in the dentinal tubules can easily be destroyed.

5. It can act as a barrier between filling materials and the canal wall and therefore compromise the formation of a satisfactory seal (Lester & Boyde 1977, White et al. 1984, Cergneux et al. 1987, Czonstkowsky et al. 1990, Foster et al. 1993, Yang & Bae 2002). Lester &

Boyde (1977) found that zinc oxide – eugenol based root canal sealers failed to enter dentinal tubules in the presence of smear. In two consecutive studies, White et al. observed that plastic filling materials and sealers penetrated dentinal tubules after removal of smear layer (White et al. 1984, 1987). Oks, an et al. (1993) also found that smear prevented the penetration of sealers into dentinal tubules, whilst no penetration of sealer was observed in control groups. Penetration in their smear-free groups ranged from 40 to 60 lm. It may be concluded that such tubular penetration increases the interface between the filling and the dentinal structures, which may improve the ability of a filling material to prevent leakage (White et al. 1984). If the aim is maximum penetration into the dentinal tubules to prevent microleakage, root canal filling materials should be applied to a surface free of smear and either a low surface activity or, alternatively, an adequate surface-active reagent must be added to them (Aktener et al. 1989). However, there are no reports of a correlation between microleakage and penetration offilling materials into dentinal tubules, whilst the basis of leakage studies remains questionable. Pashley et al. (1989) observed an extensive network of microchannels around restorations that had been placed in cavities with smear layer. The thickness of these channels was 1–10 lm. Smear layer may thus present a passage for substances to leak around or through its particles at the interface between the filling material and the tooth structure. Pashley & Depew (1986) reported that, when experimenting with class 1 cavities, microleakage decreased after the removal of smear layer, but dentinal permeability increased. Saunders & Saunders (1992) concluded that coronal leakage of root canal fillings was less in smear-free groups than those with a smear layer.

6. It is a loosely adherent structure and a potential avenue for leakage and bacterial contaminant passage between the root canal filling and the dentinal walls (Mader et al. 1984, Cameron 1987b, Meryon & Brook 1990). Its removal would facilitate canal filling (McComb & Smith 1975, Goldman et al. 1981, Cameron 1983).

Conversely, some investigators believe in retaining the smear layer during canal preparation, because it can block the dentinal tubules, preventing the exchange of bacteria and other irritants by altering permeability (Michelich et al. 1980, Pashley et al. 1981, Safavi et al. 1990, Drake et al. 1994, Galvan et al. 1994). The smear layer serves as a barrier to prevent bacterial migration into the dentinal tubules (Drake et al. 1994, Galvan et al. 1994, Love et al. 1996, Perez et al. 1996). Pashley (1985) suggested that if the canals were inadequately disinfected, or if bacterial contamination occurred after canal preparation, the presence of a smear layer might stop bacterial invasion of the dentinal tubules. Bacteria remaining after canal preparation are sealed into the tubules by the smear layer and subsequent filling materials.

Some studies provide evidence to support the hypothesis that the smear layer inhibits bacterial penetration (Pashley et al. 1981, Safavi et al. 1989). A major limitation is that the experiments were undertaken with dentine discs or root cross-sections, models with little relevance in terms of simulating the clinical conditions of root canal treatment. Drake et al. (1994) developed a more clinically relevant model to determine the effect of the presence or absence of the smear layer on bacterial colonization of root canals.

Williams & Goldman (1985) reported that the smear layer was not a complete barrier and could only delay bacterial penetration. In their experiment, using the motile, swarming bacterium Proteus vulgaris, the smear layer delayed the passage of the organisms through the tubules. Madison & Krell (1984) using ethylenediaminetetraacetic acid (EDTA) solution in a dye pene- tration study found that the smear layer made no difference to leakage. Goldberg et al. (1995) studied the sealing ability of Ketac Endo and Tubliseal in an India ink study with and without smear layer and found no difference. Chailertvanitkul et al. (1996) found no difference in leakage with or without smear layer, however the time period was short. When the smear layer is not removed, the durability of the apical seal should be evaluated over a long period. Since the smear layer is nonhomogenous and may potentially be dislodged from the underlying tubules (Mader et al. 1984), it may slowly disintegrate, dissolving around a leaking filling material to leave a void between the canal wall and sealer. Meryon & Brook (1990) found the presence of smear layer had no effect on the ability of three oral bacteria to penetrate dentine discs. All were able to digest the layer, possibly stimulated by the nutrient-rich medium below the discs.

The adaptation of root canal materials to canal walls has been studied. White et al. (1987) found that pHEMA, silicone and Roth 801 and AH26 sealers extended into tubules consistently when smear layer was removed. Gencog˘lu et al. (1993b) found removing the smear layer enhanced the adaptation of guttapercha in both cold laterally compacted and thermoplastic root fillings without sealer. Gutmann (1993) also showed that after removing the smear layer, themoplastic gutta-percha adapted with or without sealer.

A systematic review and meta-analysis by Shahra- van et al. (2007) set out to determine whether smear layer removal reduced leakage of root filled teeth ex vivo. Using 26 eligible papers with 65 comparisons, 54% of the comparisons reported no significant difference, 41% reported in favour of removing the smear layer and 5% reported a difference in favour of keeping it. They concluded that smear layer removal improved the fluid-tight seal of the root canal system, whereas other factors such as filling technique or the type of sealer did not produce significant effects.

Urethane dimethacrylate (UDMA) based root canal sealers have been introduced. Their aim is to provide a better bond to allow less microleakage and increase the fracture resistance of root filled teeth through the creation of monoblocks, when a core material such as Resilon replaces gutta-percha. Whilst some studies indicate that smear layer removal leads to higher tubule penetration, increased sealer to dentine bond strength and enhanced fluid-tight seal, a recent report concluded that smear layer removal did not necessarily equate to improved resistance to bacterial penetration along these and older types of sealers (Saleh et al. 2008).

3.3 Methods to remove the smear layer

Chemical removal

The quantity of smear layer removed by a material is related to its pH and the time of exposure (Morgan & Baumgartner 1997). A number of chemicals have been investigated as irrigants to remove the smear layer. According to Kaufman & Greenberg (1986), a working solution is the one which is used to clean the canal, and an irrigation solution the one which is essential to remove the debris and smear layer created by the instrumentation process. Chlorhexidine, whilst popular as an irrigant and having a long lasting antibacterial effect through adherence to dentine, does not dissolve organic material or remove the smear layer.

Sodium hypochlorite

The ability of NaOCl to dissolve organic tissues is wellknown (Rubin et al. 1979, Wayman et al. 1979, Goldman et al. 1982) and increases with rising temperature (Moorer & Wesselink 1982). However, its capacity to remove smear layer from the instrumented root canal walls has been found to be lacking. The conclusion reached by many authors is that the use of NaOCl during or after instrumentation produces superficially clean canal walls with the smear layer present (Baker et al. 1975, Goldman et al. 1981, Berg et al. 1986, Baumgartner & Mader 1987).

Chelating agents

Smear layer components include very small particles with a large surface: mass ratio, which makes them soluble in acids (Pashley 1992). The most common chelating solutions are based on EDTA which reacts with the calcium ions in dentine and forms soluble calcium chelates. It

has been reported that EDTA decalcified dentine to a depth of 20–30 lm in 5 min (von der Fehr & Nygaard-O¨ stby 1963); however, Fraser (1974) stated that the chelating effect was almost negligible in the apical third of root canals.

Different formulations of EDTA have been used as root canal irrigants. In a combination, urea peroxide is added to encourage debris to float out of the root canal (Stewart et al. 1969). This product (RC-Prep, Premier Dental Products, Plymouth Meeting, PA, USA) also includes a wax that left a residue on the root canal walls despite further instrumentation and irrigation and which may compromise the ability to obtain a hermetic seal (Biesterfeld & Taintor 1980). Many studies have shown that paste-type chelating agents, whilst having a lubricating effect, do not remove the smear layer effectively when compared to liquid EDTA. A recent experiment examining the addition of two surfactants to liquid EDTA did not result in better smear layer removal (Lui et al. 2007).

A quaternary ammonium bromide (cetrimide) has been added to EDTA solutions to reduce surface tension and increase penetrability of the solution (von der Fehr & Nygaard-O¨stby 1963). McComb & Smith (1975) reported that when this combination (REDTA) was used during instrumentation, there was no smear layer remaining except in the apical part of the canal. After using REDTA in vivo, it was shown that the root canal surfaces were uniformly occupied by patent dentinal tubules with very little superficial debris (McComb et al. 1976). When used during and after instrumentation, it was possible to still see remnants of odontoblastic processes within the tubules even though there was no smear layer present (Goldman et al. 1981). Goldberg & Abramovich (1977) observed that the circumpulpal surface had a smooth structure and that the dentinal tubules had a regular circular appearance with the use of EDTAC (EDTA and cetavlon). The optimal working time of EDTAC was suggested to be 15 min in the root canal and no further chelating action could be expected after this (Goldberg & Spielberg 1982). This study also showed that REDTA was the most efficient irrigating solution for removing smear layer. In a study using a combination of 0.2% EDTA and a surface-active antibacterial solution, Bra¨nnstro¨m et al. (1980) observed that this mixture removed most of the smear layer without opening many dentinal tubules or removing peritubular dentine. Bis-dequalinium-acetate (BDA), a dequalinium compound and an oxine derivative has been shown to remove the smear layer throughout the canal, even in the apical third (Kauf- man et al. 1978, Kaufman 1981). BDA is well tolerated by periodontal tissues and has a low surface tension allowing good penetration. It is considered less toxic that NaOCl and can be used as a root canal dressing. A commercial form of BDA called Solvidont (De Trey, A.G., Zurich, Switzerland) was available in the 1980s and its use as an

alternative to NaOCl was supported experimentally (Kaufman 1983a,b, Chandler & Lilley 1987, Lilley et al. 1988, Mohd Sulong 1989). Salvizol (Ravens Gmbh, Konstanz, Germany) is a commercial brand of 0.5% BDA and possesses the combined actions of chelation and organic debridement. Kaufman et al. (1978) reported that Salvizol had better cleaning properties than EDTAC. When comparing Salvizol with 5.25% NaOCl, both were found comparable in their ability to remove organic debris, but only Salvizol opened dentinal tubules (Kaufman & Greenberg 1986). Berg et al. (1986) found that Salvizol was less effective at opening dentinal tubules than REDTA.

Calt & Serper (2000) compared the effects of ethylene glycol-bistetraacetic acid (EGTA) with EDTA. The smear layer was completely removed by EDTA, but it caused erosion of the peritubular and intertubular dentine, whilst EGTA was not as effective in the apical third of root canals. EGTA is reported to bind calcium more specifically (Schmid & Reilley 1957).

Tetracylines (including tetracycline hydrochloride, minocycline and doxycycline) are antibiotics effective against a wide range of microorganisms. Tetracyclines have unique properties in addition to their antimicrobial aspect. They have low pH in concentrated solution, and because of this can act as a calcium chelator and cause enamel and root surface demineralization (Bjorvatn 1982). The surface demineralization of dentine is comparable with that of citric acid (Wikesjo¨ et al. 1986). Barkhordar et al. (1997) reported that doxycycline hydrochloride (100 mg mL-1) was effective in removing the smear layer from the surface of instrumented canals and root-end cavity preparations. They speculated that a reservoir of active antibacterial agents might remain, because doxycycline readily attaches to dentine and can be subsequently released (Baker et al. 1983, Wikesjo¨ et al. 1986). Haznedaroglu & Ersev (2001) showed that 1% tetracycline hydrochloride or 50% citric acid can be used to remove the smear layer from surfaces of root canals. Although they reported no difference between the two groups, it appeared that the tetracycline demineralized less peritubular dentine than the citric acid.

In an effort to produce an irrigant capable of both removing the smear layer and disinfecting the root canal system, Torabinejad et al. (2003) developed a new irrigating solution containing a mixture of a tetracycline isomer, an acid, and a detergent (MTAD). Their work concluded MTAD to be an effective solution for the removal of the smear layer. It does not significantly change the structure of the dentinal tubules when the canals are irrigated with sodium hypochlorite and followed with a final rinse of MTAD. This irrigant demineralizes dentine faster than 17% EDTA (De Deus et al. 2007) and bacterial penetration into filled canals is similar with both solutions (Ghoddusi et al. 2007).

Organic acids

The effectiveness of citric acid as a root canal irrigant has been demonstrated (Loel 1975, Tidmarsh 1978) and confirmed to be more effective than NaOCl alone in removing the smear layer (Baumgartner et al. 1984). Citric acid removed smear layer better than polyacrylic acid, lactic acid and phosphoric acid but not EDTA (Meryon et al. 1987). Wayman et al. (1979) showed that canal walls treated with 10%, 25% and 50% citric acid solution were generally free of the smeared appearance, but they had the best results in removing smear layer with sequential use of 10% citric acid solution and 2.5% NaOCl solution, then again followed by a 10% solution of citric acid. However, Yamada et al. (1983) observed that the 25% citric acid–NaOCl group was not as effective as a 17% EDTA–NaOCl combination. To its detriment, citric acid left precipitated crystals in the root canal which might be disadvantageous to the root canal filling. With 50% lactic acid, the canal walls were generally clean, but with openings of dentinal tubules that did not appear to be completely patent (Wayman et al. 1979). Bitter (1989) introduced 25% tannic acid solution as a root canal irrigant and cleanser. Canal walls irrigated with this solution appeared significantly cleaner and smoother than walls treated with a combination of hydrogen peroxide and NaOCl, and the smear layer was removed. Sabbak & Hassanin (1998) refuted these findings and explained that tannic acid increased the cross-linking of exposed collagen with the smear layer and within the matrix of the underlying dentine, therefore increasing organic cohesion to the tubules.

McComb & Smith (1975) compared the efficacy of 20% polyacrylic acid with REDTA and found that it was no better than REDTA in removing or preventing the build up of smear layer, thought to be as a result of its higher viscosity. McComb et al. (1976) also used 5% and 10% polyacrylic acid as an irrigant and observed that it could remove smear layer in accessible regions. Polyacrylic acid (Durelon liquid and Fuji II liquid) at 40% has been reported to be very effective, and because of its potency users should not exceed a 30 s applica- tion (Berry et al. 1987).

Sodium hypochlorite and EDTA

When irrigating a root canal the purpose is twofold: to remove the organic component, the debris originating from pulp tissue and microorganisms, and the mostly inorganic component, the smear layer. As there is no single solution which has the ability to dissolve organic tissues

and to demineralize the smear layer, the sequential use of organic and inorganic solvents has been recommended (Koskinen et al. 1980, Yamada et al. 1983, Baumgartner et al. 1984). Numerous authors have agreed that the removal of smear layer as well as soft tissue and debris can be achieved by the alternate use of EDTA and NaOCl (Yamada et al. 1983, White et al. 1984, Baumgartner & Mader 1987, Cengiz et al. 1990). Goldman et al. (1982) examined the effect of various combinations of EDTA and NaOCl, and the most effective final rinse was 10 mL of 17% EDTA followed by 10 mL of 5.25% NaOCl, a finding confirmed by Yamada et al. (1983). Used in combination with EDTA, NaOCl is inactivated with the EDTA remaining functional for several minutes.

Ultrasonic smear removal

Following the introduction of dental ultrasonic devices in the 1950s, ultrasound was investigated in endodontics (Martin et al. 1980, Cunningham & Martin 1982, Cunningham et al. 1982). A continuous flow of NaOCl activated by an ultrasonic delivery system was used for the preparation and irrigation of canals. Smear-free canal surfaces were observed using this method (Cameron 1983, 1987a,b, Griffiths & Stock 1986, Alacam 1987). Whilst concentrations of 2–4% sodium hypochlorite in combination with ultrasonic energy were able to remove smear layer, lower concentrations of the solutions were unsatisfactory (Cameron 1988). However, Ahmad et al. (1987a) claimed that their technique of modified ultrasonic instrumentation using 1% NaOCl removed the debris and smear layer more effectively than the technique recommended by Martin & Cunningham (1983). The apical region of the canals showed less debris and smear layer than the coronal aspects, depending on acoustic streaming, which was more intense in magnitude and velocity at the apical regions of the file. Cameron (1983) also compared the effect of different ultrasonic irrigation periods on removing smear layer and found that a 3 and 5 min irrigation produced smearfree canal walls, whilst an 1-min irrigation was ineffective. In contrast to these results, other investigators found ultrasonic preparation unable to remove smear layer (Cymerman et al. 1983, Baker et al. 1988, Goldberg et al. 1988).

Researchers who found the cleaning effects of ultrasonics beneficial used the technique only for the final irrigation of root canal after completion of hand instrumentation (Ahmad et al. 1987a, Alacam 1987, Cameron 1988). This is given the term passive ultrasonic irrigation and

has been the subject of a recent review (van der Sluis et al. 2007). Ahmad et al. (1987a,b) claimed that direct physical contact of the file with the canal walls throughout instrumentation reduced acoustic streaming. Acoustic streaming is maximized when the tips of the smaller instruments vibrate freely in a solution. Lumley et al. (1992) recommended that only size 15 files be used to maximize microstreaming for the removal of debris. Prati et al. (1994) also achieved smear layer removal with ultrasonics. Walker & del Rio (1989, 1991) showed no significant difference between tap water and sodium hypochlorite when used with ultrasonics, but they reported that neither solution was effective at any level in the canal to remove the smear layer ultrasonically. Baumgartner & Cuenin (1992) also observed that ultrasonically energized NaOCl, even at full strength, did not remove the smear layer from root canal walls. Guerisoli et al. (2002) evaluated the use of ultrasonics to remove the smear layer and found it necessary to use 15% EDTA with either distilled water or 1% sodium hypochlorite to achieve the desired result.

Laser Removal

Lasers can be used to vaporize tissues in the main canal, remove the smear layer and eliminate residual tissue in the apical portion of root canals (Takeda et al. 1998a,b, 1999). The effectiveness of lasers depends on many factors, including the power level, the duration of exposure, the absorption of light in the tissues, the geometry of the root canal and the tip-to-target distance (Dederich et al. 1984, O¨nal et al. 1993, Tewfik et al. 1993, Moshonov et al. 1995).

Dederich et al. (1984) and Tewfik et al. (1993) used variants of the neodymium–yttriumaluminiumgarnet (Ne:YAG) laser and reported a range of findings from no change or disruption of the smear layer to actual melting and recrystallization of the dentine. This pattern of dentine disruption was observed in other studies with various lasers, including the carbon dioxide laser (O¨nal et al. 1993), the argon fluoride excimer laser (Stabholz et al. 1993), and the argon laser (Moshonov et al. 1995, Harashima et al. 1998). Takeda et al. (1998a,b, 1999) using the erbium-yttrium-aluminium-garnet (Er:YAG) laser, demonstrated optimal removal of the smear layer without melting, charring or recrystallization associated with other laser types. Kimura et al. (2002) also demon- strated the removal of the smear layer with an Er:YAG laser. Although they showed removal of the smear layer, photomicrographs showed destruction of peritubular dentine. The main difficulty with laser removal of the

smear layer is access to the small canal spaces with the relatively large probes that are available.

Conclusion

Contemporary methods of root canal instrumentation produce a layer of organic and inorganic material called the smear layer that may also contain bacteria and their by-products. This layer covers the instrumented walls and may prevent the penetration of intracanal medicaments into the dentinal tubules and interfere with the close adaptation of root filling materials to canal walls. The data presented indicate removal of the smear layer for more thorough disinfection of the root canal system and better adaptation of materials to the canal walls. There are, however, no clinical trials to demonstrate this. Current methods of smear layer removal include chemical, ultrasonic and laser techniques none of which are totally effective throughout the length of all canals or are used universally. However, if the smear layer is to be removed the method of choice seems to be the alternate use of EDTA and sodium hypochlorite solutions. Whilst much is known about individual irrigants, their use in combination and their interactions (and in some cases precipitates) is less well understood. Conflicting reports exist regarding the removal of the smear layer before filling root canals. As several new sealer and core materials have recently been introduced, further investigations are required to determine the role of the smear layer in the outcome of treatment.

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Chapter 4

Role of Enterococcus Faecalis

Factors that may contribute to a persistent periradicular infection after root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction, and cysts containing cholesterol crystals (1). It is generally believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth (1, 2). Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms in- volved in secondary infections are composed of one or a few bacterial species (2–5). Enterococcus faecalis is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora (1). The intent of this article is (a) to describe characteristics inherent to E. faecalis; (b) to cite studies that implicate E. faecalis as an etiology of failing root canal treatment; (c) to list the mechanisms that allow E. faecalis the ability to survive and cause persistent periradicular pathosis; and (d) to discuss current treatment modalities that are effective in eliminating E. faecalis from the root canal system.

4.1 E. faecalis Characteristics and Strains

Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen (6,7). *Enterococcus* species live in vast quantities [10^5 -10^8 colony-forming units (cfu) per gram of feces] in the human intestinal lumen and under most circumstances cause no harm to their hosts. They are also present in human female genital tracts and the oral cavity in lesser numbers (8). They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and many keto acids (6). *Enterococci* survive very harsh environments including extreme alkaline pH (9.6) and salt concentrations (6, 9). They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation (6). They can grow in the range of 10 to 45°C and survive a temperature of 60°C for 30 min (9). There are currently 23 *Enterococci* species and these are divided into five groups based on their interaction with

mannitol, sorbose, and arginine. *E. faecalis* belongs to the same group as *E. faecium, E. casseliflavus, E. mundtii,* and *E. gallinarum.* These five species form acid in mannitol broth and hydrolyze arginine; however, they fail to form acid in sorbose broth (6, 10). After establishing that the gram-positive coccus is a member of one of the five groups in the *Enterococcus* genus (Table 2) (10), several conventional tests are used to identify the specific species. In group 2, *E. faecalis* can normally be identified by further testing with arabinose, tellurite, and pyruvate. *E. faecalis* is arabinose negative and except for some atypical variants, is the only member of the group to utilize pyruvate and to tolerate tellurite (11). More recently, molecular techniques have been developed that have the capability to rapidly and accurately identify the *Enterococcus* species. Techniques involving DNA-DNA hybridization, sequencing of the 16S rRNA genes, whole-cell protein (WCP) analysis and gasliquid chromatography of fatty acids have been used for taxonomic purposes. Most of these methods are nucleic acid-based involving PCR amplification assays that are followed by electrophoretic analysis of the PCR products, probing, sequencing, or both (11). Random amplified polymorphic DNA (RAPD) analysis and pulse-field gel electrophoresis (PFGE) are techniques that have been utilized to determine variations in DNA sequences and have been employed in determining various *E. faecalis* subtypes (12, 13). In fact, the Bac- teriology Collection of the ATCC (American Type Culture Collection) currently lists 69 isolates of *E. faecalis* that are commercially available (14). These isolates each have a different ATCC number and designation. The biosafety level ranges from 1 to 2 and growth conditions differ among the subtypes. Sources for these isolates include sour milk (ATCC number 376TM), meat involved in food poisoning (ATCC number 7080TM), and the root canal of a pulpless tooth (ATCC number 4083TM) (14).

Group	Species
Group I (+) acid formation in mannitol broth (+) acid formation in sorbose broth (−) arginine hydrolysis	*E. avium* *E. gilvus* *E. malodoratus* *E. pallens* *E. pseudoavium* *E. raffinosus* *E. saccharolyticus*
Group II (+) acid formation in mannitol broth (−) acid formation in sorbose broth (+) arginine hydrolysis	***E. faecalis*** *E. faecium* *E. casseliflavus* *E. gallinarum* *E. mundtii* *Lactococcus sp.*
Group III (−) acid formation in mannitol broth (−) acid formation in sorbose broth (+) arginine hydrolysis	*E. dispar* *E. durans* *E. hirae* *E. porcinus* *(E. villorum)* *E. ratti*
Group IV (−) acid formation in mannitol broth (−) acid formation in sorbose broth (−) arginine hydrolysis	*E. asini* *E. cecorum* *E. sulfureus*
Group V (+) acid formation in mannitol broth (−) acid formation in sorbose broth (−) arginine hydrolysis	*E. columbae* *Vagococcus sp.*

Table 2. Categorization of Enterococcus species and two physiologically related gram-positive cocci based on phenotypic characteristics

Attention has been turned towards *Enterococci* since the 1970s when they were recognized as major nosocomial pathogens causing bacteremia, endocarditis, bacterial meningitis, urinary tract, and various other infections (15). Sources of the bacteria in these infections have been reported as originating from the hands of health care workers, from clinical instruments, or from patient to patient (8). Studies have shown that nosocomial infections are not caused by the patient's own prehospitalization flora (16). Enterococcal infections now account for roughly 12% of nosocomial infections in the United States with the majority of those being

caused by *E. faecalis* (greater than 80%) and *E. faecium* being responsible for the majority of the remaining infections (17). Studies show *E. faecalis* is able to translocate from the root canal system to the submandibular lymph nodes of germ-free mice, suggesting this route of infection may play a role in the pathogenesis of opportunistic infections in patients (18, 19). Enterococcal urinary tract and soft tissue infections are generally treated with single drug therapy, often with penicillin or vancomycin (20). There is emerging evidence of vancomycin resistance among *Enterococcus* species and routine use of previously standard recommendations for treatment of enterococcal infections can no longer be expected to provide optimal results (21). Enterococcal strains, particularly those causing endocarditis, must be screened to define antimicrobial resistance patterns. Thirty-five vancomycin resistant *Enterococci* have demonstrated susceptibility to linezolid (antibiotic, oxazolidinone derivative), suggesting it may be the treatment of choice for multi-drug resistant enterococcal infections (22).

4.2 Prevalence in Secondary Root Canal Infections

E. faecalis is a normal inhabitant of the oral cavity. The prevalence of *E. faecalis* is increased in oral rinse samples from patients receiving initial endodontic treatment, those midway through treatment, and patients receiving endodontic retreatment when compared to those with no endodontic history (23). *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections (7). In the category of primary endodontic infections, *E. faecalis* is associated with asymptomatic chronic periradicular lesions significantly more often than with acute periradicular periodontitis or acute periradicular abscesses. *E. faecalis* is found in 4 to 40% of primary endodontic infections (7). The frequency of *E. faecalis* found in persistent periradicular lesions has been shown to be much higher. In fact, failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections (7). Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (3–5, 7, 24–31). The wide range of *E. faecalis* prevalence among studies may be attributed to different identification techniques, geographic differences, or sample size (32, 33). In some cases, *E. faecalis* has been found as the only organism (pure culture) present in root- filled teeth with periradicular lesions (4, 28). The majority of these studies have been carried out using culturing techniques; however, polymerase chain reaction (PCR) is currently a more predictable method for detection of *E. faecalis* (34, 35). This method proves to be faster, more sensitive, and more accurate than culturing methods (35). It has enabled

researchers to detect bacteria that were difficult, and in some cases impossible, to detect (35). When compared to detection of *E. faecalis* by culturing (24-70%), *E. faecalis* has been found at consistently higher percentages (67-77%) when a PCR detection method is used (7). An optical spectroscopy-based method has also been studied as a way to detect *E. faecalis* activity (36). It is possible that this detection system could be used chairside to rapidly monitor the presence or absence of *E. faecalis* in the root canal system (36).

4.3 Survival and Virulence Factors

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid (7). It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses (7, 37). *E. faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure (38). *E. faecalis* is not limited to its possession of various virulence factors. It is also able to share these virulence traits among species, further contributing to its survival and ability to cause disease (15). These factors may or may not contribute to the innate characteristics of *E. faecalis* to cause disease. Because *E. faecalis* is less dependent upon virulence factors, it relies more upon its ability to survive and persist as a pathogen in the root canals of teeth (7). *E. faecalis* overcomes the challenges of survival within the root canal system in several ways. It has been shown to exhibit widespread genetic polymorphisms (23). It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which help it bind to dentin (39). It is small enough to proficiently invade and live within dentinal tubules (37). It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available (40). Once available, the starved cells are able to recover by utilizing serum as a nutritional source (40). Serum, which originates from alveolar bone and the periodontal ligament, also helps *E. faecalis* bind to type I collagen (37). *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days (41, 42). *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms (43).

Calcium hydroxide, a commonly used intracanal medicament, has been shown to be ineffective at killing *E. faecalis* on its own, especially when a high pH is not maintained (42, 44 – 46). The following reasons have been proposed to explain why *E. faecalis* is able to survive intra- canal treatment with calcium hydroxide: (a) *E. faecalis* passively maintains pH

homeostasis. This occurs as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. (b) *E. faecalis* has a proton pump that provides an additional means of maintaining pH homeostasis. This is accomplished by "pumping" protons into the cell to lower the internal pH. (c) At a pH of 11.5 or greater, *E. faecalis* is unable to survive (1, 45). However, as a result of the buffering capacity of dentin, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques (46). Studies using the dentin powder model have shown that the presence of dentin has an inhibitory effect on various concentrations of root canal medicaments including calcium hydroxide, sodium hypochlorite, chlorhexidine, and iodine potassium iodide (47, 48). Diverse components of dentin including dentin matrix, type-I collagen, hydroxyapatite, and serum are responsible for altering the antibacterial effects of these medicaments (49).

4.4 Methods of Eradication

Many studies have been directed towards finding an effective way to eradicate and/or prevent *E. faecalis* from gaining access to the root canal space. *E. faecalis* can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed (7). Therefore, it is important to consider treatment regimens aimed at eliminating or preventing the infection of *E. faecalis* during each of these phases. Preparing the apical portion of the root canal to a larger instrument size will help eliminate intracanal microorganisms by reaching areas not normally accessible by smaller master apical files (50). In addition, larger apical preparation sizes facilitate removal of the innermost (pulpal) dentin. This provides the potential to remove intratubular bacteria and open the dentinal tubules to allow antimicrobials to penetrate more effectively. Three percent to full strength sodium hypochlorite, if used in adequate amounts and exchanged regularly, has the capability to destroy *E. faecalis* in the root canal (51). Sodium hypochlorite is an effective irrigant for all presentations of *E. faecalis* including its existence as a biofilm (52). EDTA has little antibacterial activity, but is important in its ability to remove the inorganic portion of the smear layer thus allowing other irrigants access to the dentinal tubules (53, 54). A 10% citric acid solution will remove the smear layer and, like EDTA, has little effect against *E. faecalis*. A 0.1% sodium benzoate solution added to 10% citric acid will increase the chances of killing *E. faecalis* (55). MTAD, a new root canal irrigant consisting of a mixture of a tetracycline isomer, an acid, and a detergent has shown success in its ability to destroy *E. faecalis* in preliminary studies (53, 56). Its effectiveness is attributed to its anticollagenase activity, low pH, and ability to be released gradually over time (56). The effects of MTAD are enhanced when 1.3%

sodium hypochlorite is used as an irrigant during instrumentation (57). Calcium hydroxide is relatively ineffective against *E. faecalis* because of considerations mentioned previously (1, 41). Iodine potassium iodide may be a more effective intracanal agent than calcium hydroxide (58). Chlorhexidine, in a 2% gel or liquid concentration, is effective at reducing or completely eliminating *E. faecalis* from the root canal space and dentinal tubules (59 – 61). A 2-min rinse of 2% chlorhexidine liquid can be used to remove *E. faecalis* from the superficial layers of dentinal tubules up to 100 m (59). Two percent chlorhexidine gel is effective at completely eliminating *E. faecalis* from dentinal tubules for up to 15 days (60). This may be in part attributed to its substantive antimicrobial activity (62). It is questionable as to whether 0.12% chlorhexidine is more effective than calcium hydroxide. Some studies suggest it is more effective, yet neither will completely eradicate *E. faecalis* (44, 63). Another study suggests 10% calcium hydroxide alone is more effective (64). When heated to 46°C, both 0.12% chlorhexidine and 10% calcium hydroxide have greater antimicrobial effects against *E. faecalis* than at normal body temperature (65).

Other irrigants that may be effective at eliminating *E. faecalis* include ozonated water and stannous fluoride. Ozonated water has been shown to have the same antimicrobial efficacy as 2.5% sodium hypochlorite (66). Stannous fluoride demonstrated greater antimicrobial effectiveness against *E. faecalis* than calcium hydroxide (67).

Combinations of irrigants to eliminate *E. faecalis* have also been studied. In one study, a combination of calcium hydroxide mixed with camphorated paramonochlorophenol completely eliminated *E. faecalis* within dentinal tubules (68). Metapex, a silicone oil-based calcium hydroxide paste containing 38% iodoform, more effectively disinfected dentinal tubules infected with *E. faecalis* than calcium hydroxide alone (69). The addition of stannous fluoride to calcium hydroxide is also more effective than calcium hydroxide by itself (67). Concentrations of 1 to 2% chlorhexidine combined with calcium hydroxide have also demonstrated efficacy at killing *E. faecalis* (60, 68, 70). Chlorhexidine combined with calcium hydroxide will result in a greater ability to kill *E. faecalis* than calcium hydroxide mixed with water (70). Two percent chlorhexidine gel combined with calcium hydroxide achieves a pH of 12.8 and can completely eliminate *E. faecalis* within dentinal tubules (60). 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 (60, 61). Until further studies have been conducted, an intracanal dressing of 2% chlorhexidine placed for 7 days may be the best way to eradicate *E. faecalis* from dentinal

tubules and the root canal space (60, 61). In some studies, chlorhexidine impregnated and iodoform containing guttapercha points have shown little inhibitory action against *E. faecalis* (71, 72). In another study, 5% chlorhexidine in a slow release device (Activ Point, Roeko, Langenau, Germany) completely eliminated *E. faecalis* in dentinal tubules up to 500 μm (73).

The antimicrobial activity against *E. faecalis* of various sealers 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 (74). AH Plus epoxyresin based sealer (Dentsply, DeTrey, Konstanz, Germany) and Sultan zinc oxide-eugenol based sealer (Sultan Chemists, Inc., Englewood, NJ) both exhibit good antibacterial effects against *E. faecalis* using agar diffusion and direct-contact tests (75). AH Plus and Grossman's sealer are effective in killing *E. faecalis* within infected dentinal tubules (76). Based on these studies it can be concluded that a combination of adequate instrumentation, and appropriate use of irrigants, medicaments, and sealer will optimize the chances of eradicating *E. faecalis* during retreatment of failed root canal cases.

Additional steps should be taken to prevent *E. faecalis* from re-entering the root canal space. These include having the patient rinse with chlorhexidine before treatment, disinfecting the tooth and rubber dam with chlorhexidine or sodium hypochlorite, and disinfecting gutta-percha points with sodium hypochlorite before insertion in the canal (77). Other possibilities may include using an obturating system that can provide a more effective seal. Newer obturation systems such as Epiphany (Pentron Corp., Wallingford, CT) have been designed to bond to the root canal walls and thus prevent bacterial leakage. Although research is still needed, a preliminary study shows that this system is better at preventing microleakage of *E. faecalis* than guttapercha filled canals (78). A well-sealed coronal restoration and root canal filling are important steps in preventing bacteria from entering the canal space (79).

4.5 Conclusion

Studies indicate that the prevalence of *E. faecalis* is low in primary endodontic infections and high in persistent infections. *E. faecalis* is also more commonly associated with asymptomatic cases than with symptomatic ones. Although *E. faecalis* possesses several virulence factors, its ability to cause periradicular disease stems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth. Our challenge as endodontic specialists is to implement methods to effectively eliminate this

microorganism during and after root canal treatment. Currently, use of good aseptic technique, increased apical preparation sizes, and inclusion of full strength sodium hypochlorite and 2% chlorhexidine irrigants are the most effective methods to eliminate *E. faecalis*. Recent studies have helped us better understand *E. faecalis* and the mechanisms that enable it to cause persistent endodontic infections. In the changing face of dental care, continued research on *E. faecalis* and its elimination from the dental apparatus may well define the future of the endodontic specialty.

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Experimental part

The purpose of the present thesis was to evaluate/correlate different aspects of the endodontic treatment in order to shed some light on the influence of microorganisms disinfection during root canal treatment.

Chapter 5

Final Rinse Optimization: Influence of Different Agitation Protocols

5.1 Introduction

Microorganisms and their end products are considered the main causes of pulp and periapical diseases (1), and their elimination by biomechanical procedures is crucial (2). Organic residues and bacteria located within the dentin tubules cannot be properly cleaned because of the anatomic complexities of many root canals, even after meticulous mechanical instrumentation and is a major concern for the clinical outcome (3).

Among currently used solutions, sodium hypochlorite (NaOCl) appears to satisfy most of the requirements for a root canal irrigant (4). It has the unique capacity to dissolve necrotic tissue (5) and the organic components of the smear layer (6). It also kills sessile endodontic pathogens organized in biofilms and in dentinal tubules as efficiently as chlorhexidine or iodine at a comparable concentration (7). It inactivates endotoxins (8) and also disintegrates endodontic biofilms (9, 10).

The application time of NaOCl solution is a factor that has gained little attention in endodontic studies. Even fast-acting biocides such as NaOCl require an adequate working time to reach their full potential (11). Because rotary root canal preparation techniques have expedited the

shaping process, the optimal time that NaOCl at a given concentration needs to remain in the canal system is an issue yet to be resolved.

Apart from contact time, the mode of application is a matter of concern for clinicians. Moorer and Wesselink (12) opined that mechanical agitation or fluid flow was more important in the ability of NaOCl to dissolve tissue than the initial percentage of available active chlorine. The use of an irrigant in conjunction with ultrasonic vibration is directly associated with the cleaning effectiveness of the canal space (13, 14). This could reduce the time needed for the antimicrobial efficacy of the irrigating solution.

Different techniques have been proposed for the final rinsing step to reduce the time needed for an irrigant to be effective. Huang et al (15) showed that agitation of a canal irrigant using hand files or irrigation needles could significantly remove more test album medium or allow better apical irrigant replacement. In addition, manual dynamic irrigation (push-pull agitation) with a well-fitting gutta-percha point can improve the penetration and exchange of irrigant at the apical level (16). The use of a plastic file in conjunction with sonic and ultrasonic devices has also been tested. However, a recent Cochrane review (17) revealed insufficient evidence on ultrasonic instrumentation effectiveness either when it is used alone or in conjunction with hand instrumentation (18–20).

Alizarin red is a fluorescent organic compound used in biomorphologic assays for quantifying the presence of calcific depositions (21). The purpose of this study was to assess the penetration of 5% NaOCl labeled with 0.2% alizarin red into dentinal tubules when used in root canals with different agitation protocols. The null hypothesis tested was that there is no difference in irrigant penetration using different agitation protocols.

5.2 Materials and Methods

Fifty-six recently extracted human single-rooted teeth with a straight single canal were selected for the study under a protocol approved by the University of Siena (Italy). Exclusion criteria were as follows: teeth shorter than 20 mm; apex larger than #25 before instrumentation; and presence of caries, root fissures, or fractures. All teeth were stored in saline at 4°C and used within 1 month after extraction.

To standardize canal instrumentation, crowns were removed by cutting the teeth 2 mm above the cementoenamel junction using a slow-speed Isomet saw (Buehler, Lake Bluff, IL) under

copious water cooling. A size 10 K-type file was inserted into each canal until it was seen through the apical foramen. The working length was established by reducing this length by 0.5 mm.

The canals were shaped with nickel-titanium rotary instruments (FlexMaster, VDW, Munich, Germany). A size 40, 0.06 taper was the last file used at the working length. Irrigation with 5% NaOCl was performed during instrumentation using a syringe with a 30-G needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland). Smear layer removal was achieved after irrigation with 3 mL of 17% EDTA for 2 minutes followed by 3 mL of sterile saline. The teeth were randomly divided into seven groups (N = 8 for each group). The exterior part of the apical third of each root was covered with wax to prevent irrigant from dripping through the apical foramen. This was done after placing a calibrated FineMedium guttapercha cone at the working length in order to avoid wax intrusion into the apex. The cone was removed after the wax had set.

A final rinse of each canal was performed by using 5 mL of 5% NaOCl labeled with 0.2% alizarin red using the 30-G endodontic needle at 5 mm from the working length. To standardize the procedures for all teeth, a flux of 1 mL/30 seconds was used for 90 seconds. The following Basic Research Technology

groups had a different agitation procedure during the final rinse: (1) control group: no agitation (NaOCl with Alizarin red without activation), (2) K-file group: agitation with a size 10 K-file (20 up and down movements to the working length at a frequency of 3 per second), gutta-percha group: agitation with a fine medium gutta-percha cone (20 up and down movements to the working length at a frequency of 3 per second), (3) EndoActivator group: agitation with a sonic device (EndoActivator; Advanced Endodontics, Santa Barbara, CA) 10,000 cpm for 20 seconds, (4) Plastic Endo group: agitation with F-file (Plastic Endo LLC, Lincoln-shire, IL) for 30 seconds at 500 rpm to 1 mm from the working length, (5) Satelec group: agitation with Passive Ultrasonic IrriSafe Satelec (Acteongroup, Merignac, France) with power setting at 5 for 20 seconds, and (6) EMS group: agitation with Passive Ultrasonic ESI File (EMS, Nyon, Switzerland) with power setting at 5 for 20 seconds. For guttapercha and Plastic Endo groups, the respective device was inserted to and activated at 1 mm from the working length.

After drying the canal with paper points, each specimen was cut into three 1-mm thick slabs at 1, 3, and 5 mm from the apex. Slabs were then bonded onto glass slides and ground with wet silicon carbide papers to approximately 40-mm thick. The slides were examined with a fluorescence light microscope (Nikon Eclipse; Nikon, Tokyo, Japan) at 100X with a wavelength

of 540 to 570 nm. If the whole canal could not fit completely in one image, two or more partial images were taken to produce a montage using Adobe Photoshop CS3 (Adobe Systems Italia S.r.l, Milan, Italy). Images from all specimens were evaluated by two blinded operators. In the case of disagreement between the operators, the lower score was assigned. The following set of scores was used to assess the penetration of the irrigant solution into the dentinal tubules (Fig. 1A): ‘‘0’’ = no visible alizarin red, ‘‘1’’ = minor traces of alizarin red, ‘‘2’’ = traces of alizarin red along the whole intraradicular surface of the canal, ‘‘3’’ = penetration of alizarin red in <50% of the dentinal tubules, and ‘‘4’’ = penetration of alizarin red in >50% of the tubules.

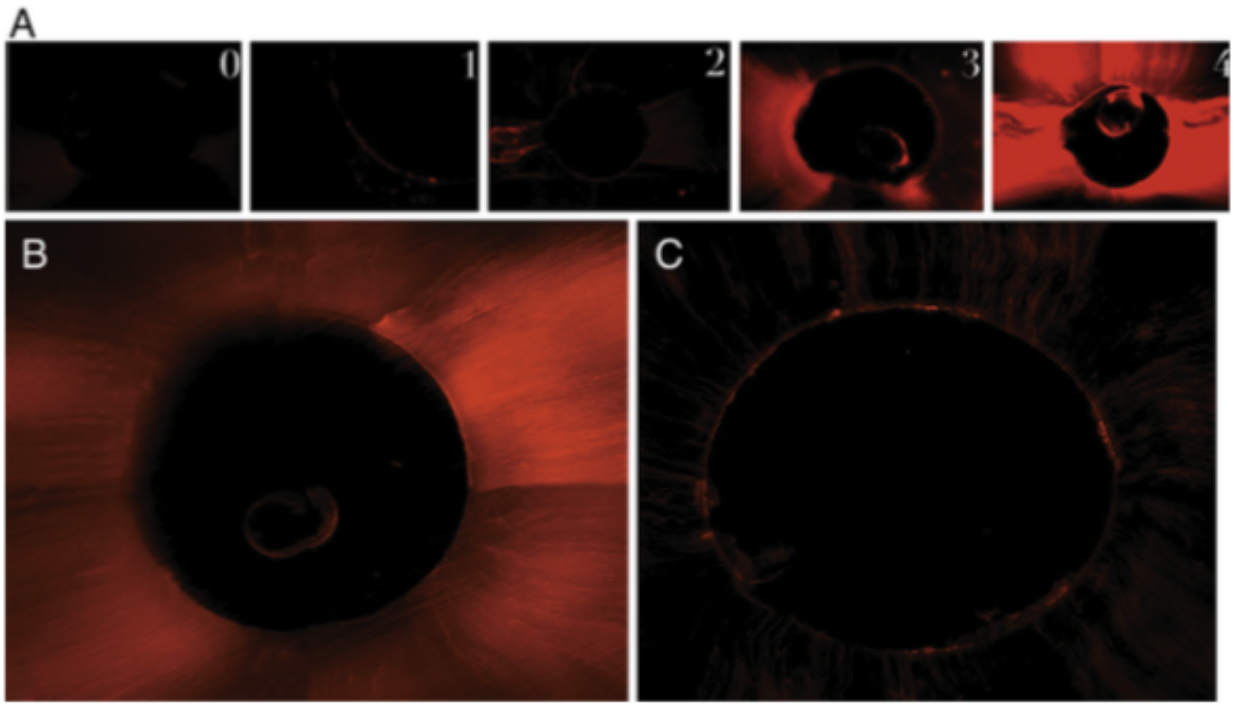

Figure 1. (A) Dye penetration scores based on the extent of fluorescence observed from dentinal tubules. ‘‘0’’: no visible alizarin red, ‘‘1’’: minor (incomplete) traces of alizarin red along the surface of the canal wall, ‘‘2’’: traces of alizarin red along the entire circumference of the canal wall, ‘3’’: penetration of alizarin red in less than 50% of the dentinal tubules, and ‘‘4’’: penetration of alizarin red in more than 50% of the dentinal tubules. (B) A representative example of fluorescence exhibited by group 7 at 1 mm from the apex. Evidence of alizarin red could be identified from the entire canal wall circumference and with penetration of the fluorescent dye into the patent dentinal tubules. (C) A representative example of fluorescence exhibited by group 4 at 1 mm from the apex. Traces of alizarin red could be partially identified along the canal wall circumference, with partial penetration into the dentinal tubules.

Additional specimens were prepared as controls as follows: (1) negative control: without adding 0.2% alizarin red to the final rinse solution and (2) positive control: 1-mm thick slabs were immersed in 0.2% alizarin red for 10 minutes to investigate dye uptake pattern. Statistical analysis was performed by using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among the groups at a = 0.05. Data were investigated either pooled together or separately with respect to the distance from the apex.

5.3 Results

Statistically significant differences were found among groups in relation to the agitation mode. For the entire canal, groups were ranked in the following order: control = K-file = gutta-percha < EndoActivator = Plastic Endo < Satelec = EMS Group (Table 1, $p < 0.05$). For different sections of the canal space, the distance from the apex (1, 3, and 5 mm) did not influence alizarin red penetration within each group ($p > 0.05$).

Analysis of the irrigant agitation modes at different locations revealed that at 1 mm from the root apex, the EMS group exhibited the highest score (Fig. 1B) and was significantly different ($p < 0.001$) from the control, K-file, gutta-percha, EndoActivator, and Plastic Endo groups (Fig. 1C). There was no difference between the Satelec groups and the EMS group (Table 2). At 3 and 5 mm from the root apex, the Plastic Endo, Satelec, and EMS groups yielded similar scores that were significantly higher than the other groups ($p < 0.001$). No fluorescence was found in negative controls, whereas the intense presence of dye tracing within dentinal tubules was recorded in positive controls.

TABLE 1. Summary of Median Scores when the Dye Penetration Scores Derived from the Coronal, Middle, and Apical Thirds of the Canal Walls Were Pooled Together

Group*	N	Median	25%	75%	*p* value
1 (control)[c]	24	1.0	0	1.0	<0.001
2 (K-file)[b,c]	24	1.0	1.0	1.0	
3 (gutta-percha)[b,c]	24	1.0	1.0	1.0	
4 (Plastic Endo)[b,c]	24	1.0	1.0	1.0	
5 (EndoActivator)[b]	24	1.5	1.0	2.0	
6 (Satelec)[a]	24	3.0	3.0	4.0	
7 (EMS)[a]	24	4.0	3.0	4.0	

*Groups with the same superscripts are not statistically significant ($p > 0.05$).

TABLE 2. Median Scores at 1, 3, and 5 mm from the Root Apex

Group	N	Median	25%	75%	*p* value
		1 mm			
Control[c]	8	0.0	0.0	1.0	<0.001
K-file[c]	8	1.0	0.5	1.0	
Gutta-percha[b,c]	8	1.0	1.0	1.0	
Plastic Endo[b,c]	8	1.0	1.0	1.0	
EndoActivator[b,c]	8	1.0	1.0	1.0	
Satelec[a,b]	8	3.0	3.0	3.5	
EMS 7[a]	8	4.0	3.0	4.0	
		3 mm			
Control[b]	8	1.0	0.5	1.0	<0.001
K-file 2[b]	8	1.0	1.0	1.0	
Gutta-percha 3[b]	8	1.0	1.0	1.0	
Plastic Endo[b]	8	1.0	1.0	1.0	
EndoActivator[a,b]	8	2.0	1.0	2.0	
Satelec[a]	8	3.0	3.0	4.0	
EMS[a]	8	4.0	3.0	4.0	
		5 mm			
Control[b]	8	1.0	0.5	1.0	<0.001
K-File[b]	8	1.0	1.0	2.0	
Gutta-percha[b]	8	1.0	1.0	2.0	
Plastic Endo[b]	8	1.0	1.0	1.5	
EndoActivator[a,b]	8	2.0	1.5	2.0	
Satelec[a]	8	3.5	3.0	4.0	
EMS[a]	8	4.0	3.5	4.0	

Group 1, control; group 2, K-file; group 3, gutta-percha; group 4, Plastic Endo; Group 5, EndoActivator; group 6, Satelec; and group 7, EMS.

[a,b,c]Groups with the same superscripts are not statistically significant ($p > 0.05$).

5.4 Discussion

Although mechanical instrumentation reduces bacteria from human root canals by approximately 50%, disinfecting irrigants are needed to eliminate the microbiota in locations where instruments cannot access (22–24). Although NaOCl is an effective disinfectant when it comes into direct contact with bacteria biofilms, it produced clean and debris-free dentin surfaces only in the coronal and middle thirds but not in the apical third of the canal wall when used in conjunction with nickel-titanium instruments (25). Consequently, different irrigant agitation techniques have been proposed to increase the efficacy of the irrigant solutions. Some of these techniques include manual agitation with hand files, manual agitation with gutta-percha cones, mechanical agitation with plastic instruments, and sonic and ultrasonic agitation (26).

In this study, alizarin red was used to label NaOCl. Because the validity of this methodology was confirmed with the control group, the protocol was used for investigating the penetration of the dye-labeled NaOCl within the root canal space after different final rinsing procedures. Tracing of NaOCl penetration into dentinal tubules with fluorescence microscopy enabled us to evaluate the effect of irrigant agitation techniques on irrigant penetration within the apical 1- to 5-mm region of the canal space. Additional studies should investigate the optimal concentration of the NaOCl to kill bacteria and deproteinize organic tissues without extracting collagen from the mineralized radicular dentin (27).

The null hypothesis tested was rejected because differences were found in irrigant penetration using different agitation protocols. Passive ultrasonic irrigation (28) improves the efficacy of irrigating solutions in removing organic and inorganic debris from root canal walls (29, 30). The term passive does not adequately describe the process because it is in fact active; however, when it was first introduced the term passive related to the ‘‘noncutting’’ action of the ultrasonically activated file. The technique relies on the transmission of acoustic energy from an oscillating file or smooth wire to an irrigant in the canal space. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming of the irrigant (31–33). A possible explanation for the improved irrigant penetration into those nonsclerotic tubules within the apical third of the canal wall is the better current flow and increased irrigant volume (34) associated with ultrasonic agitation.

Because a vapor lock exists in the apical third of the canal (35, 36) when the apical foramen is sealed with wax, it is prudent to elaborate on why better dye penetration was observed in the ultrasonic groups. Using a control and an experimental balanced design to compare the effect

of vapor lock on the efficacy of canal debridement from the apical 0 to 2 mm of the canal walls, we recently observed that the use of NaOCl and EDTA was able to remove smear layers from that region irrespective of the presence or absence of a vapor lock (ie, same ‘‘smear score’’). However, canals that simulated the presence of a vapor lock exhibited a significantly higher ‘‘debris score’’ compared with those simulating the absence of a vapor lock (37). Because this study examined only dye penetration into dentinal tubules (ie, smear layer removal), it is understandable that the ultrasonic agitation techniques produce better results. Further studies should be conducted to examine the effect of different agitation techniques on the ‘‘debris score.’’ A novel way in accomplishing this objective is to stain soft-tissue debris with phosphotungstic acid so that both hard and soft tissue debris can be simultaneously evaluated by high contrast threedimensional imaging using microcomputed tomography. Investigations with this technique are in order.

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Chapter 6

Comparison of smear layer removal using four final-rinse protocols

6.1 Introduction

The main purpose of root canal therapy in infected teeth is the elimination of debris, toxins and microorganisms by chemomechanical preparation. However, even after cleaning and shaping, total sterilization of the root canal system remains difficult to achieve (1). Studies have shown that mechanical instrumentation of root canals implies the formation of a smear layer covering the dentinal walls (2) and containing both inorganic and organic materials (2). The presence of the smear layer may considerably delay or prevent the penetration of antimicrobial agents, such as endodontic irrigants and intracanal medications, into the dentinal tubules (3), as well as interfere with the adhesion of root canal sealers to the root canal walls, thus compromising the quality of the root canal filling (4).

Keeping or removing the smear layer is a highly controversial subject. Nevertheless, it seems that the smear layer itself may be infected and may harbor bacteria within the dentinal tubules (5). This is significant in teeth with infected root canal system where the outcome of the endodontic treatment depends on the elimination of bacteria and their byproducts from the root canal system. In these cases at least, removing the smear layer appears to be of importance (6).

For effective removal of both organic and inorganic components of the smear layer, combined application of sodium hypochlorite (NaOCl) and a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), is recommended (7). The combination of these substances is capable of removing the smear layer, mainly from the middle and cervical thirds (8). However, the application of EDTA for more than 1 minute (9,10) and in volume more than 1 ml (9,10,11) has been reported to be associated with dentinal erosion. It is also noteworthy that chemical interactions between NaOCl and EDTA should be taken into account. Mixing them caused a complete loss of free available chlorine from NaOCl in less than one minute (7). This suggests that in an alternating irrigating regimen, copious amounts of hypochlorite should be administered to rinse out chelator remnants and allow the NaOCl to develop its antimicrobial and tissue dissolving potential. However, the interaction between NaOCl and EDTA makes usage of this two component difficult (12).

In 2003, Torabinejad (9) proposed the use of an irrigant to be used in association with 1.3% NaOCl to remove smear layer from canal walls and facilitate the elimination microorganism from infected dentin (13). This irrigant (MTAD, Dentsply Tulsa Dental, Johnson City, TN USA) is a solution containing a mixture of an antibiotic (doxycycline), an acid (citric acid), and a detergent (Tween-80). Citric acid works as a chelating agent in association with the lower chelating action of the antibiotic, while surfactant is able to facilitate the penetration of the solution into the root canal system. While Shabahang and Torabinejad (13) demonstrated the efficacy of this solution, other studies have shown several important limits. Tay et al. (14) demonstrated that the solution was more aggressive against intertubular dentin, leading to a reduction of collagenic matrix exposed. A new irrigant, Tetraclean (Ogna Laboratori Farmaceutici, Milano, Italy), has been developed containing a mixture of a tetracycline isomer, an acid and 2 detergents. It is recommended to be used as a final rinse after root canal preparation (15). It is similar to MTAD but with a reduced amount of doxicycline (50mg/5ml instead of 150mg/5ml for MTAD), with polypropylene glycol (a surfactant), citric acid, and cetrimide. This substance is supposedly capable of eliminating all bacteria and smear layer from the root canal system when used as a final irrigation.

This study aimed to compare the efficacy of Tetraclean and 17% EDTA in the removal of smear layer from the coronal, middle and apical thirds of instrumented root canals. The null-hypothesis tested was that there are no statistically significant differences between different protocols for smear layer removal.

6.2 Materials and Methods

Sample preparation

Forty human single-rooted teeth with a straight single canal recently extracted for periodontal reasons were selected for the study under a protocol approved by the local ethical committee. Exclusion criteria were: teeth shorter than 20 mm, apex larger than #25 before instrumentation, presence of caries, root fissures or fractures. All teeth were stored in saline at 4°C and used within one month after extraction.

To standardize canal instrumentation, crowns were removed by cutting the teeth 12 mm above the apex, using a water-cooled slow-speed Isomet saw (Buehler, Lake Bluff, IL). Size 10

K-file was inserted into each canal until it was seen through the apical foramen. The working length was established by reducing this length by 0.5 mm. The canals were shaped with nickel-titanium rotary instruments (FlexMaster, VDW, Munich, Germany). Size 30/.06 taper was the last file used at the working length. Irrigation with 5% NaOCl (Niclor 5 Dentale, Ogna, Muggio', MI) was performed during instrumentation using a syringe with a 30-gauge needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland), and the teeth were then randomly divided into four groups (N=10). The exterior part of the apical third of each root was covered with sticky wax to prevent irrigants from dripping through the apical foramen. This was done after placing a calibrated Fine-Medium guttapercha cone (Mynol Curaden Healthcare SRL, Saronno, VA) at the working length in order to avoid wax intrusion into the apex and the cone was removed after the wax had set.

After instrumentation, each group of teeth underwent a specific final irrigation protocol. For group 1 (control), 5% NaOCl was used (3ml); for group 2 (EDTA), 17% \EDTA (3ml, Ogna, Muggio', Milano, Italy) was used for 1 minute followed by 5% NaOCl (3ml); for group 3 (Tetraclean liquid, polypropylene glycol and citric acid), the liquid component of Tetraclean was used for 1 minute (3ml), followed by 5% NaOCl (3ml); and for group 4 (Tetraclean), Tetraclean (powder+liquid, 3ml, polypropylene glycol, citric acid and Doxycycline 50 mg/5 ml) was used for 1 minute followed by 5% NaOCl (3ml). The solutions were introduced into the root canals using a 30-gauge needle (Miraject, Hager Werken, Duisburg, Germany), which penetrated to 1-2 mm of the working length. The root canals were then irrigated with 5ml of distilled water and dried with paper points.

SEM observations

Two longitudinal grooves confined to dentin were prepared on the buccal and lingual surfaces of each root using a diamond disc. The roots were then immersed for 30 seconds in a bowl containing liquid nitrogen, which was sufficient for most of them to generate a separation of the two root halves, otherwise a chisel was introduced into the grooves to separate the two root halves. For each root, the half containing the most visible part of the apex was conserved and coded. The coded specimens were then mounted on metallic stubs, gold sputtered, and examined using a scanning electron microscope (SEM JSM-6060LV, JEOL, Tokyo, Japan). Pictures taken at 500X and 1000X were used to evaluate the coronal (10 mm from apex), middle (6 mm from apex), and apical (2 mm from apex) levels of each specimen. The amount of smear layer remaining on the surface of the root canal or in the dentinal tubules was scored

according to the following criteria (7): no smear layer on the surface of the root canals, all tubules were clean and open (score 1); no smear layer was observed on the surface of root canal, but tubules contained debris (score 2); and smear layer covering the entrances of the tubules (score 3) (figure 1). Approximately 250 scanning electron microscopy photomicrographs were scored by two expert endodontists who were unaware of the coding system in order to exclude observer bias. In the case of disagreement between the operators, the higher score was assigned.

Statistical analysis was performed using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among the groups at $p<0.05$.

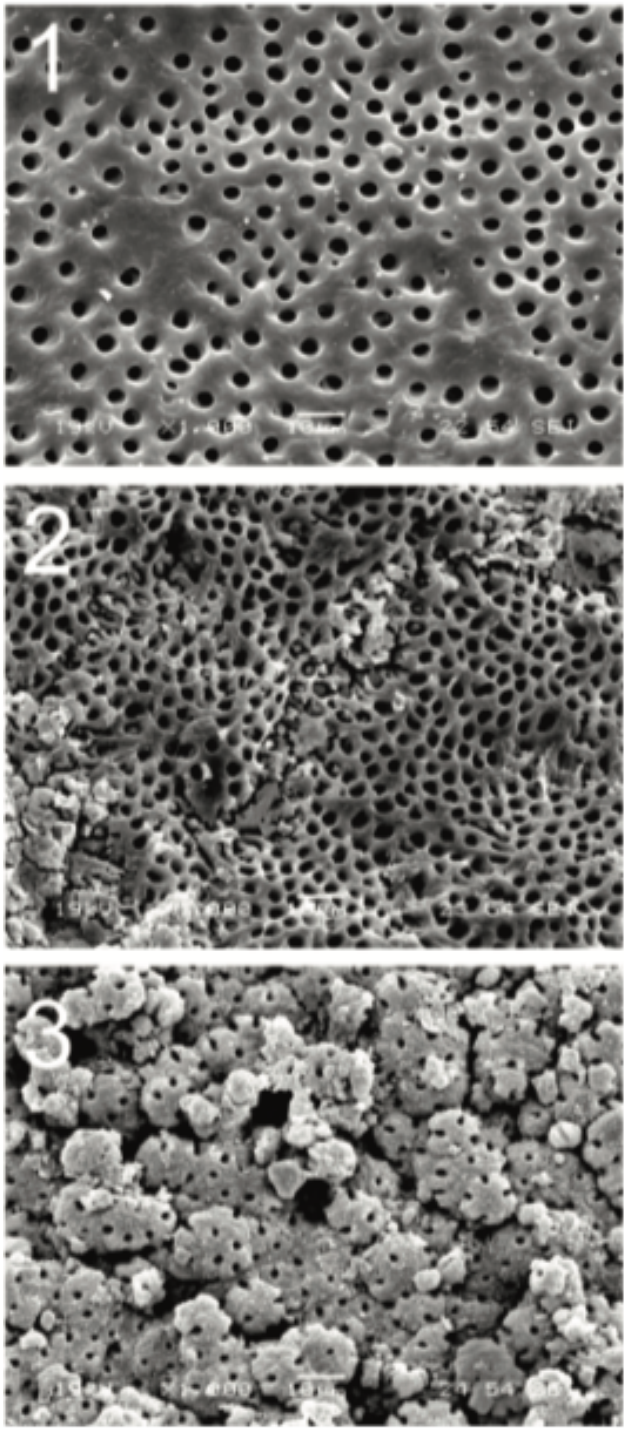

Figure 1: SEM MICROGRAPHS SCORE 1 = No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open; 2 = Moderate smear layer. No smear layer was observed on the surface of root canal, but tubules contained debris; and 3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules.

6.3 Results

One specimen in the control group and one in group 3 were excluded from the study because the canals had been perforated by the disc during the preparation for SEM evaluation. Statistically significant differences were found among the groups in relation to the irrigant

used. When the levels were compounded, groups were ranked in the following order: 1>2≥3=4 ($p<0.05$). For different sections of the canal space, the distance from the apex (2, 6 and 10 mm) influenced the smear layer removal within each group ($p<0.05$).

Analysis of the smear layer removal at different locations revealed that at 10 mm from the apex, the control group showed the highest score without significant differences with group 2. Groups 3 and 4 revealed the lowest scores ($p<0.05$). At 6 mm the result obtained were similar to those at 10 mm but group 4 performed significantly better than group 2 (fig.2) ($p<0.05$). At 2 mm from the apex the control group showed the highest score with a statistical significant difference with all the other groups ($p<0.05$).

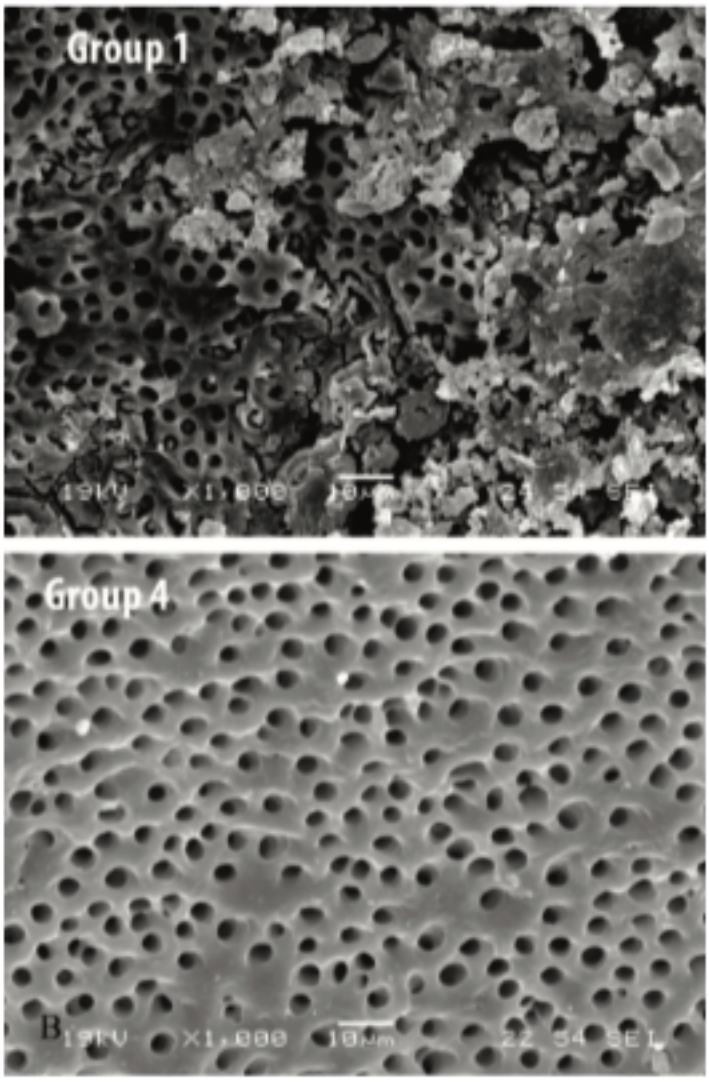

Figure 2: SEM micrographs representing different smear layer removal ability at 6 mm from the apex between group 1 and 4.

6.4 Discussion

The null-hypothesis tested in the study had to be rejected since there were statistical differences between the smear layer removal ability of the different irrigation protocols.

In the present study, 3ml of chelating solutions were used. There is no agreement in the literature concerning the volume of chelating agent or the contact time required in final rinse protocols. (7,9,11) EDTA and Tetraclean were not used according to usually recommended durations but according to experimental ones. As it has been shown that EDTA is effective in removing smear layer without affecting intra and peritubular dentin (11) 1min application of EDTA was chosen as protocol, and tetraclean application time was mirrored to that of EDTA. It is noteworthy that different application times might yield different results.

The results of the present study are in accordance with other studies showing that NaOCl is not effective in removing the smear layer (7,9,11) when used without a chelatant. When considering the whole root canal it was evident that the use of a chelatant was imperative for removing the smear layer. Tetraclean is a helpful solution for the removal of the smear layer when used as a final rinse ex vivo: it promotes clean canal walls, with absence of smear layer and opened dentinal tubules, without changing the structure of dentine (16). In this study, a final rinse of each canal was performed by using 3 ml of 5% NaOCl for all the experimental groups to standardize final irrigation protocols. Because this study examined only the efficacy of different protocols for smear layer removal, further studies should be conducted to examine the effect of 5% NaOCl final rinse on antimicrobial effectiveness of doxycycline component in Tetraclean and its substantivity. The liquid component of Tetraclean has been proposed for the final rinsing step, followed by 5%NaOCl (group 3), for understanding the chelating action when citric acid works with surfactants, estimating an optimal time-effect relationship for the clinical application. De Deus et al.(17) reported that demineralization kinetics promoted by 10% citric acid is faster than for 17% EDTA as demineralizing substance: real-time observation of the demineralization process in radicular dentine 17% EDTA promoted much weaker demineralization and caused less peritubular and intertubular dentine erosion when compared with 10% citric acid. The association of a powder and a liquid (group 4) is even more effective in cleaning the root canal walls. This is possibly due to the presence of an antibiotic with chelating action in the powder. Doxycycline has been used in periodontal treatments because of its antibacterial and chelating ability as well as its substantivity (18). Barkhordar et al (19) and Haznedaeroglu and Ersev (20) recommended

the use of tetracycline hydrochloride to remove the smear layer from the surface of instrumented canals and root-end cavity preparations.

At 6 mm from the apex, groups 2 and 3 gave better results than control group, and group 4 revealed statistically significant differences with all the other groups: this can be explained by the addition of a powder containing a tetracycline isomer which has a chelating action and improves the penetration ability of the solution into this narrow region of the root canal. However at 2 mm from the apex, groups 2, 3 and 4 were not statistically different, and gave lower scores when compared to the control group. At this level, the presence of the surfactant agent should have improved the penetration of the solution into dentinal tubules however, no significant differences were detected. Although images from groups 4 revealed better smear layer removal than group 2, the sample size was probably too small to allow detection of differences between these groups. The current study showed that the process of smear layer removal was more efficient in the coronal and middle thirds than in the apical third of the canals. This finding is in agreement with the results of various studies that have shown an effective cleaning action in the coronal and middle thirds of the canals even when different irrigation times and volumes of solutions were investigated (7). A larger canal diameter in the coronal and middle thirds exposes the dentin to a higher volume of irrigants, allowing a better flow of the solution and, hence, further improving the efficiency of smear layer removal (7). Consequently, it is important to use other methods, such as ultrasonic devices, for improving the efficiency of low-volume chelating agents used for a short application time (22). From another standpoint, Mancini et al (21) showed that the apical third is always the least cleaned as it is likely to receive less volume of irrigant when compared to the more coronal portion of the canal. In a recent study Poggio et al (16) investigating by SEM image analysis the endodontic dentinal surfaces after canal shaping with Ni- Ti instruments and irrigating with 5.25% NaOCl + different irrigating solutions as final rinse showed that NaOCl+Tetraclean group had significantly lower scores than other groups were in accordance with present study.

It is evident that increasing the instrument taper will allow a deeper penetration of the irrigation needle and improve the flushing of debris (23). Shuping et al (24) found a better antibacterial effect using nickel-titanium (NiTi) instrumentation when NaOCl was used, but only after instrumentation exceeded ISO size #30 to #35. To overcome the potential limited irrigation in the apical area, enlargement of this area has been advocated for better cleansing (25). For this reason it was decided to prepare the apical foramen of the samples to #30 in

order to be able to compare the outcome of the present study with other studies in literature.

It is noteworthy that when an antibiotic is included in the formulation of the irrigant, the possibility of increasing the microbial resistance to that antibiotic should be taken into account. Several mechanisms including oxygen limitation, antibiotic penetration, and the presence of a small subpopulation of 'persister' cells, could be responsible of antiobiotic susceptibilities (26).

Therefore it can be concluded, within the limitation of this ex-vivo study, that the use of a chelating agent leads to a higher removal of smear layer from the root canal walls. Differences between EDTA and Tetraclean were only evident at 6 mm from the apex, whereas at 2 mm both protocols had similar performances in smear layer removal from the root canal system of single-rooted permanent teeth.

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Chapter 7

Influence of surfactant and PUI on the effectiveness of NaOCl for final rinse optimization

7.1 Introduction

The elimination of microorganisms from infected root canal systems (RCS) is a complex task involving the use of various instrumentation techniques, irrigation regimens and intracanal medicaments. Obtaining a bacteria-free root canal system is difficult mainly due to the complex anatomy of the root canal system (1). In fact, *ex vivo* and clinical evidence has shown that mechanical instrumentation leaves significant areas of the root canal walls untouched (2) and complete elimination of bacteria by instrumentation alone is thus unlikely to occur (3). Therefore chemical disinfection using irrigation is necessary to remove residual tissues and to kill microorganisms. Among currently used solutions, sodium hypochlorite (NaOCl) appears to satisfy most of the requirements for a root canal irrigant (4). It has the unique capacity to dissolve necrotic tissue (5) and the organic components of the smear layer (6). It also kills sessile endodontic pathogens organized in biofilms and in dentinal tubules as efficiently as chlorhexidine or iodine at a comparable concentration (7). It inactivates endotoxins (8) and also disintegrates endodontic biofilms (9,10). Despite its excellent tissue-dissolving and antimicrobial abilities, NaOCl possesses some drawbacks such as high surface tension limiting its penetration into canal irregularities or dentinal tubules. Stojicic *et al* (11) have shown that hypochlorite with added surface active agent had the lowest contact angle on dentin and was most effective in tissue dissolution in all experimental situations. In this spirit, a recently a new modified sodium hypochlorite solution was introduced (Hypoclean, OGNA, Laboratori Farmaceutici S.p.A. Muggiò, Italy) composed of 5.25% NaOCl and two detergents with addition of surfactant. In addition, the use of an irrigant in conjunction with ultrasonic vibration is directly associated with the cleaning effectiveness of the canal space (12,13). This could reduce the time needed for the antimicrobial efficacy of the irrigating solution. The purpose of this study was to assess the penetration of a modified sodium hypochlorite solution labelled with 0.2% alizarin red into dentinal tubules when used in root canals with

PUI activation. The null hypothesis tested was that modification of the irrigant solution or PUI does not change in irrigant penetration.

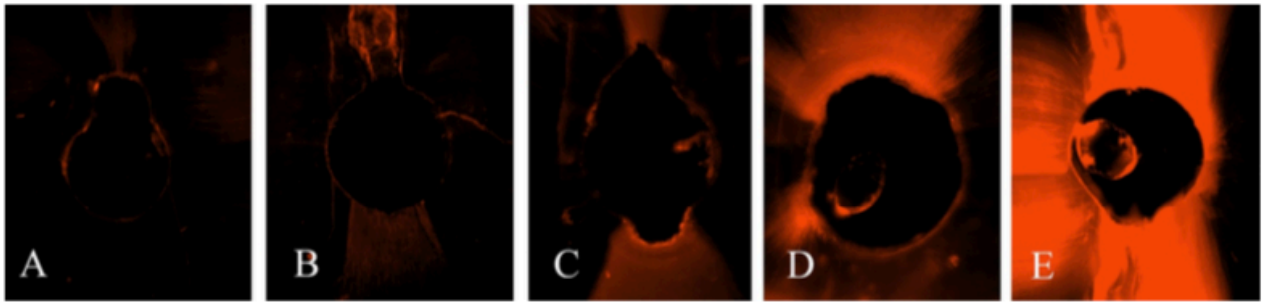
7.2 Materials and Methods

Forty recently extracted human single-rooted teeth (maxillary central incisor) with a straight single canal in 35 patients 40 to 50 years old were selected for the study under a protocol approved by the University of Siena (Italy). Exclusion criteria were as follows: teeth shorter than 20 mm; apex larger than #25 before instrumentation; and presence of caries, root fissures or fractures. All teeth were stored in saline at 4°C and used within 1 month after extraction. To standardize canal instrumentation, crowns were removed by cutting the teeth 2 mm above the cementoenamel junction using a water-cooled slow-speed Isomet saw (Buehler, Waukegan Road, Lake Bluff, IL). The working length was established visually by inserting size 10 K-type file (Dentsply, Maillefer, Verger 3, Ballaigues, Switzerland) into each canal until its tip was seen at the apical foramen and reducing this length by 0.5 mm. The canals were shaped with nickel-titanium rotary instruments (FlexMaster, VDW, Munich, Germany) to size 30/.06 taper. Irrigation with 5% NaOCl was performed during instrumentation using a syringe with a 30 gauge needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland) at 2 mm from the working length. Smear layer removal was achieved after irrigation with 3 mL of 17% EDTA (OGNA) for 2 minutes followed by 3mL of sterile saline. The teeth were randomly divided into four groups (n=10). The exterior part of the apical third of each root was covered with wax to prevent irrigant from dripping through the apical foramen. This was done after placing a calibrated fine medium gutta-percha cone (Mynol, Ada Products Company, Milwaukee, Wisconsin) at the working length in order to avoid wax intrusion into the apex. The cone was removed after the wax had set. A final rinse of each canal was performed by using 5 mL of 5% NaOCl (OGNA) labeled with 0.2% alizarin red using the 30 gauge endodontic needle at 2 mm from the working length for group 1 and 2, and 5 mL of Hypoclean labeled with 0.2% alizarin red using the 30-G endodontic needle at 2 mm from the working length for group 3 and 4. To standardize the procedures for all teeth, a flux of 1mL/30 seconds was used for 90 seconds. The following groups had a different agitation procedure during the final rinse: (1) control group: no agitation (NaOCl without activation), (2) NaOCl+PUI group: agitation with Passive Ultrasonic ESI File (EMS, Nyon, Switzerland) with power setting at 5 for 20 seconds at 2 mm from the working length, (3) Hypoclean group: no agitation, (4) Hypoclean +PUI group: agitation of Hypoclean with Passive

Ultrasonic ESI File (EMS, Nyon, Switzerland) with power setting at 5 for 20 seconds, at 2 mm from the working length.

After drying the canal with paper points, each specimen was cut into three 1mm thick slabs at 1, 3, and 5 mm from the apex, the thickness of the root slices was verified using a digital caliper. Slabs were then bonded onto glass slides and ground with wet silicon carbide papers to approximately 40μm thick. The slides were examined with a fluorescence light microscope (Nikon Eclipse; Nikon, Tokyo, Japan) at 100x with a wavelength of 540 to 570 nm. If the whole canal could not fit completely in one image, two or more partial images were taken to produce a montage using Adobe Photoshop CS3 (Adobe Systems Italia S.r.l, Milan, Italy). Images from all specimens were evaluated by two operators that were blinded to the procedure. In case of disagreement between the operators, the unfavorable score was assigned. The penetration of the irrigant solution into the dentinal tubules was assessed semi-quantitavely (Fig. 1): ''A''= 0 = no visible penetration, ''B''= 1 = minor traces of dye, ''C'' = 2 = superficial traces of dye on the whole periphery of the section, ''D'' = 3 = penetration of dye <50% of the dentinal tubules, and ''E'' = 4 = penetration of dye >50% of the tubules. Additional specimens were prepared as controls: negative control without adding 0.2% alizarin red to the final rinse solution and positive control: immersed in 0.2% alizarin red for 10 minutes to investigate dye uptake patterns. Statistical analysis was performed using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among groups at a=0.05. Data were investigated either pooled or separately.

Fig.1 Dye penetration scores based on the extent of fluorescence observed from dentinal tubules. "A" = (Score 0) : no visible alizarin red; "B" = (Score 1): minor (incomplete) traces of alizarin red along the surface of the canal wall; "C" = (Score 2): traces of alizarin red along the entire circumference of the canal wall; "D" = (Score 3): penetration of alizarin red in less than 50% of the dentinal tubules; "E" = (Score 4): penetration of alizarin red in more than 50% of the dentinal tubules.

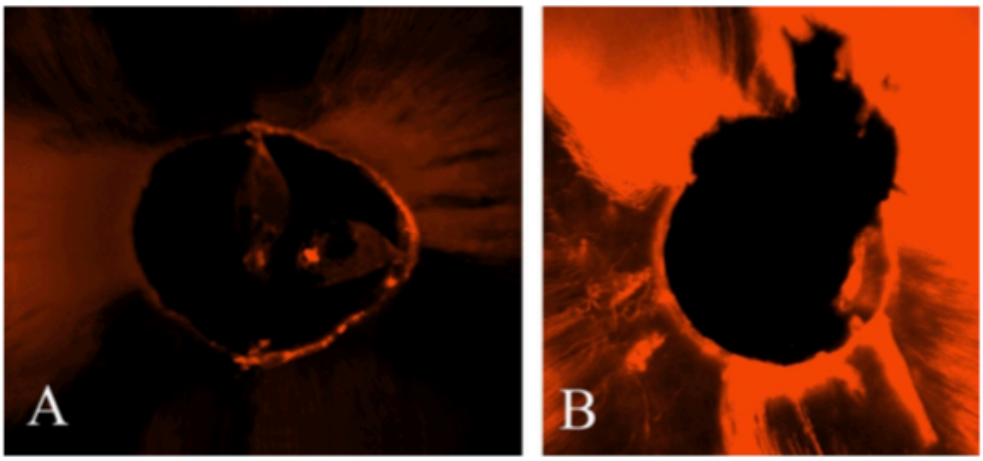


7.3 Results

No fluorescence was found in negative controls, whereas the intense presence of dye tracing within dentinal tubules was recorded in positive controls. Statistically significant differences

were found among experimental groups in relation to the irrigation regimen used. When results from the 3 sections were pooled, groups ranked in the following order: control = Hypoclean < control+PUI = Hypoclean+PUI ($p<0.05$). When taken separately, non-PUI groups displayed similar alizarin red penetration irrespective of the distance from the apex ($p>0.05$). Results also revealed that at 1 mm from the root apex, the PUI groups were significantly different ($p<0.001$) from non PUI groups (Fig 2), however, there was no difference between PUI groups. While the control groups exhibited the lower score. At 3 and 5 mm from the root apex, both PUI groups and Hypoclean group yielded similar scores that were significantly higher than the control groups ($p<0.001$).

Fig.2 A representative example of fluorescence exhibited by Group 1 (A) and Group 4 (B) at 1 mm from the apex. In group 4, evidence of alizarin red could be identified from the entire canal wall circumference and with penetration of the fluorescent dye into the patent dentinal tubules. In group 1 traces of alizarin red could be partially identified along the canal wall circumference, with no penetration into the dentinal tubules.



7.4 Discussion

In this study, alizarin red was used to label NaOCl. This method was validated and used in a previous study (14). Tracing NaOCl penetration into dentinal tubules with fluorescence microscopy enabled us to evaluate the effect of chemical modifications and agitation techniques and the degree of penetration of the irrigant inside the dentinal tubules in the apical region of the root canal space. The null hypotheses tested were partially rejected because differences were found in irrigant penetration using the modified sodium hypochlorite solution without activation but no differences were found when irrigants were used in combination with PUI.

Adequate application mode and sufficient volumes are required to reduce working time in the main canals, but antimicrobial effect of NaOCl depends on its ability to reach the irregularities of root canal systems and penetrate inside dentinal tubules (5). Moreover, its wettability on solid dentin (15) is strictly correlated to its surface tension (16). The surface tension is defined as "the force between molecules that produces a tendency for the surface area of a liquid to decrease" (17). This force tends to limit the ability of the liquid to penetrate a capillary tube, which is why endodontic irrigants should have a low surface tension. It was reported that to achieve optimal wettability, the surface energy of the substrate must be as high as possible and the surface tension of the liquid contacted with substrate must be as low as possible (18,19). The spreading of root canal irrigants, therefore, appears to change depending on the properties of dentine surfaces. Dentine is composed of 2 different substrates: collagen, which has a low surface energy, and hydroxyapatite, which has a high surface energy (20). It should also be noted that when using irrigant solutions on a dentin surface is succession or alternation, the first conditioning solution will change the surface properties of the substrate and therefore affect the wettability properties of subsequent irrigating solutions (21).

By improving the wettability, an irrigant antimicrobial solution could increase its protein solvent capability and enable better activity in uninstrumented areas of the root canal system (22). Abou-Rass and Patonai (23) found that Polysorbate 80 reduced the surface tension of distilled water, NaOCl, EDTA, and alcohol by 15 to 20%, thereby enhancing the flow and penetration of the test solutions into the irregularities of the root canal system. Furthermore, Mohammadi *et al* (24) compared the antimicrobial efficacy of modified NaOCl and 5.25% NaOCl solution against *Enterococcus faecalis* in bovine root dentin *in vitro*, at all experimental periods, modified NaOCl demonstrated more effective antibacterial action than 5.25% NaOCl:

modified NaOCl group showed no bacterial growth after treatment. However, reducing the surface tension in irrigants used during instrumentation may also cause an increased penetration of smear material into the dentinal tubules: Aktener *et al* (25) indicated that a solution or an agent with low surface activity should not be used in the root canals during instrumentation, otherwise the smear material would show deeper penetration into the dentinal tubules. The results of the present study did not show any effect by modifying the surface tension of the irrigant. This could be due to the wettability of dentin described by Perdigao (26). This is agreement with the results obtained by Zehnder (4). As for the absence of penetration of the irrigant in the apical 1mm in non-PUI groups, it could be due to the vapour lock effect described by Senia *et al* (27), and Tay *et al* (28). It is also noteworthy to indicate that absence of penetration in dentin tubules can also be attributed to the presence of sclerotic dentin (29,30).

Passive ultrasonic irrigation has been shown to improve the efficacy of irrigating solutions by dislodging organic and inorganic debris from root canal walls (31). The technique relies on the transmission of acoustic energy from an oscillating file or smooth wire to an irrigant in the canal space, and these ultrasonic waves could in turn induce acoustic streaming of the irrigant (32). This could possibly explain the improved irrigant penetration into nonsclerotic tubules within the apical third due to better flow and increased irrigant volume (27).

Because of the vapor lock phenomenon that occurs in the apical third of closed-end root canals (33), it is prudent to elaborate on why better dye penetration was observed in the ultrasonic groups. Using a control and an experimental balanced design to compare the effect of vapor lock on the efficacy of canal debridement from the apical 0 to 2 mm of the canal walls, we recently observed that the use of NaOCl and EDTA was able to remove smear layers from that region irrespective of the presence or absence of a vapor lock (ie, same ‘‘smear score’’). However, canals that simulated the presence of a vapor lock exhibited a significantly higher ‘‘debris score’’ compared with those simulating the absence of a vapor lock (28). Because this study examined only dye penetration into dentinal tubules (ie, smear layer removal), it is understandable that the ultrasonic agitation techniques produce better results.

Within the limitations of the present study, Hypoclean showed a better result when was used without activation, but no differences were found between the solutions when PUI was used. Further investigations regarding intratubular penetration and antibacterial power of new low surface tension endodontic irrigants should be performed.

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Chapter 8

The influence of PUI in eradicating EF in chemomechanical root canal disinfection

8.1 Introduction

The presence of microorganisms in the root canal system (RCS) is a key factor in the development of periapical lesions (1), hence, endodontic treatment procedures aim at completely eliminating the microorganisms from the root canal. However, this proves to be very difficult not to say impossible to achieve due to the anatomic complexites and the subsequent limitations of both instruments and irrigants to reach all aspects of the RCS (2). Chemomechanical detersion techniques more realistically aim at reducing bacterial populations below the threshold required to induce or sustain disease (3). Sodium hypochlorite (NaOCl) has been widely used since its introduction in endodontics by Walker in 1936 (10). Besides its bleaching, deodorant, and tissue dissolution effect, NaOCl has been proven to be an effective disinfectant (11). Furthermore, several studies have also suggested the use of ultrasonics as a mean to improve the effectiveness of the irrigant (12). Passive ultrasonic instrumentation (PUI) has been shown to be more effective than other irrigation systems in removing tissue remnants and dentinal debris from the main root canal as well as from irregularities (13).

A member of the endodontic flora, Enterococcus faecalis, displays resistance to endodontic treatment. E. faecalis has been frequently selected for experimental studies of endodontic infection (4) because it is often associated with treatment failure (5) and commonly expresses multiple drug resistance (6). E. faecalis is a gram-positive facultative anaerobic coccus commonly found in cases of failure in endodontic therapy (7). Its prevalence is higher in persistent infections than in primary infections (8). This can be explained by its ability to withstand prolonged periods of nutrient deficiency, allowing it to persist as a pathogen within the root canal (9).

Therefore, E. faecalis was chosen to evaluate the residual antimicrobial activity of five final irrigation regimens in root canals contaminated with E. faecalis biofilms. The null hypothesis tested in this study is that there is no difference between the five irrigation regiments.

8.2 Materials and methods

Sample Preparation

Forty freshly extracted single-rooted teeth were obtained and autoclaved individually at 121°C for 15min. To prepare the root canals, the crowns of all teeth were removed, and the root lengths standardized to 15mm. Then, patency of each canal was established with a size 10 K-File (Dentsply Maillefer, Ballaigues, Switzerland). The canals were prepared with reciproc R25 files (VDW, Dentsply Maillefer, Ballaigues, Switzerland) to the full working length. Irrigation with 5% sodium hypochlorite solution was performed throughout the instrumentation. The canals were rinsed copiously with sterile saline solution to flush away residual irrigants and each root was placed into a block of freshly mixed silicone impression material (Elite HD, Zhermack, Via Bovazecchino 100, 45021 Badia Polesine (RO), Italy) and molded inside a glass specimen jar ensuring that the coronal end of the prepared root was flush with the surface of the silicone block. Each root was then sectioned longitudinally through the root canal using a 0.3mm-thickness diamond wafering blade mounted on an Isomet low-speed saw (Buehler, Lake Bluff, IL).

One half with the most uniform visible canal from each root was selected for biofilm growth. The root halves were placed back into their corresponding silicone index, to ensure good reapproximation. Both silicone blocks and the chosen root halves were marked, so that the correct orientation of each root in its block could be ensured. The marked root halves were then removed from the silicone indices and immersed in a 17% solution of EDTA for 1min to remove the smear layer, after which they were washed thoroughly with water. The corresponding root halves were then re-autoclaved as previously described. Following autoclaving, the root halves that were not used for biofilm growth were left hydrated in labelled sterile flasks.

An E. faecalis biofilm (ATCC29212) was grown on each selected root half using a standardized biofilm growth protocol. The strain was cultured anaerobically at 37°C on Fastidious Anaerobe Agar (LabM, Bury, UK) supplemented with 5% defibrinated horse blood. Starter cultures were set up in filter-sterilized modified fluid universal medium (mFUM), which were incubated anaerobically at 37°C for 3h, until the growth appeared moderately turbid. The turbidity was adjusted with fresh mFUM to an optical density of 0.5 at 540nm (Labsystems iEMS Reader MF, Basingstoke, UK). Each root was incubated with 3mL of the E. faecalis culture in 24-well trays, and the medium was replaced after 24, 48, 72, 96, 120, 144,

168, 192h. After the final replacement, the tray was removed from the anaerobic cabinet and stored at 4°C. Preliminary studies had shown that after 72 h, the biofilm was uniformly present over the surface of the root canal. The 40 root half pairs were reapproximated and divided into 5 groups, each consisting of 8 roots. Group A was irrigated with 4.5mL of 5% sodium hypochlorite solution energized with size 25 ultrasonic file (VDW). Irrigation sequence in this group was performed in 3 sequences of 1.5mL irrigation and 20s passive ultrasonic activation. Experimental group B was irrigated by 4,5 mL of 5% sodium hypochlorite solution energized with a size 25 ultrasonic file. The irrigation sequence for this group was performed in 2 sequences of 2.25mL irrigation, and 20s passive ultrasonic activation. Group C received 6mL irrigation of 5% sodium hypochlorite solution. Negative control groups D and E received respectively irrigation with 6mL of sterile saline solution and no irrigation. Irrigation was carried out with a 27-gauge side-venting irrigating needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland) and 3mL syringe (Monoject, Tyco Healthcare, Gosport, UK). A stabilized 5% sodium hypochlorite solution (Ogna, Muggio', Milano, Italy) was used for the experimental groups. Penetration of irrigating needles was controlled to 12mm using a silicon stopper. Irrigation was performed using digital pressure with the forefinger only, and the needle was gently moved back and forth in the canal ensuring that the needle did not bind in the canal itself. Irrigation time was 2 minutes in all relevant groups. Power setting on the ultrasonic unit (VDW Ultra) was kept at one quarter of the maximum setting and the file was inserted 1.5mm shy of the working length. All shaping and irrigation procedures were performed by the same operator.

After completion of the respective irrigation protocols, roots were immediately immersed in a phosphate-buffered glutaraldehyde fixative for 4h followed by glutaraldehyde wash solution. The samples were subsequently immersed in ethanol for 10min at successive concentrations of 10%, 50%, 70%, 90% and completed with two cycles at 100%. Specimens were then immersed for 15min in one part of hexamethyldisilazane (HMDS) (Panreal S.A.U., Castellar de Vallès, Barcellona, España) to two parts of absolute ethanol, followed by a 15min immersion in one part HMDS to one part 100% ethanol. This was also followed by 15min in one part 100% ethanol to two parts HMDS, and finally two cycles of 20min in 100% HMDS. The specimens were dried on clean lint-free tissue. The root halves were then mounted on stub plates and gold-sputtered (Polaron E5100; Quorum Technologies, Ringmer, UK). Three scanning electron microscopy (SEM) images were taken for each sample along the midline of the canal at 9mm from the apex (coronal area), 6mm from the apex (middle area), and 3mm from the apex (apical area) at x700 and at x2000 magnification.

Scoring system

Images from all specimens were evaluated by three operators, who were not aware of the purpose of the study. A 4-point scoring system was devised to allow the E. faecalis biofilms to be assessed semi-quantitatively: 1= less than 5% biofilm coverage of the root canal walls, 2= biofilm coverage between 5–33% of the root canal walls, 3= biofilm coverage between 34–66% of the root canal walls, and 4= biofilm coverage between 67–100% of the root canal walls. The 3 examiners were calibrated using several images obtained during preliminary studies. In the case of a disagreement between the operators, the lower score was assigned.

Data alalisys

Statistical analysis was performed using Kruskall-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among the groups at a=0.05.

8.3 Results

The scores obtained for each group are given in tables 1, 2 and 3.

No significant differences were observed in the scores between the three levels (coronal, middle and apical) of observation in any of the five groups.

There were no significant differences between the scores for group A (conventional syringe irrigation with 4,5 mL of 5% sodium hypochlorite solution energized with a size 25 ultrasonic file, three cicles of PUI/20s), for group B (4,5 mL of 5% sodium hypochlorite solution energized with a size 25 ultrasonic file, two cicles of PUI/20s) and group C (conventional syringe irrigation with 6 mL of 5% sodium hypochlorite solution) at any of the three levels (Tables 1, 2, 3)

There was a significant difference between experimental groups (groups A, B & C) and group E ($P < 0.001$) at all three levels (Tables 1, 2, 3). Both conventional syringe irrigation and passive ultrasonic irrigation with 5% sodium hypochlorite were more effective at removing the biofilm than group not exposed to any irrigant.

At 3, 6 and 9mm from the root apex, Group A exhibited the highest score and was significantly different ($p<0.001$) from Groups D and E. There was no difference between Group A and Group B (Tables 1, 2, 3). At 6 and 9mm from the root apex, Group B yielded similar scores that were significantly higher than groups D and E ($p<0.001$). groups where PUI with 5% sodium

hypochlorite was used were more effective at removing the biofilm than conventional syringe irrigation with sterile saline solution.
No significant differences were observed in the scores between group C and group D at the three levels (coronal, middle and apical).
Control group E (no irrigation) proved to be effective in biofilm covering at all three levels of the root canal in the eight observed specimens.

8.4 Discussion

This in vitro study investigated the ability of five final irrigation regimens used after chemo-mechanical procedures to disinfect root canals contaminated with E. faecalis biofilms.
The model of biofilm formation used in this study has already been reviewed and reported in previous articles that focused on antimicrobial strategies against biofilms. However, there is still no consensus in the literature regarding the formation time of this biofilm. Some studies used 24 hours (14–17), whereas others used 48 hours (18), 72 hours (19), 21 days (20), and even 6 weeks (21). In this study the teeth were incubated with E. faecalis for 8 days to ensure the penetration of the bacteria into the dentinal tubules, which was confirmed by SEM evaluation.
Scanning electron microscopy has frequently been used to observe intraradicular biofilms (22, 23, 24). One limitation of the SEM may be that only a topographic localization of the structures observed is possible. The resulting images are only pseudo-three dimensional, and since the biofilm is stratified on various levels, the SEM is not suitable to identify the depth of such structures. For this reason, with this technique you can only make qualitative and semi-quantitative observations. Clearly, although this method is not representative of the true distribution of the biofilm on the entire root canal surface, the high number of observations made in this study should have compensated for this limitation. The irrigation effect for each protocol was evaluated at three levels for each sample. The reference points were taken at 3, 6 and 9 mm from the apex, these points were referring respectively to the apical, middle and coronal root canal.
There were no observed differences between the three levels in any of the experimental or control groups.
In the experimental group (A & B) subjected to passive ultrasonic irrigation with 5% sodium hypochlorite solution, the total irrigation time was two minutes for each root, but included three 20 s cycles for group A and two 20 s cycles for group B of passive ultrasonic irrigation.

The protocol for conventional syringe irrigation used in this study was again two minutes in duration, with a volume of 6 mL of 5% sodium hypochlorite solution being used for each root.
Two control groups were used in the study. The first control group (D) was exposed to 6 mL of conventional syringe irrigation with sterile saline solution, with the same protocol being used as for the syringe irrigation with sodium hypochlorite. The purpose of this control group was to assess the mechanical flushing effect of an irrigant with no antibacterial or tissue-dissolving properties. The second control group (D) was not exposed to any irrigant. The purpose of this group was to demonstrate the reliability of the biofilm growth at all three levels of the root canal. The assessed SEM images were acquired at a magnification of X700. This was the lowest magnification at which the biofilm images could be accurately evaluated. Use of this magnification permitted the maximum possible surface area to be assessed with each image.
Conventional syringe irrigation with sterile saline solution was only partially effective at biofilm removal, with persistent bacteria frequently evident not only on the root canal wall, but also within the dentinal tubules.
The use of ultrasonics as an aid in root canal irrigation has been suggested as an alternative to improve cleaning and disinfection of the root canal system (25, 26, 27). However, in this study, no significant differences were found between the groups that used NaOCl with or without ultrasonic agitation. The ability of both conventional syringe and passive ultrasonic irrigation with sodium hypochlorite to completely remove the intraradicular biofilm demonstrates that both of these protocols were effective in obtaining clean root canal walls.
These results are consistent with the findings of Siqueira et al (11) and Bhuva et al (19), who also found no difference between conventional irrigation with NaOCl and the ultrasonic passive irrigation using this irrigant.
The efficacy of ultrasonic passive irrigation in cleaning areas unreachable by endodontic instruments has been tested in other studies using simulated lateral canals (28, 29) and irregularities (30) created in human teeth. The artificial production of these inaccessible areas may help to explain the superiority of ultrasonic irrigation found in these studies because irregularities or artificially created lateral canal are larger than dentinal tubules, which benefit from the irrigating solution and ultrasonics. However, it must be considered that these studies only assessed the efficacy of the irrigation techniques on the visual cleanliness of the artificial grooves rather than the removal of bacteria, particularly those within a biofilm (28, 31).

Within the limitations of this in vitro study, both conventional syringe irrigation and passive ultrasonic irrigation with sodium hypochlorite solution were completely effective at removing the E. faecalis biofilm from the root canal walls of extracted human teeth. Conventional syringe irrigation with sterile saline solution was only partially effective at removing the biofilm.

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Chapter 9

Summary and future directions

The purpose of the present thesis was to evaluate/correlate different aspects of the endodontic treatment in order to shed some light on the influence of microorganisms disinfection during root canal treatment.

Although mechanical instrumentation reduces bacteria from human root canals by approximately 50%, disinfecting irrigants are needed to eliminate the microbiota in locations where instruments cannot access (1-3). Although NaOCl is an effective disinfectant when it comes into direct contact with bacteria biofilms, it produced clean and debris-free dentin surfaces only in the coronal and middle thirds but not in the apical third of the canal wall when used in conjunction with nickel-titanium instruments (4). Consequently, different irrigant agitation techniques have been proposed to increase the efficacy of the irrigant solutions.

In our research Passive ultrasonic irrigation (PUI) was found to be more effective in delivering sodium hypochlorite inside the dentinal tubules. (5) PUI improves the efficacy of irrigating solutions in removing organic and inorganic debris from root canal walls (6, 7).

Another aspect to consider for the success of root canal therapy is the presence of the smear layer. However, even after cleaning and shaping, total sterilization of the root canal system remains difficult to achieve (8). Studies have shown that mechanical instrumentation of root canals implies the formation of a smear layer covering the dentinal walls (9) and containing both inorganic and organic materials (9). The presence of the smear layer may considerably delay or prevent the penetration of antimicrobial agents, such as endodontic irrigants and intracanal medications, into the dentinal tubules (10), as well as interfere with the adhesion of root canal sealers to the root canal walls, thus compromising the quality of the root canal filling (11).

In our research the association of a chelating agent, such as Tetraclean, was found to be effective in leading to a higher removal of smear layer from the root canal walls.

Microbiota are found in highly organized and complex entities, known as biofilms, the characteristics of which are fundamentally different from microbes in planktonic suspensions. Root canal infections are biofilm mediated. The anatomical complexity of the root canal

system, together with the multi-species nature of biofilm, make disinfection of this system extremely challenging. Microbial persistence appears to be the most important factor for failure of root canal treatment and this could further have an impact on pain and quality of life. Biofilm removal is accomplished by a chemo-mechanical process, using specific instruments and disinfecting chemicals in the form of irrigants and/or intracanal medicaments. Endodontic research has focused on the characterization of root canal biofilms and the clinical methods to disrupt the biofilms in addition to achieving microbial killing. Ultrasonic agitation can cause deagglomeration of the bacterial biofilm, thus re-suspending the bacteria in planktonic form which are then, more susceptible to antimicrobial irrigants (12, 13).

Also, any cavitation that may be produced, would cause temporary weakening of the cell membrane, thereby increasing the bacterial cell permeability to antimicrobial irrigants (14).

In our research passive ultrasonic irrigation with sodium hypochlorite solution has proven to be completely effective in the removal of the E. faecalis biofilm from the root canal walls of extracted human teeth.

Despite the increasing knowledge of the microbial status of root canal systems, much still remains unknown. The reported success rates of root canal treatment have not undergone significant improvement.

From the clinical perspective, it is important to understand the etiopathogenesis of periradicular periodontitis as a disease caused by microbial infection of the root canal system. Even though it is known that root canal biofilms are complex, the literature unfortunately does not seem to offer due credence to understanding the dynamics between the components of a biofilm. Crosstalk between bacteria is a paradigm that has not be sufficiently studied thus far in the context of endodontic disease.

Further studies should be conducted to examine the effect of different agitation techniques on the ‘debris formation and on the “Enterococcus Faecalis”.

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Roma, XIII Congresso Nazionale SIDOC, 19-20-21 Febbraio 2009
Comunicazione Scientifica VII Premio Pietro De Fazio:
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3) **Paragliola R**, Chazine M, Porciani PF, Ferrari M, Grandini S
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dichiaro che

tutto quanto è contenuto nel curriculum vitae soprariportato corrisponde a verità ai sensi delle norme in materia di dichiarazioni sostitutive di cui agli artt. 46 e seguenti del D.P.R. 445/ 2000.

In fede,

Raffaele Paragliola

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