Quantitative assessment of covariants of root canal treatment efficacy in human teeth

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	Chapter 2 (Biofilm attachment)	**Chapter 3 (File motion)**	**Chapter 4 (File design)**	**Chapter 5 (File reuse)**
Study design	VSM, RS, PG	VSM, RS, PG	VSM, RS, PG, AJM	VSM, RS, PG
Experiments	VSM, PG	VSM	VSM	VSM
Statistical analysis	VSM	VSM, RS	VSM, RS, AJM	VSM, RS
Thesis and paper preparation	VSM	VSM	VSM	VSM
Thesis and paper review	RS, PG	RS, PG	RS, PG, AJM	RS, PG

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Abstract

Clinically relevant cofactors that can demonstrate aspects of root canal treatment quality are of importance to clinicians, researchers and dental instrument manufacturers. Endodontics has been one of the most developing fields of dental science in recent years. There have been new instruments, materials, and methods introduced, which have been very rapidly adopted since most facilitate the root canal treatment process. Considering the current rate of technological developments and the long-term follow-ups required for clinical evaluation of root canal treatment success, clinical trials are not feasible for assessing every variable in treatment. In search of cofactors that could be used to demonstrate the efficacy and quality of a root canal treatment, the effect of surface roughness was investigated in the present thesis.

Clinical relevance of surface roughness and its effect on endodontic treatments was assessed in the second chapter. This aim was achieved by comparing biofilm formation on rough and smooth dentine surfaces. *Enterococcus faecalis* was the microorganism tested to form biofilms on these surfaces because of its role as one of the most important endodontic pathogens in persistent endodontic infections. A novel methodology utilizing flow cytometry to quantify bacteria attached to the surfaces was designed for this experiment. The results showed that rough surfaces harboured a significantly higher number of bacteria compared to smooth surfaces. This indicated that achieving a final smooth surface in root canal treatment reduces the chance of bacterial biofilm formation. Considering the wide range of instrument designs and functions that are used in endodontic treatments, the results demonstrated the necessity for further investigations into their effect on a treated canal's final surface quality.

Practical aspects of root canal treatment that may be effective on the canal surface roughness were the focus of the next experiments of this thesis. The third chapter compares the effect of two different filing motions, continuous rotary and adaptive reciprocation, on root canal surface roughness. Continuous rotation and reciprocation are the two most frequently used

filing techniques in root canal instrumentation. In this experiment, a filing system that was compatible to work in both rotary and adaptive reciprocation modes was used to answer whether filing motion can affect surface roughness of a root canal. Experiments showed that surface roughness was significantly higher overall in the root canals of teeth prepared with adaptive reciprocation compared to continuous rotary. The results of this chapter showed that roughness of the root canal is a cofactor that can be modified by the clinician. Treatment strategies with different techniques can be implemented even while using identical instruments to achieve smoother treated surfaces. Based on the findings of this study, using a continuous rotary system to prepare canals or to finish the cleaning and shaping stage of a root canal treatment can be beneficial to reducing roughness of the canal surface.

Differences between filing systems consists of differences in a mixture of variables including alloy, surface treatment, cross-section, taper, motion, design, etc. The fourth chapter in this series was aimed to evaluate the effect of three different filing systems with different concepts, on the final root canal surface quality. Cleaning and shaping was carried out on teeth with either a single-file reciprocating (Reciproc), continuous rotary (HyFlex EDM) or oscillating self-adjusting file (SAF) system. The results from this chapter showed that the three completely different filing systems resulted in similarly rough root canal surfaces. The high level of roughness in all groups suggested that the three filing systems tested in this experiment were relatively aggressive.

File wear results in reduced cutting efficiency and aggressiveness. Since each file undergoes a life cycle and it is eventually worn out, the fifth chapter of this thesis was designed to assess how the effect of file wear translates into changes on the treated root canal surface roughness. In order to evaluate the impact of file wear effectively, Reciproc single-file reciprocating instruments were used for this study. Reciproc files endure the same stress that is usually distributed among a number of files in multi-file systems. This study showed that the

amount of wear during three uses, which is within the range of use recommended by the manufacturer, does not affect the final root canal surface roughness. Without consideration of safety of these files in terms of file separation risk, these files can be used up to three times while expecting a similar treatment outcome. However, similar to the previous study, these files left a relatively rough surface in all cases.

The key findings in the present thesis were that root canal surface roughness is an effective and modifiable cofactor that can be used to determine the quality of root canal instrumentation and the performance of the instruments used. The two new methodologies developed can be used to test other available endodontic instruments and techniques. These methods can provide a foundation for generating comparable and quantitative data regarding the roughness values and thresholds associated with biofilm formation and different endodontic instruments. Standard levels can be set for future instrument designs once enough research is available regarding the performance of the current instruments and the ideal levels of surface roughness.

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Glossary

Ra	Roughness average. Mean height deviation from the mean plane surface that represents the average distribution of height values.
Rz	A ten-point extreme value parameter calculated by measuring the mean difference between the five highest peaks and the five lowest valleys of the surface, used to demonstrate average maximum profile and roughness depths of a surface.
Rq	Root mean square deviation roughness. An average between the mean line and the height deviations. This parameter is mostly used to demonstrate the skewness and kurtosis properties of roughness.
Sa	Arithmetical mean height of an area or in other words Ra (roughness average) extension onto a surface. It is the difference in height of every point compared to the surface's arithmetical mean.
Radial lands	Presence of neutral cutting angles in the filing instrument. These types of instruments tend to burnish the cut debris onto the surface.
Rake angles	Angle used to describe the cutting segment of a file. The angle is subtended between the line from the cutting tip to the centre of the instrument and the line from the cutting tip that is tangential to the cutting face.

Chapter 1 Introduction and literature review

1.1 Introduction

Root canal treatment failures occur in a significant portion of cases and researchers aim to reduce risk of failure by identifying the factors that can decrease chances of reinfection. Success rates of root canal treatment based on strict assessing criteria range from 31 to 96 percent, which reflects the significantly heterogenic distribution of the results. Variable combination of factors assessed in these studies, different follow-up periods and study designs make the comparison and interpretation of these studies difficult. Although randomized controlled trials are considered the gold standard, similar to many other areas of medical research endodontics is in shortage of such level of evidence.[1] Clinical and radiographic evaluation of endodontic treatments require at least 1 year and in many cases up to 4-5 year follow-up,[1,2] which has made it fall behind with the rate of advancements in technology used in root canal treatment. The effect of new instruments and materials that are introduced for clinical use is unclear apart from the advantages that are claimed in their mechanical properties, efficiency and working times. Therefore, it is of critical importance to evaluate how these changes may affect the treatment quality and outcome. Success of a root canal treatment is determined based on long-term clinical and radiographic assessments that provide evidence of healing. Controversy regarding the factors and thresholds indicating treatment success has led to different “strict” and “loose” criteria in reports.[1] Meta-analysis of the clinical studies from the last five decades shows the success rates have not improved. Pooled data even suggests the highest success rates were reported during 1960-80.[1] Although the efficiency of the chemicals and instruments used in treatment have improved over the years, the unaltered success rates suggests that overall these advances have not affected the outcome.[1] Another possibility may be that although the effect of the combination of these

technological advances has been comparable to older treatment methods, some of these innovations may be having detrimental effects while others have been improving the treatment quality.

Pre-operative clinical factors such as periapical lesions have been widely researched and their effect on success of root canal treatments has been established. However, intra-operative factors which are in control of the clinician have been poorly researched. Meta-analyses of these factors identified fillings within 0-2 mm of the apex and absence of voids in root canal fillings to be effective in increasing the treatment success rate. However, data regarding variables of the instrumentation stage such as preparation size and taper is not sufficient for meta-analysis.[3] Individual studies on these factors have conflicting results. Hoskinson et al. reported a decreasing trend in success rates with larger master apical file sizes, although this was not statistically significant. They also found no difference between 0.05 and 0.10 tapered canals.[4] In contrast, Smith et al. reported higher taper to be associated with higher success rates.[5]

Cofactors of clinical treatment efficiency and success which represent the quality of work can act as a much needed bridge between clinical and laboratory research. Currently, only few quantitative cofactors are available that are used to reflect treatment quality. Recent years has seen some of the previously reliable factors such as root canal seal questioned because of the errors seen in the methodologies used in their studies.[6-8] The identification of relevant cofactors requires a deeper look into the dynamics of root canal infections.

Root canal treatment failures occur when the persistent microorganisms in the root canal or invading microorganisms from the outside find a way to grow again.[9] Effectiveness of chemical antimicrobials in eradicating bacteria has been of research interest for decades. The antimicrobial activities of many disinfectants are weakened in contact with the chemical structures and microanatomy in a root canal.[10] Biofilm formation and antimicrobial resistance

of the microorganisms involved also complicate their eradication.[11,12] Recent concepts of infection suggest that low levels of microorganisms may be present at sound sites but do not cause a clinical threat. In this model, disease can occur when environmental changes disturb the balance of the existing flora towards growth of better adapters to the new conditions.[13] Much research is now being carried out to discover means of reducing chances of bacterial growth.

Root canal infections are biofilm-mediated infections, meaning that the bacteria are not floating freely in the tissue.[14] This explains much about how they develop to mature communities over time and become resistant to treatment. Biofilms have a different path of formation, growth and maturity.[11] Use of the agar diffusion method for testing antimicrobial susceptibility has been discouraged since it does not replicate the growth mode and resistance of bacteria in clinical conditions.[6]

Biofilm formation is initiated with attachment of microorganisms to the substrate surface, known as adhesion. Many factors have been identified to alter chances and modes of biofilm growth. Surface energy, charge, stiffness, chemistry, and roughness of the surface are some of the factors that are effective in biofilm formation. These factors can have a different effect magnitude based on the type of microorganisms involved.[15] There is currently little information about the effects of these factors in endodontic treatments and the effect that they may have on microbial species involved in root canal infections.

Surface roughness has been shown to be dominant factor among the substrate's properties that can affect biofilm formation. Surface charge and surface energy have a less significant effect in rough surfaces.[16] In the oral cavity, roughness can increase the amount and maturity of the biofilm formed by oral microorganisms on dental implants.[17] Moreover, roughness is a surface property that could be modified by both the chemical erosion caused by irrigants and the physical abrasion caused with mechanical instrumentation during root canal treatment.

Irrigants and antimicrobials used in the root canal system can cause changes in the physical properties of dentine.[18,19] Chemical erosion and surface changes have been experimented over the last decades with the available irrigants and also the newer chemicals that have been introduced. Although most these experiments showed a significant difference in the amount of roughness that various irrigants left, these differences are in a nanometre scale.[19-21]

Roughness caused by mechanical instrumentation has not been thoroughly researched. The few reports available use qualitative or semi-quantitative methods that make them incomparable to other instruments outside of the study.[22-26] Even so, the difference in surface roughness after using different filing systems seems so obvious that some researchers such as Barthel et al. compared surfaces without magnification.[22]

In conclusion, the present research was designed towards achieving two goals. The first goal was to develop a method to quantitatively investigate the effect of roughness on endodontic bacteria and determine the clinical relevance of roughness in root canal infections. The second goal of this series of experiments was to develop a quantitative method to evaluate roughness of root canals and determine whether the current methods and instruments used for filing canals can affect the root canal surface roughness.

In the following section of this chapter, the available research on this topic, the knowledge gaps and the methodologies used in previous experiments will be reviewed in detail.

1.2 Literature review

1.2.1 Oral diseases and oral microbiology

The oral cavity can support the growth of one of the most complex and divergent communities of microorganisms in the human body with over a thousand species.[27] These microorganisms are constantly subjected to a wide range of physical and chemical changes. The oral cavity is the only part of the body that has externally exposed hard tissue (teeth). Bacteria can adhere to the teeth and create a biofilm known as dental plaque. Keratinized and non-keratinized soft tissues of gingiva, tongue, palate, mucosa, and floor of the mouth also provide environments for various types of microorganisms.[28,29]

Overall, the oral microbiota is believed to have major health benefits for the human body under normal conditions[28]. The commensal microbiota can prevent exogenous infection by multiplying and covering the binding sites for exogenous pathogens, which is known as colonization resistance.[30] However, commensal microorganisms may also become the cause of oral disease if normal conditions change. Environmental stresses that alter the haemostatic mechanisms of the oral biofilms are the main reason that start the pathogenic cycle.[28]

Dental caries is the most prevalent cause of pulpitis and pulpal infection.[31] Caries and infectious disease in the oral cavity occur when the environmental conditions of the oral microflora change. These stresses can cause an impediment to the equilibrium between remineralisation and demineralisation of teeth. If the progress of these events is not stopped or reversed, it can promote further selective development and multiplication of the acidogenic and acidophilic bacteria in dental plaque. This process can eventually lead to extensive carious lesions in enamel and dentine, pulpal inflammation and infection.[29] Bacterial by-products can stimulate the pulpal immune response through dentinal tubules and the bacteria can infect the root canal system once the caries lesion reaches the pulp.[31]

1.2.1.1 Oral biofilm

Oral epithelium sheds around 3 times daily which significantly reduces the amount of bacterial adhesion and biofilm formation on its surface.[15] Exposed hard tissues in the oral cavity have a very different interaction with the oral environment, especially with saliva and the oral microbiota. This interaction can start within seconds upon the exposure of the enamel to saliva. Saliva usually coats all hard and soft tissues in the oral cavity creating a conditioning film. Salivary proteins are absorbed to the enamel hydroxyl apatite and form what is known as the acquired enamel pellicle (AEP). This pellicle matures with the absorption of other proteins, lipids, carbohydrates and adhesion and colonization of microorganisms. [32]

The adapting potential of bacteria gives them unlimited mechanisms to overcome the barriers that prevent them from colonizing inside the oral cavity. The addition of saliva, especially with its protein content, to this environment, adds further complexity to the system.

The growth mode of oral bacteria is much more complex than the growth of single or multiple species. Microorganisms in the oral cavity grow in biofilms. The biofilms consist of a polymer-rich matrix, which have the microorganisms colonizing both inside and on the surface of it. Dental plaque is a very well-known form of biofilm that is present in the mouth. Aside from normal oral tissues, microorganisms can also adapt and form biofilms on dental materials used inside the mouth. Biofilms develop on surfaces of restorative, prosthodontic and endodontic materials and can cause many problems in treatments. This has led to interest in anti-biofilm properties in dental materials. [33]

Interspecies associations develop in biofilms and help the bacterial community's nutrition, adherence and stability. However, these interactions may change with alterations to the oral environment and become pathogenic. The most noted example would be a change in the diet that can lead to caries (tooth decay). Intake of a high level of carbohydrates can lead to higher level of acid production by cariogenic bacteria. The produced acid lowers the pH level of both

the plaque and subsequently the oral cavity. The altered pH inhibits the growth of some of the other non-cariogenic bacterial species that are acid-sensitive.[28,34]

Biofilm bacteria are more resistant to antimicrobials. Antibiotics have not been designed to eradicate biofilm populations.[35] Therefore, treatment of a biofilm-mediated infection is more difficult.[36] Many oral diseases including post-treatment root canal infections are biofilm-induced infections.[37] The maturity level of a biofilm is also effective in its resistance to antimicrobials. This resistance is believed to be have a major role in the persistence of infections and recolonization of microorganisms after antimicrobial treatments.[38] Biofilms have a higher chance of being associated with longer standing lesions. Slower metabolism of microorganisms in a biofilm and the presence of an extracellular matrix, that may act as a barrier itself, reduces the effectiveness of antimicrobials.[39]

1.2.1.2 Biofilm formation and bacterial adhesion

Development of a biofilm initiates with attachment and adhesion of the microorganisms to the substrate surface.[40] This stage is also believed to be the most important stage of biofilm formation. After the initial adhesion to the surface, the bacteria start forming ligand-receptor binding to the surface which makes the adhesion irreversible.[36] Attachment of microorganisms is followed by development of micro-colonies and microbial growth.[41]

Initial interaction between bacteria and the surface, which is known by adhesion, is the foundation of biofilm formation. Adhesion mainly takes place between the bacterial cell wall and the extracellular components of the substrate or the medium covering it. The dynamic nature of the bacteria's response and how it adapts in response to the surface also adds more complexity to the infinite number of combinations that are possible in this interaction. Although the mechanisms that bacteria use in these cases are not yet completely understood,

but they have systems in place to sense their environments and then respond accordingly or adapt to changes.

Streptococcus mutans has the most prominent role in caries etiology which is rooted in its high adhesion capability to dental tissues, even though it is less acidogenic and pathogenic than some other species involved in caries development.[42] Susceptibility to bacterial attachment is considered one of the most important factors in determining a restorative material's longevity.[42] Therefore, it is logical that a wide range of research has been done on factors that have the potential to prevent or limit their attachment.

Surface charge, surface energy (hydrophobicity), roughness, topography, stiffness and chemistry of the surface are some of the most important substrate properties found to affect adhesion,[15] which are reviewed in the following section.

1.2.1.2.1 Surface charge

The negative charge present in most bacterial cell walls adheres better to surfaces with a positive charge. Negative charge of a surface on its own factor cannot always prevent adhesion because some bacteria have mechanisms to attach to these surfaces too.[15] In addition, different environmental ions, proteins and mediums such as saliva that coat the substrate surface, have an important effect on the final role of surface charge.[43]

1.2.1.2.2 Hydrophobicity and surface energy

Superhydrophobic and superhydrophilic materials are both used to create non-fouling surfaces because of their non-adhesive properties. This demonstrates the different role surface energy has on bacterial adhesion. An average range of hydrophilicity or hydrophobicity can affect bacterial adhesion based on the bacterial species and also the dynamic state of the

environment (e.g. saliva film covering the surface).[15] In subgingival areas of the mouth where saliva flow is less significant, surface energy has less effect on biofilm formation. However, on supragingival areas of the mouth, shear stresses caused by saliva flow seems to detach biofilms easier from hydrophobic surfaces compared to the hydrophilic ones.[44,45]

1.2.1.2.3 Surface topography

Topographic patterns of certain shape and size can inhibit biofilms. These patterns that are mostly in nanometre or micrometre scales can be used to create non-fouling surfaces or even surfaces that can kill bacteria upon contact. This may be one of the only instances where a surface with roughness is less suitable for biofilm formation compared to a flat and smooth surface.[15] Although some of these properties may be someday used in prefabricated treatments, it is highly unlikely to apply directly inside the oral cavity.

1.2.1.2.4 Surface stiffness

Softer materials allow better adhesion and more rapid growth of biofilms. Stiffness is one of the most recent and least known surface properties that affects bacterial response and physiology. This effect has only been investigated on a limited number of bacterial types and requires much more research. However, there is evidence that saliva forms different viscosity films on intraoral surfaces.[15,46] These differences may influence the surface stiffness properties too.[15]

1.2.1.2.5 Surface roughness

Roughness has a very distinct yet variable effect on biofilm formation. This effect has been the most widely investigated surface property compared to the other factors.[15] Roughness can

multiply the amount of surface area available for adhesion by a factor of 2-3.[47] These areas can also trap bacteria or provide shelter and shield for them against shear forces that can detach the biofilm. The magnitude and threshold of this effect relies on the bacteria type and species. Roughness in nanometre scale can significantly increase the biofilm formation of certain bacteria while others might be much less affected by the same scale roughness.[15]

Teeth and various dental materials' roughness attract formation of different types and amounts of biofilm. The first bacterial attachments and biofilm formations on dental tissues and restorative materials occur in irregular and rough surfaces such as cracks and grooves.[42] Intraoral plaque formation on polymer surfaces increases significantly with a 2 μm increase in surface roughness. [47] Experimentation of subgingival microbiological changes based on roughness differences have been technically challenging. These studies require surgical interventions and alteration of subgingival hard surfaces[48] that were not common before introduction of dental implants.

Dental implants' optimal roughness properties have been extensively experimented to achieve lower levels of biofilm formation. Early studies showed plaque accumulation can be as much as 25 times on rough intraoral surfaces compared to smooth ones.[48] Implant research has focused on surface roughness extensively since the implant surface characteristics is important to both osseointegration of the implant and adhesion of the microorganisms. Peri-implantitis, the inflammation of the tissues surrounding the implant, caused by plaque and microorganisms can result in implant failure. Rough implant surfaces harbor and colonize more bacteria, therefore, increase the risk of peri-implantitis.[49,50] On the other hand, moderate roughness of the implant surface has been reported to promote bone response and osseointegration.[51] An ideal surface would have a balance in which it is rough enough to provide osseointegration but not too rough to significantly boost plaque accumulation and cause peri-implantitis.

Quirynen et al. showed in 1993 that there were 25 times more microorganisms on subgingival rough surfaces compared to smooth ones. In addition, more motile bacteria and a larger proportion of spirochetes were seen on rough surfaces. This effect was especially seen in supragingival plaque after 3 months which suggests the effect of roughness on plaque maturity as well as bacterial quantity. This effect was seen by only increasing the surface roughness average (Ra; for more information regarding roughness parameters please refer to section "1.2.3.3.5.1 Surface roughness characterization and measurement parameters") of the implant abutments from Ra=0.35 µm to Ra=0.81 µm.[17]

Roughness threshold of 200 nm was suggested for implants. Further reduction of roughness beyond this point is expected to cause no change in biofilm formation. Clinical assessment of patients that had implant abutments that were highly polished (Ra=0.05 µm) with standard (Ra=0.21 µm) abutments showed no significant difference in the biofilms formed on them after 3 months. This meant that roughness values lower than 200 nm have less clinical significance and do not impact the biofilm composition. Furthermore, the standard abutments showed less probing depths, which suggests better attachment gain for the gingival cells in this group.[48]

Long-term effects of roughness below the 200 nm threshold on biofilm formation and composition was experimented in a split-mouth study by Bollen et al. in 1996. Implant abutments made of machined titanium (Ra=200 nm) were compared to polished ceramic abutments (Ra=60 nm). Clinical examinations, differential phase-contrast microscopy and bacterial cultures were carried out for the implants in 3 and 12 months after abutment placement. The results in general show that the two types of surfaces did not differ significantly in quantity or quality of their biofilms.[52]

Xing et al. found a strong correlation between the amount of biofilm accumulation on TiZr surface discs and nano-roughness ranging from 29 to 214 nm. Polymicrobial biofilms were

being tested in this study since TiZr discs were placed in a removable splint inside the mouth of the ten participants for 11 hours. The roughness of each disc was assessed with 50× magnification on four areas of 255 µm × 191 µm using a blue light laser profilometer. Biofilms were stained using safranin and released from the discs using acetic acid. The amount of bacteria from each sample was tested with spectrophotometry.[53]

Atomic force microscopy (AFM), scanning electron microscopy (SEM), fluorescence in situ hybridization (FISH), and confocal laser scanning microscopy (CLSM) were carried out on 6 different implant surface disks and bovine enamel slabs in an in vivo study by Al-Ahmad et al. in 2010. The average surface roughness (Ra) of the surfaces were calculated with AFM on a surface area of 50 × 50 µm. Twelve volunteers wore the splints containing the disks for 3 and 5 days. After 3 days, the biofilm thickness in 6 groups out of 7 was found to be correlated to the surface roughness. The correlation between surface roughness and the biofilm thickness decreased significantly after 5 days. The biofilm composition assays with FISH and CLSM showed no difference between enamel slabs and the implant material. The materials did not have a significant effect on the bacterial composition. This was assumed to be due to the fact that the acquired pellicle has a more dictating role in the biofilm composition than the materials.[54]

Quantitative assessments of the effect of surface roughness (Ra) and surface free energy on the amount of biofilm formation were done by Burgers et al.[55] They used fluorescent microscopy and an automated multi-detection fluorescence reader to examine the amount of biofilm formation which was more sensitive than the methods previously introduced. Surface free energy and surface roughness (Ra) of the two types of titanium materials were calculated with a goiniometer and perthometer, respectively. Splints that carried the rough (Ra=0.15 µm) and smooth (Ra=0.95 µm) titanium specimens were worn by volunteers for 12 hours. Observations of the relative fluorescence intensity (RFI) and surface area covered by biofilm

on the titanium specimens showed that both were significantly higher for the rough specimens.[55]

Lin et al.[56] showed that 0.3-1.4 µm range of roughness (Sa) of titanium disks does not have an impact on the quantity of biofilms developed by *Streptococcus mutans* or *Porphyromonas gingivalis* species after 1 and 3 days. The two levels of roughness that were experimented in this study with low (Sa=0.3 µm) and moderately (Sa=1.4 µm) roughened titanium disks were both above the 200 nm threshold mentioned earlier that was described earlier for titanium implants and the range of microorganisms involved on their trial.[48] Roughness did however have an effect on how effective chlorhexidine was on the biofilms. The colony forming unit (CFU) counts of both 1 day and 3 day biofilms reduced significantly less after treatment on the rougher surfaces,[56] which suggests a more resistant biofilm on rougher surfaces.

Saliva can also affect the topography and roughness of oral and dental surfaces by its uneven distribution in a nanometre scales.[32] Once the pellicle is formed inside the oral cavity, the proteins and enzymes in the extracellular matrix can affect the pellicle's surface properties. The polysaccharides that are produced by the exoenzymes that come in contact to sugar can change the surface topography and create high affinity binding sites for bacteria on the pellicle.[57]

Pellicle formation and its effect on the substrate surface properties has been controversial. Although some research point out the role that saliva has in masking some of the qualities of the substrate's surface, there is some evidence that the substrate surface properties are also effective in presence of saliva.[42] Research shows surface roughness can enhance *S. mutans* binding to parotid saliva on composite resins and glass ionomers.[58] Therefore, it seems that the presence of saliva is another variable partially affecting bacterial attachment in the oral cavity. This effect can be minimized in endodontics if the root canal environment is sufficiently isolated.

Interaction between the biofilm and substrate that alters surface properties is dependent on the type of substrate material and microorganisms.[42] *S. mutans* biofilms can increase surface roughness on resin composites in vitro. This effect can in return accelerate bacterial attachment and biofilm formation and therefore, start a cycle that eventually compromises the restoration.[59]

The overall trend of research seems to show that roughness affects the amount and characteristics of biofilm formed on implants.[60] This effect that roughness shows is different depending on the types of microorganisms and substrates tested. The magnitude of effect varies in studies based on the methods utilized and the types of roughness parameters used to describe surfaces; e.g. Ra, Rz and Sa.

1.2.2 Endodontic microbiology

Endodontology represents the study of the diseases of the pulp-dentine complex and periapical tissues. The dental pulp is a sterile and protected tissue surrounded by dentine and enamel. The embryonic origin of dentinal and pulpal tissues is similar. These tissues form a functional organ that is responsible for producing dentine and tooth sensitivity. Dentine is in contact with the enamel (dentinoenamel junction or the DEJ) or cementum (dentinocemental junction or DCJ) on its outer surface. During the development of dentine, the odontoblasts form a porous structure with the dentinal tubules running from the DEJ and DCJ to the inner surface.[61] When the integrity of the tooth is somehow compromised (caries, trauma, periodontal disease, etc.), an inflammatory response will occur. Traditionally, endodontic disease is a sequel to caries, and non-infectious pulpal inflammation is much less common compared to infectious conditions. The infectious diseases affecting the pulp also exhibit a progressive nature.[62] Reversible pulpitis can transform into an irreversible state where pulp extirpation and root canal filling would be necessary.[62] The pulp complex has limited defence

against infection because it is surrounded by mineral tissue and lacks collateral circulation.[63] The temperature, humidity, available nutrition, anaerobic conditions in the root canal system, which are largely inaccessible to the host defence, are ideal for many microorganisms to colonize.[64] This leads to a rapid loss of vitality in the tissue which is believed to be "higher than any other tissue in the body".[63]

The landmark research of Kakehashi et al.[65] in 1965 revealed the pathogenic nature of pulpal inflammation. Their research demonstrated this for the first time by examining pulpal exposure in germfree and conventionally-reared rats. The report showed that exposed pulpal tissue in germ-free rats could initiate repair by creating dentine bridges.[61,65] The vital role of microorganisms in this process provides an understanding of why endodontic treatment largely focuses on eliminating infection and preventing reinfection.[66]

Prognosis of root canal treatment in cases that are associated with preoperative infection are lower than of teeth with vital pulps. Ng et al. reported a success rate of above 80% for primary and secondary root canal treatments in a prospective study on 2484 roots. However, presence of a preoperative periapical lesion decreased the odds of success by 49% compared to roots without a lesion (OR=0.51, 95% Confidence Interval 0.32-0.80).[67] Another large-scale prospective cohort study on 1369 roots by Riccuci et al. reported a success rate of 93.1% for vital roots. The success rates for roots with necrotic pulps and roots with a combination of pulp necrosis plus apical periodontitis were 92.3% and 84.1%, respectively.[68]

Invasion of dentinal tubules with microorganisms or exogenous substances can initiate from exposed dentine in the oral cavity. This process can be initiated by bacteria that are common in dental plaque but obligate anaerobic bacteria are dominant in the infected root canals. Although the pulp-dentine complex has some defensive mechanisms, if the source of infection is not eliminated, it may result in pulpitis, pulp necrosis, and pulp infection that may eventually lead to periapical disease. Bacterial invasion of dentine can also lead to persistent

root canal infections. These findings are consistent with the research that show some species like *Enterococcus faecalis* can resist periods of starvation, sodium hypochlorite, heat, hydrogen peroxide, and highly alkaline conditions (that could be caused by calcium hydroxide dressings). Survival through these stages can provide a bacterial source that could cause failure in root canal treatment.[61]

1.2.2.1 Bacteriology of endodontic related infections

Root canal infections are usually endogenous, where oral bacteria contaminate the root canal and cause the infection.[69] Given the highly complex and diverse nature of the microbial communities in the oral cavity,[28] a polymicrobial community is seen in most oral infections.[30] The organisms that often invade the root canal are opportunistic pathogens. These pathogens are not the most virulent or invasive species, e.g. *E. faecalis*. However, these organisms are the more resistant to antimicrobial agents and pH fluctuations.[69-71] Development of mature biofilms and also the ability of *E. faecalis* to invade dentinal tubules, where it is protected from antibacterial irrigants, can make its eradication even more difficult.[38]

Studies investigating the species of bacteria associated with certain infections in the oral cavity have been hindered by the fact that approximately half of the oral bacteria cannot grow on a conventional culture media.[30,72] Recent research using molecular techniques, PCR, and anaerobic culture methods for defining the bacterial composition of endodontic infections have revealed contradictory results.[66,71,72]

Dentine tubule invasion with microorganisms is important for understanding the mechanism of root canal infection and treatment. From the hundreds of bacterial species in the oral microflora, only a small number can invade dentine and cause infection in the root canal. Microflora that are involved in caries development are from the streptococci, lactobacilli and *Actinomyces* species family.[31,73] Streptococci and lactobacilli have both been shown to be able

to bind to collagen type I and invade dentinal tubules. The superficial layers of caries are mostly populated with Gram-positive rods. The deeper layers of caries in dentine harbour more anaerobic species of the Gram-positive rods.[31,74] Streptococci which are more dependent on the nutrients in saliva have less chance of thriving in deeper depths of the lesion.[31]

Coronal dentinal tubules are wider in deeper levels that are close to pulp (approximately 4.3 µm diameter) and narrower in superficial depths of dentine close to enamel (approximately 2.4 µm diameter).[75,76] The size of the tubules both in the surface and depths close to the canal decreases towards the apical region of the tooth.[77] However, it is important to note that these sizes are still large enough to harbour the bacteria that invade tubules.

Sampling errors from root canals infections are inevitable amid the different techniques used by researchers. The samples are categorized based on their recovering site, which is usually either the pulp chamber (non-vital or containing some vital tissue) or apical tissues. Maintaining the integrity of the samples acquired and preventing oral and saliva contamination of the sample remains a challenging task.[14,66] Sample collection is usually done with use of paper points which is believed to be biased towards collecting free-floating bacteria rather than the biofilms attached to canal walls. In addition, this method cannot specify which part of the canal the microorganisms are acquired from.[14,63]

The microbial communities that are recovered from the primary and secondary infections of the root canal are different. Bacteria which are recovered from a primary root canal infection are usually polymicrobial communities of 2-8 species. Obligate anaerobes are dominant in these communities. The flora of secondary infections of the root canal (failed cases) usually consists of 1-2 species per canal. In these cases, mainly gram positive facultative anaerobes are recovered. *Enterococcus faecalis* is the predominant species in failed root canal treatments,[61,70,71] whereas this species has shown to be less commonly recovered from the primary infection.[70]

E. faecalis has a number of potential virulence factors that give it the opportunity to thrive in root canal reinfections. This microorganism has low sensitivity to antimicrobial agents and chemicals used in the root canal. In addition, it has the ability to invade dentinal tubules where it can be sheltered from the medications and chemicals used in treatment until the conditions are suitable for it to reinfect the root canal system.[78]

1.2.3 Treatment of endodontic-related infections

Eliminating microorganisms and their by-products from the root canal system and preventing reinfection are the primary objectives of root canal therapy.[79,80] After endodontic access preparation, debridement of the root canal system is carried out. During and after debridement, the root canals are shaped in a way that can be filled. Reinfection may occur due to coronal penetration of oral bacteria or the remaining bacteria in the root canal system after cleaning and shaping. The root canals are therefore filled to prevent reinfection by sealing the remaining space (against bacterial penetration from the oral cavity) and also entombing the remaining bacteria.[80,81]

A range of endodontic instruments, techniques and materials have been experimented to optimize the results that are achieved. Different filing systems, sonics, ultrasonics, irrigation solutions, smear layer removal methods, and intracanal medicaments are some of the different options that a clinician may consider at this stage. The ideal result of this treatment stage would be a root canal and pulpal chamber that are free of microorganisms and would be ready to be filled. These conditions would allow periapical healing and osseous regeneration. However, these conditions cannot always and practically be met due to the complexities of the root canal anatomy.[80]

1.2.3.1 Microbiological considerations in root canal debridement

Debridement of the root canal is defined as elimination of the substances (organic and inorganic) and microorganisms from the root canal.[82] This stage of treatment is referred to as the foundation of a successful endodontic treatment and the importance of it has been emphasized since 1931.[83]

Debridement is achieved by cleaning and shaping the root canal system. Cleaning, which is done before and during shaping, is often carried out with a combination of chemical and mechanical approaches.[83] Studies show that large areas of the canal remain intact during sole instrumentation and emphasize the importance of adequate irrigation. Even though mechanical instrumentation does reduce the number of microorganisms infecting the canals, combining instrumentation with irrigation has shown to result in 100-1000 times more reduction in the number microorganisms compared to instrumentation alone.[83] Irrigation also allows chemical disinfection and elimination of bacteria from the canal, which are key to root canal treatment success.[84,85]

Time, physical restrictions and the complex morphology of the root canal system do not allow complete disinfection and removal of the smear layer and debris.[86-90] In practice, the aim is towards minimizing the number of microorganisms. The root canal debridement is usually limited to the main canal, which also has some remote areas that might be unprepared at the end of treatment.[91]

Residual microorganisms that are infecting dentinal tubules are one other source of bacteria that may jeopardise the final clinical outcome.[86] Since these microorganisms grow in biofilms, root canal biofilm resistance to various irrigation solutions and medicaments has also been a focus of research in this field. The efficacy of these treatments has been tested on biofilms formed in wells, membrane filters and dentine.[92-94]

New instruments have been introduced to reduce the procedural errors of instrumentation. Much effort has been made also to reduce the chances of instrument fractures.[95] There have been changes in the type of materials used with the introduction of more flexible Ni-Ti instruments instead of stainless-steel. Instrument designs and cutting efficiency have also seen many improvements.[95]

1.2.3.2 Microbiological considerations in the obturation of the root canal

None of the current techniques employed in endodontics can entirely eliminate root canal bacteria.[96] The aim of a root filling is to achieve a seal against bacteria and their by-products. The result of an ideally debrided root canal would be a disinfected hollow space in the tooth. However, even in such conditions, this space is in proximity of the bacteria of the oral cavity. This space is not accessible by the host's immune system if contaminated, and would therefore be a potential site for reinfection. Incomplete filling of the root canal is suggested to be associated with up to 60 percent of endodontic treatment failures.[97,98]

Orstavik et al.[99] carried out a multivariate analysis on the factors influencing the final outcome of endodontic treatment. They reported that root filling density and other technical qualities of the filling such as the apex-to-filling distance have a significant effect on the clinical outcome.[99]

Current filling materials cannot entirely seal the root canal space and they do allow leakage. Gutta-percha does not bond to the canal walls and is often used with a sealer to help fill this gap. If the sealer has bonding qualities, it may also help prevent dislodgment of the root filling.[100] More recently, heated and pre-heated gutta-percha methods have been introduced that are suggested to enhance the root filling.[101]

1.2.3.3 Cofactors that influence root canal treatment

The possible combinations that can be made from the wide range of instruments, chemicals and techniques to carry out a root canal treatment may seem almost infinite. However, many of these combinations may not have been thoroughly tested. Rapid technological developments and innovations in the available materials and instruments also need to be experimented against conventional treatments. Although clinical studies provide the highest level of evidence, they require long-term follow-ups that are difficult to achieve with the rate of advancements. The quality of a root canal treatment is assessed by evaluating various factors that directly or indirectly affect the treatment outcome. The following section will review the factors that have been utilized to test instruments and methods used in the cleaning and shaping stage of root canal treatments.

1.2.3.3.1 Dentinal integrity, defects, cracks and craze lines

Vertical root fractures are one of the relatively common reasons (8.8-13.4%) for extraction of teeth after root canal treatments.[102,103] This has led to a large amount of research regarding the factors that could create craze lines, cracks and ultimately fractures in dentine.[104]

Root canal preparation and obturation were both shown effective in creating dentinal defects in a study by Shemesh et al.[105] Horizontal teeth sections were observed under a stereomicroscope with x12 magnification after different treatments. The number of teeth with defects were significantly higher after preparation with Gates Glidden drills and rotary files compared to unprepared teeth. Obturation of the canals with lateral condensation technique also created more dentinal defects compared to the prepared teeth without any filling.[105] In contrast to this study, which found significantly more dentinal defects in teeth using a lateral condensation technique compared to no compaction of gutta-percha, Onnink et al. reported the obturation technique does not affect incomplete root fracture occurance.[106]

Exposure of dentine to sodium hypochlorite, which is frequently used in root canal irrigation, decreases its flexural strength. This effect is more significant when 5.25% NaOCl is used compared to the 0.5% concentration solution. Endodontically treated teeth were previously claimed to be more susceptible to fracture because of loss of dentinal tissue and changes in proprioception and nociception. The findings regarding the changes in dentine's physical properties further supported this idea.[107]

Calcium hydroxide is another chemical that decreases dentine fracture resistance especially when exposed to it long-term. This is of importance since calcium hydroxide is used as a root canal dressing and should therefore be applied with caution considering this effect.[18,108]

Post space preparation and post placement generally weakens the endodontically treated tooth structure. [109] Recent developments in adhesive luting of posts and availability of materials other than the rigid metal posts have decreased the chances risk of root fracture failures,[109,110] however, they should still only be placed when essential.[109]

Micro-computed tomography methods that were developed to research dentinal cracks allowed comparison of defects before and after procedures. Some studies utilizing the new methods question the clinical relevance of previous research and the effect of micro-cracks as a cofactor in root canal treatments. The recent studies using micro-computed tomography indicate there is no causal relationship between canal instrumentation and formation of microcracks.[111-113] It is also important to interpret the results from in-vitro experiments with caution since they usually require the tooth to be in dehydrating conditions and without the support of the periodontal tissues.[114]

1.2.3.3.2 Apical debris extrusion

Apical debris extrusion may complicate a root canal treatment outcome by causing pain, swelling and flare-up. The type of filing technique and instrument seem to affect the amount of extruded debris.[115] However, it is difficult to compare data between studies since they have conflicting results while using the same instruments. An example for this is the different results obtained for HyFlex CM and ProTaper Next files in reports from Capar et al.[116] and Kocak et al.[115] Filing motion has also been linked to the amount of debris extrusion. Rotary filing leads to less debris extrusion compared to certain modes of reciprocation.[117]

Clinical relevance of *in-vitro* studies of apical extrusion is questionable since it is not clear what amount of debris would actually cause clinical complications. The threshold may vary in each case depending on the infectious potency of the debris. Debris extrusion in a clinical scenario would be greatly influenced by the amount of resistance from periradicular tissues and the dimension of apical preparation too, which is difficult to simulate in laboratory settings.[118]

1.2.3.3.3 Smear layer and root canal surface debris

Smear layer is a 1-5 µm thick surface film of debris that is formed on the root canal surface after instrumentation with endodontic files.[119] The smear layer may harbour bacteria and their byproducts and it can also be packed as far as 40 µm deep into dentinal tubules.[120] It has been a focus of research since it can prevent irrigants from reaching deeper parts of dentine and reduce the bond of adhesives and sealers to the root canal wall.[119] Various chemical,[121] mechanical, ultrasonic[122] and laser methods[120] have been tested to remove the smear layer.

EDTA is the most common solution used for removal of the smear layer in endodontic treatment.[123] It is also referred to as the gold standard for removal of the smear layer in research.[121] Multiple studies regarding the effect of EDTA on the final seal of root canal treatments exist that have found no change in leakage with removal of the smear layer.[123]

However, the meta-analysis by Shahravan et al. in 2007 concluded an improvement in the seal of the root canal system when the smear layer is removed.[124] The use of ultrasonic activation of irrigants in combination with EDTA to achieve cleaner root canal surfaces has been claimed to be beneficial in some studies.[122,125] In contrast, a randomized clinical trial by Beus et al. showed no difference in achieving bacteria free canals when comparing use of only 1% NaOCl with use of a passive ultrasonic multi-irrigant (1% NaOCl, 17% EDTA and 2% chlorhexidine) protocol to clean the canals.[126] Evidence from another randomized clinical trial by Liang et al. indicates that periapical healing of endodontically treated teeth also seems to be similar with or without ultrasonic activation of 5% NaOCl.[127] The methods used in these studies widely vary and the effect of smear layer as a cofactor has not been completely clear. There has also lately been more debate regarding whether some experiments may be biased due to not differentiating sclerotic dentine from the smear layer.[121]

1.2.3.3.4 Biofilms and bacterial infection

Microbial infection is an integral part of pulpal and periapical pathosis.[65] This role has led to the development of various research regarding the microbial status in root canals[128] and study models to test the effect of treatments on their reduction and elimination. Disc diffusion methods and testing antimicrobial effects of chemicals on bacteria grown on agar plates that were common earlier are now questioned for their clinical relevance and the level of evidence that they provide.[6] Experiments on the antimicrobial efficacy of irrigants on planktonic cultures is also of limited value since bacteria express different phenotypes and have much higher resistance to antimicrobials in biofilms compared to when they are in a suspension.[129] Plastic, glass and stainless steel, which are often used as the substrate in biofilm experimental models, can result in different amounts of biofilm formation. This difference has been

attributed to the ability of the microorganism to adhere to the substrate as the first stage in biofilm formation.[130]

Bacteria generally do not adhere well to mineral tissue.[131] A biofilm model with a substrate that resembles dentine with a combination of organic and inorganic components was introduced by Shen et al.[129] Disks made of collagen-coated hydroxyapatite (C-HA) were used as the substrate in this study. The results showed that multi-species biofilm in this experiment was killed faster using CHX-Plus (Vista Dental Products, Racine, WI), which has surface modifiers in addition to chlorhexidine, compared to 2% chlorhexidine gluconate.[129]

Mono-species biofilm models have been more popular in endodontic research. Although the number of species in the root canal infections are much less than the oral microbial flora, poly-microbial biofilm models and their characteristics better resemble root canal infections.[132]

Quantification of bacteria in relation to treatments and disinfectants has remained a major challenge in endodontics. Starved biofilms, similar to the populations in the root canal, may be viable according to staining patterns and microscopy but in most cases cannot be cultured on media. Enumeration of Colony Forming Units (CFU) is one of the most prevalent means of quantification bacteria in endodontic biofilm studies,[132] which could be affected by the cultivability of bacteria.

Environmental Scanning Electron Microscopy (ESEM), Confocal Laser Scanning Microscopy (CLSM), flow cytometry, fluorescent protein tagging and fluorescence in situ hybridization (FISH) techniques have made better characterization of biofilms possible.[132]

Bacterial communities that are associated with endodontic infections have a high degree of variability. Different species and abundance are associated in individuals with similar clinical symptoms. This individual-to-individual variability is higher when comparing people from

different geographic locations. Bacterial communities residing in the apical, middle and coronal thirds of the root in an individual are also each diverse and significantly different.[14]

The role of bacterial infection as a cofactor in root canal treatment efficacy has long been proven with both clinical and laboratory evidence. Clinical testing of root canals before filling show the success rates of treated canals that have negative cultures before filling are higher.[133] However, it is agreed that the testing methods available until now have their shortcomings because of the difficulties in access to root canals, sampling and culturing.[134] Bacterial detection and characterization methods have improved in the last decades, which are helping researchers reach a better and more accurate understanding of the role of infection in the root canal environment.

1.2.3.3.5 Root canal surface roughness

Search in the PubMed database with the terms “("dental pulp cavity"[MeSH Terms] OR ("dental"[All Fields] AND "pulp"[All Fields] AND "cavity"[All Fields]) OR "dental pulp cavity"[All Fields] OR ("root"[All Fields] AND "canal"[All Fields]) OR "root canal"[All Fields]) AND roughness[All Fields]” dated 26 August 2017 was performed to obtain 48 results. No language restrictions were applied but to have at least an English abstract (2 articles dated before 1981 were omitted for this reason). After initial screening of the these articles and exclusion of unrelated research that were focused on the surface properties of anything other than the root canal surface (e.g. instruments, root filling material, outer root surface, etc.), 17 original research papers remained which are described in this section.

Chemical erosion caused by using different irrigants is the most common type of research regarding the surface qualities of the root canal after treatment. Farshad et al.[135] measured roughness of polished dentine surfaces (1200 grit polishing paper) that had been exposed to NaOCl 5.25%, chlorhexidine 2%, EDTA 17%, an imidazolium- based irrigant with nanosilver

particles or distilled water for 10 minutes. Atomic force microscopy (AFM) showed roughness average (Ra) mean values ranging from 95 nm (distilled water) up to 187 nm (chlorhexidine 2%).[135]

Simezo et al.[136] compared the chemical erosion after passive ultrasonic irrigation (PUI) against irrigation with reciprocating activation. Their roughness analysis was done using environmental scanning electron microscopy (ESEM) with the Phenom ProX (Phenom-World BV, Eindhoven, the Netherlands) and the 3D Roughness Reconstruction program (Phenom-World). The two methods of irrigation were similar in terms of causing dentinal roughness. The median values of roughness (Rz) ranged 0.31-0.54 µm and 0.44-0.99 µm for the PUI and irrigation with reciprocating activation groups, respectively.[136]

Ballal et al.[19] used AFM to compare chlorine dioxide (ClO2) 13.8% to other common root canal irrigants and chemicals. The roughness of polished dentine surfaces were tested after exposure to root canal irrigants. Mean Ra values for the ClO2 group (Ra approximately 200 nm) was lower than NaOCl (Ra approximately 300-400 nm), EDTA (Ra approximately 300-400 nm) and maleic acid (Ra>500 nm).[19]

Confocal laser scanning microscopy (CLSM) is another method which has been used to compare roughness created by smear layer removal methods. De Macedo et al. compared the effect of using Nd:YAG (1064 nm) and diode laser (980 nm) with EDTA to conventional EDTA use on polished bovine dentine. Roughness assessments by comparing “Sa” values of the groups revealed that the lasers cause a significantly rougher surface compared to conventional EDTA treatments for the removal of the smear layer. [137]

Cold plasma treatment has been considered as an option for disinfection of canals and eradication of *E. faecalis* biofilms. However, to evaluate its safety, the effect it had on dentine roughness and microhardness was studied by Li et al.[138] Polished dentine surfaces that were treated with cold plasma for up to 12 minutes were examined under a 3D Profile

Measurement Laser Microscope. Ra means were similar and at approximately 1.5 µm after up to 12 minutes of treatment.[138]

Endodontic regeneration protocols with calcium hydroxide, diluted triple antibiotic paste (DTAP) and triple antibiotic paste (TAP) can increase dentine roughness. Yassen et al.[139] calculated surface Ra and Rq from data acquired with an optical profilometer. Ra values of the polished dentine surfaces used in this study increased from approximately 0.3 µm in the untreated group up to 1 µm in samples treated with TAP.[139]

Semi-quantitative comparison of root canal roughness after using two different files was done first by Sabet and Lufty.[23] They used two commercially available filing systems (ProTaper and NRT) in combination with irrigants to chemomechanically prepare root canals. The method used to evaluate roughness on root canals was by CCD digital imaging and software analysis of the amount of darkness in each area with a grayscalescore (a score from 0 to 255). Although the method was unconventional in endodontic research, it showed that the ProTaper files created smoother surfaces in the apical third compared to NRT files.[23] The semi-quantitative method used to report roughness are difficult to compare to data outside of this study.

CLSM has also been used by Oliveira et al. to measure “Sa” of dentine surfaces after exposure to calcium based (Ca(OCl)2) hypochlorite solutions as an irrigant.[140]

Profilometer testing was used on polished primary teeth pulp chamber surfaces after exposure to different irrigants and chelating agents to determine the effect they could have on the bonding of restorative materials used to seal endodontically treated teeth. The roughness average (Ra) of surfaces treated with NaOCl 1% + EDTA 17% were the highest (1.117 µm) compared to the non-treated samples (0.254 µm).[141] Tartari et al.[142] also used a profilometer measuring Ra to test polished dentine surfaces after different irrigation regimens including etidronate (HEBP), EDTA, citric acid and NaOCl. Patil et al. used a similar design to calculate Ra after use of hydrogen peroxide 3% compared to conventional irrigants.[143] Ballal et al.

employed the same method to compare Ra of polished dentine samples exposed to maleic acid 7% with EDTA 17% as a chelating agent.[144] Profilometry was used by Eldeniz et al. to compare the effect of citric acid and EDTA.[21] Ra was determined using a profilometer to evaluate the effect of common irrigants on grounded root dentine surfaces for 15 minutes by Ari et al.[145]

Intracanal silicone impressions were the method that Barthel et al. used to compare the roughness left after using three different filing systems. Although they reported significant smoother surfaces in the hand filing and ProFile groups, their criteria to detect roughness was subjective and only by grading roughness as present or not present by the researcher.[22]

AFM analysis was used to compare the effect of irrigants on dentine specimens earlier by Hu et al.[20] This was one of the earliest studies using this method to compare roughness average (Ra) after use of hydrogen peroxide 3%, EDTA 17% and NaOCl 5.25%. These tests were accompanied with wettability evaluations of the surfaces to better understand how it may affect adhesion of biofilms to treated surfaces. It was concluded that EDTA created the roughest surfaces and NaOCl created surfaces with the smallest water contact angles.[20]

Overall, the majority of reports regarding roughness of the root canal surface have evaluated the effects of chemical erosion caused by irrigants and antimicrobials used during treatment. Although a wide range of methods have been used, roughness average (Ra) is the most common parameter calculated in these experiments. The roughness average (Ra) of root canal surfaces that have been chemically treated generally seem to be under 1 µm.

1.2.3.3.5.1 Surface roughness characterization and measurement parameters

Quantitative description of roughness has been the norm in dental research since it is fundamental for comparing data from studies. There are a wide range of measurement

methods and amplitude parameters used to report roughness. None of the parameters can represent a comprehensive view of the surface and each have their benefits and limitations.[146]

Amplitude parameters, which describe the surface based on height values, are the most common means of surface analysis in biological research.[147] Among these parameters, roughness average (Ra) has been the most commonly reported roughness measurement in dental studies.[146] Ra is the mean height deviation from the mean plane surface, which represents the average distribution of height values.[147] Other specific parameters are recommended in some cases based on their ability to provide information on certain aspects of roughness, e.g. use of bearing curves to show potential for surface wear in restorative dentistry. However, use of unconventional parameters often has the disadvantage of being difficult to interpret and compare with other studies.[146]

Extreme value parameters, such as Rz, can represent the surface characteristics with less evening out of the peaks and valleys of the surface through average calculation. Rz is a ten-point parameter calculated by measuring the mean difference between the five highest peaks and the five lowest valleys.[147] Extreme value parameters such as Rz are more sensitive to outlier values and reflect these measurements better than roughness average (Ra).[148] Rz provides a clearer understanding of the depth of the irregularities present in the surface.[149] It has been recommended to use other parameters such as Rq or Rz together with Ra to better specify the surface.[150,151] As an example in dental research, Rimondini et al. studied biofilm formation on titanium disks associated with both different Ra and Rz values that were inside patients mouths for 24 hours. Surfaces with Ra means less than 0.088 µm and Rz values lower than 1.027 µm had less plaque accumulation after the study period.[152]

1.2.4 Evolution of nickel-titanium endodontic filing systems

Rotary instruments were widely accepted after their introduction because of their flexibility and ability to negotiate through canal curves. The evolution of filing systems can be summarized into five generations based on the alterations in their design, metallurgy and motion.[153] Due to testing and comparison of multiple filing systems in our experiments, the evolution of nickel-titanium (NiTi) instruments and the data available on them has been reviewed in the following section.

1.2.4.1 First generation

Uniform taper and passive cutting radial lands were machined into a NiTi wire to manufacture the first generation of files. Their passive cutting design with neutral or negative rake angles made them less aggressive files. Therefore, in practice a large number of files would be required for each canal.[153]

Lightspeed files (Discus Dental, Culver City, CA, USA) had a different approach to other systems in this generation of files. Their unique design had a short cutting part and a long shank. These files were mainly used for apical preparation since most of the file length is non-cutting and smooth.

1.2.4.2 Second generation

Design changes in the second generation of files included having multiple tapers and active cutting edges with a positive rake angle that increased the file's efficiency. This improvement reduced the number of files in these systems. These instruments also had less risk of the screw effect compared to the first generation. Aside from design modifications, methods such as ion

implantation and electropolishing were used to improve the mechanical properties and cutting efficiency of this generation of files.[153]

The ProTaper filing system (Dentsply Tulsa) was initially introduced with 6 instruments.[153] These files have an increasing taper, a positive rake angle and a non-cutting tip. The cross-section is somewhat similar to a reamer with three cutting edges.[154]

1.2.4.3 Third generation

Thermomechanical processing of NiTi generated the third generation of endodontic instruments. M-wire and CM (Controlled Memory) wire, which are created by thermomechanical treatment of NiTi, were used in a wide range of files. Alongside the advancements in metallurgy, SybronEndo introduced the first NiTi file built by twisting and plastic deformation instead of machining a wire.

Twisted Files (SybronEndo) were manufactured with the technological advances in metallurgy and development of the R-phase NiTi alloy. Twisting NiTi wires was made possible by a heating and cooling process and modification of the crystalline structure of NiTi (R-phase).[155] Twisted Files had higher cyclic fatigue resistance compared to files that were manufactured by a machining process (RaCe, ProTaper and Helix).[156] Bacterial reduction in teeth that had *E. faecalis* cultured in them for a period of 30 days was similar among the Twisted File, Reciproc and self-adjusting file systems.[157]

HyFlex CM (Coltene/Whaledent AG, Altstatten, Switzerland) files and the newer HyFlex EDM (Coltene/Whaledent) files were both manufactured by heat treatment of CM-wire alloys.[158] HyFlex CM files are softer and have a lower proportion of nickel (52% weight) compared to other NiTi alloys used in endodontic instruments. Heat processing of the alloy also make it more elastic and resistant to cyclic fatigue.[159] This characteristic of the file translates to

significantly less canal straightening when using HyFlex CM files compared to the off-centred ProTaper Next system.[160] The softer alloy in these files results in deformation in 31% of the files during use but the majority of these deformed instruments recover most of their shape change after heat sterilization.[161]

Electrical discharge machining (EDM), which is used in manufacturing HyFlex EDM files, is a process changing the alloy shape by means of an electric potential and non-contact thermal erosion.[158,162] The surface of the file melts and vaporizes in this process, which hardens and roughens it. These files have a high cyclic fatigue resistance compared to single-file reciprocating Reciproc and WaveOne files that are made with M-wire.[158] The same pattern of having a higher cyclic resistance and lower torsional resistance was seen when comparing HyFlex EDM to ProTaper Gold files. These experiments all suggest the better suitability of the HyFlex EDM system for preparation of severely curved canals. Both the taper and cross-section shape of HyFlex EDM files change from apical towards the coronal. File taper is 0.08 at the apical 4 mm of the file but decreases to 0.04 in the coronal part. The cross-section of the file is rectangular-shaped in the apical region and changes to trapezoidal shapes in the middle and coronal segments.[163]

1.2.4.4 Fourth generation

Continuous rotation gave its place to other forms of motion in this generation of filing systems. Reciprocation reduces risk of the file locking into the canal by a counterclockwise (CCW) rotation while the clockwise (CW) rotation cuts and moves the file forward.[164] The angles of CW and CCW rotation in the first systems introduced were mostly small and equal. However, different cutting cycles with varying CW and CCW angles developed with each system that was introduced to enhance their cutting efficiency and debris removal. These innovations led to the introduction of using a single file for the instrumentation of a canal.

Reciproc and WaveOne are both examples practicing this concept with different designs and cutting cycles.[153]

Reciproc files benefit from the synergistic effect of reciprocating motion and M-wire technology to achieve a relatively high cyclic fatigue strength.[165] Micro-CT analyses show these files have a similar degree of apical transportation, centring ratio and canal volume increase compared to manual K-files and the rotary Protaper Next system.[166] However, there have been controversy regarding the quality of debridement with reciprocating instruments.[164] The amount of apically extruded debris associated with Reciproc files is higher than the multiple-file rotary Mtwo system and single-file rotary OneShape and F360 files.[167]

WaveOne files are a single-file reciprocating system similar to Reciproc files with differences in their cross sections (S-shape in Reciproc and concave triangular in WaveOne files) and reciprocation cycle angles (150° CCW and 30° CW at 300 rpm for Reciproc; 170° CCW and 50° CW at 350 rpm for WaveOne). WaveOne files have a higher torsional strength compared to Reciproc files but lower number of cycles to failure, indicating a lower cyclic fatigue resistance.[168] Micro-CT comparisons show WaveOne files lead to higher debris accumulation compared to preparation with a series of rotary ProTaper instruments.[164]

Twisted Files Adaptive (TF Adaptive; SybronEndo) were introduced by SybronEndo to operate in “hybrid reciprocation” using its Elements motor (SybronEndo). This meant that the files operate in rotary motion (600° CW and 0° CCW) but the motor can switch to reciprocation (370° CW and up to 50° CCW) if the torsional stresses build up on the file shaft.[169]

The Self-Adjusting File (SAF) was also introduced in this generation with a novel concept in file motion and design.[153] This system consists of a single file that vibrates in-and-out of the canal with 3000-5000 vibrations per minute at an amplitude of 0.4 mm. The file itself has a hollow and lattice design that can compress and adapt to the shape of the canal. The rough surface of the file abrades the root canal surface instead of the cutting action seen in conventional files

with blades.[170] Micro-CT evaluations show SAF leaves less untreated dentine surface in oval canals compared to preparation with ProTaper rotary instruments that have a round metal core.[171] Histology of oval-shaped root canals after instrumentation with SAF also traces less residual pulp tissue compared to the conventional rotary ProTaper system.[172] SAF performs more effectively in reduction of bacteria compared to hand instrumentation.[86]

1.2.4.5 Fifth generation

The offset design of the file is the major change in the fifth generation of files. In these systems, the centres of mass and rotation may each or both be offset while functioning. This modification creates less engagement between the instrument and dentine. Limitation of the active portion of the file also means risk of the screw effect is reduced.[153]

The first continuous rotary single-file system, One Shape (Micro-Mega, Besancon, France), was introduced in this generation of files. This file has a variable cross-section along its length that transitions from a three-edge design in the apical tip of the file to a two-edge design in the coronal segment.[153] This design feature has been suggested to be responsible for the lower apical bacterial extrusion of this system compared to ProTaper rotary instruments.[173] One Shape files also have less apically extruded debris compared to the single-file reciprocating Reciproc files.[167] However, the amount of bacterial reduction (*E. faecalis*) after instrumentation with One Shape system, the single-file reciprocating WaveOne system or manual filing are not significantly different.[174]

The TRUShape 3D Conforming file system (Denstply Tulsa Dental Specialties, Tulsa, OK, USA) has an S-shape in its longitudinal axis which allows it to have a larger surface of revolution.[175] These files have a variable taper and are manufactured by heat treatment methods.[176] When sterile saline is used as the irrigant, these files can remove more bacteria from oval-shaped canals compared to Twisted Files (SybronEndo), which are a conventional rotary system.[175]

TRUShape files also leave less unprepared areas overall in oval-shaped canals compared to Reciproc files though this difference is not significant when the apical area areas of the canals are compared.[177] Recent micro-computed tomography research found no difference in dentinal micro-cracks formed by using this system compared to the conventional rotary (BioRace), single-file reciprocating (Reciproc) and self-adjusting files.[113]

The XP-Endo Finisher file (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is another file with an offset design but different approach. It has an ISO 25 core size but no taper. Although the file can be straightened when cool, it has a C-shape curve in its apical half at body temperature. Its offset rotating design allows it to reach a diameter of 6 mm in function. This file is recommended to be used after root canal instrumentation to enhance cleaning by filing hard to reach areas of the canal.[178,179] XP-Endo finisher file's efficiency in removal of calcium hydroxide from straight canals is comparable to ultrasonic irrigation and significantly higher than needle irrigation.[179] Its ability to remove smear layer and debris in combination with EDTA is also higher than that of conventional rotary file (BT-Race, FKG Dentarie) agitation or rinsing without file agitation.[178]

1.3 Research questions

An overall shortage of clinically relevant cofactors that can determine the quality of a root canal treatment is obvious in the science and practice of endodontics. Experimenting with one of the only relevant factors, bacterial contamination, is a challenging task in practice using the current methods. Therefore, the search for cofactors that can evaluate different aspects of treatment quality continues. Surface roughness was found to be a potential cofactor considering the role it has been shown to have in other parts of dental research.

The knowledge gaps identified led to designing five research questions. The first question attempts to determine the clinical relevance of roughness in root canal treatments by evaluating the effect that surface roughness has on biofilm formation of *E. faecalis*, as one of the most important endodontic pathogens. The next four questions attempt to assess some aspects of clinical practice that may affect surface roughness of a treated root canal. Thus, the research questions that guided this thesis are as listed below:

1. Is the mean number of *E. faecalis* bacteria from biofilms formed on rough and smooth dentine surfaces different? (Chapter 2)

2. Is the mean surface roughness of canals after instrumentation with adaptive reciprocation and continuous rotary motions different? (Chapter 3)

3. Is the mean surface roughness of canals after instrumentation with a continuous rotary, single-file reciprocating or self-adjusting filing system different? (Chapter 4)

4. Is the mean surface roughness of canals after instrumentation with new files and files that have been reused once or twice different? (Chapter 5)

5. Is the mean surface roughness of canals after instrumentation in the apical, middle and coronal thirds different? (Chapters 3, 4 and 5)

My proposed research would lead to an understanding of whether surface roughness can be used as a reliable cofactor to assess the efficacy of root canal treatments. If surface roughness is proven effective, comparison of the potential effect that different instruments and techniques have could give insight into how they can be used to improve and optimize treatments. This can lead to changes in clinical practice and treatment strategies where the clinician can use this information to provide better quality treatment. Furthermore, quantitative results can provide a foundation where standards can be set for future instruments that are being designed. This would make practical and efficiency testing of instruments before introduction into clinical practice possible.

1.4 Hypotheses

The null hypotheses proposed for this thesis that were all later rejected in the following chapters are:

1. The mean number of *E. faecalis* bacteria from biofilms formed on rough and smooth dentine surfaces would not be significantly different. (Chapter 2)
2. The mean surface roughness of canals after instrumentation with adaptive reciprocation and continuous rotary motions would not be significantly different. (Chapter 3)
3. The mean surface roughness of canals after instrumentation with a continuous rotary, single-file reciprocating or self-adjusting filing system would not be significantly different. (Chapter 4)
4. The mean surface roughness of canals after instrumentation with new files and files that have been reused once or twice would not be significantly different. (Chapter 5)
5. The mean surface roughness of canals after instrumentation in the apical, middle and coronal thirds would not be significantly different. (Chapters 3, 4 and 5)

Chapter 2 Quantitative comparison of biofilm formation on rough and smooth root canal surfaces using flow cytometry

2.1 Chapter overview

Establishing the link between dentine surface roughness and biofilm formation with bacteria that are involved in root canal infections is what this chapter is aiming to achieve. The results from this study can demonstrate the clinical relevance of dentine surface quality and its potential effect on treatment failure. The research question for the following experiment is "Is the mean number of *E. faecalis* bacteria from biofilms formed on rough and smooth dentine surfaces different?"

This chapter contains material that has been used for the following paper, which is currently under review for publication in the International Journal of Endodontics:

Sakhaei Manesh V, Giacomin P, Stoll R. Quantitative comparison of biofilm formation on rough and smooth root canal surfaces using flow cytometry.

This study was supported by the James Cook University Graduate Research Scheme Grant (grant numbers JCU-QLD-537191, JCU-QLD-565281 and JCU-QLD-602531).

2.2 Introduction

Primary apical periodontitis and post-treatment endodontic disease are both considered biofilm-induced diseases.[134,180,181] Persistent apical periodontitis that occurs after endodontic treatment is considered more complex in terms of its aetiology and treatment compared to the primary infection.[181] This suggests that the endodontic treatment may be altering the environmental balance in the root canal. Biofilm formation on any surface including dental

tissues depends on the characteristics of both the bacteria and the surface.[16] In endodontic treatment, this translates to the characteristics of the dental tissues and the bacteria, including *Enterococcus faecalis* as one of the most common species.[182]

Microbial growth-positive results are found in up to 33% of patients' root canals after their chemomechanical preparation has been completed.[183] However, bacteria need to reach a certain number (load) to be able to cause tissue damage and disease. This threshold can vary with differences in microbial virulence and host defence. Therefore, the goal of root canal treatment is to eliminate or minimize bacterial numbers to a level below this threshold. Determining the bacterial load required to cause disease or result in healing in endodontic diseases has been mostly investigated with older culturing studies and needs further research with more modern and quantitative methods.[182]

Culturing methods had been considered the gold standard for microbial detection and assessments in endodontic research.[63,64] Even though this method is still preferred for antimicrobial sensitivity tests and phenotypic assessment of bacteria,[63] it does not provide an accurate reflection of all species that infect the root canal system. This is because it is not possible to culture almost half of oral microbiota with the methods used.[63,184] This effect may even be amplified in endodontic infections since microorganisms are often exposed to antimicrobials which also interfere with their growth.[63] Advances in culturing methods have also made obtaining cultures of previously uncultivated microorganisms possible. Strategies that simulate the natural environment for the microorganisms such as adding little or no nutrition to the media or culturing for periods more than 30 days have proven to be effective in some cases.[185] Although being costly and time-consuming, culturing methods are still a feasible option for viability assessment and quantifying of species that are cultivable.[63,64]

Advances in imaging and microscopy methods allow accurate localization of microbial cells and biofilm structures. Fastidious microorganisms can be detected using microscopy. In addition,

vitality staining can be useful to determine live and dead cells.[63] However, the sensitivity and specificity of microscopy is still relatively low compared to molecular biology methods. Interpretation of results are often subjective and a great number of microorganisms are required to be visible using the microscope.[64]

Immunological methods such as the enzyme-linked immunosorbent assay (ELISA) and immunofluorescence tests can be used to target specific microorganisms. These tests are often quick, cost-effective and standardized which can be used to detect dead cells too. However, they also have lower sensitivity compared to molecular biology methods and their specificity largely depends on the antibody that is applied in the test.[64]

Novel molecular biology methods have made detection and activity analysis of a much wider range of root canal microorganisms possible. Molecular methods allow accurate classification of microorganisms with a very high sensitivity.[63] Many microorganisms which were previously unknown have been identified in the past decades with the help of molecular biology. Various methods based on the detection of DNA, RNA and proteins have been developed. These tests have higher sensitivity, specificity and provide rapid results and diagnosis.[64] Genetic analysis can determine the virulence, antibiotic resistance, functions and taxonomy of microorganisms. Proteomic analyses help identify which proteins have been expressed and provide a better understanding of microbial activities.[63]

Variations of polymerase chain reaction (PCR) and DNA-DNA hybridization techniques can be used to test presence of target a broad range or specific species. Fluorescence in situ hybridization (FISH) techniques can help quantify microbial species and determine how they are spatially distributed in the host tissue. Currently, small subunit genes, specifically the 16S rDNA which is an evolutionary conserved macromolecule in all living organisms, are the most frequently used sequences for identifying microorganisms.[64] However, PCR-derived methods also have many limitations and use of each variation requires careful consideration based on

the application. Although real-time PCR methods can provide quantitative information, most PCR methods are qualitative assays of one or a limited number of the target species. Broad-range PCR assays which are costlier are more suitable for detecting unknown or a wider range of species. The high sensitivity of PCR means that in case of a non-quantitative assay, species that may be of little clinical significance because of low numbers are still identified. In addition, identification of dead cells with PCR may further complicate interpretation of the role of microorganisms.[64]

Flow cytometry is one of the few methods that can provide real-time information about microorganisms and their physiological status. The advantage of this method is that it provides rapid results and is not dependent on culturing of the microbial cells. Need for high cost and complex equipment and expertise to carry out flow cytometry has been the limiting factor for its use. However, more biological reagents and antibodies are being developed into kits that can be used to selectively label microbial cells. Advances in fluorescent markers that can label variables from phylogeny to enzymatic activity have made flow cytometry into a powerful tool in studying microorganisms.[186]

Flow cytometry use in endodontics is limited to only a few research experiments. Live/dead staining, detection of cells and membrane damage have been the main applications of flow cytometry in these reports. Kesler Shvero et al. used flow cytometry and a BacLight Bacterial Membrane Potential kit (Molecular Probes, Invitrogen, Eugene, OR, USA) to assess the antibacterial effect that a modified epoxy resin based sealer may have on biofilms formed on its surface. It was shown that introduction of cationic nanoparticles into the sealer damaged the bacterial cell membrane integrity because the cell membrane potential for *E. faecalis* had decreased.[187] Noites et al. also used flow cytometry to assess the mechanism of action for multiple antimicrobials that can be used in disinfection of root canals. The cell membrane permeability of *E. faecalis* and *C. albicans* after treatments were tested with fluorescent

markers. Flow cytometry assays showed that cell membrane permeability changed after use of ozone but not chlorhexidine. The combination of chlorhexidine and ozone gas was proven to have a synergistic antimicrobial effect which was understandable since their mechanisms of action were different.[188] Pirnat et al. used flow cytometry with the Cell Viability Kit with Liquid Counting Beads (BD Biosciences) enumeration and viability assessment of *E. faecalis*. Results showed that sub-second laser-generated heat pulses were as effective as continuous-mode in disinfection. The proposed model created shows efficiency of these pulsed mode lasers would be higher in root canals since they would lower chances of thermal damage to tissues. The results from conventional plate counting of the same samples with *E. faecalis* showed agreement with the data from flow cytometry in this study.[189]

Elimination of *E. faecalis* from the root canal system with root canal treatment seems to be infeasible in most cases.[190] However, it is important that the bacteria are reduced as much as possible and that their chance of regrowth is minimized. Factors that may have the potential to eliminate biofilms inside root canals or prevent their formation after treatment have always been of research interest. These experiments have mostly been on the efficacy of chemicals and irrigants used in root canal treatment,[38] use of hand or rotary instruments,[191] number of visits to complete treatment and use of intra-canal medications in between treatment stages.[92,192]

Roughness is the dominant surface characteristic affecting bacterial adhesion, which itself is the first step in biofilm formation.[16] Surface roughness of implant abutments has been shown to increase adherent bacteria up to 25 times.[17] In dental research, roughness has been one of the most thoroughly investigated properties of dental materials and tissues in regards of how it can affect the attachment of microorganisms. Investigations in this field have also shown how surface quality may even differently affect the balance of subgingival and supragingival microorganisms involved in creating biofilms on dental materials and implants.[16,56] Surface

characteristics of dental implants, including their roughness, have been shown to be of significant importance in preventing peri-implantitis and implant failure.[49,50] These findings have justified research into the effect of roughness on different dental treatments and determining the ideal roughness levels to achieve the best clinical outcome.

Physical properties of a surface, such as roughness, surface charge and wettability, can have a different magnitude of effect on biofilm formation. The significance of each of these factors widely depends on the type of microorganisms involved. There is a noticeable gap in the research regarding how *E. faecalis* and other microorganisms of the root canal interact with these surface features. The effect of roughness as the most significant surface factor and how it may effect microorganisms that cause root canal failures is the focus of this study.

2.3 Materials and methods

2.3.1 Sample preparation

Nine maxillary canine teeth with a straight root, mature apices and free of decay were collected from JCU Dental Clinic. Sample size was estimated based on a pilot study and using the following formula (considering the sample size would suffice for tests with a significance level of 0.05 with a power of 80%):

$$\boldsymbol{n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{d^2}}$$

$$\boldsymbol{\alpha = 0.05 \Rightarrow Z_{1-\alpha/2} = 1.96}$$

$$\boldsymbol{1 - \beta = 0.80 \Rightarrow Z_{1-\beta} = 0.84}$$

$\boldsymbol{\sigma_1 = 100000}$ $\boldsymbol{\sigma_2 = 100000}$ (Standard deviations of approximate cell counts calculated in pilot tests)

$$\boldsymbol{n} = \frac{(1.96 \quad + 0.84 \quad)^2(100000^2 \ + 100000^2 \ )}{180000^2} \ = \frac{15.86}{3.24} = \mathbf{4.89} \cong \mathbf{5}$$

Ethical approval of the study was obtained from the James Cook University Human Research Ethics Committee (H5798). The teeth were decoronated and cut to a root length of 18 mm. The root canals were instrumented with K-files sized 15 to 25. The roots were mounted into an acrylic resin cylinder mould and numbered 1-5. After 48 hours storage in water to allow setting of the resin, the samples were cut in half vertically using a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA; Figure 2-1). One half was randomly assigned to the "Rough" group while the other half was assigned the same number in the "Smooth" group.

Figure 2-1 Precision saw used for sectioning teeth and preparing dentine blocks.

Extra-coarse finishing discs (OptiDisc, Kerr, Bioggio, Switzerland) were used with low speed hand instruments to grind the surface of the root until a flat surface was achieved. The coarse-medium finishing discs were used next in the sequence. This was the final preparation stage

to achieve a final rough surface for the Rough group. For the Smooth group samples, the fine and extra-fine discs were also used in the sequence to achieve a final smooth root canal surface. Prior to this experiment, pilot studies using 3D roughness reconstruction (Phenom G2 Pro SEM System and the Phenom Pro Suite, Phenom-World, Eindhoven, Netherlands) had shown that surfaces prepared with the coarse-medium and extra-fine finishing discs had Rz mean values of approximately 35 µm and 15 µm, respectively (Figure 2-2). Seven root halves were prepared using the same method as the Rough group to serve as the Control group (n=7) that would later undergo the same experimental procedures but without the bacterial contamination stage.

The surfaces of all groups were covered with 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute followed by irrigation with 1% sodium hypochlorite to remove the smear layer and achieve a final surface similar to a treated root canal (Figure 2-2). The surface of each sample was then rinsed with distilled water. Samples were sterilized in an autoclave set to 121°C and 15 psi, for 15 minutes. They were then placed in a six-well cell culture plate with the prepared side facing up in a laminar flow hood.

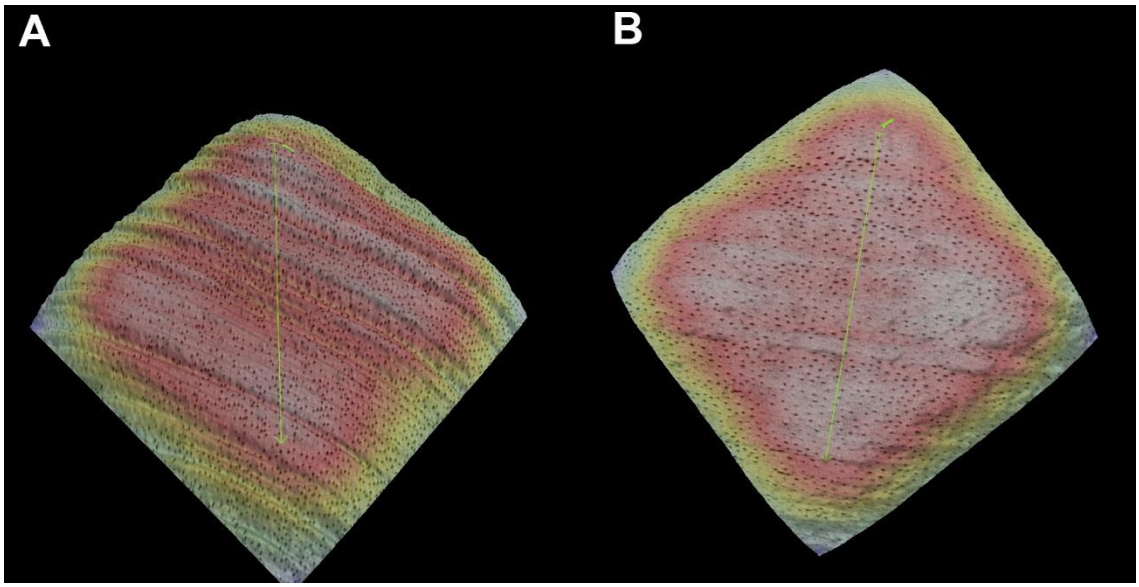

Figure 2-2 Scanning electron microscope surface height map of an (A) rough (Rz=35.10 µm) and (B) smooth (Rz=16.74 µm) sample obtained using 3D roughness reconstruction.

2.3.2 Bacterial contamination

E. faecalis bacteria (ATCC 29212) were obtained from -80 degrees stock culture. Bacteria were inoculated into Todd Hewitt Broth (THB) agar plates and incubated for 24 hours at 37°C. A single colony was taken from the plate and grown overnight in Tryptone Soy Broth (TSB) at 37°C in a shaking incubator. The bacteria solution was tested with a spectrophotometer to achieve an optical density of 1 at 600 nm (OD_{600}=1). Inoculation of *E. faecalis* into the six-well culture plates with 10 µl of the suspension was done under sterile conditions in a laminar flow hood. Each well was supplemented with 6 ml of sterile TSB. The plates were kept in a shaking incubator for 48 hours at 37°C. Additional broth was added to the wells every 12 hours.

After the 48 hour incubation period, samples were gently rinsed with phosphate buffered saline (PBS). A dentine block (1 × 1 × 0.8 mm) was cut out from the centre of the surface of the sample, so that the dentinal tubules would be directed vertically as they would in a root canal, using the precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) along with PBS as its coolant. After gentle rinsing of the acquired dentine block using PBS, they were each placed into a vial with 500 µl of PBS. The vials were placed in a vortex shaker (TissueLyser II, Qiagen, Hilden, Germany) for 60 seconds to dislodge the attached biofilm (Figure 2-3). The fluid was stained with a cell viability kit with liquid counting beads (BD Biosciences, San Jose, CA, USA; Figure 2-4) according to the manufacturer's instructions. Briefly, thiazole orange (TO) and propidium iodine (PI) from the kit were added to the vials. Samples were vortexed and stored in the dark at room temperature for 15 minutes. Liquid counting beads were added to the solution and the total number of bacteria in each solution were assessed using flow cytometry (BD FACSCanto II, BD Biosciences; Figure 2-5). Flow cytometric analysis was performed using FlowJo software (Treestar, Ashland, OR, USA).

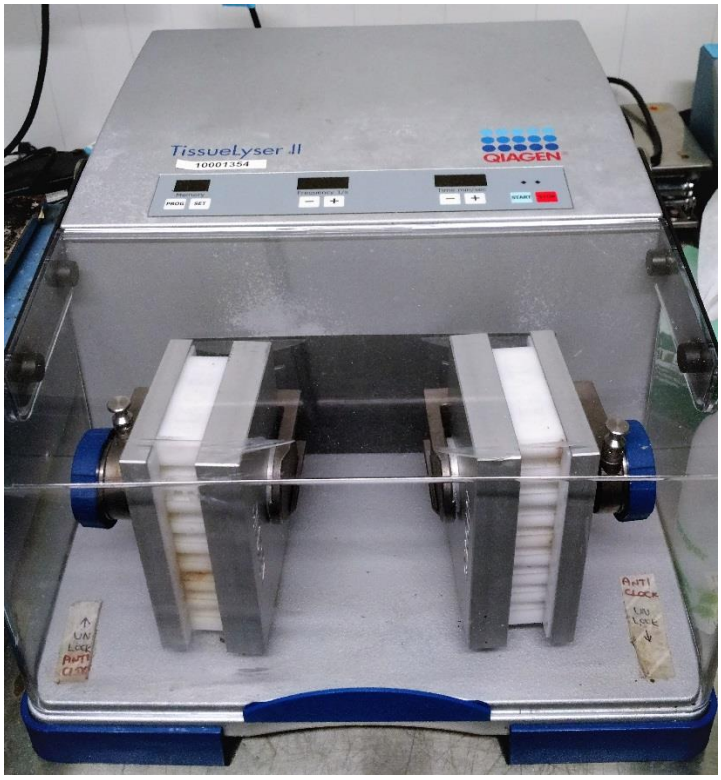

Figure 2-3 Vortex shaker that was utilized to dislodge the attached biofilm.

Figure 2-4 Cell viability kit and liquid counting beads used to carry out flow cytometry assay. Solutions from left to right contain Propidium Iodine (PI), Thiazole Orange (TO) and BD Liquid Counting Beads.

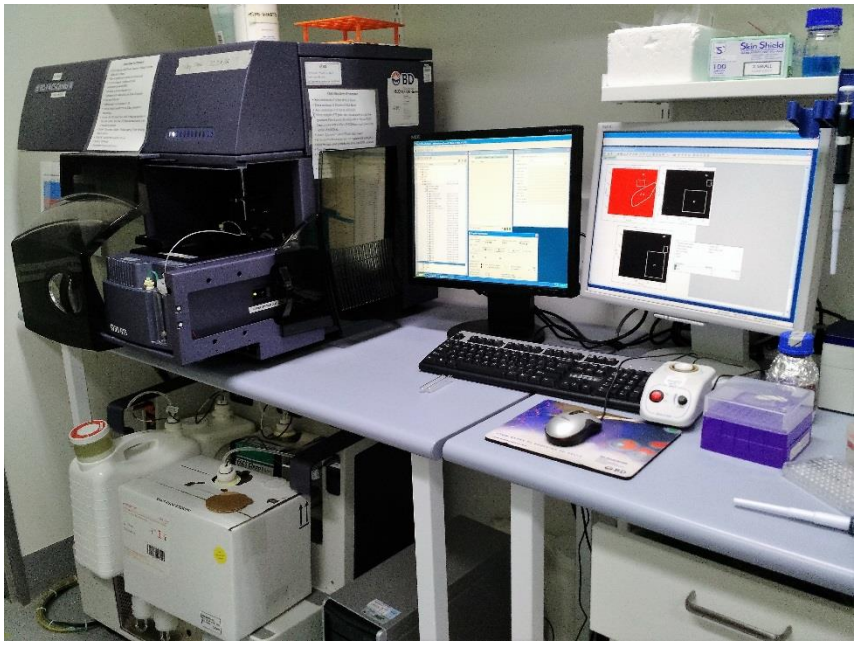

Figure 2-5 BD FACSCanto II flow cytometer.

Data was entered into IBM SPSS Statistics for Windows Version 23.0 (IBM Corp., Armonk, NY, USA) and the bacteria counts of the Rough and Smooth groups were compared using a paired-sample T-test. One-way ANOVA was also performed for the three groups (Rough, Smooth and Control). Significance level of 0.05 was considered for all tests.

2.4 Results

Paired samples T-test showed a significant difference between mean bacterial count of the samples in the Rough group and their counterparts in the Smooth group ($p=0.025$; Figure 2-6). One-way ANOVA revealed a statistically significant difference among the Smooth, Rough and control groups ($p<0.001$). Post-hoc Tukey tests demonstrated that the Rough group had a significantly higher mean bacteria count than the control group ($p=0.007$) but the difference between the Smooth and control groups was not statistically significant ($p=0.256$; Figure 2-7).

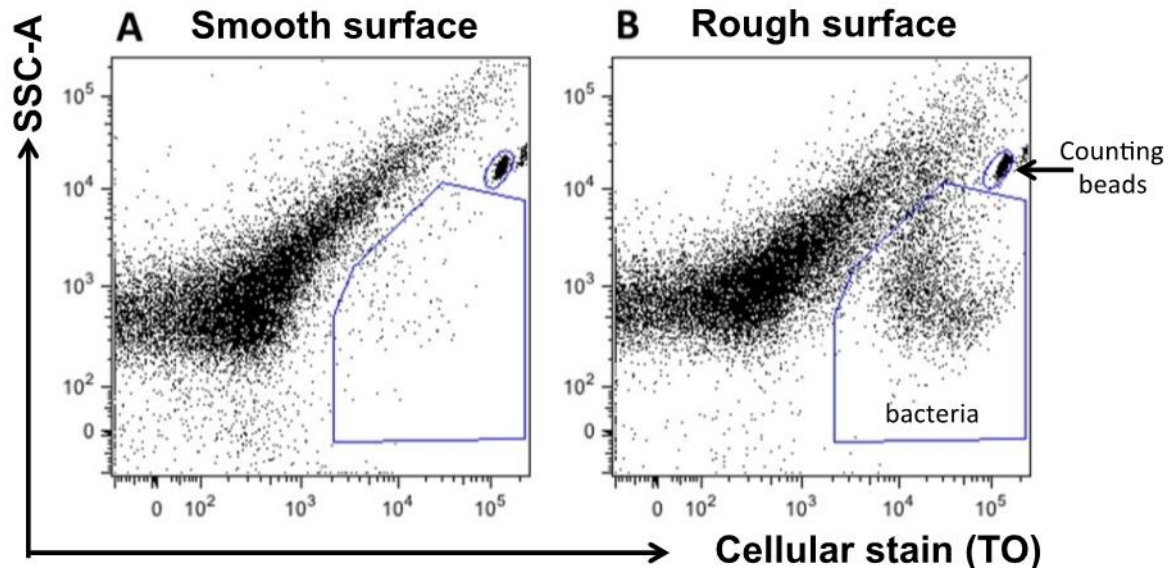

Figure 2-6 Representative flow cytometric plots of bacterial samples derived from a smooth (A) and rough surface (B). Number of bacterial cells were assessed by analysing the frequencies of gated TO-positive bacteria relative to gated counting beads. SSC-A denotes side scatter area.

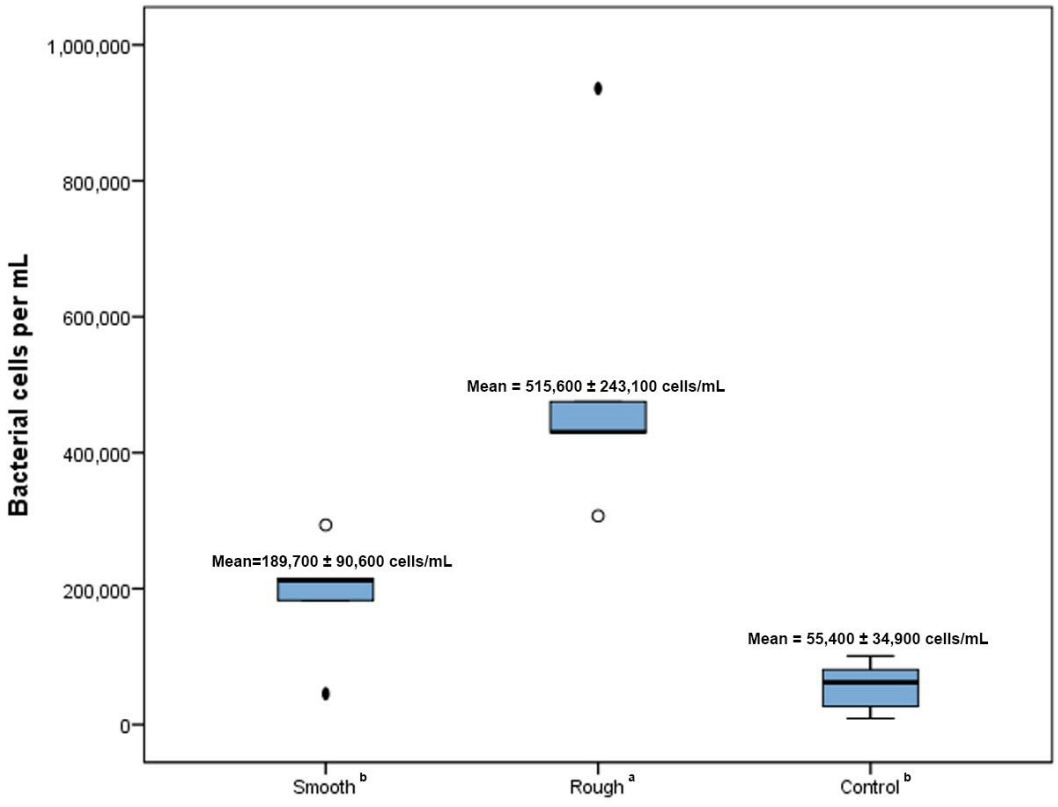

Figure 2-7 Box plot of bacteria count per mL displaying median and distribution of results. Conventional mean bacteria count per mL ± Standard deviation indicated in writing based on experimental group. Statistical comparison by post-hoc Tukey tests. Different superscript letters indicate statistically significant difference between groups ($p<0.05$).

2.5 Discussion

Reducing bacterial load to below a level that can no longer be detected by culturing is the goal of root canal treatment based on the current limitations of research methods. This bacterial level is arguably estimated to be 10^3-10^4 cells but it is also a challenging task to calculate because of the difficulty of culturing anaerobic microorganisms. More advanced methods such as quantitative polymerase chain reaction assays and fluorescence in-situ hybridization may help gain a more accurate understanding of the interactions between the host and microorganisms but little research is currently available with these methods.[182] Treated teeth with a negative culture before obturation have been shown to have a better prognosis.[133]

Flow cytometry had previously shown to be a reliable and rapid method for acquiring quantitative data on *E. faecalis*.[189] The results from flow cytometry are comparable with plate counts by culturing. The sensitivity of flow cytometry to detect microorganisms, its ability to detect microorganisms that are not cultivable or dead and its speed are some of the advantages that can be used in experiments.[189,193] This is especially of importance because of the starvation and "viable but not culturable" state that bacteria may enter when they are in biofilms.[194] Root canal bacteria show similar mechanisms to survive the effects of chemicals such as calcium hydroxide dressings used endodontic treatments.[195]

Sampling from canals has been a main concern in previous experiments. Using a paper point to collect root canal microorganisms is one of the most common methods. The acquired sample should ideally be representative of microorganisms in the canal, dentinal tubules and the attached biofilms. In practice, the sample acquiring tool does not touch most surfaces of the root canal and captures free-floating bacteria[63] since their function is based on the paper's capillary effect.[17] The use of files to disrupt the canal biofilm is also limited to the surface microorganisms in the canal and only those that are touched by the file[63] and may have a high sampling error based on the operator. High-speed shaking of the sample which was used in

the present method allows access to a better representation of bacteria that are present in different parts and depths of the sample without being affected by the operator. Vortex and shaking methods used to dislodge biofilms have been well documented in previous research.[93,196,197] Dentine blocks were cut with a relatively large size surface area in the present experiment. It was assumed that the biofilm may be disrupted at the edges of the block where the sample was cut but the conditions would be similar for both groups. However, assessing a larger surface area covered with biofilm was believed to further reduce the effect of any factors related to sample preparation.

Biofilm composition and quantity are in relation with the substrate's surface qualities. Supragingival plaque that adhere to rough surfaces in the oral cavity after a period of 3 months have been shown to contain less coccoid cells and more spirochetes and motile bacteria. This indicates higher maturity of the biofilm in rough surfaces.[17] Higher pathogenicity and growth rate of plaque in a 96-hour experiment[17,47] also show that the effect that surface characteristics have on the microorganisms starts early in the biofilm formation stages. These results may provide better insights as to why bacterial species that are less common in primary root canal infections are seen in persistent endodontic infections.

E. faecalis was used in this study since it is one of the most common species found in persistent endodontic infections.[71] This bacteria has been shown to be able to invade dentinal tubules[95], survive through nutritional deprivation,[71] form biofilms[39,198] and cause persistent infections that are resistant to treatment.[71,95] However, since root canal infections are known to be polymicrobial,[199] assessing the effect of roughness and other surface quality attributes with multispecies samples would also be the next step in further understanding how to benefit from these effects. Based on research that demonstrates the relationship between surface roughness and biofilm composition,[17,47] if smoother surfaces could lead to less mature and

resistant biofilms in root canals, this could be used as an advantage in treating root canal infections.

Biofilm formation in 48 hours was 2.7 times more in rough surfaces compared to smooth ones in the present study. Although not much quantitative data is available, very few factors that affect adhesion of microorganisms to the root canal surface to this extent have been reported in previous research. Smear layer presence has been shown to inhibit bacterial colonization with some species such as *Streptococcus gordonii* and *Streptococcus anginosus*.[200,201] On the contrary, elimination of smear layer has been associated with the decrease of *E. faecalis* and *Prevotella nigrescens* adhesion, which are active bacteria in root canal infections.[202,203] The contradictory results regarding the elimination of smear layer has raised some questions as to whether its effect is due to exposing dentinal collagen (which bacteria can bind and adhere to) or a combination of factors including the effect that its removal has on physical surface properties.[203]

Roughness has been shown to even increase in the same filing system when different movements are implemented. Reciprocation of the file results in a significantly rougher (higher Rz) surface compared to continuous rotation of a file. This has been suggested to be associated with the multiple cuts a reciprocating instrument makes while changing directions.[204] Although there is not much research available regarding the surface roughness of root canals after instrumentation, an attempt was made to achieve dentine surfaces with roughness close to that of treated canals. The Rz values of approximately 15 µm and 35 µm achieved with finishing discs were considered after initial testings of files on root canal surfaces (Chapters 4 and 5) as they were close to the low and high thresholds after filing. However, further research is required to gain a better understanding of how different levels of roughness can affect biofilm formation. Future experiments on the different instruments and treatment methods available would also reveal what levels of smoothness are practically

achievable. Research also shows that use of different instruments and irrigants in the preparation of a root canal can translate into irregularities that impacts the apical and coronal parts of a root canal differently.[19,154] These experiments designate the importance of the quality of a cleaned and shaped root canal surface especially when the performance of a filing system is being considered.

Dentinal tubules can shelter bacteria deep inside and make them difficult to eliminate. The remaining bacteria may regrow and be the source of reinfections.[205] These microorganisms in addition to the cells detached from the tissue may also partly be the source of the cells that are counted even in the control group. Provided that enough nutrients are present, *E. faecalis* has been found to reach a mean of 1166-1483 μm depth into dentinal tubules after only 21 days.[206]

Root canal surfaces can be considered to exhibit a unique topographic pattern with their dentinal tubules and therefore, root canal dentine was used as the substrate in this experiment. This may be a matter of concern when experimenting bacterial attachment on artificial surfaces. Surface topography could have a significant effect on bacterial attachment when other characteristics of surfaces such as surface roughness, surface energy and chemistry are similar.[207]

Incubation times of 48 and 72 hours have shown to have no significant difference in biofilm formation but bacterial levels decrease after 7 days. This timing has been suggested as when the bacterial growth is maximum and has entered a stationary phase.[190,192] Two day culturing has also been utilized in previous studies.[190,199]

Chemicals used in root canals lose their potency over time. However, physical characteristics such as roughness are more stable and can cause the same effect over time. Roughness can remain and continue to increase the chances of biofilm formation when conditions allow bacterial growth. This is particularly important in endodontic treatments since cleaning and

shaping of canals consists of physical cutting of dentinal tissues, which alters the surface. Further research is required to determine the magnitude of the effect that instrumentation of canals has on their surface roughness.

2.6 Conclusion

Surface roughness of the root canal after treatment can affect the amount of biofilm formation after treatment. The results from this chapter helped establish the clinical relevance of surface roughness in root canal treatments. Therefore, treatment methods, instruments and chemicals that can promote smoothness of the root canal surface are recommended to decrease chances of biofilm formation. In order to achieve this, the next step is to determine the range of surface roughness that results from root canal treatment and whether the clinician can alter the treated root canal surface towards achieving a smoother finish. Identifying practical variables in treatment that can be used to alter the root canal surface roughness is attempted in the following chapters.

Chapter 3 Quantitative evaluation of root canal surface roughness after filing with adaptive reciprocating and continuous rotary instruments

3.1 Chapter overview

After establishing the role of dentine surface roughness on biofilm formation, this chapter is designed to study the effect of practical treatment techniques on dentine surface roughness. Continuous rotation and reciprocation are the two most frequently used filing motions used in root canal instrumentation. In the present experiment a filing system that was compatible to work in both rotary and adaptive reciprocation modes was used to answer the following research questions:

- Is the mean surface roughness of canals after instrumentation with adaptive reciprocation and continuous rotary motions different?
- Is the mean surface roughness of canals after instrumentation in the apical, middle and coronal thirds different?

This chapter contains material that has been presented in a scientific congress and published in a journal article listed below:

Sakhaei Manesh V, Giacomin P, Stoll R. Quantitative evaluation of root canal surface roughness after filing with adaptive reciprocating and continuous rotary instruments. Microsc Res Tech. 2017;80:657–661. https://doi.org/10.1002/jemt.22845

Sakhaei Manesh V, Giacomin P, Stoll R. Quantitative evaluation of root canal surface roughness after filing with adaptive reciprocating and continuous rotary instruments. SIE (Societa Italiana di Endodonzia) International Congress 2016, Rome, 10-12 November 2016.

This study was supported by the James Cook University Graduate Research Scheme Grant (grant number JCU-QLD-602531). The author would like to thank SybronEndo for their donating of the files used in this study and loaning of the motor used during the study.

3.2 Introduction

The ideal canal preparation is difficult to achieve with inflexible steel instruments since canals that undergo root canal treatment are typically curved. Instruments made out of nickel-titanium (NiTi) alloys are superelastic and better suited to shape curved canals.[208] Unfortunately, these instruments fracture due to their continuous rotation that causes fatigue, and the torque forces created during function. Compared to hand instruments, these files also undergo more rotation when used with rotary instruments which makes them more prone to fracture.[169,209]

Different approaches such as reciprocating rotation instead of continuous rotation have been suggested to increase the safety and performance of engine driven NiTi files.[209] This means that the file rotates a specified amount in one direction (where most of the cutting is carried out) and then rotates in a reverse direction to disengage the instrument. Some filing systems can cut in both of the rotation directions.[210] Although initially the NiTi files used in reciprocation were the ones that were designed for rotary use, the improved mechanical performance of the files led to introduction of new filing systems that were designed to work in reciprocation. The file design, reciprocating angles and speed of these systems were optimized to achieve an acceptable cutting efficiency and improve their progression into the canal.[169]

Stationary reciprocation is a type of reciprocating motion in which the angle of rotation in both directions is equal. This results in the instrument returning to the same position after every cycle. Since the file may be under more stress in some areas, stationary reciprocation

can lead to fatigue damage localization which reduces its fatigue life. Progressive reciprocation is a type of reciprocating movement which periodically progresses the file forward in order to change the position of the file relative to the canal. This means that the angles in forward and reverse motion of the cycle are not equal. Both progressive shifting and the degree of progression in each reciprocating cycle can affect the fatigue life of NiTi instruments.[211]

Reciprocation can improve the fatigue resistance of a NiTi file and improve its life span compared to continuous rotary motion.[212] Interest in the effect of movement mechanics on cyclic fatigue of files begun when research showed that reciprocation extended the cyclic fatigue life of the rotary ProTaper files.[213] Yared described a technique in which the whole canal preparation could be done with one ProTaper file in reciprocating motion. The clockwise and counterclockwise movement angles, which were four-tenth and two-tenth of a circle respectively, were calculated based on the torsional fatigue profile of the files.[210] Later research on newer rotary files such as the Twisted File,[212] RaCe and MTwo[214] systems also confirmed that using them in reciprocation mode results in higher cyclic fatigue resistance. However, utilization of reciprocation should be done with caution since reciprocation may cause torsional distortion in files that are designed for use in continuous rotation. Damage and unwinding of the file occurs when the reciprocating angles are not within the file's elastic limit.[215]

The cutting efficiency of Twisted File instruments are not significantly different between rotary filing and adaptive reciprocation.[216] Single-file reciprocating Reciproc files also show no significant change in cutting efficiency when their functioning mode is altered from reciprocating to rotary.[217] Reciproc instruments demonstrate higher cutting efficiency on Plexiglas blocks compared to WaveOne files, even though both are single-file reciprocating systems. This outlines the effect of a file's design and cross section in its cutting behaviour. The smaller cross-section of a Reciproc file has been linked to its better cleaning effectiveness,

which has been attributed to why it can cut more efficiently while displacing the debris that are created during the process.[218]

Cleaning effectiveness of reciprocating instruments is affected by the shape of the root canal. Using a single reciprocating F2-sized ProTaper instrument results in similar debridement of pulp tissue in round canals compared to a full set of ProTaper instruments in rotary motion. However, reciprocating with a single file in oval canals leads to a considerably higher percentage of residual pulp tissue.[219] Comparison of the cleaning efficiency of single-file reciprocating Reciproc and WaveOne files with the previously established Mtwo and ProTaper rotary systems shows that overall they are both as efficient as Mtwo and significantly better performing than ProTaper files. The efficiency seems to heavily depend on the file design since the cleanliness of the apical third of canals that have been filed with Reciproc or Mtwo are significantly better than WaveOne and ProTaper.[220]

The introduction of single-file systems that work in reciprocation has also simplified the instrumentation process and made it possible to achieve centred preparations while being less dependent on user experience.[221] Multiple studies support the ability of reciprocating files in preserving the root canal anatomy and preventing transportation.[222-224] SEM evaluations also show that defects appear on Reciproc files after being reused in nine canals compared to six canals for Twisted Files, which work in continuous rotary motion.[225]

Dentine defect formation in result of root canal instrumentation has been a controversial topic. The findings of studies in this field are variable depending on the experimental method. Scanning electron microscopy (SEM) studies indicated a lower frequency of microcrack formation in roots that are instrumented with reciprocating files.[226] However, more recent studies utilizing non-destructive micro-CT methods question the effect of filing on crack formation.[112]

Bacterial elimination in oval canals is comparable between the Reciproc single-file reciprocating system and the BioRaCe rotary system. Both instrumenting systems are able to eliminate over 99.9% of bacteria.[227] Marinho et al. also compared the efficiency of Reciproc files in eliminating *Escherichia coli* bacteria and endotoxins in comparison to rotary systems (Mtwo, ProTaper and FGK Race). Their study also found that all systems were able to remove over 99% of the bacteria and an average of 79-92% of their endotoxins, but no significant difference was seen among filing groups.[228] Comparison of Reciproc, self-adjusting file and Twisted Files shows they are all effective in reduction of bacteria load from the root canal. However, it is noteworthy that a high number of canals still have positive cultures after chemo-mechanical preparation of root canals with the various filing systems.[157]

Twisted Files (TF) were introduced with a heat treatment in their manufacturing process, known as the R-phase, that allowed twisting of the NiTi to create the cutting edges.[155] This process in combination with surface conditioning of the files is believed to be responsible for improvement in file flexibility, strength and fatigue resistance.[229] TF instruments were initially designed to be used in rotary motion. However, with the introduction of the technique of using a single F2 ProTaper file in reciprocation mode for canal preparation,[210] other systems such as TF were also tested in reciprocation. The cyclic fatigue fracture resistance of TF files in simulated canals with 60° curvature increases if used in reciprocation instead of rotary. The increase cyclic fatigue life compared to rotary motion is observed in both 30°CW/150°CCW and 150°CW/30°CCW modes of reciprocation.[212]

Adaptive reciprocation is one of the more recently introduced modes of file motion and has been referred to as “hybrid reciprocation”. It is a combination of rotary and reciprocating motions in which the motor alters the amount of rotation in each direction and the angles of reciprocation based on the torsional stresses and torque on the file.[169] When the load is applied on the file or it is engages dentinal walls, the motor switches to reciprocal motion.[230]

The amount of reciprocation and the angles of clockwise (CW) and counterclockwise (CCW) motion is altered by the dedicated Elements Adaptive Motor (SybronEndo) depending on the amount of stress on the file.[231] Micro-CT evaluations of the canal tomography shows that adaptive reciprocation with TFA files have less canal transportation and higher centring ratio compared to reciprocating single-file systems (Reciproc and WaveOne).[232] According to micro-CT scans, adaptive reciprocation with TFA files has also shown to be more effective than reciprocating Reciproc files in removing root fillings from oval canals.[233] Surface strain analysis using electrical gauges in simulated curved root canals shows adaptive reciprocation with TFA files caused significantly less surface strain compared to reciprocating WaveOne and rotary ProTaper Next files. This difference in maximum surface strain that files induce was strongly correlated to their mean canal transportation in the apical and coronal regions.[234]

A smooth and clean root canal surface is the ideal clinical outcome of the cleaning and shaping stage of a root canal treatment,[235,236] since rough surfaces can enhance adhesion of bacteria.[20,142] Chemo-mechanical approaches consisting of mechanical instrumentation along with chemical irrigation and disinfection are used to achieve this goal.[236] The sole effect of different irrigation solutions on the root canal surface roughness has been thoroughly investigated.[142] However, quantitative measurement of surface roughness has been rarely used as a descriptor in experimenting the performance of different filing systems. Methods that allow evaluation of samples with less preparation and provide quantitative results are more favourable for experimenting surface roughness. Atomic force microscopy (AFM),[19,20] scanning electron microscopy,[154] confocal laser scanning microscopy (CLSM),[137,140] surface roughness testers,[21,144,145] and digital imaging devices[23] have been used in previous reports.

New technologies in SEM allow for creating a 3D surface image. Traditional measures of roughness can be calculated from these images. These scans could be a perfect way to retrieve both qualitative and quantitative data on surface properties even on curved surfaces. SEM

methods are considered as a gold standard for assessing root canal cleanness.[237] However, most methods used in previous studies in this field are semi-quantitative and focus on the smear layer and debris.

Roughness of root canal surfaces instrumented with two traditional rotary NiTi systems with different blade geometry was reported by Sabet and Lutfy.[23] According to this study, reamer-shape files produced a smoother surface compared to the quasirectangular files. The influence of blade geometry may be simpler to determine during continuous rotation but in the situation where the direction of movement changes during a reciprocating motion, a cutting blade functions in a different manner. This study compares the surface roughness of root canal surfaces instrumented with a NiTi filing system in adaptive reciprocating (AR) and continuous rotation (CR).

3.3 Materials and methods

Sample size estimations based on the most relevant previous research using "Ra" measures determined an approximate of seven samples needed for statistical analysis.[21] However, since the Rz measures were larger, 12 samples per group was considered after a pilot study and using the following formula (with the condition that the estimated size for each group would suffice for tests with a significance level of 0.05 with a power of 80%):

$$\boldsymbol{n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{d^2}}$$

$$\boldsymbol{\alpha = 0.05 \Rightarrow Z_{1-\alpha/2} = 1.96}$$

$$\boldsymbol{1 - \beta = 0.80 \Rightarrow Z_{1-\beta} = 0.84}$$

$\boldsymbol{\sigma_1 = 170}$ $\boldsymbol{\sigma_2 = 170}$ (Standard deviations of Rz values in pilot experiments)

$$\boldsymbol{n} = \frac{(1.96 \quad + 0.84 \quad)^2(170^2 \ + 170^2 \ )}{200^{\mathbf{2}}} \ = \frac{226576}{20000} = \mathbf{11.32} \cong \mathbf{12}$$

Extracted first molar teeth with mature apices and curved roots were collected from the JCU Dental Clinic. Ethical approval of the study was obtained from the James Cook University Human Research Ethics Committee (H6199). Teeth were stored in a 0.1% thymol solution. The overall storage time for all teeth was less than 8 weeks.

3.3.1 Sample preparation and root canal treatment

Teeth were decoronated and the moderately curved roots (20-25° curve and 4-5 mm radius) were separated from the other roots. Mesial roots of the lower molars and the mesiobuccal roots of the upper molars were used for this study. Root lengths were measured and roots that were 11-13 mm long were cut to the standard length of 11 mm. Roots shorter than 11 mm or longer than 13 mm were discarded. Twenty four roots were collected and randomly assigned to two groups.

Twelve roots were prepared using a conventional continuous rotary movement (300 rpm) and 12 roots were prepared using adaptive reciprocating movement, both according to the manufacturer´s instructions using an Elements motor (SybronEndo, Glendora, CA, USA).

Working length was determined by entering a size #10 K-file into the canal until the tip was visible and subtracting 1 mm from that measurement. After hand instrumenting and establishing an apical glide path with a #10 and #15 K-file, the same type small procedure pack (SM) of Twisted File (TF) Adaptive instruments (SybronEndo, Glendora, CA, USA) was used for both groups. Each of the procedure packs consisted of 3 files: SM1 (#20/.04), SM2 (#25/.06), and SM3 (#35/.06) and was discarded after one use. All samples were irrigated after each instrument change with 2 ml of 5.25% NaOCl, 2 ml of 17% EDTA, and 3 ml of distilled water. The final irrigation step after the last file consisted of 2 ml of EDTA for 1 minute followed by

rinsing with copious amount of distilled water. After completing root canal preparation, two 0.5 mm deep cuts were made on the opposite sides of the root surface, parallel to the root curve with a diamond disc. Roots were split longitudinally, to expose the root canal surface.

3.3.2 Sample scanning and surface roughness evaluation

Root halves were blinded with a random three letter code. Specimens were dried overnight and then sputter coated (Figure 3-1) and analysed in a Phenom G2 Pro SEM System (Phenom-World, Eindhoven, Netherlands; Figure 3-2). An overview image at 20x magnification with the optical magnification of the SEM was taken of each half root and the root canal curvature was recorded [238] and analysed for any differences between groups. Each half root was then imaged 6 times at 550x magnification; twice at every third of the root canal (2 apical, 2 middle, and 2 coronal). A total of 12 images were taken from each sample (4 apical, 4 middle, and 4 coronal). Phenom Pro Suite software was used at each scan area to conduct 3D roughness reconstruction. Surface roughness was calculated based on the height maps created. Rz measurements were calculated after filtering out wavelengths higher than 13.06 µm and lower than 106 nm. The measurements were made at three different parts of the height maps (total of 36 calculations for each sample) for scanning directions parallel to the root canal axis.

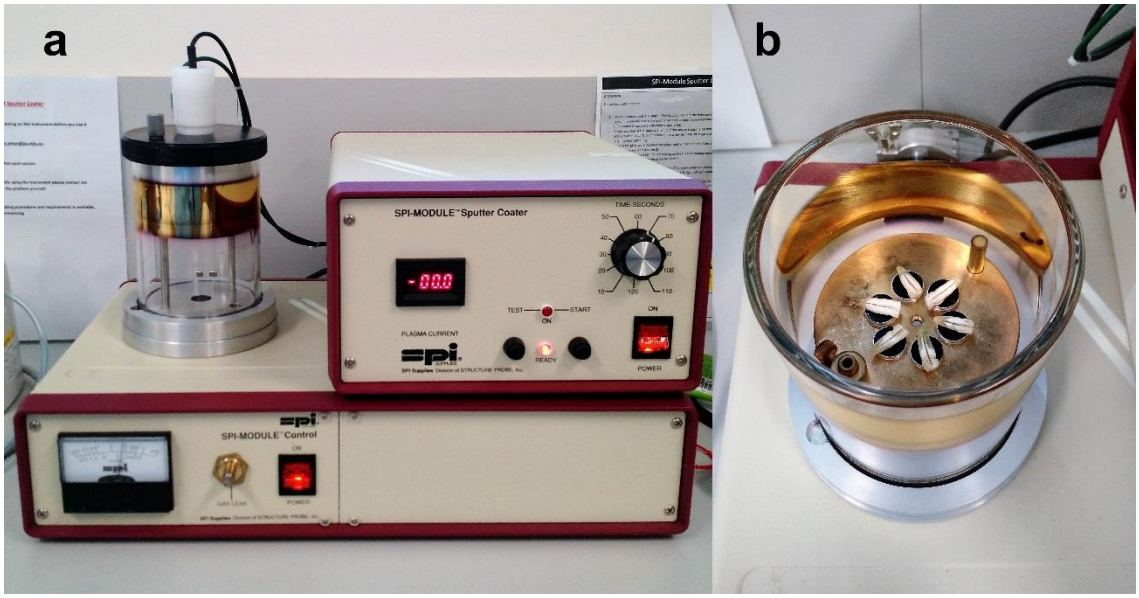

Figure 3-1 (a) SPI-MODULE sputter coater (b) Tooth samples loaded into the chamber before sputter coating.

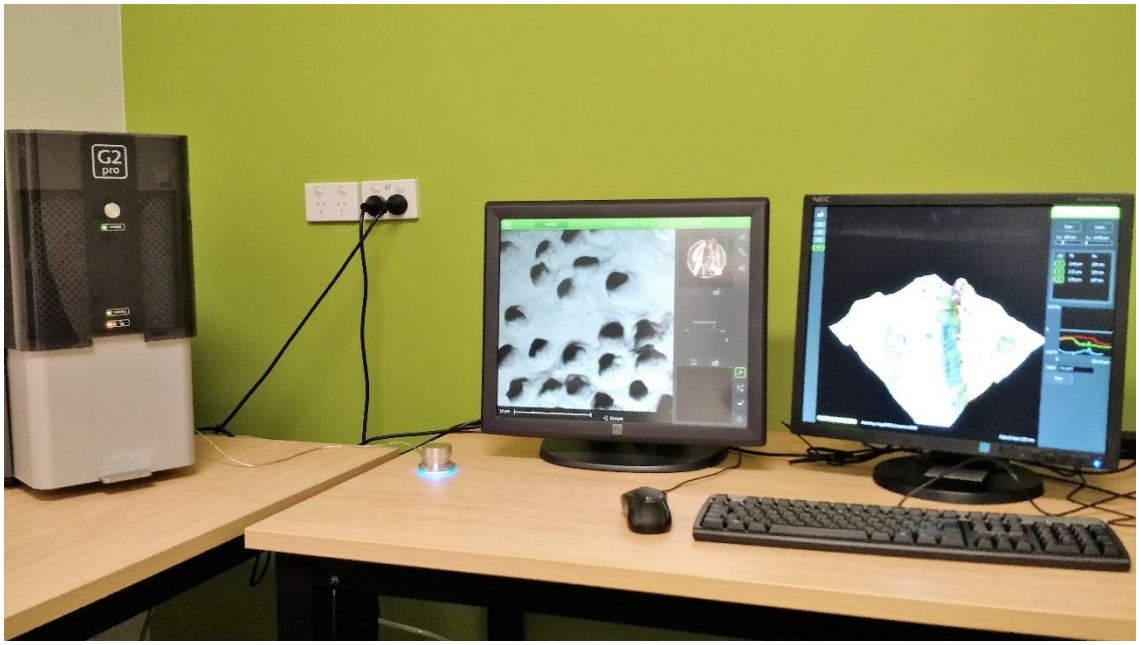

Figure 3-2 Phenom G2 Pro scanning electron microscope.

Data was imported into IBM SPSS Statistics for Windows Version 22.0 (IBM Corp., Armonk, NY, USA). Height map Rz mean was calculated from the three Rz values obtained from each height map. The apical, middle and coronal third mean Rz was calculated from the four height map Rz means of each third of the root canals. The two experimental groups were compared

statistically with Mann-Whitney tests. Differences between the apical, middle and coronal thirds of the samples were compared using a General Linear Model. Significance level of 0.05 was considered for all tests.

3.4 Results

One sample from the CR group was lost at the preparation stage after splitting. Mann-Whitney tests showed that surface roughness was significantly higher overall in the AR group compared to the CR group ($p=0.044$; Table 3-1). The AR group samples also had higher surface roughness means in apical, middle and coronal thirds, although these separate third comparison differences were not statistically significant ($p>0.05$; Figure 3-3).

The roughness generally increased from apical towards the coronal third in both the AR and CR group. General Linear Model of the Rz changes between the root thirds showed a statistically significant difference ($p<0.001$). This decreasing trend had no interaction with the filing motion and was similar for both CR and AR groups ($p=0.238$). No file separations occurred during the experiment (Figure 3-4).

Table 3-1 Rz (nm) means ± standard deviation by experimental groups and root thirds.

Rz (nm)	n	Apical	Middle	Coronal	Overall
Adaptive reciprocating	12	752.24 ± 362.68	979.97 ± 366.85	1169.50 ± 473.70	**967.23 ± 250.28**
Continuous rotary	11	599.62 ± 204.55	742.14 ± 355.61	877.44 ± 298.91	**739.73 ± 239.74**
Total	**23**	**679.25 ± 301.43**	**866.22 ± 373.53**	**1029.82 ± 418.05**	**858.43 ± 266.36**

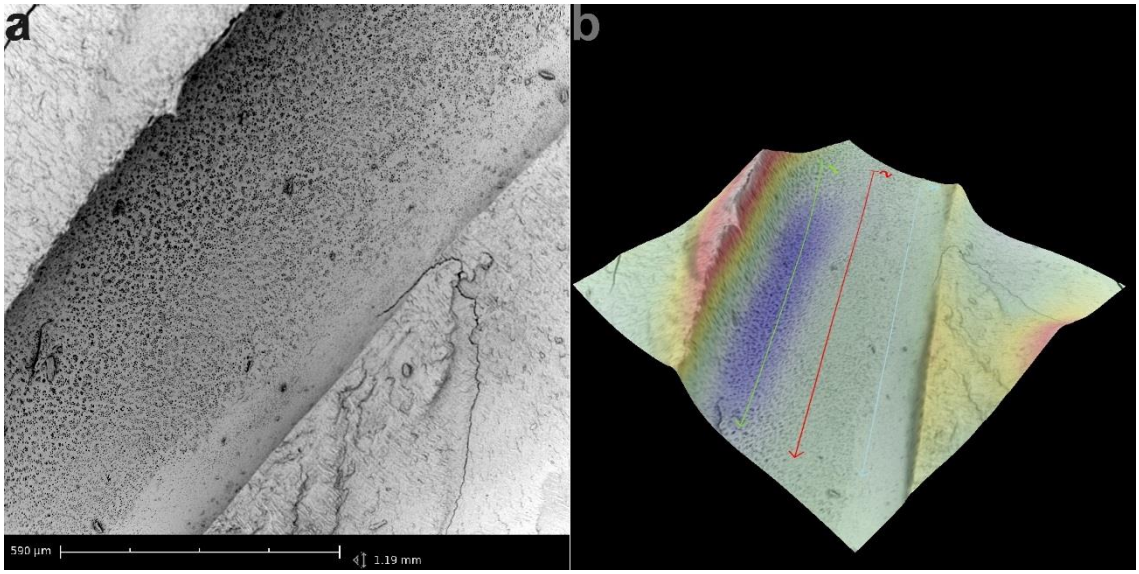

Figure 3-3 a) Scanning electron microscope image of a filed root canal surface at 550x magnification and b) its surface height map.

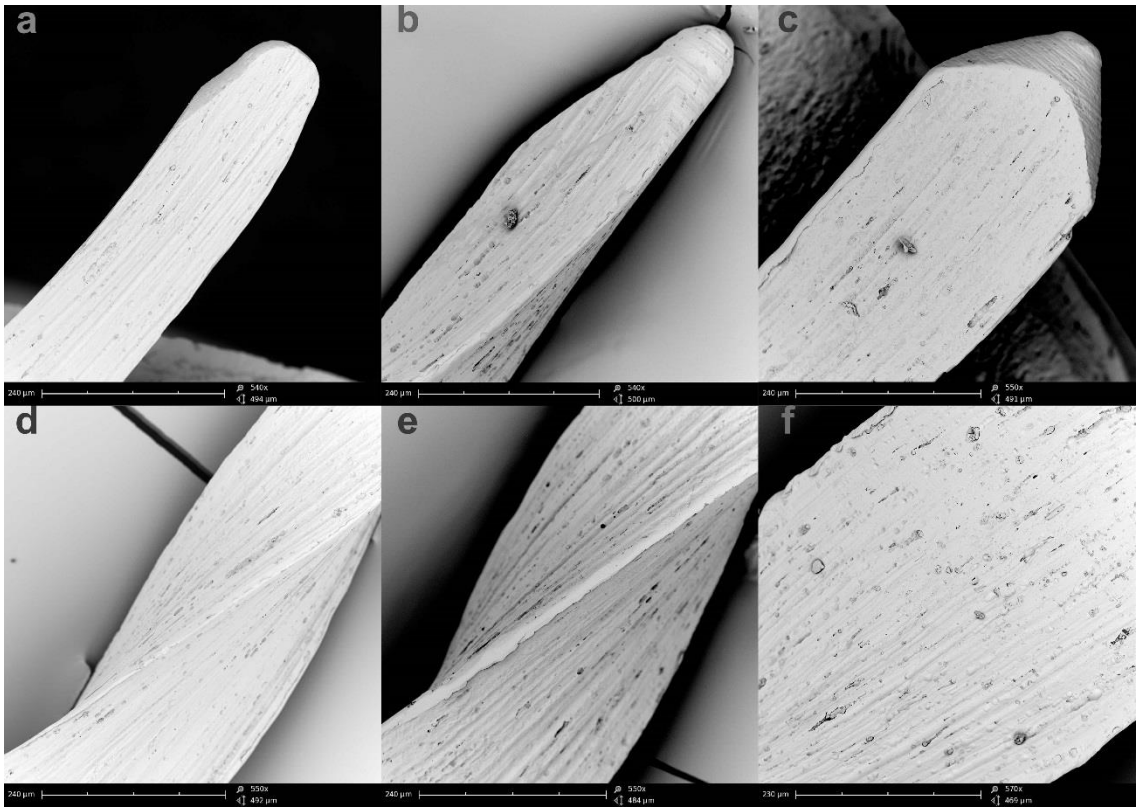

Figure 3-4 Scanning electron microscope images of the Twisted Files. a) The tip and d) middle third of the SM1 (#20/.04) file. b) The tip and e) middle third of the SM2 (#25/.06) file. c) The tip and f) middle third of the SM3 (#35/.06) file.

3.5 Discussion

In the present study, a relatively large field (~1 mm^2) was chosen to evaluate roughness in each height map. This would allow a roughness evaluation of more surface area, and therefore, a more representative mean calculation. A high number of scans were considered for each sample to make sure that the means were reflective of the roughness of each sample. Since the canals were 10 mm long and 12 areas (approximately 1 mm wide) of each root was scanned, more than half of the total root length was included in the roughness calculation. Separately scanning each root half also doubled the amount of root surfaces that were scanned for each tooth. This was made possible by the use of a non-destructive method that allowed each sample to be imaged multiple times.

Rz is an extreme value amplitude parameter representing roughness. This ten-point parameter calculates the mean difference between the maximum peaks and summits, in five points.[147] This makes it a suitable parameter for root canal assessments since it does not even out the major irregularities caused by cutting motion with the overall smoother parts of the root canal surface. These major irregularities would still cause problems in adaptation with root fillings even if the majority of the canal surfaces are smooth.

Extracted teeth were selected for the present study. Although simulated canals can be better standardized in terms of their root curvature and morphology,[239] they cannot simulate the same range of hardness and surface resistance of natural teeth. Therefore, their surface would not be filed similar to a natural root.

The increase of roughness with reciprocating movement may be associated with the many engaging and disengaging phases of the file during function. Because the reciprocating file can cut in both directions, the bidirectional movement allows for interrupted cutting where it stops at a certain location and resumes form a different location. Although this process

reduces the stress on the file, it seems to leave a less smooth surface because of the larger number of cuts.

Roughness, irregularities, grooves and less-instrumented surfaces were previously reported in association with rotary instruments.[23,154] However, in contrast with the present study, Foschi et al. found the apical third to be the least homogenous and the coronal to be the smoothest.[154] The method in the mentioned study is subjective and semi-quantitative as it requires scoring of the surface profile based the irregularities and non-instrumented areas seen on the SEM scans. The root roughness gradient in the present study may be attributed to the increase in flute size of the apical section of the file towards the coronal.

Surface roughness of root canal surfaces after treatment with different irrigants has been examined by Ballal et al. They believed that increased roughness caused by the irrigants may be favourable for the adherence of some restorative materials.[19] This effect is partly related to the irregularities caused by the exposure of dentinal tubules.[240] The effect of different irrigation protocols on the bond strength of resin sealers has also been investigated and the importance of other factors such as presence of oxygen-rich dentine layer and hydrophilic characteristics of the surface should also be considered.[241] However, the threshold of the amount of roughness that would be beneficial is of critical importance. Irregularities which may assist in bonding process are in a very smaller scale than wide-ranging waviness of the surface. For example, the threshold reported to minimize adhesion of bacteria in abutments is 200 nm.[15] Large irregularities are not only unhelpful in adhesion of the obturating material and may prevent less adaptation of the obturating material in a location where the clinician does not have proper access and control over, but they also promote bacterial adhesion.[15] This problem can be amplified if the materials and techniques used for obturation are less fluid e.g. cold gutta-percha lateral compaction.

Surface roughness can promote bacterial adhesion by increasing contact area for microorganisms (by a factor 2-3) and also protection from shear forces.[15,242] This effect can vary based on the size and shape of bacteria.[15] Therefore, further research needs to be carried out on the exact roughness effect and threshold for specific microorganisms that are active in the root canal region.

Creating a smooth surface without the formation of cracks is important for the longevity of root canal treatment. It can be assumed that rough surfaces created by instruments will also contain more microcracks and these surfaces will be difficult to obturate in a way that the final filling is tight and resistant to bacterial reinfection. This study may be important for the valuation of modern reciprocating systems and also important for the development of future file designs. The role of smoothness is especially important for conventional root canal obturation methods. Further research to evaluate the amount of irregularities that different endodontic filling materials can adapt to is also recommended.

3.6 Conclusion

Surface roughness of treated root canals can be modified by clinicians based on their technique. Surface roughness of root canals that have been instrumented with reciprocating motion is higher compared to canals prepared with the same files in continuous rotary. In case of using reciprocating files in root canal instrumentation, treatment strategies implemented to use continuous rotary motion towards the end of the treatment may be beneficial in achieving a smoother final finish on the canal surface. Further research can show whether use of a different instrument or chemical can also lead to smoother canal surfaces. The next step in this series of experiments compares the effect of using three different filing systems in terms of the roughness they cause on root canal surfaces.

Chapter 4 Quantitative evaluation of root canal surface roughness after filing with conventional rotary, single-file reciprocating or self-adjusting filing systems

4.1 Chapter overview

The previous chapter showed how a single factor such as filing motion can significantly affect the quality of a treated root canal surface. Following on, this chapter was aimed to evaluate the effect of a mixture of variables (e.g. alloy, cross-section, taper, motion, design, etc.) that make filing systems with different concepts on the final root canal surface quality. To do so, three contemporary filing systems were compared. HyFlex EDM, a conventional continuous rotary system that has been more recently introduced with improved mechanical properties was used for filing the first group of roots. This system consists of multiple files similar to traditional filing methods. The self-adjusting file, which employs a completely new file design, movement and abrading action to prepare canals, was used for the second group. Reciproc, a single-file reciprocating system, was used for the third group. In this experiment, these systems were tested to answer the following research questions:

- Is the mean surface roughness of canals after instrumentation with a continuous rotary, single-file reciprocating or self-adjusting filing system different?
- Is the mean surface roughness of canals after instrumentation in the apical, middle and coronal thirds different?

This chapter contains material that has been used for the following paper, which is currently under review for publication in the Journal of Endodontics:

Sakhaei Manesh V, Giacomin P, Jablonski-Momeni A, Stoll R. Quantitative evaluation of root canal surface roughness after filing with conventional rotary, single-file reciprocating or self-adjusting filing systems.

This study was supported by the James Cook University Graduate Research Scheme Grant (grant number JCU-QLD-602531). I would like to thank Coltene-Whaledent, ReDent-NOVA and VDW (Gunz Dental) for their donating of the files used in this study and loaning of the motors used for each filing system.

4.2 Introduction

Smooth and clean root canal walls is the ideal outcome of root canal cleaning and shaping.[235,236] Filing systems used to achieve this goal have been going through a rapid revolutionary stage. File movement, materials, shape and surface texture have all been modified in newer filing systems to achieve better, quicker or more cost-efficient results.[243]

The number of microorganisms remaining in a prepared root canal space after treatment should be low enough to enable healing, if the bacterial burden is too high it can cause disease. Since complete eradication of the microorganisms in all parts of the root canal system and dentinal tubules is not practically possible in most cases, minimizing their chance of colonizing after treatment is of upmost importance.[182]

Intracanal microorganisms are the main cause of root canal reinfections after treatment.[180] The prepared root canal surface is the largest potential site for these organisms to colonize after removal of the pulp tissue. Therefore, the physical and biological state of the root canal surface which chemomechanical preparation results in is of key importance in how likely it will be a recolonization site after treatment. The physical qualities of this prepared surface, which

are also stable over time, can be used to minimize chances of bacterial adherence and biofilm formation.

Surface roughness is a physical quality that can increase biofilm formation. Implant abutments that had an Ra mean approximately 0.5 μm higher than their smooth counterparts were shown to have 25 times more bacteria adhered to them subgingivally.[17] Roughness of the root canal surface increases by using reciprocation compared to continuous rotation when the same file is used in root canal instrumentation.[204] This raises many questions regarding whether other aspects of a file such as design, alloy or surface texture may influence the final surface finish too.

Rotary files cause less grooves on the root canal surface and produce a smoother and more even surface compared to hand files.[244] Conventional rotary filing systems have been evolving with different numbers of files and designs. Finishing files with a modified taper, design or material and that are used in the final stages of canal instrumentation, have been incorporated into some filing systems. Some finishing files such as the F-file, a plastic file with abrasive diamond particles embedded into it, have the potential to change the root canal surface qualities.[245]

HyFlex EDM is also a continuous rotary filing system with improved mechanical properties and a slightly rough surface. The mechanical improvements of the HyFlex EDM are claimed to allow it to be used as a single-file system in some cases.[246] HyFlex EDM files are an evolutionary step forward from the HyFlex CM files previously introduced. Both these files are manufactured from CM (Controlled Memory) wire. NiTi alloys have an austenite/maternsite transition temperature when the physical properties of the metal changes. The heat-treated CM wire shifts the transition temperature and allows the NiTi files to be in a maternsitic state at body temperature. The maternsitic state makes these files softer, more ductile and flexible compared to conventional NiTi alloy files.[162] HyFlex EDM instruments have a rectangular cross-

section towards their tip that makes them more resistant to torsional forces and they have a triangular cross-section towards the shaft, which makes them more flexible and resistant to fatigue.[247]

The Self-Adjusting File (SAF) system uses vertical vibration of an abrasive nickel-titanium (NiTi) lattice combined with constant irrigation during filing to clean and shape the canals.[170] The file has been claimed to remove a uniform layer of dentine from the root canal surface rather than machining the root canal into a round cross-section.[248] Since the SAF can be compressed, it can contact asymmetrical, flat and oval shaped sections of canals more efficiently that conventional files with a metal core.[170,249] Despite the better adaptability of the SAF to some canal shapes and its more conservative approach, fracture strength of roots instrumented with SAF are not significantly different to those prepared with rotary ProTaper files.[250] Results regarding the efficiency of the SAF depend on study design and the factors analysed but are in general comparable or in some cases better than traditional files. Biofilm removal inside a premade groove with SAF had previously been reported to be more efficient compared to instrumentation with rotary or hand filing.[191] The SAF removes debris from the apical third of oval-shaped canals more efficiently and has better contact to the canal walls compared to K3 (SybronEndo) rotary files.[251] In contrast, when the mesial canals of mandibular molars were assessed, the bacterial reduction and shaping ability was similar to rotary Twisted File (SybronEndo) and reciprocating Reciproc (VDW) files.[157]

Single-file reciprocating filing systems simplify the root canal treatment process. Although they are substantially comparable to conventional rotary files, they function based on a modified file design and reciprocating movement of the file. Research regarding the efficiency of these files and their cleaning and shaping abilities show results similar to conventional multi-file rotary systems.[157] Reciproc files are a single-file system manufactured with M-wire alloy for high torsional strength. They have a relatively large S-shaped cross sectional area and two

cutting edges.[229] Instrumentation of severely curved root canals with Reciproc files leads to similar results in terms of canal straightening and changes in surface area compared to rotary instruments. However, these files remove more dentine compared to the OneShape, ProTaper Universal and Twisted Files Adaptive systems.[230]

The aim of the present study was to compare the surface roughness of the root canal after cleaning and shaping with three different filing systems (conventional rotary, single-file reciprocating and SAF).

4.3 Materials and methods

4.3.1 Study design and ethics

Sample size estimations based on previous research using "Ra" measures suggested an approximate of seven samples for each group. However, since the Rz measures were in a larger scale and were also assessed for this study, 12 samples per group was considered after a pilot study to determine Rz value ranges and using the following formula (significance level of 0.05 with a power of 80%):

$$\boldsymbol{n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{d^2}}$$

$$\boldsymbol{\alpha = 0.05 \Rightarrow Z_{1-\alpha/2} = 1.96}$$

$$\boldsymbol{1 - \beta = 0.80 \Rightarrow Z_{1-\beta} = 0.84}$$

$\boldsymbol{\sigma_1 = 3.5}$ $\boldsymbol{\sigma_2 = 3.5}$ (Standard deviations of Rz values estimated based on pilot tests)

$$\boldsymbol{n} = \frac{(1.96 + 0.84)^2(3.5^2 + 3.5^2)}{4^2} = \frac{192.08}{16} \cong \boldsymbol{12}$$

Fifteen roots were prepared for each group to account for sample loss. Extracted teeth with mature apices and straight roots were collected from the JCU Dental Clinic. Ethical approval of

the study was obtained from the James Cook University Human Research Ethics Committee (H6199). Teeth were stored in a 0.1% thymol solution at 5 °C. All teeth were stored for less than 8 weeks.

4.3.2 Sample preparation and root canal treatment

Incisors with straight roots were used for this study. Extracted teeth were checked for having a single canal and apical foramen with radiographs taken from their buccal and proximal sides. Canals were accessed and the working length of the teeth were determined using a #10 K-file and subtracting 1 mm from where the file tip was visible at the apical foramen. Canals were examined with hand files and the ones allowing an initial size 15 file to bind apically were kept. Crowns of teeth were cut to standardize root sample lengths to 16 mm. Forty-five roots matching this criteria were collected and randomly assigned to three groups to account for sample loss. The roots were embedded in a cylinder of acrylic resin so that the tip of the root and orifice of the canal would be standing out. A wax cylinder with approximately 3 mm height to simulate the pulp chamber was placed on the orifice before the embedding to be removed after the acrylic resin had set. This was done for all groups since a chamber to keep the irrigating solution was required for the SAF system to function according to the manufacturer's instructions (Figure 4-1).

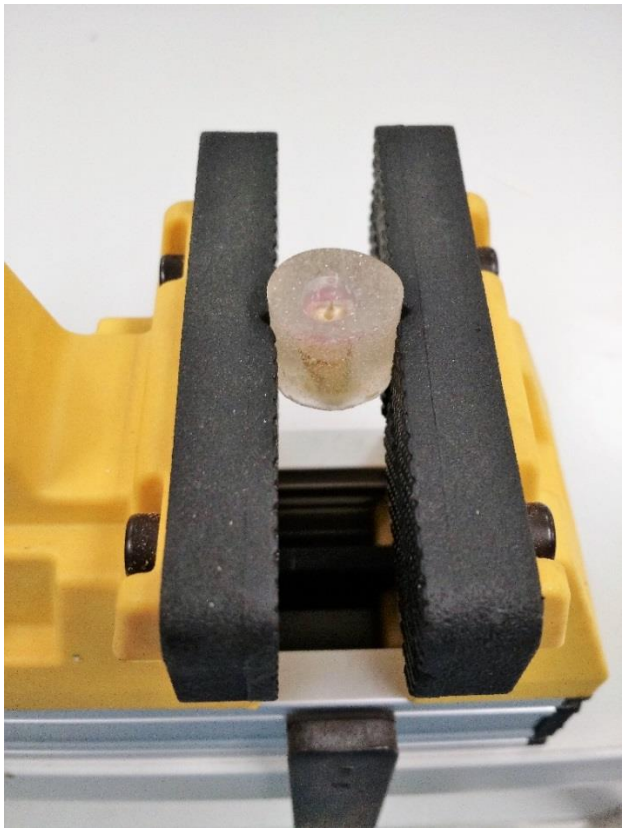

Figure 4-1 Root embedded in an acrylic cylinder with a hollow space designed above the orifice to simulate the pulp chamber.

Instrumentation of each group was done with a different filing system according to the manufacturers' instructions. Each set of files was used once for a single canal and discarded.

4.3.2.1 Group 1 (HFEDM): Continuous rotary filing system (Hyflex EDM, Coltene/Whaledent GmbH + Co. KG, Langenau, Germany)

Each root was filed in sequence with a complete set of the HyFlex EDM system (Figure 4-2) consisting of an Orifice Opener (#25/0.12), Glidepath file (#10/.05), HyFlex OneFile (#25/~) and a Finishing file (#40/.04). Files were operated using a CanalPro CL motor handpiece (Coltene Endo, Coltene/Whaledent) set to continuous rotary motion (400 rpm for all HyFlex EDM files except for the Glidepath files which were operated at 300 rpm). Irrigation with a total of 10 ml of 2.5% NaOCl was carried out in between file changes.

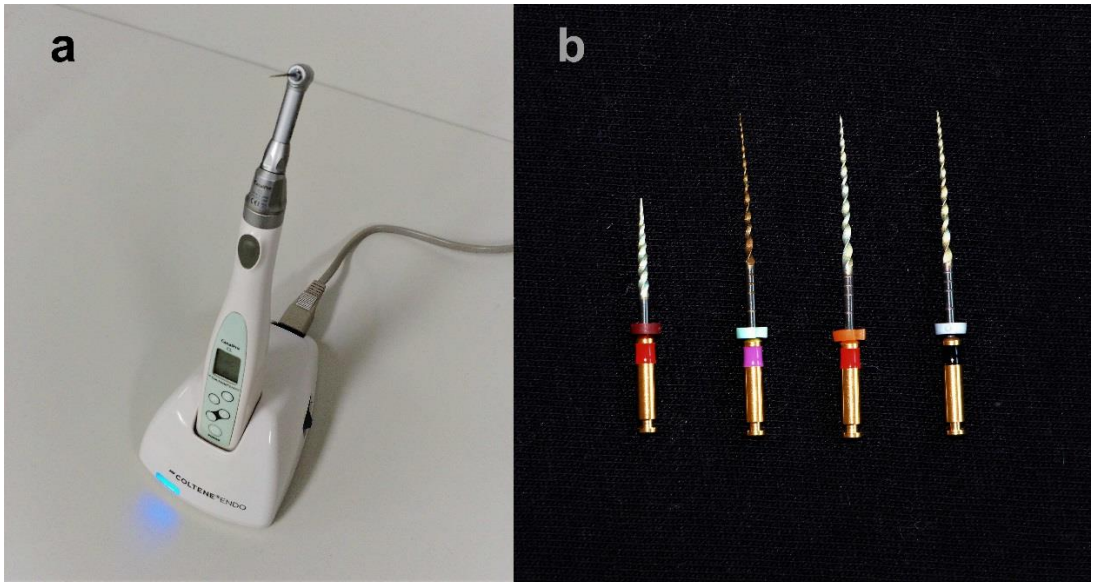

Figure 4-2 Continuous rotary filing system consisting of the (a) CanalPro CL motor handpiece and (b) HyFlex EDM files [Left to right: Orifice Opener (#25/0.12), Glidepath file (#10/.05), HyFlex OneFile (#25/~) and Finishing file (#40/.04)].

4.3.2.2 Group 2 (SAF): Self-adjusting filing system (ReDent-NOVA, Ra'anana, Israel)

Pre-SAF-OS (#40/0.10), Pre-SAF-1 (#15/0.02) and Pre-SAF-2 (#20/0.04) files were used in sequence according to the manufacturer's instructions to achieve a glide path that allows the SAF 1.5 to reach working length. Irrigation in between each file change consisted of 2 ml of 2.5% NaOCl. A 21 mm long SAF 1.5 mm was inserted manually to assure it reaches working length. The file was taken out and attached to the RDT3-NX hand piece (ReDent-NOVA) connected to an EndoSTATION motor (ReDent-NOVA; Figure 4-3). Instrumentation of the canal with SAF was carried out for a total of 4 minutes. Irrigation with 2.5% NaOCl was continuous at a rate of 4 ml/min using the EndoSTATION for 3 minutes of operation. The pump was then deactivated and the canal was filed while it was filled with EDTA for 30 seconds. After this, the EndoSTATION NaOCl pump was activated again and the canal was filed for another 30 seconds.

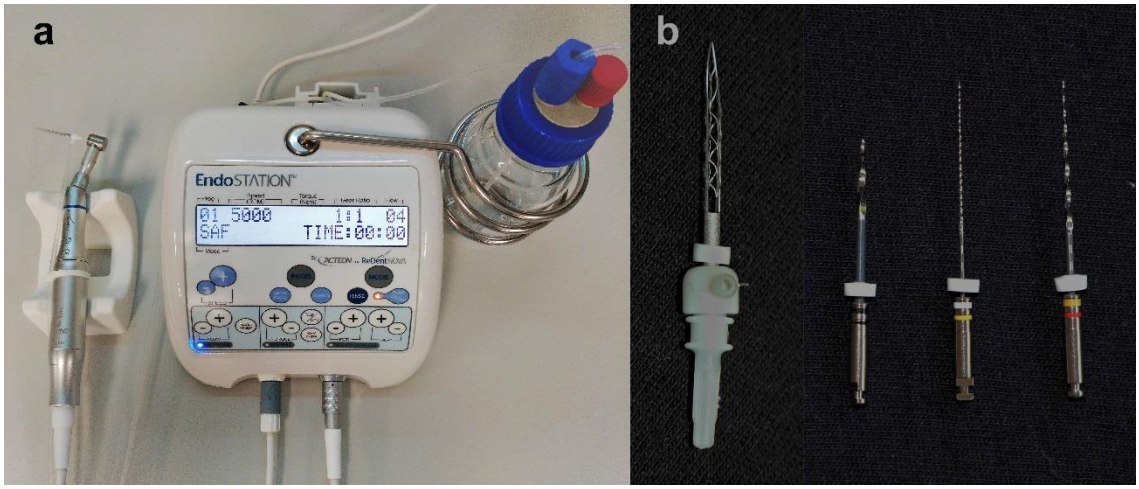

Figure 4-3 Self-adjusting filing system consisting of the (a) EndoSTATION motor and the (b) SAF SYSTEM file set [Left to right: SAF 1.5, Pre-SAF-OS (#40/0.10), Pre-SAF-1 (#15/0.02) and Pre-SAF-2 (#20/0.04)].

4.3.2.3 Group 3 (RCP): Single-file reciprocating system (Reciproc, VDW GmbH, Munich, Germany)

Each canal was filed with a single R40 file to working length according to manufacturer's instructions. The RCP group were prepared using Reciproc 40 (R40) NiTi files (VDW GmbH) and a VDW.Silver Reciproc motor (VDW GmbH; Figure 4-4) set to reciprocating motion (RECIPROC ALL). Three slow pecking motions were applied in each insertion of the file into canal before cleaning and reinsertion. The file was progressed up to a maximum of 3 mm in each insertion. Irrigation was carried out between file cleaning with a total of 10 ml of 2.5% NaOCl.

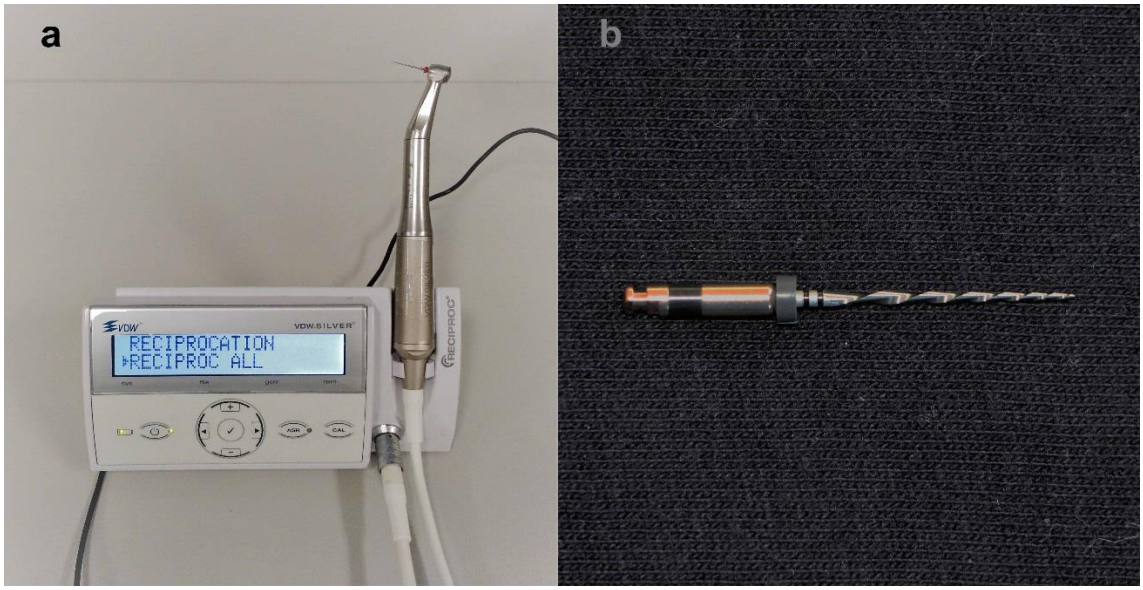

Figure 4-4 Reciprocating single file system consisting of the (a) VDW.Silver Reciproc motor and the (b) Reciproc (R40) file.

All samples were irrigated after the final instrumentation step with 1 mL of EDTA for 1 minute followed by irrigation with 1 mL of 2.5% NaOCl and then rinsing with 5 mL of distilled water.

After completing root canal preparation, the acrylic cylinders were mounted in a precision saw to be cut in half longitudinally. Any grinding material remaining on the exposed root canal surfaces were water and air blasted for 3 seconds to clean and prepare samples for scanning electron microscopy.

4.3.3 Sample scanning and surface roughness evaluation

Root halves were blinded with a random three-letter code. Specimens were dried overnight and then mounted on aluminum stubs to be sputter coated with gold. The prepared samples were scanned and analyzed in a Phenom G2 Pro SEM System (Phenom-World, Eindhoven, Netherlands). Each half root was imaged 6 times at 550x magnification; twice at every third of the root canal (2 apical, 2 middle, and 2 coronal). A total of 12 images were taken from each sample (4 apical, 4 middle, and 4 coronal). Phenom Pro Suite software was used at each scan

area to conduct 3D roughness reconstruction. Surface roughness was calculated based on the height maps created. Rz and Ra measurements were calculated after filtering out wavelengths higher than 1060 µm and lower than 20 nm. The measurements were made at three different parts of the canal height maps (total of 36 calculations for each sample) for scanning directions parallel to the root canal axis.

Data was imported into IBM SPSS Statistics for Windows Version 23.0 (IBM Corp., Armonk, NY, USA). Height map Rz and Ra means were calculated from the three values obtained from each height map. The apical, middle and coronal third means were calculated from the four height map Rz and Ra means of each third of the root canals. Normal distribution in groups was tested with Kolmogorov-Smirnov tests and the homogeneity of variances were verified using the Levene test. The three filing systems were compared in each third using analysis of variance (ANOVA) tests. Changes in the apical, middle and coronal thirds of the samples were compared using a general linear model while testing the interaction with the filing system used. Significance level of 0.05 was considered for all tests.

4.4 Results

Two samples from the RCP group and one sample from each of the other two groups were lost during the sectioning stage. ANOVA tests showed Ra means of the three filing systems were not significantly different in the apical ($p=0.335$), middle ($p=0.759$) or coronal ($p=0.954$) thirds (Figure 4-5). Ra overall means of the three thirds also did not significantly differ among the files ($p=0.685$). In all three filing systems, Ra decreased from the apical towards the coronal third. General Linear Model of the changes among the three thirds of the roots showed a statistically significant difference ($p<0.001$). This decreasing pattern from apical towards the coronal was similar among the filing systems and no interaction was seen with the filing system used ($p=0.598$).

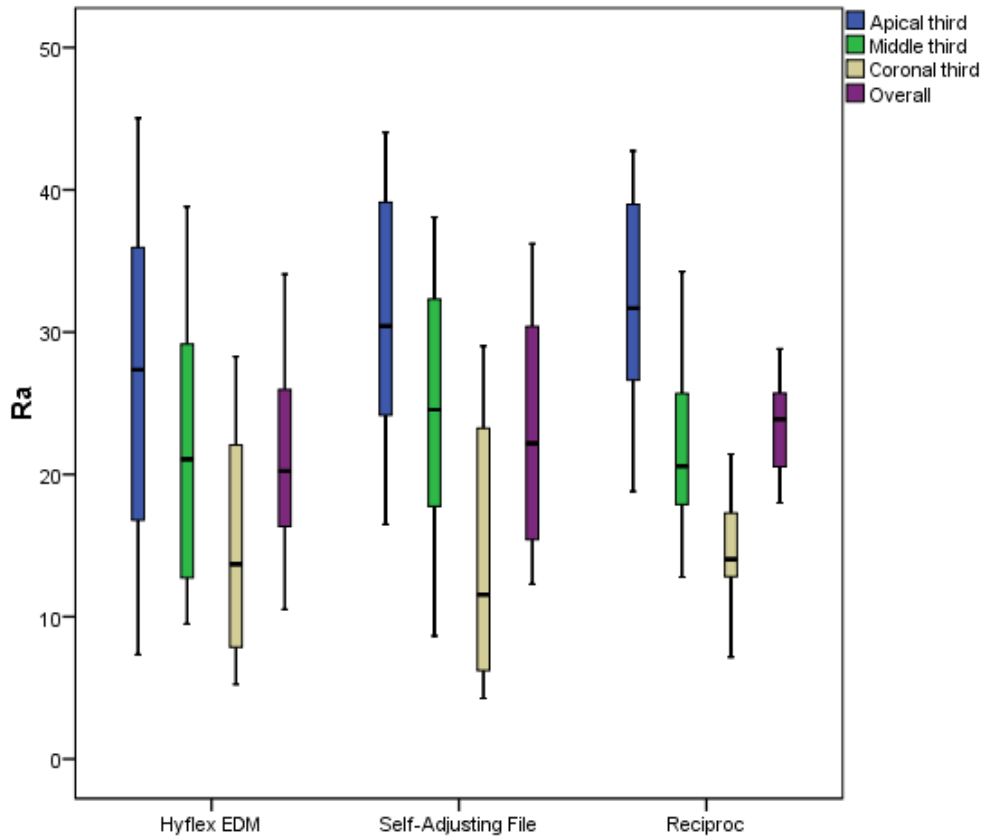

Figure 4-5 Box plot illustrating the median and distribution of the Ra (µm) of canal surfaces in different thirds of the root after cleaning and shaping with each filing system.

ANOVA tests showed that Rz means of the three filing systems were not significantly different in the apical (p=0.683), middle (p=0.182) or coronal (p=0.511) thirds (Figures 4-6 and 4-7). Rz overall means of the three thirds also did not significantly differ among the files (p=0.577). In all three filing systems, Rz peaked in the middle third and decreased towards the apical and coronal thirds. General Linear Model of the changes among the three thirds of the root showed a statistically significant difference (p=0.005). This pattern was similar among the filing systems and no interaction was seen with the filing system used (p=0.175). None of the files used in the experiment separated during the experiments (Figures 4-8, 4-9 and 4-10).

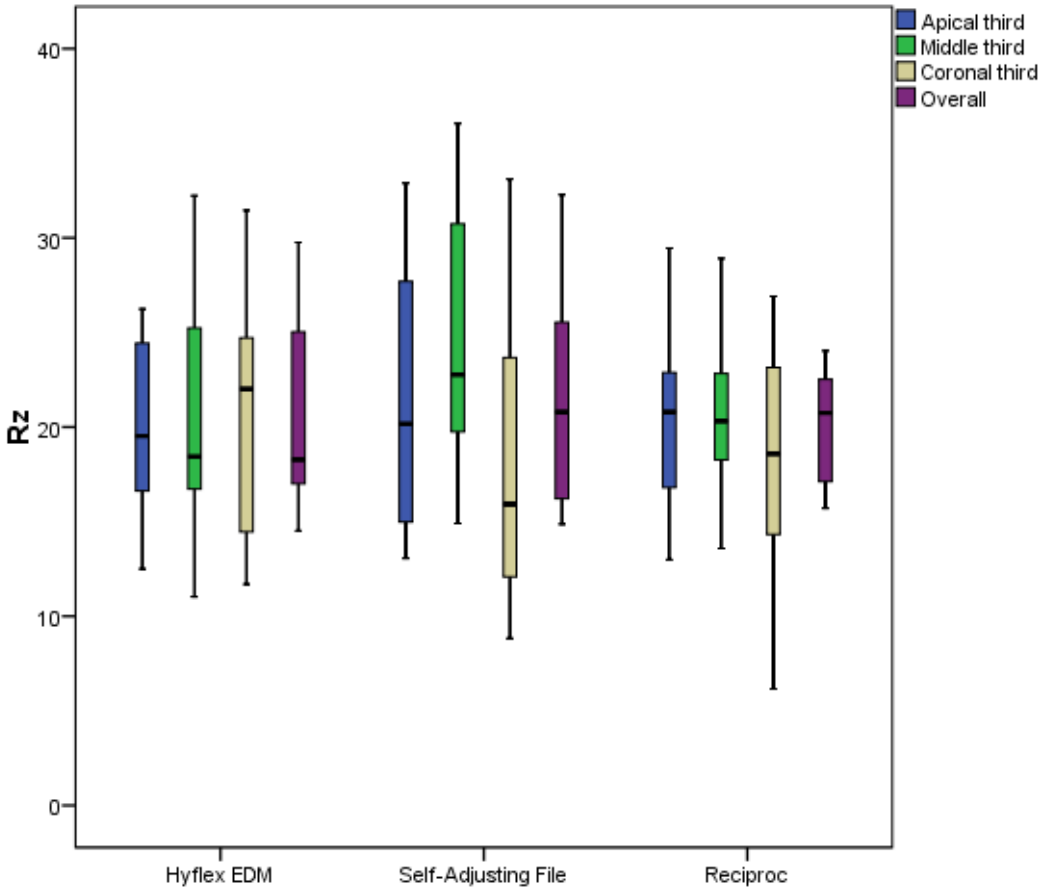

Figure 4-6 Box plot illustrating the median and distribution of the Rz (µm) of canal surfaces in different thirds of the root after cleaning and shaping with each filing system.

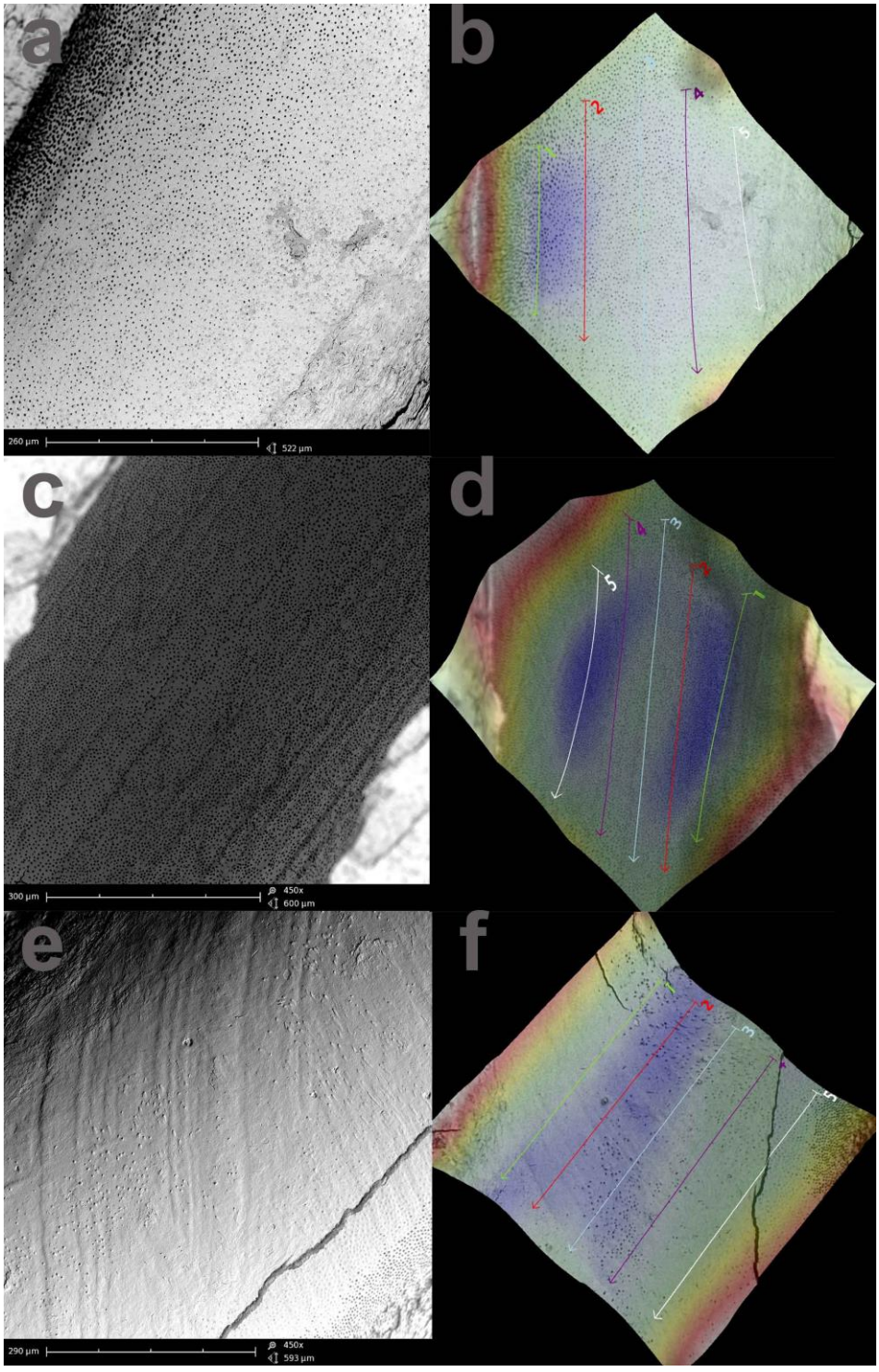

Figure 4-7 Scanning electron microscope images of canals instrumented with a) HyFlex EDM c) self-adjusting and e) Reciproc (R40) files. Height maps and roughness parameter calculations of the scans performed for the b) HyFlex EDM d) self-adjusting file and f) Reciproc (R40) group surfaces.

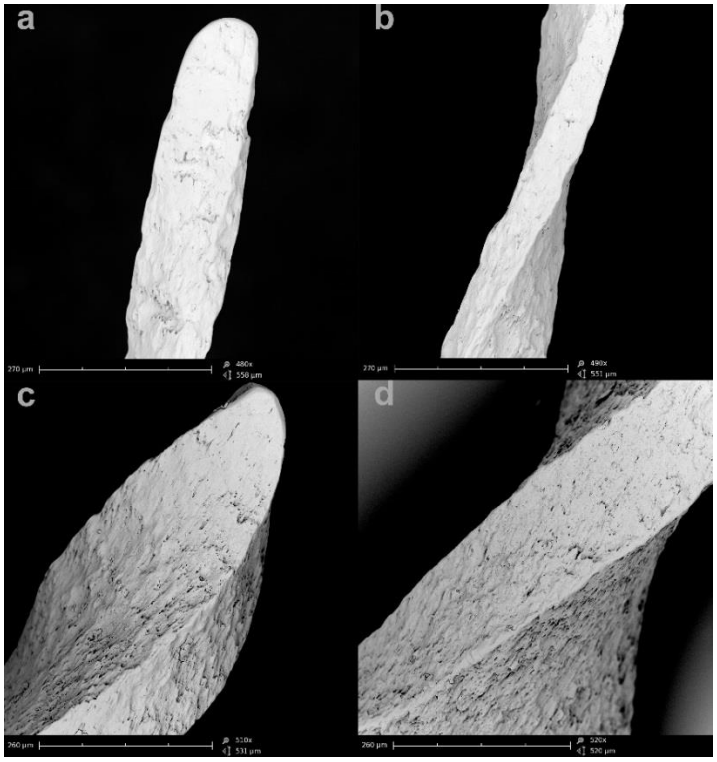

Figure 4-8 Scanning electron microscope images of HyFlex EDM files. a) Apical tip and b) middle third of the HyFlex Glidepath file (#10/.05). c) Apical tip and d) middle third images of the HyFlex Finishing file (#40/.04).

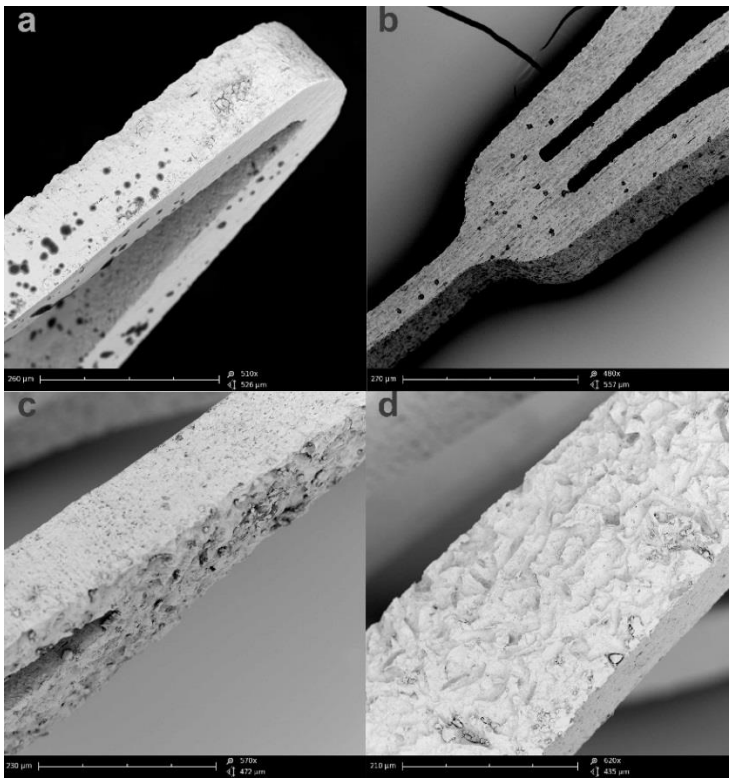

Figure 4-9 Scanning electron microscope images of the self-adjusting files. a) Tip, b) and c) mesh design connections and the d) abrasive outer surface of the file.

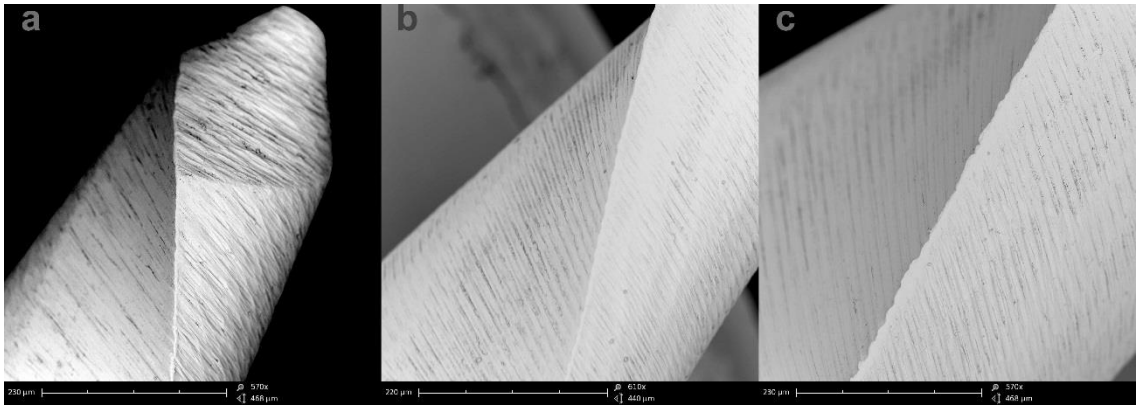

Figure 4-10 Scanning electron microscope images of the Reciproc (R40) files. a) Tip, b) apical third and c) middle third surfaces of the file.

4.5 Discussion

The filing system used did not affect the Ra and Rz means of the cleaned and shaped canals in the present study. The type of movement that a file has been shown to be an influential factor in final roughness according to previous research.[204] However, in the present study the final root canal surface of the reciprocating group (Reciproc) is similar to the continuous rotating (Hyflex EDM) and oscillating (SAF) files. The reciprocating motion factor in the Reciproc system seems to be evened out by the other properties of the files in the other systems. Although continuous rotation in the Hyflex EDM system may be helpful to achieving a final smooth canal surface, these files have a coarse surface which is the result of the electrical discharge machining (EDM) process.[158] This roughness of the file may be contributing to roughening the final surface. The surface of the SAF is also rough since it works by scrubbing the root canal wall.

Bacterial reduction after instrumentation with SAF, Reciproc or Twisted File systems does not differ significantly.[157] This similarity among the three filing systems is in line with the similarity of their root canal surface quality in the present study. Every filing system is a complex combination of properties e.g. movement, shape, cross section, blade angles, surface treatment, alloy, etc.[252] These properties may each affect the final bacterial load reduction if

everything else in that file is kept identical. Each of these variables can then be individually optimized in developing every filing system by identifying and understanding the underlying effect they have on the effectiveness of that filing system.

Method of roughness evaluation in this study was similar to a previous study linking reciprocation to more roughness compared to continuous rotation.[204] Similarly, large height maps each covering a wide area (~1 mm^2) were evaluated in this study to be representative of the whole root canal surface roughness. The high magnifications used in previous research and the relatively small areas visualized with SEM methods were one of the main concerns in traditional SEM studies.[136]

Rz and Ra were both calculated in this experiment. Ra is an average value parameter that describes amount of deviation from the mean plane. Ra has been the most commonly used roughness parameter in biological research. Rz is a ten-point extreme value amplitude parameter.[147] Rz has been suggested to be suitable for endodontic research and studying biofilm formation since it does not even out the peaks and summits of the root canal surface with mean calculation.[204]

Extracted teeth were used in this study to simulate clinical conditions. Resin blocks with simulated canals may be able to standardize confounding factors better but they do not simulate the microstructure of dentine and the effect that a file would have on it.

The use of medicaments and irrigants has been a focus of research to minimize the chance of microbial recolonization. However, as with any chemical, the stability of the active agent over time and the long-term efficacy remains a challenge. Substantivity of chlorhexidine and its ability to prevent bacterial adhesion has been reported to be from a few days up to a maximum of months, but never permanent. This effect is also dependent on the irrigant's concentration and duration of application, which may limit its practicality in clinic.[253]

The dominant role of surface roughness on bacterial attachment has been suggested to sometimes mask the effect of other factors such as surface energy and hydrophobicity.[16] Even so, chemicals used in the root canal can partially influence these physical properties of the root canal surface too. NaOCl 5.25% causes more dentine erosion when used as an irrigant compared to lower concentrations.[254] In the present study NaOCl 2.5% was used to limit the effects of chemical erosion.

Irrigation may be a factor contributing to the final surface quality because of the erosive effect that irrigants can have on dentine. Simezo et al. performed 3D roughness reconstruction and calculated roughness on root canals irrigated with two different methods but reported much lower median roughness values. This may be due to the multiple exposure of dentine to irrigants during the experiment and also sample preparation, e.g. washing of specimens in ultrasonic baths with NaOCl and EDTA, which may have caused the erosion and smoothening of larger irregularities caused from filing.[136] Irrigation is especially different in the SAF system compared to other systems since it requires a longer instrumentation time and constant flow of the irrigant while operating. The surface of the SAF is abrasive and the mode of function for it is described as a "sandpaper effect". Although this scrubbing of the canal wall would theoretically be expected to leave some sort of roughness, it does not seem to be significantly different to the machined surfaces left by conventional cutting files. Roughness of the SAF surface has been determined 2.8 µm ± 10%.[249] Therefore, whether there would be traces of roughness in the nanometre scale is a question that would require further tests. Since biofilm formation has been shown to increase with surface roughness thresholds of around 200 nm in implants,[48] comparing the irregularities of under 1 µm that each system leaves is recommended in future research.

Apical thirds of samples have the highest Ra means in all three filing system and the same decreasing trend is apparent towards the coronal in all groups. Previous experiments reported

presence of more dentine depressions and grooves in the apical third after filing with Mtwo or Protaper rotary instruments.[154] SEM evaluation of canal wall cleanliness and presence of smear layer also indicate relatively better and cleaner surfaces towards the coronal side of the canal.[248] It would be interesting to see if there is an association between root canal cleanliness and roughness average (Ra). Rz changes in the root thirds are different compared to Ra. Rz tends to be fluctuating less among the thirds in the HFEDM and RCP groups but increases in the middle third of the SAF group. This is in agreement with previous research showing that SAF left more untreated surfaces (with less than 20% of the root canal perimeter treated) in the middle third (35% of samples) compared to coronal (8%) and apical (15%) thirds of the canal.[255] It may be due to less uniform contact and abrasion of the SAF in the middle third compared to the other thirds of the canal but would require further investigation. The file design seems to affect the Rz values, which is a better representative of the depth of the irregularities, differently compared to average roughness (Ra). Unlike HFEDM and RCP files that are both tapered files, SAF has a cylinder shape in the middle of the instrument towards the shaft.

Biofilm formation has been shown to have a positive correlation with surface roughness. The threshold of roughness that effects the attachment of bacteria varies among different species. Little information is currently available regarding the optimal and desired surface qualities that would reduce attachment of bacteria involved in root canal infections such as *Enterococcus faecalis*.

4.6 Conclusions

The filing systems experimented in this study left an almost similarly rough root canal surface after cleaning and shaping. Much development can be made in instrument designs to achieve smoother canals that would be less prone to biofilm formation. The level of roughness caused

by every one of the three filing systems tested were very high. This indicates that these files are relatively aggressive in cutting. Future research can show whether files with less aggressive designs or files with lower cutting efficiency due to their wear can create smoother surfaces upon use in canals. The next chapter looks at how file wear caused from its reuse may affect the surface roughness it leaves on the treated root canal.

Chapter 5 Quantitative evaluation of root canal surface roughness after repeated use of files with a reciprocating single-file system

5.1 Chapter overview

The previous chapter showed how the effect of a mixture of variables (e.g. alloy, cross-section, taper, motion, design, etc.) that made three completely different filing systems resulted in similarly rough root canal surfaces. Since each file undergoes a life cycle and it is eventually worn out, the present study was designed to assess how the effect of file wear translates into changes on the treated root canal surface quality. In order to evaluate the impact of file wear effectively, Reciproc single-file reciprocating instruments were used for this study. Reciproc files endure the same stress that is usually distributed among a number of files in multi-file systems. In this experiment, these files were tested to answer the following research questions:

- Is the mean surface roughness of canals after instrumentation with new files and files that have been reused once or twice different?
- Is the mean surface roughness of canals after instrumentation in the apical, middle and coronal thirds different?

This chapter contains material that has been used for the following paper which is currently under review for publication in the International Journal of Endodontics:

Sakhaei Manesh V, Giacomin P, Stoll R. Quantitative evaluation of root canal surface roughness after file reuse with a reciprocating single-file system.

This study was supported by the James Cook University Graduate Research Scheme Grant (grant number JCU-QLD-602531). The author would like to thank VDW (Gunz Dental) for their donating of the files used in this study and loaning of the motor used during the study.

5.2 Introduction

Torsional failure of endodontic files reduces with use of reciprocation instead of continuous rotary motion. The advancements in file designs and metallurgy have further increased flexural fatigue resistance. The combination of these two factors has made using a single-file a safe approach and a practical choice for root canal treatment.[169,243,256]

Single-file reciprocating systems are simpler compared to the conventional use of multiple files in sequence and therefore, they are easier to learn and adapt to. Although at first they seem to be more cost-effective since the system consists of a single instrument compared to multi-file systems, their cost increases because manufacturers recommend they should only be used on a single case. A single case might have a single or multiple canals and the question remains as to what number of canals is the limit of reuse and how safe it would be to use the same file in multiple canals.[256,257] A common issue that is present with many filing systems is that there is little consensus regarding the recommended number of uses of a file based on the canal curvature or the type of tooth being treated.[258] This issue becomes more complicated with single-file systems since the single-file used in these systems is under the same stresses that is distributed among a number of files in multi-file systems. Therefore, it may be more prone to deformation or wear after use in the same number of canals.[168,169]

Multiple use of these single-file system instruments has become quite common in both practice and research.[257] Analysis of the surface and composition of these files after three uses showed no significant change or plastic deformation that was correlated with the number of uses.[256] Clinical use of WaveOne and Reciproc files has showed that file separations do not

increase after using the files in treatment of three posterior teeth.[257] The lifespan of each file depends on various factors. For comparison, the F2 ProTaper file which is designed to be utilized in rotary mode can be used safely in up to six curved canals in reciprocation.[259] However, reuse of a file exposes it to a range of chemicals, sterilization cycles, physical wear and deforming stresses that can alter its performance.

File wear that is caused by dentine removal and instrument autoclaving reduces the cutting efficiency of a NiTi file during its usage cycle.[260,261] Reuse of files can increase the surface roughness of rotary files.[262] Surface fatigue wear such as flaking and pitting can be seen in the SEM images of all ProFile instruments after their first use.[263] Microcracks are visible on Reciproc files after they are used in canals for up to five times.[264] Research also shows that when file separation occurs as a result of fatigue failure, it is accompanied with microscopic surface defects such as dimples, striations and cracks initiations. Files with torsional failures are characterised with signs of circular abrasion, unwinding, bending and rollover.[265]

Aside from a higher chance of file separation with wear and cyclic fatigue build-up,[243] a worn out file may also leave a final root canal with surface qualities different to that made by a new and sharp file. This may especially be more apparent in systems that are more heavily worn in each use such as a single-file system. Files that have higher cutting efficiency tend to lose their efficiency and wear quicker.[266] Reuse of rotary ProTaper Universal files for instrumenting resin simulated canal showed that after three uses there is a significant decrease in the volume of preparation sizes calculated with micro-CT,[267] but this number may differ in every filing system and also varies based on the type of canal it has been used in.[256] Park et al. showed there is correlation between the number of times a Reciproc file has been used and their working time to prepare a canal.[264] Blunt edges can be observed in SEM images of over 73% of reciprocating Reciproc files that have been used in nine canals.[225]

File usage has been shown to reduce the cutting ability in NiTi ProFile instruments that have not gone through sterilization cycles. This experiment by Rapisarda et al. also revealed 20% and 50% reduction in file cutting efficiency after 7 and 14 autoclave cycles, respectively.[268] Patterns of change in cutting efficiency and mechanical properties as a result of sterilization cycles and repeated use is not identical in all NiTi instruments. The cutting ability and flexibility of HyFlex CM files does change after the first use and sterilization cycle. However, then there seems to be a drop in their cutting efficiency and mechanical behaviour before they return to a normal state (at 4 cycles), followed by another decrease in their performance at 7 sterilization cycles.[269] Torsional fracture resistance and mean angular deflection values of files before separation is not affected after 7 autoclaving cycles in files made with M-wire (ProFile Vortex), R-phase (Twisted Files) and CM Wire (10 Series Files) technology.[270]

Roughness is the dominant physical surface characteristics that can affect biofilm formation. Recent research shows that filing motion can significantly affect the root canal roughness. Reciprocation results in rougher canal surface compared to continuous rotary filing.[204] No evidence is currently available regarding how reuse of files and the resultant wear on the instrument can affect the treatment outcome apart from its effect on file deformation and separation. The aim of the present study was to compare the surface roughness (Ra and Rz) of a root canal after filing with a single-file reciprocating system after being reused for up to three times.

5.3 Materials and methods

5.3.1 Study design and ethics

Sample size estimations based on the most relevant previous research using “Ra” measures determined an approximate of seven samples needed for statistical analysis. However, since the “Rz” measures were larger and were also used for this study, 12 samples per group was

considered after a pilot study and using the following formula (designed to show a difference of 0.05 with a power of 80%):

$$\boldsymbol{n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{d^2}}$$

$$\boldsymbol{\alpha = 0.05 \Rightarrow Z_{1-\alpha/2} = 1.96}$$

$$\boldsymbol{1 - \beta = 0.80 \Rightarrow Z_{1-\beta} = 0.84}$$

$\boldsymbol{\sigma_1 = 3.5}$ $\boldsymbol{\sigma_2 = 3.5}$ (Standard deviations of Rz values based on pilot studies)

$$\boldsymbol{n = \frac{(1.96 + 0.84)^2(3.5^2 + 3.5^2)}{4^2}} = \frac{192.08}{16} \cong \boldsymbol{12}$$

Fifteen roots were prepared for each group to account for sample loss. Extracted teeth with mature apices and straight roots were collected from the JCU Dental Clinic. Ethical approval of the study was obtained from the James Cook University Human Research Ethics Committee (H6199). Teeth were stored in a 0.1% thymol solution at 5 °C. The overall storage time for all teeth was less than 8 weeks.

5.3.2 Sample preparation and root canal treatment

Mesio-buccal canals of the lower molars and the mesiobuccal roots of the upper molars with a moderate or severe curvature were used for the first and third usage groups of this study, while straight lower incisor canals were used for the second usage group. Access cavity was made on each tooth and the working lengths were determined. Working length was determined by entering a size #10 K-file into the canal until the tip was visible and subtracting 1 mm from that measurement. Teeth that had a working length of 18-20 mm long were cut at the crown to the standard length of 18 mm. Teeth shorter than 18 mm or longer than 20 mm were discarded. Buccolingual and mesiodistal radiographs were taken of each sample to

evaluate canal curvature, radius and length according to the Schafer et al. method [271] (Table 5-1; Figure 5-1). Thirty molar teeth were collected and randomly assigned to one of two groups. Fifteen lower incisors were assigned to the second group.

Table 5-1 Mean ± Standard deviation of curvature degree, radius and length for roots according to their groups.

	n	Curve (Degree)	Radius of curve (mm)	Length of curve (mm)
First use group	15	31.60±9.71	7.21±6.97	11.03±3.39
Third use group	15	30.60±7.97	6.25±5.65	10.68±2.78
p-value*		0.760	0.683	0.760

** Statistical comparison by independent samples t-test (α=0.05).*

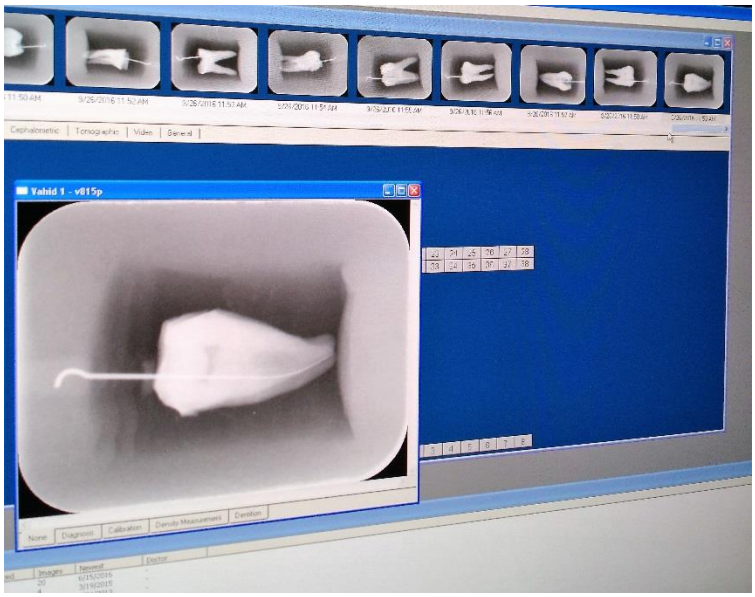

Figure 5-1 Radiography of samples to determine canal curvatures.

Each canal was filed with a Reciproc 25 (R25; VDW GmbH, Munich, Germany; Figure 5-2) file to working length using a VDW.Silver Reciproc motor (VDW GmbH) set to reciprocating motion ("RECIPROC ALL") according to manufacturer's instructions. Three slow pecking motions were applied in each insertion of the file into canal before cleaning and reinsertion. The file was

progressed up to a maximum of 3 mm in each insertion. Irrigation was carried out between file cleaning with a total of 10 ml of 2.5% NaOCl.

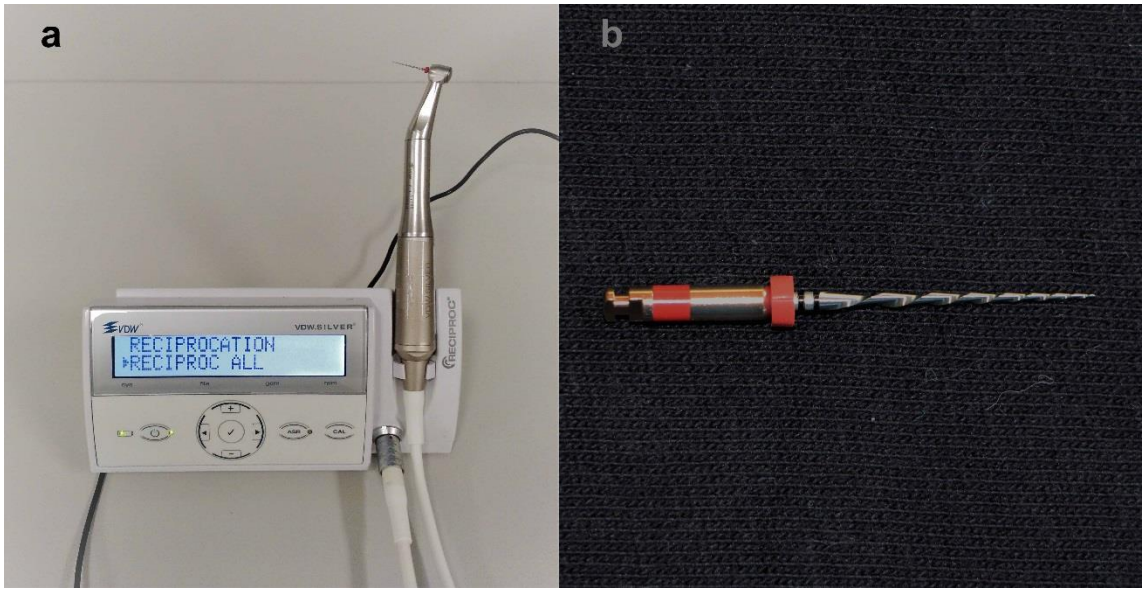

Figure 5-2 Reciprocating single file system consisting of the (a) VDW.Silver Reciproc motor and the (b) Reciproc (R25) file.

All samples were irrigated after the final instrumentation step with 1 mL of EDTA for 1 minute followed by irrigation with 1 mL of 2.5% NaOCl and then rinsing with 5 mL of distilled water.

After completing root canal preparation, two 0.5 mm deep cuts were made on the opposite sides of the root surface, parallel to the root curve with a diamond disc. Two mark cuts were also made at 5 mm and 10 mm away from the apex to record where the middle third would be after sectioning the roots. To prevent loss of samples due to the curvature of the roots, they were first sectioned into two halves horizontally using a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Then each half was split longitudinally, to expose the root canal surface.

5.3.3 Sample scanning and surface roughness evaluation

Each sample had 4 root pieces (2 apical halves and 2 coronal halves) at this stage that were blinded with a random three letter code. Specimens were dried overnight and then put on aluminum stubs to be sputter coated with gold. The prepared samples were scanned and analyzed in a Phenom G2 Pro SEM System (Phenom-World, Eindhoven, Netherlands). Each root piece specimen was imaged 3 times at 550x magnification. A total of 12 images were taken from each sample (4 apical, 4 middle, and 4 coronal). Phenom Pro Suite software was used at each scan area to conduct 3D roughness reconstruction. Surface roughness was calculated based on the height maps created. Rz and Ra measurements were calculated after filtering out wavelengths higher than 1060 µm and lower than 20 nm. The measurements were made at three different parts of the canal height maps (total of 36 calculations for each sample) for scanning directions parallel to the root canal axis.

Height map Rz and Ra means were calculated from the three values obtained from each height map. The apical, middle and coronal third means were calculated from the four height map Rz and Ra means of each third of the root canals. Normal distribution of data was verified with Kolmogorov-Smirnov tests and the Levene test was used to examine the homogeneity of variances. To compare the three uses in each third of the root canal, repeated measures general linear models were done for Ra and Rz. Differences between the apical, middle and coronal thirds and the effect of file use on it was tested using a General Linear Model. Significance level of 0.05 was considered for all tests.

5.4 Results

General linear models with repeated measures showed Ra means of the root canal surface in the apical ($p=0.499$), middle ($p=0.575$) and coronal ($p=0.498$) thirds were not significantly different based on the number of times usage of the file (Figure 5-3). Ra overall means of the

three uses of the file did not significantly differ among the file reuse groups (p=0.608). In case of all three uses of the files in the canals, Ra decreased from the apical towards the coronal third. General linear model with repeated measures of the changes of among the three thirds showed a statistically significant difference (p<0.001). This pattern was similar after file use and no interaction was seen with the number or file reuse (p=0.657).

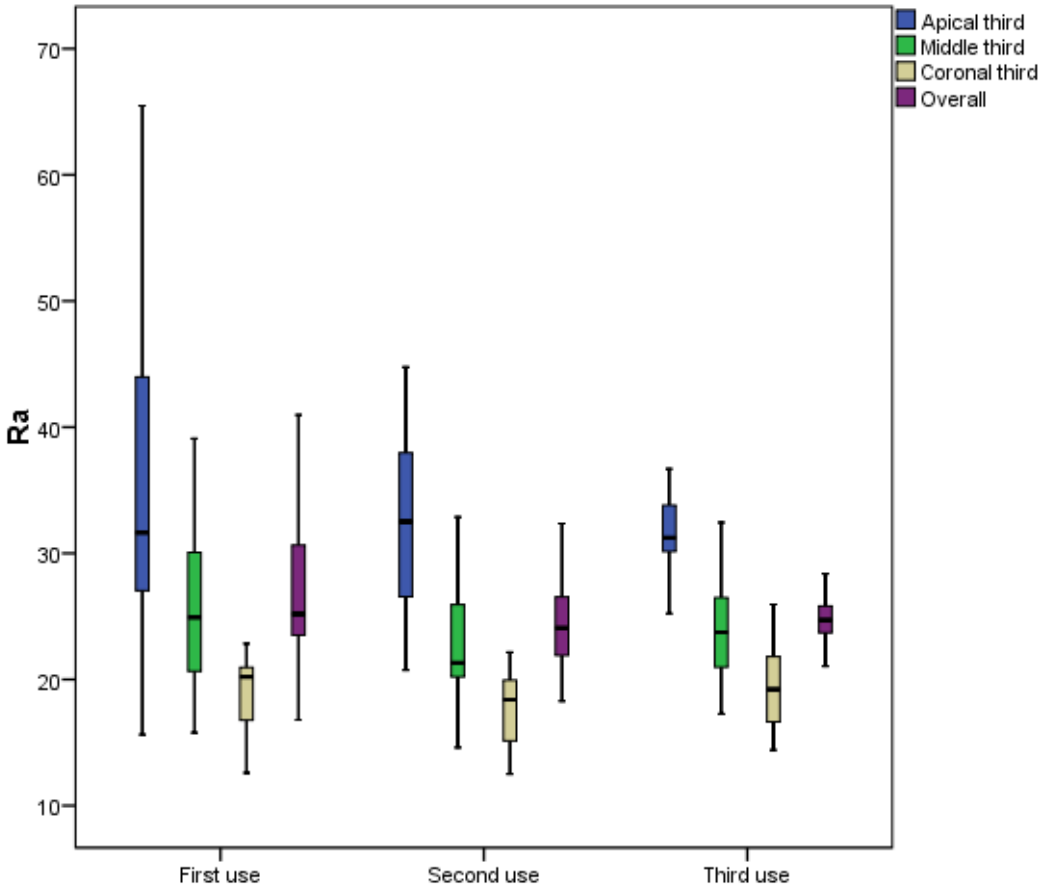

Figure 5-3 Box plot illustrating the median and distribution of the Ra (µm) of canal surfaces in different thirds of the instrumented root after use of files for the first, second and third times.

General linear models with repeated measures showed Rz means of the root canal surface in the apical (p=0.429), middle (p=0.772) and coronal (p=0.229) thirds were not significantly different based on the number of times usage of the file (Figures 5-4 and 5-5). Rz overall means also did not significantly differ after reuse of the files (p=0.513). In case of all three uses of the files in the canals, Rz generally increased from the apical towards the coronal third.

General Linear Model for repeated measures of the changes among the three thirds showed a statistically significant difference (P=0.001). This pattern was similar after file use and no interaction was seen between the effect of these two factors (p=0.492). No file separations occurred during the experiments (Figure 5-6).

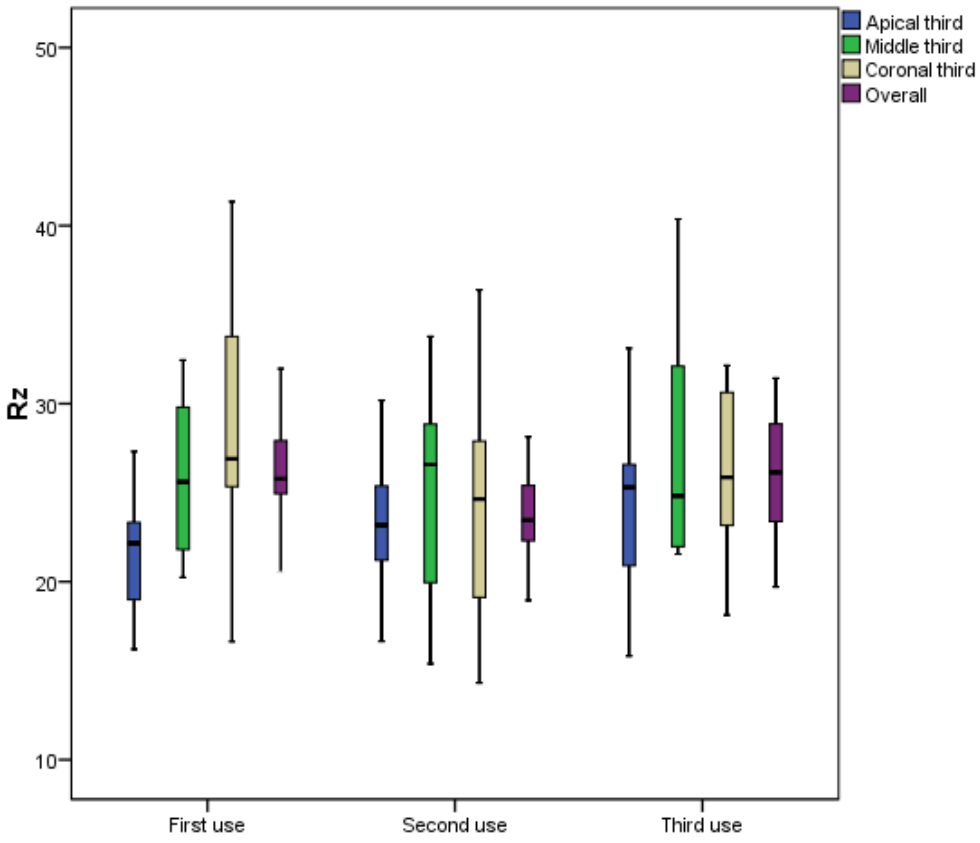

Figure 5-4 Box plot illustrating the median and distribution of the Rz (µm) of canal surfaces in different thirds of the instrumented root after use of files for the first, second and third times.

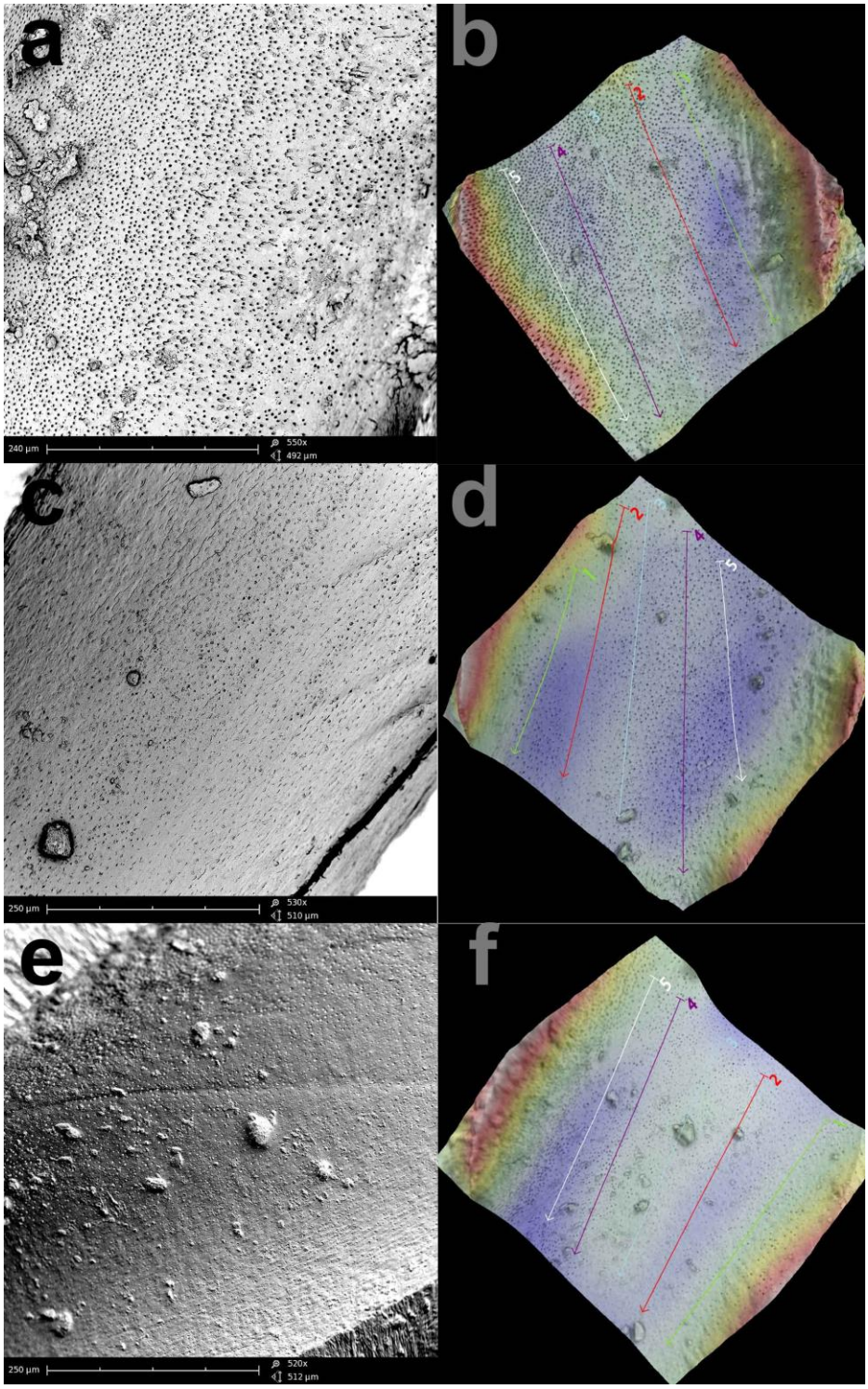

Figure 5-5 Scanning electron microscope images of canals surfaces instrumented from the a) first use, c) second use and e) third use groups. The height maps, Ra and Rz calculations of the scans performed for samples from the b) first use, d) second use and f) third use groups can be seen in the images on the right.

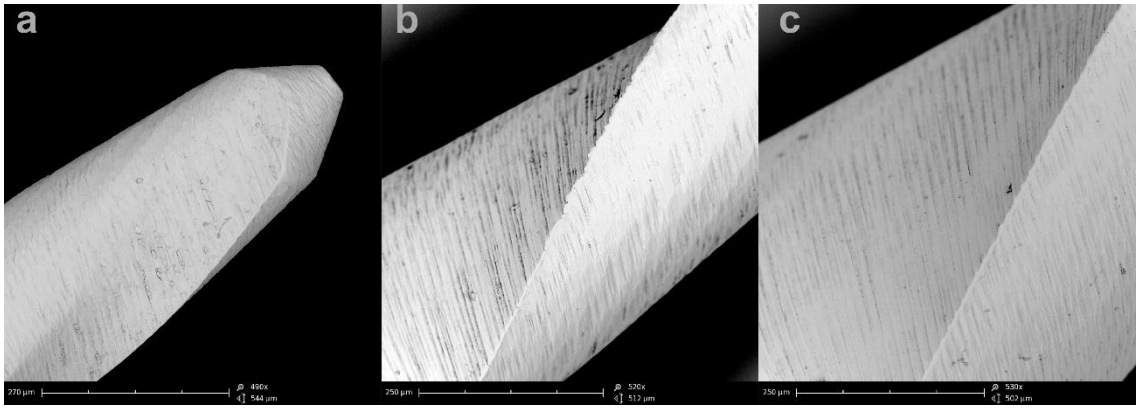

Figure 5-6 Scanning electron microscope images of the Reciproc (R25) files with wear after the third use. a) Tip, b) apical third and c) middle third surfaces of the file.

5.5 Discussion

Reciproc files are recommended to be used for a single case. This suggests that the file wears heavily after use in a limited number of canals, which would increase risk of file separation. The current results show that the amount of wear after use of the file in up to three canals, which could be a multi-root case in practice, does not significantly affect the treated surface quality in terms of Ra and Rz. Reuse of Reciproc files in practice and research is common despite the files having measures such as the silicon ring to prevent their reuse after being autoclaved.[257] Since the use of Reciproc was shown to be safe for up to 3 posterior teeth after sterilization,[257] further research to investigate the effect of that amount of file wear on the treated canal surface is recommended. Although in the present experiment use of the file in three canals did not significantly affect the treated root canal surface quality, the effect of file wear on the treated canal surface quality may become apparent with further use. In addition, sterilization cycles under autoclave have been shown to affect the in-depth and surface chemical composition of ProFile NiTi files and decrease their cutting efficiency.[268] Therefore, using Reciproc files for more than one case would not only add to the wear from extended use but also the effect of sterilization cycles.

Surface flaws and irregularities on a filing instrument can result in file separation during treatment.[272,273] Exposure of files to multiple autoclave cycles[274], contact with chemicals such as sodium hypochlorite[273] and wear as a result of file use [275,276] can all cause changes to the surface quality of the file. However, it is unclear when these changes start translating into significant changes in the treated root canal surface. The cutting efficiency of the file after prolonged use may be an indicator of when file wear may affect the treated root canal's surface quality. Reduced cutting efficiency has previously been linked to undermining bacterial elimination by diminishing adequate dentine removal.[267] However, this may be partly due to rougher surfaces that can result from blunt instruments. There is a lack of evidence on how much cutting efficiency is lost due to use in the every filing system. However, some research such as the report by Gambarini et al. shows that cutting efficiency of Twisted File instruments in both reciprocating and rotary motion does not decrease after 10 uses on Plexiglas plates.[216] It is important to acknowledge whether the limit in which the root canal surface quality is affected is reached during the life cycle of a file or if files are discarded before that due to increased risks of file separation. Based on the present results, file separations did not occur and the root canal surface was not affected after three uses of the Reciproc files, which are a single-file system.

Extracted teeth were used to simulate the effect of file wear and also assess how filing affects dentine. Dentine microstructure and hardness varies among different teeth and among different sections of the root.[260,266,277] An adequate sample size can assure that these variations among different teeth are distributed normally among groups. Other materials such as polymethyl methacrylate may be easier to standardize, but have been shown to have little effect on instrument wear.[266] In addition, poor canal smoothness had been reported to be more common in highly curved canals (>40°).[278] In the present study, curved roots were used for the first and third-use groups while straight roots were used for the second-use group. This study design was considered to compare first and third use groups with similar circumstances,

while being able to compare them to a straight canal. However, results showed no significant difference in roughness levels among the different use groups.

Biofilm formation rises with both nanoscale and macroscopic increases in surface roughness. Larger scale roughness can increase the contact area for biofilm formation and sheltering bacteria from shear forces. Nanoscale roughness can affect the first interactions between the surface and the attaching microorganism. The threshold roughness which can reduce attachment of each specific microorganism is different. Although this nanoscale threshold has been reported to be around 200 nm for oral microorganisms which attach to implant abutments,[15] no evidence is currently available on microorganisms active in root canal infections.

Roughness averages (Ra) of the canal surfaces after reuse did not change significantly in this study. However, the amount of deviation from the means decreased with more use of the files. This indicates more consistent cuts are achieved as the cutting efficiency of a files decreases. Although no standard has been set regarding the cutting efficiency and sharpness of endodontic files,[279] there has been tendency towards developing files with higher efficiency since it can reduce working times.[264] Many variables such as file design, cross-section, blade angle and metallurgy can affect its cutting efficiency.[279] Since a Reciproc file is a highly efficient instrument with only two cutting edges and prepares the whole canal alone,[218] it is expected to have relatively high wear upon use compared to other filing systems.

The relatively high range of both Ra and Rz values in treated root canals shows that these surfaces are generally rough. Previously, studies using the “Cardiff experimental design” had been testing smoothness of root canal surfaces.[118] Although the method used was by grading the root canal impression as either “poor” or “good”, in some filing systems such as the Naviflex, up to 45% of canals had poor smoothness in the apical half.[280] The roughness from using the Reciproc system may partially be caused by reciprocation that has been suggested to

leave more cut marks on the final surface compared to continuous rotary motion.[204] Sabet and Lufty compared the roughness of canals after using ProTaper and NRT files using a method that quantified roughness on a scale from 0 (roughest) to 255 (smoothest). In contrast to the present study, the mean roughness they reported was 253.51 and 251.29 for the ProTaper and NRT groups, respectively. This may cause the an initial impression that the surfaces may be smooth. However, since the scaling unit is not common, it is difficult to interpret or compare to any other findings outside the study.[23]

Reciprocation has been shown to create a more centred root canal instrumentation especially in the apical third of the root,[281,282] which may be responsible for more machined and rough surfaces too. Reciproc instruments have been associated with formation of more complete dentinal cracks compared to rotary instruments. These files have high cutting efficiency and sharp edges due to their S-shape cross section.[283] Their aggressive cutting properties leads to removal of a larger volume of dentine and increases the surface area of the root canal more compared to Twisted File and WaveOne filing systems.[252] Reciprocation in this system along with its cutting ability has been suggested to contribute to transporting more debris to the apex.[284] Understanding the role of each of these factors on the root canal surface quality and means of using them to reach a smooth surface requires more investigations.

Roughness evaluation in this study was similar to previous research.[204] Both an extreme value amplitude parameter (Rz) and an average value parameter (Ra) were calculated in our experiments to cover more aspects of roughness. Rz changes in root thirds is similar to a pervious experiment with the Twisted File Adaptive (TFA) system.[204] It was suggested that the increased Rz in the coronal third may be due to the fact that the file flutes are bigger in the coronal third and therefore the cuts are deeper in this third.[204] However, in this study the Ra means tend to increase from coronal to apical, which shows the average roughness increases towards the apical third. Previous research shows Reciproc files create more incomplete

dentinal cracks in the apical section of roots compared to rotary files[283], which might be better reflected in Ra than Rz. In addition, dentine microstructure changes from coronal to apical. The mineral content and nano-hardness of dentine decreases towards the root apex.[277] These factors can lead to a different interaction with the filing system in each part of the root. The softer dentine towards the apical third of the tooth seems to be better machinable and rougher.

5.6 Conclusions

Root canals are similar in terms of surface roughness after instrumentation with new and reused Reciproc files. The amount of wear after using these files in three canals, which is recommended by the manufacturer, does not create smoother surfaces. Considering increased chances of file separation with overuse of files, it is not recommended to use file wear as a means of reducing the cutting efficiency and aggressiveness of Reciproc files. However, future research would reveal whether the amount of wear endured in files that are not single-use could result in changes in treatment quality. Furthermore, more data is required on the effect that other filing systems with their different designs and variable cutting efficiencies have on root canal surfaces.

Chapter 6 Conclusions and future directions

Root canal treatment success rates have not changed in the last decades.[1,285] This outcome is despite the technological revolution in instruments, materials, and techniques used in treatment.[285] Many of these advancements such as rotary instruments have been widely adopted in clinical practice since they simplify the treatment process and reduce working times.[286] Although more of these new treating options are becoming available, they are very few factors that are tested against before their introduction. The clinical relevance of many of these test factors have been questioned which makes proper assessment of these innovations even more difficult.[6] Reliable and clinically relevant cofactors are important in developing new technology before they are introduced to dental practitioners. They provide a bridge between the clinics and dental industries since it is not feasible to conduct clinical trials for every variable in treatment. There are no clear standards available for many of the endodontic instruments that are being used by clinicians[279] and having clinically relevant cofactors can be a starting point to provide a scale to evaluate their performance.

Complete elimination of bacteria inside the root canal system has shown to be practically impossible in most cases. This is because the adapting potential of bacteria gives them unlimited mechanisms to survive antimicrobials and elimination methods. Therefore, reducing the amount of bacteria and gaining control of each of the influential factors that can reduce the chances of their growth is of upmost importance. Developing a surface which reduces the number of initial adherent bacteria is of great importance since the microorganisms are much more difficult to remove once they have formed biofilms and matured.[54,129]

Roughness was established as an effective cofactor on root canal treatment quality by means of a novel study design utilizing biofilm formation. The methodology introduced had the benefit of providing quantitative results. *E. faecalis* was used as the testing species in this experiment because of its prominent role in root canal infections.[180] Single-species biofilm

models have less variables confounding their results and their biofilm growth rates can be better compared.[56] However, *E. faecalis* is not the only pathogen in endodontic infections. The next step towards understanding the role that roughness plays in root canal infections would be to experiment other species in addition to multi-species biofilms. The same experimental design could also be used to evaluate the potential effect that smoothness of surface may have on the biofilm composition and preventing maturation of root canal biofilms. If smoother surfaces can tip the biofilm balance to bacteria that are less pathogenic than *E. faecalis*, it may itself provide a means to increase the current treatment success rates. Furthermore, maturity of a biofilm is correlated to its resistance to antimicrobials.[129] Having less mature biofilms in root canals with persistent infections may mean that they are easier to eliminate and treat. The results from these experiments can help develop new treatment strategies that would contribute less to antimicrobial use and resistance. However, after reaching this level of evidence, it is necessary to also see how roughness can interact with other variables in the oral cavity, such as saliva and the normal flora. After identifying instruments and techniques to achieve smoother canal walls, clinical trials can show to what extent they can effect long-term success rates compared to conventional or rough treated surfaces.

Extremely high roughness levels achieved in this study by using the available files are alarming at least. File manufacturers have been successful in improving file efficiency and mechanical properties, but the final root surface quality is far from ideal. The direction of technological advancements in filing systems has led to producing stronger instruments and reducing the working time for dental practitioners. The higher strength of the files also translates to less treatment complications such as file separations. However, up until now there has been very little focus on how these changes can affect the surface quality of the treated root canal. Further research into the effects of different filing systems can reveal what variables can affect the final canal surface roughness. File design, size, motion, surface treatment and alloy are only some of the factors that can be tested among the available systems. Having adequate

quantitative and comparable information on the available instruments and the ideal roughness levels can lead to developing standard levels for future products.

Changing the filing motion was one factor that improved treatment results in terms of root canal surface smoothness. This means that roughness is a factor that the clinician is able to modify during treatment. More research into how other factors such as materials, instrument design and techniques could affect surface roughness can give a guide on developing treatment strategies to use each in order to smoothen surfaces. This information can also be utilized in developing and designing new files that use these factors in favour of achieving smoother surfaces.

Variability in cutting effect of a file decreases with its wear, according to the trends seen in the data obtained from our experiments. Therefore, having high cutting efficiency seems to have the side effect of achieving less consistent surface roughness values. Similar to the restorative procedures where less cutting and finer grit instruments are used towards the finishing stages of a filling, it may be beneficial to apply comparable principles in root canal treatments. The current research has established a reliable method to assess root canal surface roughness and has tested a few of the variables that had the potential of affecting it. This can act as the foundation towards building enough research that could eventually generate practical improvement in treatment methods and strategies.

Surface quality characteristics that can affect bacterial adhesion are not only limited to surface roughness.[15] Although surface roughness seems to be a dominant factor that the clinician also has control over, there are other surface characteristics that may be modifiable during root canal treatment with novel methods. Surface chemistry, charge and energy[15] are some of the factors that require more research. The range of methods and materials that can be used in root canal treatments are much wider compared to other parts of the body since the

treatment field is relatively separated from the surrounding vital tissues by highly mineralized dentine.

The current methods used in treatment may have underlying effects on surface qualities that have yet not been completely understood. Sodium hypochlorite has been used as one of the most common disinfectants in root canal treatments for decades. Aside from its disinfecting ability, its high performance and desirability in clinic is partly due to its ability to dissolve organic tissues such as the tooth pulp.[287,288] However, the same dissolving effect exists for the non-mineralized collagen on root canal surfaces[289] that is the main binding site for endodontic pathogens.[290] Further research into this field and developing new means to block or eliminate bacterial binding sites can lead to a novel line of defence against root canal pathogens. Similar to the effect of roughness, bacteria are not the direct target in this mechanism of action. Therefore, these methods have the additional benefit of not causing antimicrobial resistance since they also prevent bacterial attachment and biofilm formation.

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