Evaluation of Dentine Damage after rotary NiTi preparation

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Dissertation submitted in fulfilment of the requirements for the degree of Master of Science (Rest.) 2017
in the Department of Restorative Dentistry, Faculty of Dentistry; University of the Western Cape

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SUMMARY

NiTi rotary instruments have shape memory and are highly flexible and super-elastic. These properties of the metal alloy allows for ease of root canal preparation to ultimately result in a root canal preparation that has a continuous taper, while canal shape and curvature is maintained. It must be noted that the NiTi rotary instrumentation may have an effect on root canal dentine, which may manifest as dentine damage. Different NiTi rotary systems on the market vary with regards to their design features and kinematics, which may influence dentine damage.

The purpose of this *in-vitro* study was to compare the effect of four different NiTi rotary systems, as well as stainless steel files on root canal dentine.

One hundred and eighty permanent human mandibular molar mesial roots were used for the study. The total samples were randomly divided into six groups, where one group (n=30) was left unprepared to serve as the control group. The remaining five groups were randomly assigned to a nickel-titanium rotary instrumentation system and one stainless steel hand file group.

Group 1: Control group

Group 2: Stainless steel files group

Group 3: Wave One (Dentsply Maillefer) rotary group

Group 4: ProTaper NEXT (Dentsply Maillefer) rotary group

Group 5: iRaCe (FKG Dentaire) rotary group

Group 6: BT-Race (FKG Dentaire) rotary group

The root canal preparations were carried out according to the manufacturers' recommendations, after decoronation of the tooth crowns. Sodium hypochlorite (5,25%) and 17% EDTA was used as a root canal irrigant and a chelating agent during canal preparation. Each root segment was sectioned at 3mm, 6mm and 9mm from the apex. The root segments were observed under a stereomicroscope at 12x magnification and digital camera at 40 x magnification for the appearance of dentine damage. The images were observed by the author and an impartial second observer. Root segments were observed for the appearance of dentine damage (microcracks, craze lines or fractures), and samples were described as having 'dentine defect' or "no dentine defect'.

Data for the different groups were collected and results were calculated and the total incidence of dentine damage was as follows:

Control group = 0%

Stainless steel hand files group = 0%

Wave One group = 56, 67%

ProTaper NEXT group = 60%

iRaCe group = 60%

BT-Race group = 56, 67%

There are no significant differences between the groups: WaveOne, ProTaper NEXT, iRACE and BT-Race. However, there is a significant difference between the four groups and the stainless steel group ($p<0.0001$).

DECLARATION

I, Suwayda Ahmed, declare that this dissertation entitled, “Evaluation of dentine damage following NiTi rotary canal preparation”, which I herewith submit electronically to the University of the Western Cape in fulfilment of the requirements for the degree MSc (Restorative Dentistry); is my own original work and has neither been submitted for any academic award to this University, nor to any other institution of higher learning.

SIGNATURE **DATE:** 11th November 2016

QUOTATION

(Moses) said: O my Lord! Expand my chest

And ease my task for me,

And loosen the knot from my tongue,

That they may understand my saying.

(Surah Ta-Ha 20: 25-28)

ACKNOWLEDGEMENTS

It is with the greatest appreciation and gratitude that I would like to express my thanks to:

Almighty Allah, for granting me the strength and ability to complete this study.

Dr CM Saayman (Dept of Restorative Dentistry, Faculty of Dentistry, University of the Western Cape), my mentor and supervisor, whose guidance in this dissertation was invaluable.

Prof Madsen (Statistician, University of Missouri)

My sister and colleague, Dr Rukshana Ahmed, for providing invaluable support and guidance.

My mother, Khadija Sablay, for her many sacrifices to ensure that I received a tertiary education; and to continue to encourage and inspire me to pursue knowledge.

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LIST OF ABBREVIATIONS

µm= nanometres

mm= millimetres

P= Probability

rpm= rotations per minute

EDTA= ethylenediaminetetra-acetic acid

s= seconds

mm^2= millimters squared

MPa= megapascal

GPa= gigapascal

%= percentage

Ca= Calcium ion

P= Phosphorus ion

ºC= degrees celcius

NiTi= nickel-titanium

n= number

Ncm= newton centimetre

CHAPTER 1: LITERATURE REVIEW

1. INTRODUCTION

1.1 The Objective of Endodontic Treatment

The objective of endodontic treatment is to restore an affected tooth with an inflamed or infected root canal system to its proper form (aesthetics) and function in the masticatory apparatus, in an acceptable state of health (Peters, 2004; Weine, 1996).

1.2 Principles of Endodontic Treatment

Endodontic treatment is the combination of mechanical and chemical preparation of the root canal space, to allow for the placement of a biocompatible material that seals the root canal system in its entirety. The success of root canal treatment is achieved by forming a correct diagnosis; developing a suitable plan of treatment; applying knowledge of tooth structure, anatomy and morphology; and thereby allowing proper cleaning, shaping and obturation of the entire root canal system (Torbinejad and Walton, 2009).

1.3 Basic concepts of Endodontic Treatment

Endodontic treatment involves the treatment of any infection associated with the dental pulp and surrounding periapical tissues. The basic concepts of endodontics involve:

- The removal of necrotic pulp tissue, debri, vascular tissue and most micro-organisms. During this debridement phase; instrumentation is introduced into the root canal system and intracanal medicaments and irrigants are used. Optimal debridement of the root canal system is of paramount importance in the success of endodontic treatment (Cohen and Hargreaves, 2011).
- Prevention of the proliferation of micro-organisms in the root canal system by disinfecting the root canal to eliminate bacteria and to prevent the regrowth of bacteria (Young, Parashos and Messer, 2007).
- Optimal cleaning and shaping of the canals to obtain a well-tapered root canal space which remains centred and does not transport the apex. This will allow for adequate irrigation and medicament placement (Torabinejad and Walton, 2009).

- The maintenance of health of the surrounding peri-apical tissues, by avoiding over irrigation, over-instrumentation and caustic intracanal medicaments being placed or extruded (Cohen and Hargreaves, 2011; Weine, 1996).
- A well-shaped, sterile canal that allows for a superior root filling (obturation) being placed (Torabinejad and Walton, 2009).
- Successful obturation constituting a hermetically sealed canal and the placement of a high quality coronal restoration to prevent leakage in the coronal area of the root filling (Cohen and Hargreaves, 2011).

2. DENTINE (Human Body)

2.1 Structure of dentine

Dentine is a highly mineralized hydrated hard tissue located between the enamel and inner pulp of tooth structure. Dentine acts as a semi-permeable buffer between the enamel layer and the pulp (Arola and Reprogel, 2006; Bertassoni, Stankoska and Swain, 2012). Dentine forms a resilient foundation to aid in the prevention of crack propagation from the enamel (Giannini, Soares and de Carvalho,2004; Imbeni *et al*. 2003). The mechanical properties of dentine; although crucial for the prediction of caries, fractures and sclerosis, is not widely understood and there is not a clear indication in literature on the structural performance of dentine. Although research has been conducted on dentine, there is no consistency in results pertaining to the structural performance of dentine (Imbeni *et al.* 2003). Resistance to fracture is an important structural performance characteristic of dentine; as dentine structure may undergo changes due to various physiological, age-related and disease processes. These changes in the dentine structure complicate the study of dentine; as various forms of dentine are created through these processes (Kinney, Marshall SJ.and Marshall GW, 2003; Marshall Jnr *et al.* 1997).

2.1.1 Microstructure of dentine

In order to investigate the effect of caries, sclerosis and ageing on tooth structure, it is essential to study the mechanical properties of dentine. Dentine is a hydrated mixed material consisting of a nanocrystalline carbonated apatite mineral phase (50% by volume), a felt-work of Type I collagen fibrils (30% by volume) and fluid (Giannini *et al.* 2004; Kinney *et al*. 2003; Marshall

Jnr *et al.* 1997). The size of the collagen fibrils are approximately 50-100μm in diameter. Within this collagen platform, the mineral phase is located and can either be intrafibrillar (inside the spaces in the collagen fibril) or extrafibrillar (in the spaces between collagen fibrils) (Kinney *et al.* 2003).The mineral is scattered in the collagen platform as 5μm thick crystallites (Imbeni *et al.* 2003). The mineral crystallites have needle-like shapes close to the pulp (Kinney *et al.* 2003). At the dentino-enamel junction toward the pulp, an arrangement of cylindrical tubules (1-2μm in diameter) is found. These dentinal tubules contain the odontoblasts during tooth formation and they are encompassed by peri-tubular dentine. Peri-tubular dentine is highly mineralized, and approximately 1μm thick. There is also a softer intertubular matrix present (which is filled by organic material in the form of collagen fibrils and noncollagenous proteins and phosphorylated proteins). These tubules are enclosed within a softer intertubular matrix of mineralized collagen which makes up intertubular dentine (Arola and Reprogel, 2006; Imbeni *et al*. 2003). The tubules provide an important role in dentine development, function and pathology (Earl *et al*. 2010). The mineralized collagen fibrils (50-100nm in diameter) form a plane which is orientated perpendicular to the tubules (Giannini *et al*. 2004; Imbeni *et al.* 2003; Kinney *et al.* 2003).
The value of hardness and elastic modulus of peri-tubular dentine is greater than intertubular dentine (Arola and Reprogel, 2006; Imbeni *et al*. 2003). Dentinal tubules run continuously from the dentino-enamel junction to the pulp in the coronal dentine; and runs from the cemento-enamel junction to the inner pulp (Kinney *et al.* 2003).The tubule density and lumen diameter decreases with greater distance from the pulp. As dentine has distinctive elements, combined with the distinctive orientation of fibril structures; it is an anisotropic material (Arola and Reprogel, 2006; Kinney *et al.* 2003; Lertchirakarn, Palamara and Messer, 2001).

2.2 Properties of dentine

2.2.1 Elastic properties of dentine

To understand or evaluate tooth strength, the elastic properties of dentine are an important factor. Elastic constants are usually described in terms of stiffness or compliance matrix, including Young's modulus of elasticity. There is limited understanding of the structural performance of dentine, which is crucial when evaluating resistance to fracture (Imbeni *et al.* 2003).To evaluate the elastic properties of dentine, the symmetry of dentine also needs to be investigated. Dentinal tubule orientation influences the elastic modulus and dentine strength (Kinney *et al.* 2003). Dentine displays the greatest elastic modulus in a perpendicular direction to the tubule axis (Arola and Reprogel, 2006).

2.2.1.1 Young's modulus of elasticity

Young's modulus of elasticity is described as "The elastic deformations of a solid are related to the associated stresses by quantities called elastic modulus" (Imbeni *et al.* 2003).

2.2.1.2 Tensile and compressive measurements of Young's modulus

Numerous attempts have been made to quantify the Young's modulus of dentine. Numerous investigative research projects have been conducted in either tension or compression. One of the earliest attempts to quantify the Young's modulus of elasticity was conducted in 1962, and Bowen and Rodriquez recorded a mean modulus of elasticity at 19.3 GPa (Bowen and Rodriguez, 1962). During an experimental study in 1967, Lehman recorded the tensile properties of dentine and achieved a result of 11.0 GPa. In 1958, Craig and Peyton documented a compressive Young's modulus of 18.5 GPa. Measurements of the modulus are sensitive to specimen preparation, study design, stress relaxation and study variables (Imbeni *et al.* 2003). The differences in Young's modulus values may be ascribed to the structural configuration of the dentine (i.e. dentinal tubules, peritubular and intertubular dentine). The specific characteristics of dentinal tubules (i.e. density, direction, and dimension) are location dependent, and can therefore have an effect on the mechanical properties of dentine (Kinney *et al.* 2003, Plotino *et al*. 2007).

2.2.1.3 Viscoelastic properties of dentine

Although experimental studies on the visco-elastic properties of dentine have been conducted and acquired in both the tensile and compressive measurement, the visco-elastic nature of dentine is not clear (Plotino *et al.* 2007). In biological tissues; at a continuous stress, material continues to distort with time (creep). Thus, if a continuous strain is to be sustained, the applied stress needs to be constantly decreased (stress relaxation). Any material samples that reveal a time-dependant response, is a viscoelastic material. If the time dependence of the relaxation is independent of the amount of the applied stress, then the material demonstrates linear viscosity. If the time response deviates with the applied stress then the material sample displays nonlinear viscosity (Kinney *et al.* 2003). The elastic properties of dentine have been indirectly acquired in either tensile or compressive measurement (Imbeni *et al.* 2003; Kinney *et al.* 2003; Kishen, Kumar and Chen, 2004).

2.2.2 Tensile strength of dentine

The definition of tensile strength is the resistance of a material to fracture. Strength of dentine is dependent on anatomical location. Both the ultimate tensile strength and shear strength (ability of dentine to resist shear force) of dentine is influenced by the orientation of dentinal tubules. A higher measure of tensile strength is observed when the dentinal tubules are perpendicular to the direction of loading (Giannini *et al.* 2004). Strength increases as the distance from the pulp is increased, in other words the strength of coronal dentine will be greater than that of root dentine. The difference in strength may be ascribed to the increase of tubule density toward the pulp (Arola and Reprogel, 2006; Lertchirakarn *et al.* 2001).

2.2.2.1 Tubule orientation and tensile strength

Dentine fractures occur frequently across dentinal tubules when the tensile force is in a perpendicular direction to the dentinal tubules and greater strength is displayed in dentine (Watanabe, Marshall Jnr and Marshall SJ, 1996). This is due to the relation of collagen fibrils direction with respect to dentinal tubules. Collagen fibrils are interwoven and perpendicular to the dentinal tubules. The crystal apatite is situated parallel to the long axis of collagen fibrils. Therefore any fracture perpendicular to the dentinal tubules will happen within the plane of the collagen network (Rasmussen *et al.* 1975). Fractures parallel to the dentinal tubules will require interference of the collagen fibril network (Arola and Reprogel, 2006; Rasmussen *et al.* 1975; Watanabe *et al.* 1996).

2.2.3 Shear strength of dentine

Shear strength is measured by punching or lap shear. Studies investigating shear strength using shear punch apparatus obtained values from 64 to 132 MPa (Cooper and Smith 1968), while studies using a single-plane lap shear produced a result of 36MPa (Gwinnett 1994). Less mature dentine, closer to the pulp demonstrated low values of lap shear strength, while the highest value was recorded in mature cuspal dentine (Imbeni *et al.* 2003). Watanabe *et al.* (1996) showed that the difference in shear strength values could be influenced by the dentinal tubule orientation and the location within the tooth. Dentinal tubules are arranged in a radial arrangement from the pulp toward the dentino-enamel junction. Thus, anisotropic (directionally dependent) properties could have an effect on shear strength. The shear strength values of the study conducted by Watanabe *et al.* (1996) ranged from 38.2 to 100.3MPa. The researchers also determined that differing methods of load exertion lead to differing stress distributions.

2.2.4 Flexural strength of dentine

Flexural strength is defined as the ability of a material to resist deformation under load (Pascon *et al.* 2009). Plotino *et al.* (2007) found that the flexural strength of dentine was found to be 17,5 GPa. The flexural strength measurement defines the resistance to fracture of a sample. Increased values of flexural strength indicate that a sample is more resistant to fracture, while a lower value indicates that the sample has a decreased ability to resist fracture. Flexural strength is thus measured by the greatest load that a sample can withstand before fracture. Flexural modulus parameter defines the flexibility of a material and the higher the value, the greater the stiffness; while a lower value indicates greater flexibility. Thus flexural strength of a material is calculated by the greatest load a sample can withstand (Plotino *et al.* 2007; Xu *et al.* 1998).

2.2.5 Hardness of dentine

Hardness of dentine is defined in units of pressure or the force per unit area of indentation with a stylus. The resistance of dentine to distortion produced by penetration of an indenting stylus measures the hardness of dentine. The Vickers and Knoop hardness tests are examples of this

(Kinney *et al*. 2003; Marshall Jnr *et al.* 1997). Dentine hardness depends on both the mineral concentration as well as the location within the tooth. Dentine hardness gradually decreases closer to the pulp. A study by Kinney *et al.* (1996) showed that the decreased hardness of dentine near the pulp may be due to the decrease in hardness of the intertubular matrix. The intertubular dentine matrix close to the pulp is possibly less mineralized (Kinney *et al.* 1996; Kinney *et al.* 2003; Marshall Jnr *et al.* 1997).

2.2.6 Fracture properties of dentine

Fracture strength is very important in the mechanical performance of dentine. During mastication, cyclic loading of teeth occur (Arola and Reprogel, 2006). The fracture toughness of dentine is greater when a crack within dentine is directed parallel to the dentinal tubules (Rasmussen and Patchin, 1984; Rasmussen *et al*. 1975). In this direction the collagen fibrils are arranged perpendicularly to the direction of the crack expansion, and they may be responsible for dispersing fracture energy. Fracture strength is also age dependant, as there is a significant decrease in fracture strength noted as patients' age (Arola and Reprogel, 2006; El Mowafy and Watts, 1986).

2.2.7 Fatigue properties of dentine

The response of tooth structure to cyclic loading as during mastication demonstrates the fatigue behaviour of tooth structure. Normal masticatory forces as well as temperature changes in the oral cavity give rise to cyclic loading of teeth. Fatigue is not a factor in tooth failure under normal masticatory loading. In instances where no inherent flaws are present in tooth structure, it seems that no fatigue failure in human teeth will occur (Arola and Reprogel, 2006). If there is inherent pre-existing flaws in dentine (flaws can be *intrinsic*: change in tooth mineralization or *extrinsic*: wear, damage, cavity preparation); tensile failure can occur (Rasmussen *et al.* 1975). The age of a patient can have an effect on fatigue strength, as fatigue strength decreases significantly with increased age. Cyclic forces of mastication do not necessarily result in failure within dentine; unless there is pre-existing flaws in dentine and failure will occur with small cyclic loading (Arola and Reprogel, 2006; Imbeni *et al.* 2003).

2.2.8 Stress-strain response in dentine

Dentine is calcified tissue with a collagen-rich organic matrix which is strengthened by calcium phosphate mineral particles. Mineralization and the value of elastic modulus of dentine are determined by the functional stress pattern and strain distribution (Kishen *et al.* 2004). The higher mineralization in the outer dentine results in a larger elastic modulus, compared to less mineralization and lower modulus of elasticity in the inner core dentine. In structural dentine, greater strains are found on the innermost dentine, and increased stresses are found on the surface. This indicates that energy exposed in the structure, will dissipate through the inner dentine with decreased localised stress at the outer surface. Localised stresses at the external surface have the potential to develop into fractures at that surface. The stress-strain reaction of dentine is responsible for the interchangeable dentine response to improve its fracture resistance (Kishen *et al.* 2004). The inner dentine matrix (especially close to the root canal) is less mineralised, and an increased density of dentinal tubules is evident in this area. Dentinal tubules in this area are filled with odontoblast processes, dentinal fluid, water and pulpal nerves (Mjör *et al.* 2001). Collagen is a durable material that stores energy while the water in the structure increases plastic deformation and fracture energy in dentine. This biological consequence implies that the protection of inner dentine in a clinical situation is of paramount importance, as the inner core of dentine is important to provide toughness and fracture resistance to tooth structure. This is an especially important factor to bear in mind during clinical procedures where inner dentine is removed (Giannini *et al*. 2004; Kishen *et al.* 2004; Mjör *et al.* 2001).

3. ROOT CANAL DEBRIDEMENT

3.1 Principles of Root Canal Debridement

The aim of root canal debridement is to eliminate the microbial population from the root canal system, to eradicate any necrotic pulpal tissue that may cause proliferation of bacteria and to prevent the extrusion of debris through the apical foramen (Young *et al.* 2007).

3.2 Clinical and Biological Objective of Debridement

The clinical objective of root canal debridement is the removal of root canal contents (necrotic pulp, microorganisms and debris) and elimination of infection (Cohen and Hargreaves, 2011).

Adequate root canal debridement combined with efficient anti-bacterial irrigation is the foundation of successful endodontic treatment. The mechanical preparation of the root canal system contributes to the shaping and centring of canals (Yoldas *et al.* 2012). Shaping of canals allow for introduction of antibacterial irrigants into the root canal to complete the asepsis of the root canal system, to enable medicament placement and to provide optimal shape of the canal. The canal shape after preparation and the efficacy of antibacterial irrigants is closely linked, through the procedure of infected dentine removal and irrigant access into root canal (Young *et al.* 2007). During shaping and cleaning, the original shape of the root canal and position of the apex should be maintained (Cohen and Hargreaves, 2011). Debridement is followed by placement of an effective root canal filling material during obturation and a well sealing, functional coronal restoration to prevent further entry of bacteria. This also aids in entombing remaining bacteria so that proliferation of these micro-organisms cannot take place (Young *et al.* 2007). However, debridement on its own will not cause significant clinical reduction in bacterial growth and therefore antibacterial irrigation is an important co-factor to mechanical preparation in effectively debriding the root canal systems (De-Deus and Garcia-Filho, 2009; Young *et al.* 2007).

3.3 Mechanical Objectives of Root Canal Debridement

The following mechanical factors are highlighted as criteria for effective mechanical preparation:

i. Continuous tapered, funnel shape of the root canal from the access cavity to apical foramen

ii. The preparation of the root canal system should maintain the path of the original canal

iii. The apical foramen should remain in its original position

iv. The apical opening should be kept as small as possible

v. Creating smooth canal walls

vi. Preservation of radicular root dentine to prevent weakening of the root structure (Cohen and Hargreaves, 2011; Torabinejad and Walton, 2009; Young *et al.* 2007).

3.4 Factors influencing the success of root canal debridement

- Precise working length determination of root canal system
- Acceptable apical enlargement
- Adequate shaping and taper of canal

- Optimal root canal disinfection
- Retaining the integrity of radicular structures (Cohen and Hargreaves, 2011; Fornari *et al.* 2010; Usman, Baumgartner and Marshall, 2004).

Although the outcome of canal debridement is greatly influenced by the nickel-titanium rotary system used (with regards to design features and alloy of the nickel-titanium instruments); the tactile skills of the clinician also influences canal debridement quality (Fornari *et al.* 2010).

3.5 Glide path during debridement

Glide path is defined as a smooth radicular tunnel from root orifice to the physiological terminus of the canal (West, 2010) This ensures that there is a free passageway for rotary NiTi instrumentation (Saber and Schäfer, 2015). By creating an effective re-producable glide path, the success of root canal preparation is greatly increased. By maintaining a glide path, a smooth direct passage is created towards the apical area of the canal; and also decreases any coronal resistance of the nickel-titanium files. This constant smooth path makes it easier to introduce successive files in the root canal (Cassim and Van der Vyver, 2013; Elnaghy and Elsaka, 2014). Coronal enlargement and the creation of an effective glide path are necessary for the safe use of nickel-titanium instruments (Arias, Singh and Peters, 2015.; Berruti *et al.* 2012a), by preventing taper locks, fracture of instruments and shaping aberrations (D'Amario *et al.* 2013; Topçuolglu *et al*. 2015). The absence of glide path establishment and glide path enlargement is often noted as the cause of ledge formation, transportation, blockage of root canals which results in canal obturation short of the apical constricture (Van der Vyver, 2011).

3.6 Canal Anatomy and Complexities during Debridement

Although debridement is fairly straightforward in a root canal with straight canals, physical constraints may be experienced during the preparation of curved, oval and flattened canals (Barbizam *et al.* 2002; Siqueira, Alves, Almeida, de Oliveira and Rôças 2010; Topçuolglu *et al.* 2015). Nickel-titanium rotary instrumentation offers a positive response to these various anatomical canal complexities. The distinctive properties of the nickel-titanium alloy; like shape memory, superelastic nature, and resistance to torsional fracture, greatly improve root canal preparation especially in the cases of anatomical complexities (Hartmann *et al.* 2007). Research has indicated that nickel-titanium rotary instrumentation cause less canal transportation and created a centred and tapered preparation (De-Deus and Garcia-Filho, 2009). Canal preparation greatly influences the outcome of canal obturation (Peters, 2004). Although great advancement has been made in debridement with nickel-titanium instrumentation, canal preparation may still be influenced by the inconstant nature of root canal anatomy (Cohen and Hargreaves, 2011; Fornari *et al.* 2010).

3.6.1Apical preparation during debridement

The apical constriction is an important anatomical landmark in terms of success of canal preparation. Instrumentation through the apical constriction may delay healing or have a negative effect on the outcome of root canal treatment. With the extrusion of obturating materials beyond the apical constriction, a negative prognosis will be acquired. Thorough debridement of the apical area is an important aspect of canal preparation and is a critical step in the instrumentation procedure (Baugh and Wallace, 2005). Various studies have differing opinions related to the ideal apical preparation size. In the apical area, root canal shapes usually tend to have a round cross-section. Oval canals tend to change in diameter in the apical area. Nickel-titanium rotary instruments are able to produce a cleaner canal compared to hand instruments due to the ability of nickel-titanium rotary instruments to remain centred and make contact with most surfaces of the root canal wall (Fornari *et al.* 2010). Most researchers advocate the termination of the debridement and obturation at the apical constriction (at the cemento-dentinal junction), but this is not always possible due to the variation in position of the cemento-dentinal junction in human teeth. The accurate determination of the cemento-dentinal junction is also difficult to determine from radiographs. Terminating the working length short of the apical constriction may lead to the accumulation of debri and apical blockage. The use of an apex locator aids in this clinical impasse (Cohen and Hargreaves, 2011).

3.7 Mechanical debridement and effective canal irrigation

Mechanical preparation is required to allow for the chemical debridement (irrigation) of root canals. Mechanical preparation of root canals should remove bacterial population, debri and predentine (De-Deus and Garcia-Filho, 2009). Studies have shown that mechanical debridement alone decreases the microbial population of canals to a certain extent (Baugh and Wallace, 2005). However, the combination of mechanical debridement and the use of an adequate canal irrigant, results in an even greater decrease of the microbial population (Baugh & Wallace, 2005;

Young *et al.* 2007). The irrigant is responsible for the dissolution of organic pulpal tissue (Gulabivala *et al.* 2005; Topcu *et al.* 2014). As soon as canal debridement is initiated, the microbial population in the coronal area may be eradicated by antibacterial irrigation. However, the apical area and less accessible areas of the root canal may only be disinfected after the canal preparation (shaping) (Cohen and Hargreaves, 2011).

3.7.1 Effective Canal irrigation

The use of an effective antibacterial irrigant is a vital aspect of the chemo-mechanical debridement of root canals. The important objective of antibacterial irrigation is effective bacterial disinfection as well as the promotion of debridement of necrotic pulpal tissue and debri from the root canal. For this reason, the taper of the root canal is a significant feature to promote effective canal irrigation. In a study by De-Deus and Garcia-Filho, (2009) investigating the cleanliness of root canals after debridement, it was found that none of the nickel-titanium rotary systems investigated cleaned the root canal completely even when an adequate taper was obtained. This finding indicated debridement quality is dependent on the irrigation protocol.

3.7.2 Root Canal Taper and Canal Debridement and Irrigation

During mechanical canal preparation, the root canal space that has been prepared is where irrigating solutions are introduced. The efficacy of the irrigating solution is dependent on the dimensions of the prepared canal space, as it determines the irrigant's volume (Arvaniti and Khabbaz, 2011).

4. MICROBIOLOGY OF INFECTED ROOT CANALS

4.1 Infections of the root canal system

Apical periodontitis involves the microbic pathological infection of the pulpal system of an affected tooth. The infected root canal with its necrotic pulp is a reservoir for the microbiota which grows in biofilms, aggregated masses and co-aggregates which are enclosed in an extracellular matrix (Nair *et al.* 2005).

4.2 Etiology of root canal infections

The etiology of primary endodontic infections, apical periodontitis and pulpal necrosis involves bacteria and their toxic by-products (Mohammadi and Abbott, 2009). The eradication of these bacteria and their by-products is a critical step in endodontics. Failure or secondary reinfection occurs when endodontic treatment has not successfully eradicated or controlled the microbial population. Proliferating microorganisms can remain in canals after surviving biomechanical procedures; and can be located in inaccessible or missed canals or in un-instrumented areas of the canal (Pinheiro *et al*. 2003).

4.3 Bacterial access to pulpal tissues

The common access for microorganisms to the pulp is from the normal oral flora via the expansion of an open cavity due to dental caries in the crown of a tooth. This opens up the dentinal tubules to allow bacterial access (Baumgartner and Falkler, 1991). The infected pulp is shielded from the normal immune response of the body and therefore healing of the pulp cannot take place. The root canal becomes a haven for microbial pathogens and their toxic by-products. Access to pulp can also be achieved via accessory canals and apical foramina, in the case of periodontal disease. Dentinal exposure via erosion or enamel microcracks, restorative procedures, anachoresis and pulpal exposure during cavity preparations can all facilitate bacterial access (Gomes *et al.* 2004).

4.4 Microbial population in infected canals

4.4.1 Biological selection

Biological selection will determine the type of microbial species in the root canal. All bacteria within the oral cavity have comparable potential to infiltrate the root canal system, but only a select group of microbial species is identified within the root canal system. The microbial population is influenced by an anaerobic environment, and the interchange between the bacteria and accessibility to nourishment. At the start of root canal infection the number of bacterial species is typically low, and it is perceivable that long standing larger chronic infections usually have more bacterial species and also have a greater mass of bacterial species in the root canals. Oxygen and oxygen products play an important role in the configuration of root canal microflora (Figdor and Sundqvist, 2007).

4.4.2 Bacterial biofilms

Bacterial cells which attach themselves onto a surface via an extracellular matrix are defined as a biofilm. It is dependent on free-floating bacteria in an aqueous solution. By definition biofilm refers to the thin layer of condensed microbes (bacteria, fungi, protozoa) on a surface (Svensäter and Bergenholtz, 2004). Evidence of biofilms is found in infected pulps of both primary and secondary root canal infections. These biofilms within the root canal system needs to be eliminated via both mechanical and chemical disinfection (Mohammadi and Abbott, 2009; Ordinola-Zapata *et al.* 2012). As mentioned previously debridement with instrumentation alone will not remove all infected tissue in the canal system and thus antimicrobial irrigation is of utmost importance. Although biofilm can be found on external root surfaces, the possibility of biofilms on inner root canal walls complicates endodontic treatment. The formation of biofilm in root canal walls may be responsible for endodontic infections that resist treatment (Ordinola-Zapata *et al.* 2012).

4.4.3 Bacterial species in untreated, infected canals

As mentioned previously, during the preliminary phase of infections, the number of species in root canals is low. As the infection progresses the population increases. The initial count of bacterial species in an infected canal may differ from one to greater than twelve in number. Where teeth have been left untreated, the bacterial species will be higher in number and the density of bacteria in a canal will subsequently increase. Untreated infected canals allow microorganisms to access the pulp, to colonise the pulpal tissue and diminish pulpal function (Figdor and Sundqvist, 2007). Root canal infections are polymicrobial. These infections are overly influenced by anaerobic bacteria. Commonly associated microorganisms associated with root canal infections include gram negative anaerobic rods, gram positive anaerobic cocci, gram positive anaerobic and facultative rods, *Lactobacillus* species, and gram positive facultative *streptococcus* species. The bacterial species often isolated from necrotic pulps are *Peptostreptococcus, Prevotella, Porphyromonas, Fusobacterium, Eubacterium, Actinomyces and Streptococcus* (Gomes *et al.* 2004).

4.4.4 Bacterial species in endodontically treated root canals

Microbial growth in endodontically treated root canals can occur due to a wide variety of factors such as; missed canals, inadequate isolation, dislodgement of restoration, etc. In these situations certain bacteria can survive under harsh conditions such as lack of nutrition (Figdor and Sundqvist, 2007). Microorganisms are able to resist biomechanical debridement or regain access to the root canal via coronal leakage, and are able to survive and proliferate in the canal system. A definite difference is noted between the bacterial population in primary and secondary infected canals. The species of *Lactobacilli, Actinomyces, Peptostreptococcos and Enterococci* are positively identified in secondary infected root canals (Gomes *et al.* 2004).

5. ROOT CANAL IRRIGANTS

5.1 Aim of canal irrigation

Successful endodontic treatment is achieved by a combination of factors which include acceptable instrumentation, optimal irrigation and disinfection of the root canal system (Mohammadi, 2008). Due to the complex anatomy of root canal systems, host defences and different levels of virulence in microorganisms, irrigating solutions must have essential tissue dissolving ability as well as antimicrobial action. Although root canals are shaped mechanically either by hand or rotary instrumentation, the elimination of microorganisms is completed by adequate irrigation. As mentioned previously, mechanical debridement on its own will not eliminate the microbial population, and a chemical antimicrobial agent is required to assist with elimination of the microbial population (Estrela *et al.* 2002). With current instrumentation techniques, 40-50% of the root canal may remain untouched and tissue is left behind for microorganisms to survive and flourish. The aim of irrigation is to optimize root canal disinfection (Slutzy-Goldberg, Hanut, Matalon, Baev and Slutzky, 2013).

5.2 Ideal properties of a root canal irrigant

An antibicrobial agent should have the following properties:

- Have a broad antimicrobial spectrum and capacity to eliminate anaerobic and facultative microorganisms

- Inhibit bacterial growth
- Ability to penetrate the infection site
- Have a low toxicity level
- Dissolve necrotic pulp tissue remnants
- Inactivate the endotoxin
- Should have adequate concentration to have an antimicrobial effect
- Microorganisms should not be able to develop resistance to the antimicrobial agent
- Prevent smear layer formation during instrumentation and/or possess the ability for dissolution of the smear layer once it has formed (Estrela *et al.* 2002; Mohammadi, 2008; Moorer and Wesselink, 1982; Zehnder, 2006).

5.3 Sodium hypochlorite as a root canal irrigant

Sodium hypochlorite is a popular choice as an endodontic irrigant because of its solvent activity as well as its antimicrobial action (Estrela *et al.* 2002; Mohammadi, 2008; Moorer and Wesselink, 1982). While anaerobes are easily eliminated, the eradication of facultative bacteria like *streptococci*, *eneterococci and lactobacilli* proves to be more difficult (Zehnder, 2006). Therefore it is vital that a suitable antimicrobial agent be used during irrigation and sodium hypochlorite emerges as the optimal agent to be used as it complies with most of the above criteria, compared to other irrigant solutions (Zehnder, 2006).The tissue dissolving ability of sodium hypochlorite depends on its concentration, volume, contact time of solution with tissue remnants, and surface area of exposed tissue (Slutzy-Goldberg *et al*. 2013).

5.4 The sodium hypochlorite reaction

The dynamic balance of sodium hypochlorite is demonstrated by the following reaction:

$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$

Sodium hypochlorite acts as a solvent by degrading fatty acids, converting the fatty acids into fatty acid salts (soap) and glycerol (alcohol); which causes the reduction of surface tension of the remaining solution (Estrela *et al.* 2002; Mohammadi, 2008; Zehnder, 2006). Sodium hypochlorite neutralizes amino acids by forming water and salt. Due to the exit of the hydroxyl ions, there is a decrease in pH. Hypochlorous acid, which is present in sodium hypochlorite solution, acts as a solvent when interacting with organic tissue. This reaction results in the release of chlorine which combines with the protein amino group to form chloramines. Hypochlorous acid and hypochlorite ions results in degradation of amino acids and hydrolysis. Chloramines are formed due to the reaction between chlorine and the amino group, which then interfere with cell metabolism. Chlorine will lead to antibacterial action by suppressing bacterial enzymes which leads to irreversible oxidation of sulfhydryl groups of essential bacterial enzymes. Sodium hypochlorite (with a pH of more than 11) is found to have detrimental biological effects on bacterial cells. Enzymatic sites are located in the cytoplasmic membrane of bacteria and are essential for functions such as metabolism, cell division and growth. The high pH when hydroxyl ions are released changes the integrity of the cytoplasmic membrane which leads to chemical death. Thus, the key mechanism of sodium hypochlorite depends on the saponification reaction (formation of soap and alcohol); amino acid neutralization and chloramination reactions that occur when microorganisms are present which progresses to the antimicrobial and tissue dissolution effect (Mohammadi, 2008; Estrela *et al.* 2002).

5.5 Efficacy of sodium hypochlorite

The path of the sodium hypochlorite reaction is determined by the amount of the organic matter present and the amount and concentration of sodium hypochlorite used (Moorer and Wesselink, 1982; Zou, Shen and Haapasalo, 2010). The second characteristic of the reaction is that there is an initial fast reaction, which is then followed by a slower second reaction. This means that an excess of organic matter can diminish the irrigant of most of its activity and cause a great drop in pH within minutes (Moorer and Wesselink, 1982). The efficacy of sodium hypochlorite means that it needs to respond rapidly and be in excess of the organic matter. Therefore to ensure the efficacy and maintain a greater ratio of irrigant to organic matter; there should be regular use of fresh irrigant and/or increasing the concentration of the sodium hypochlorite solution (Moorer and Wesselink, 1982).

5.5.1 Bactericidal action of sodium hypochlorite

The penetration of dentine by sodium hypochlorite is confirmed by the bleaching action of sodium hypochlorite on dye-impregnated dentine. Wong and Cheung, (2014) reported that the bactericidal effect of sodium hypochlorite was observed at depths of 300µm. At deeper layers 3% sodium hypochlorite was able to reduce the amounts of viable bacterial cells when compared to 0.5% sodium hypochlorite, thus the bactericidal effect of sodium hypochlorite reached a greater depth in dentinal tubules at a higher concentration.

<u>5.5.2 Factors which influence the efficacy of sodium hypochlorite</u>

As mentioned, the tissue dissolving ability of sodium hypochlorite depends on its concentration, volume, contact time of solution with tissue remnants, and surface area of exposed tissue. Efficacy of sodium hypochlorite for dissolution of tissues can be increased by activation with sonics or ultrasonics, increasing the pH and temperature of the sodium hypochlorite solution and increasing the working time (Slutzy-Goldberg *et al.* 2013).

i) *Altering /adjusting the pH*

When sodium hypochlorite is added to water, hypochlorous acid is formed and dissociates into a hypochlorite ion (OCl^-). At a pH of 10 most of the available chlorine is in the hypochlorite ion form and at a pH of 4.5; most available chlorine is in the hypochlorous form (Kandaswamy and Venkateshbabu, 2010).

ii) *Temperature of sodium hypochlorite*

An increase in the temperature of sodium hypochlorite can increase its effectiveness. A preheated solution of sodium hypochlorite has the following positive effects: short-term stability of sodium hypochlorite, improved tissue dissolving capacity and antimicrobial efficacy (Mohammadi, 2008; Sirtes *et al.* 2005). Preheated sodium hypochlorite solution removed organic debri from dentine shavings more effectively than an unheated solution. The tissue dissolving capability of sodium hypochlorite is increased by increasing the concentration, volume and contact time and by maintaining a temperature of 36°. Studies done by Sirtes *et al.* (2005) found that the ability of tissue dissolution of 1% sodium hypochlorite at 45˚C was equal to that of 5.25% sodium hypochlorite at 20˚C.

iii) *Agitation of sodium hypochlorite*

The efficacy of sodium hypochlorite has been proven by its anti-bacterial activity when it comes into contact with bacterial biofilms, especially in the coronal and middle third of the root canal. The apical third of the root canal has always been challenging with regards to sodium hypochlorite penetration and efficacy (Paragliola *et al.* 2010). The agitation of irrigant solutions is used to increase the efficacy of irrigants. These techniques can involve manual agitation with hand instrumentation, manual agitation with gutta percha points, mechanical agitation with plastic instruments and sonic and ultrasonic agitation (Al-Ali, Sathorn and Parashos, 2012; Paragliola *et al.* 2010). A study conducted by Paragliola *et al.* (2010) found that the use of ultrasonic activators to agitate 5.25% sodium hypochlorite within the canal, especially the apical third increased the efficacy of sodium hypochlorite.

iv) *Concentration and Time*

Although sodium hypochlorite is widely used; no consensus has been reached with regards to the ideal concentration to be used. The ideal concentration should have a low toxicity level and adequate antibacterial action. Canal preparation is done in a short window period, and therefore the antibacterial efficacy of the irrigant will be influenced by the concentration of the solution. Organic matter present in canals that come into contact with sodium hypochlorite will consume the available chlorine and the antibacterial efficacy is reduced. With the use of sodium hypochlorite at a lower concentration, this phenomenon is evident; and with a higher concentration of sodium hypochlorite; a reserve would be created to maintain the antibacterial activity (Siqueira *et al.* 2000). The most effective regimen is found to be a sodium hypochlorite concentration at 5.25% at 40 minutes; especially to remove *Enterococcus Faecalis.* Irrigation with sodium hypochlorite at a lower concentration for an equivalent period of time period is less effective in removing *Enterococcus Faecalis*. Antimicrobial agents require adequate exposure time in the root canal system to yield results (Retamozo *et al.* 2010).

5.5.3 Capacity to dissolve organic matter

Tissue dissolving ability depends on the frequency of agitation, the volume of organic matter in proportion to the amount of irrigant and surface area of remaining tissue. Greater concentrations of sodium hypochlorite provide faster dissolution of tissues. Sodium hypochlorite is a strong proteolytic agent, which demonstrates maximum tissue dissolution (Mohammadi, 2008).

6. THE EFFECT OF SODIUM HYPOCHLORITE ON DENTINE STRUCTURE

6.1 The effect of sodium hypochlorite on the dentine matrix

Irrigation is currently the best method for the removal of necrotic tissue and dentinal debri. During irrigation, radicular and coronal dentine and enamel are exposed to solutions used during irrigation (Ari, Erdemir and Belli, 2004; Karunakaran *et al*. 2012). Antibacterial effect, tissue dissolution ability, cleaning and chelating are important features of irrigation during root canal preparation. Irrigation with sodium hypochlorite during debridement may result in changes to the mineral content of dentine, as it has an effect on the chemical composition, physical and mechanical properties of dentine structure. Irrigants can thus alter surface characteristics of dentine. These changes may influence the integrity and longevity of the tooth (Karunakaran *et al.* 2012; Oliviera *et al.* 2007; Slutzy-Goldberg *et al.* 2004; Ulsoy and Görgül, 2013). The impact of sodium hypochlorite on the dentine matrix is of particular importance when investigating changes in the dentine matrix. Due to the alterations in dentine structure and mechanical properties of dentine; the effect of sodium hypochlorite can affect the interactions of these surfaces with obturation materials and as well as coronal restorations (Ari *et al*. 2004; Oliveira *et al.* 2007; Pascon *et al.* 2009; Ulusoy and Görgül, 2013; Zaparolli *et al.* 2012). Irrigation solutions allow for ease of root canal instrumentation and preparation by lubrication of the root dentine walls. The ability of sodium hypochlorite to alter the chemical structure of dentine and thereby effecting change in the microhardness of the dentine structure; facilitates the ease of canal preparations (Tartari *et al.* 2013).

6.2 Flexural strength of dentine

The ability of a material to resist deformation under load is defined as flexural strength. A decrease in flexural strength would indicate that a decreased force is needed for the cohesive bonds within dentine to fragment. Irrigation with sodium hypochlorite has an effect on mechanical effects of dentine including: microhardness, flexural strength, and elasticity (Pascon *et al*. 2009).

6.2.1 Effect of sodium hypochlorite on flexural strength

Exposure of dentine to sodium hypochlorite results in decreased flexural strength and modulus of elasticity of dentine. The overzealous use of sodium hypochlorite may increase the risk of fracture in endodontically treated teeth. Marending *et al.* (2007a) found that the dentine surface structure was degraded after sodium hypochlorite exposure and this could contribute to the decrease of flexural strength. The researchers in this particular study exposed dentine to sodium hypochlorite (concentration of 3% and higher) for one hour, after which changes in flexural strength was noted.

6.3 Microhardness of dentine

Determining the hardness of a material is a non-destructive mechanical simulation method. Hardness of a material is measured as the resistance of the material to penetration of an indenter, which is harder than the material being studied (Kalluru *et al.* 2014). The mineral content as well as the hydroxyapatite concentration in the intertubular spaces determines the hardness profile of dentine. A positive relationship is present between dentine hardness and mineral content of dentine. Evaluating the microhardness of dentine can impart information pertaining to mineral loss or gain in dentine. Microhardness values may vary with regard to location; with the value decreasing as the indenter moves closer to the pulp. This can be due to the presence of open dentinal tubules closer to the pulp, and these tubules would have less resistance to the indenter (Aslantas *et al.* 2014; Ari *et al.* 2004; Oliveira *et al.* 2007). Dentine microhardness (which is determined by the amount of calcified matrix per mm^2) is inversely correlated to tubular density. Determination of microhardness only provides indirect evidence of mineral loss or gain in dental tissues. No consensus/agreement is available in the current literature on the ideal amount of dentine microhardness reduction to facilitate mechanical instrumentation and at the same time avoid excessive mineral loss which could lead to weakening of dentine structure (Ulusoy and Görgül, 2013).

6.3.1 Effect of sodium hypochlorite on microhardness of dentine

The reduction of microhardness is caused by a reduction of stiffness of the intertubular dentine matrix. This is caused by varied distribution of the mineral phase within the collagen matrix (Slutzky-Goldberg *et al.* 2004). A study conducted by Slutzky-Goldberg *et al.* (2004) found that the dentine microhardness evaluated next to the root canal lumen was higher (dentinal tubuli are dense compared to the peripheral area where the tubuli are less crowded). The degree of mineralisation and the hydroxyapatite content in the intertubular substance affects the intrinsic dentine hardness (Ari *et al.* 2004). In the study by Ari *et al*. (2004) a decrease in dentine microhardness was observed as well as an increase in surface roughness of the root canal dentine. This was observed with concentrations of sodium hypochlorite of 2.5% to 5.25%. Oliveira *et al.* (2007) concluded that 1% of sodium hypochlorite decreased dentine microhardness. A Vickers hardness test was used and researchers found that lower Vickers hardness values were obtained at 500μm from pulp. Dentine microhardness is location related, and the value of dentine microhardness decreased as the indentations were closer to the pulp. This can be attributed to the open dentinal tubules (free of peritubular dentine), which are closer to the pulp (Oliviera *et al.* 2007). Tartari *et al*. (2013) also confirmed that sodium hypochlorite significantly decreased dentine microhardness. It was found that although the different areas of the root (cervical, middle and apical) are structurally different, all the root thirds displayed the same results with regards to decrease in dentine microhardness (Tartari *et al.* 2013).

6.3.2 Concentration of sodium hypochlorite and microhardness

The greater efficacy of sodium hypochlorite at greater concentrations has influenced clinicians to use higher concentrations of sodium hypochlorite during root canal preparation. However this may have a deleterious effect on dentine properties (Sim *et al*. 2001). The concentration of sodium hypochlorite has an effect on microhardness as demonstrated by Slutzy-Goldberg *et al.* (2004), where both concentrations of 2.5% and 6% sodium hypochlorite rendered a decrease in microhardness; but 6% sodium hypochlorite rendered a greater decrease in microhardness than 2.5%. Weight loss of dentine after immersion in sodium hypochlorite was greater at a higher concentration of sodium hypochlorite (Oliveira *et al.* 2007).

6.3.3 Contact time of sodium hypochlorite and dentine microhardness

The decalcifying effect of sodium hypochlorite is influenced by the irrigation period, and will therefore have an effect on dentin microhardness (Butt and Talwar, 2013; Zaparolli *et al.* 2012). Slutzky-Goldberg *et al.* (2004) demonstrated that during contact with sodium hypochlorite a reduction in dentine microhardness was found in the first 10 minutes. After 20 minutes contact time there was no statistical significance. The initial decrease may be due to the initial removal of the organic matrix from the dentine during the first 10 minutes.

6.4 Effect of sodium hypochlorite on mineral composition of dentine

Dentine is comprised of approximately 22% organic material which is made up mostly of type I collagen, and this constituent influences the mechanical properties of dentine. Sodium hypochlorite is a non-specific oxidising agent and deconstructs long peptide chains and chlorinates protein terminal groups; which leads to the breakdown of N-chloramines into other species. This leads to adverse consequences for dentine structure (Marending *et al.* 2007b). Sodium hypochlorite dissolves both collagen components of dentine and magnesium and phosphate ions, and increases dentinal carbonate (Aslantas *et al.* 2014; Pascon *et al.* 2009). Pascon *et al.* (2009) found that sodium hypochlorite solutions with concentrations varying from 1% to 6% caused a reduction in dentine microhardness. Sodium hypochlorite can also alter the calcium to phosphate ratio of root dentine. The authors concluded that these changes in the mineral content can affect the hardness profile of dentine. In a study by Aslantas *et al*. 2014, the exposure of dentine with 6% sodium hypochlorite for a period of 5 minutes decreased the dentine microhardness. The decalcifying effect of sodium hypochlorite largely depends on application time, the pH and concentration of the solution as well as the hardness of dentine (Butt and Talwar, 2013). In this particular study by Butt and Talwar (2013) sodium hypochlorite demonstrated maximum reduction in microhardness compared to other acids like carbonic citric and tartaric acid. 5.25% Sodium hypochlorite caused the maximum reduction of microhardness, which could be attributed to the degradation of the organic dentin components.

6.5 Effect of sodium hypochlorite on the modulus of elasticity of dentine

Sodium hypochlorite has the ability to deproteinize and disintegrate the organic dentine matrix. Disintegration of the organic dentine matrix, results in a reduction in the elastic modulus and flexural strength of dentine. Sodium hypochlorite also increases the permeability of the altered intertubular dentine with a 5% sodium hypochlorite concentration altering the peripheral dentine matrix (Marending *et al.* 2007b). The modulus of elasticity of dentine after irrigation with sodium hypochlorite can be determined using ultrasonic wave propagation measurements. A study by John, Löst, and Elayouti (2013) determined (through ultrasonic investigation and the use of the 3-point bending test) that changes to the modulus of elasticity in dentine exposed to sodium hypochlorite could be measured. The results demonstrated a reduction of the modulus of elasticity by 3%. As dentine is anistropic and varies in thickness, a reduction of 3% can have an effect on dentine. At regions of stress concentrations this reduction in elasticity can lead to the propagation of microcracks. Grigoratos *et al.* (2001) found the fracture loads were much less with significant deformation of the dentine bars before complete fracture. Although a range of disparity in the behaviour of dentine bars were observed, there was enough significant statistical difference to indicate that both 3% and 5% sodium hypochlorite caused a decrease in the modulus of elasticity and flexural strength (Grigoratos *et al.* 2001).

6.6 Effect of sodium hypochlorite on tooth surface strain

Tooth surface strain is measured at the cervical margin of a tooth by using electrical strain gauges during cyclic loading. A study by Sobhani *et al.* (2010) found that 5% sodium hypochlorite demonstrated an increase in strain value under cyclic loading. The actions of sodium hypochlorite may produce surface flaws in the dentine, and subsequent cyclic loading during function may allow crack propagation through fatigue. Tooth tissue loss (demineralisation) causes a reduction of the force required by the tooth to strain and this may lead to crack development and fractures. Sim *et al.* (2001) also found that dentine exposed to 5.25% sodium hypochlorite had a significant decrease in flexural strength, rigidity and decrease in elastic modulus. This could be attributed to the decrease of the organic matrix within dentine. Also noted in the same study by Sim and co-workers, there was an increase in tooth strain, and as a result changes in stiffness of the tooth may predispose the tooth to fracture. Increase in tooth strain after sodium hypochlorite irrigation was 15.9% tensile strain and 3.5% compressive strain (Sim *et al.* 2001).

6.7 Dentine permeability

The effect of irrigating solutions may be affected by the permeability of dentine, which may favour or decrease their effect. The floor of the pulp chamber consists of primary and secondary dentine which allows for more uniform penetration of ions. Alternatively, reparative dentine is more amorphous in structure, less tubular and the route of fluids may be obstructed. Thus the dentine permeability will have an effect on sodium hypochlorite penetration (Zaparolli *et al.* 2012).

6.8 Dentine penetration

Knowledge of the depth of sodium hypochlorite penetration into dentine and factors which may influence the depth of penetration can be beneficial when practicing one appointment endodontic treatment. A study conducted by Zou *et al.* (2010) outlined the penetration of sodium hypochlorite into dentine by measurement in micrometres. The depth of sodium hypochlorite penetration into the dentinal tubules was recorded as between 77 and 300µm. Factors such as sodium hypochlorite concentration, exposure time and temperature have an effect on sodium hypochlorite efficacy, and it stands to reason that these variables can impact sodium hypochlorite penetration (Zou *et al.* 2010).

7. ETHYLENEDIAMINETETRAACETIC ACID (EDTA) USE IN ENDODONTICS AND THE EFFECT ON DENTINE

7.1 EDTA and debridement

During canal debridement (with hand or rotary techniques) a smear layer and calcium plugs are formed on the canal wall which needs to be removed (Kalluru *et al.* 2014; Poggio *et al.* 2012). As mentioned previously, the success of root canal treatment is dependent on removing the contents of the root canal, cleaning and shaping as well as the removal of the smear layer and the calcium plugs. EDTA is used in endodontics as a chelator, for smear layer removal and increased dentine permeability (Kalluru *et al.* 2014). EDTA is used during rotary preparation, to assist instrumentation procedures and decrease the incidence of file separation (El-Sayed *et al*. 2015).

7.2 Smear layer formation on root canal dentine

The smear layer is described as a thin amorphous layer that forms on the root canal walls; and has both an organic and inorganic component. It contains dentine particles, necrotic debri, odontoblastic processes and may occlude the dentinal tubule openings (Kaya, Yiğit-Özer and Diyarbakir, 2011; Marending *et al*. 2007b; Taneja, Kumari and Anand, 2014). The smear layer has 2 regions: the first is made up of organic matter and dentinal debris and is usually 1-2µm thick; and the second region extends into the dentinal tubules up to a depth of 40µm (smear plugs) (Teixeira, Felippe and Felippe, 2005).

7.3 Removal of the smear layer

Complete removal of the smear layer during canal debridement is encouraged, as this action will enable improved adhesion of root canal dentine and sealants and consequently decrease apical and coronal microleakage (Aranda-Garcia *et al*. 2013; Eldeniz, Erdemir and Belli, 2005; Pérez-Heredia *et al.* 2008). The smear layer removal encourages the diffusion of intra canal medicaments and irrigants into the root canal dentine (Dineshkumar *et al.* 2012). The potential of bacterial survival and proliferation is also greatly reduced by the removal of the smear layer, as attached microorganisms and their toxins will be dissolved. Failure to remove the smear layer will result in incomplete disinfection of the root canal system (Marending *et al.* 2007b; Taneja *et al.* 2014).

The removal of the smear layer requires an irrigant that can effectively remove the organic component as well as the inorganic component (Çalt and Serper, 2002; Kalluru *et al.* 2014). Sodium hypochlorite is used in the removal of the organic component of the smear layer; and EDTA is used to remove the inorganic component (Aranda-Garcia *et al*. 2013). Sodium hypochlorite is an excellent antibacterial and proteolytic agent and dissolves organic tissue so that the combination of a chelating agent (EDTA) and sodium hypochlorite removes the smear layer more effectively (De-Deus, Paciornik and Mauricio, 2006). Thus, the synergistic effect between sodium hypochlorite and EDTA is required for the removal of the smear layer (Akcay, Erdliek and Sen, 2013).

7.4 Mechanism of action of EDTA as a chelator

Chelation is a physico-chemical process that results in the uptake of positive ions, reactions with the Ca^{2+} ions in the hydroxyl apatite crystal of dentine This results in a change in the microstructure of dentine as well as change in the calcium: phosphorus (Ca:P) ratio of the dentine surface. When there is a change in the Ca:P ratio, the permeability and solubility of dentine may change (Kalluru *et al.* 2014). By changing the Ca:P ratio, EDTA may change the original ratio between organic and inorganic components. This may also contribute to a change in the permeability and solubility (Eldeniz *et al.* 2005).

7.5 Efficiency of EDTA as a chelator

The decalcifying action of the chelating agents depends on:

- Length of the root canal
- Penetration depth of material
- Dentine hardness/diffusion in dentine
- Time period of application
- pH of solution
- Concentration of solution (Kaya *et al*. 2011; Pérez-Heredia *et al.* 2008; Serper and Çalt, 2002; Tartari *et al.* 2013).

7.5.1 The effect of root canal length and anatomy on smear layer removal

The removal of the smear layer is more effective in the coronal and middle third of the root canal. The apical third of root canals have a smaller diameter and this may compromise smear layer removal as less volume of irrigants are able to access the apical third of the root canal (Dineshkumar *et al.* 2012). In a study by Texeira *et al.* (2005), it was found that the smear layer removal was effective in the coronal and middle third of the canal (after 5 minutes with EDTA and sodium hypochlorite); whereas the smear layer removal of the apical third of the canal was not completely successful.

7.5.2 The concentration and pH of EDTA

A neutral pH of EDTA solution is recommended (Pérez-Heredia *et al.* 2008). With a neutral pH, EDTA is more effective than when the pH was increased. The pH of the EDTA solution influences its effect on the demineralisation of dentine. Effective demineralisation can be achieved with EDTA at a pH between 5.0 and 6.0. The pH of EDTA affects Ca^{2+} availability (Serper and Çalt, 2002). The concentration of EDTA is preferred at 17% (Aranda-Garcia *et al.* 2013; Cohen and Hargreaves, 2011). In the above study by Serper and Çalt, (2002) the amount of phosphorus free from dentine was increased with increased EDTA concentration and exposure time.

7.5.3 Time exposure

Poggio *et al.* (2012) established that the percentage of Ca^{2+} ions that were extracted by 17% EDTA after 10 minutes was not significantly different to the percentage of Ca^{2+} ions extracted after a 15 minute exposure. Essentially 10 minutes of EDTA application was sufficient time exposure to cause maximum release of Ca^{2+} ions. This can be attributed to the organic matrix of dentine limiting the dissolving action on the inorganic component (after time); thereby reducing the decalcifying action of EDTA over an extended time period. Serper and Çalt, (2002) found that phosphorus release was rapid after the first minute of EDTA application and further application resulted in slower phosphorus release. This could be due to the rapid action of EDTA in removing the smear layer initially and thereafter the chelating agents start to affect the dentine structures and cause a slower release of phosphorus. Another study by Pérez-Heredia *et al.* (2008) found that an increased percentage of Ca^{2+} ions were released during the first 5 minutes of dentine immersion in 15% EDTA. After the initial 5 minutes, the decalcification is reduced and significant differences in percentage of Ca^{2+} ions, were found between 5 minutes, 10 minutes and 15 minutes immersion. The organic matrix of dentine may limit the further dissolution of the inorganic components with time; thus causing a reduction in the decalcifying action of chelators. 15% EDTA produced dentine decalcification especially during the initial 5 minutes of action. The decalcifying process was found to be self-limiting.

7.5.4 Dentine diffusion

Chelating solutions are effective only through direct contact with the dentine surface. For this reason wetting agents/surfactants are added to the chelator to increase its ability to penetrate dentine (Aranda-Garcia *et al*. 2013).

7.6 Effect of EDTA on dentine

As mentioned previously, the hardness of a material is measured as the resistance to penetration of an indenter that is harder than the sample material to be investigated (Kalluru *et al.* 2014). The hardness profile of dentine structure is determined by the degree of mineral content and the amount of hydroxyapatite in the intertubular substance. A positive association exists between hardness and mineral content of teeth. The measure of hardness can indirectly indicate degrees of mineral loss or gain (Aslantas *et al.* 2014). The chelating action of EDTA results in the softening of calcified components of dentine and ensuing decrease in microhardness of dentine (Eldeniz *et al.* 2005). The altered irrigation regimen of EDTA and sodium hypochlorite affects the hardness of dentine (Taneja *et al*. 2014). The demineralizing effect of EDTA facilitates dentinal tubules enlargement, softening of dentine and denaturation of collagen fibres (Çalt and Serper, 2002). The effect of decreasing the microhardness of the superficial dentine layer in the root canal lumen by chelating agents facilitates the ease of preparation during endodontic instrument use (Poggio *et al.* 2012).

7.6.1 Dentine microhardness

Chelating agents may have an effect on the microstructure of dentine by changing the ratio of calcium and phosphorus. The hardness and mineral content of dentine structure is connected and any change in the mineral content ratio may have an effect on the mechanical and physical properties of dentine, i.e. microhardness, permeability and solubility (Tuncer, Tuncer and Siso, 2015). The use of EDTA during debridement may reduce the microhardness of radicular dentine, which may contribute to dentine defect formation (El-Sayed *et al.* 2015). This change in dentine microhardness can have an impact on the obturation quality of endodontic treatment, as the bond between the root canal dentine and canal sealers may be affected (Tartari *et al.* 2013). Studies by Aranda-Garcia *et al.* (2013) and Dineshkumar *et al.* (2012) found that 17% EDTA resulted in decrease in dentine microhardness. A study by Taneja *et al.* (2014) showed a decrease in dentine microhardness after exposure of dentine to 17% EDTA and 5% sodium hypochlorite, whereas studies by Tuncer *et al* (2015) and Tartari *et al.* (2013) found that 17% EDTA and 2.5% sodium hypochlorite, (at varying time and sequence) caused a decrease in dentine microhardness.

7.6.2 Dentine roughness

A change in the surface roughness of dentine was observed in a study conducted by Ari *et al.* (2004). This revealed that dentine surface roughness was increased with the use of 17% EDTA as well as causing a reduction in dentine microhardness. In a study by Eldeniz *et al.* (2005) it was also reported that a regimen of EDTA and sodium hypochlorite (17% EDTA and 5.25% sodium hypochlorite) caused a reduction in dentine microhardness as well as increasing the roughness of dentine.

7.6.3 Flexural strength and modulus of elasticity

A study by Marending *et al.* (2007b) found that the combination of sodium hypochlorite and EDTA did not reduce flexural strength and modulus of elasticity. This may be due to exposure time (which was at 3minutes). The remaining EDTA on dentine could have caused a reduction in sodium hypochlorite volume and thereby preventing its proteolytic effect.

7.6.4 Dentine erosion

The combination of 17%EDTA and 5% sodium hypochlorite may be responsible for the erosion/dissolution of peritubular and intertubular dentine, as the alternating action of sodium hypochlorite and EDTA dissolves both the inorganic and organic components. As the peritubular dentine has low collagen content, erosion by EDTA and sodium hypochlorite is easier (Darda *et al.* 2014). A study by Çalt and Serper, (2002) reported that the smear layer was removed with EDTA (after one minute application of EDTA) followed by sodium hypochlorite application. When dentine was exposed to EDTA for 10 minutes, the peritubular and intertubular dentine exhibited signs of erosion. Kaya *et al*. (2011) found that radicular dentine can be demineralised by EDTA and with prolonged use root canal wall erosion can occur. Similar findings were observed by Tuncer *et al.* (2015) where the combination of 17% EDTA and 2.5% sodium hypochlorite showed both peritubular and intertubular erosion. The erosion of dentine can be due to the hyper-decalcifying effect of EDTA (Tuncer *et al.* 2015). Aranda-Garcia *et al.* (2013)

established that 17% EDTA also showed the ability to cause dentinal erosion; and caused excessive peritubular and intertubular erosion when used for a prolonged time period.

7.6.5 Stresses in Dentine

A study by Belli *et al.* (2014) found that the use of 17% EDTA on root canal dentine during preparation resulted in increased stresses within the root structure. Thus, EDTA increased the risk of fracture in roots, especially in flared canals as a result of the increased stresses within root dentine.

7.7 Effect of surfactant in EDTA and the effect on dentine

The addition of a surfactant or wetting agent to EDTA solution may be encouraged to reduce surface tension, enable increased penetration of the solution into dentinal tubules and lateral canals and finally increase the clinical performance of the chelator. The combination of EDTA with a wetting agent (surfactant) may increase its bacteriocidal properties (De-Deus *et al.* 2006). In a study by Aslantas *et al.* (2014) it was reported that EDTA (with and without added surfactant) caused a significant decrease in dentine microhardness. A study by De-Deus *et al.* (2006) also found that EDTA caused a significant reduction in dentine microhardness. It was also observed in the same study that the addition of a wetting agent (surfactant) did not improve the effectiveness of Ca^{2+} removal (De-Deus *et al.* 2006).

7.8 Irrigation Regimen with EDTA and sodium hypochlorite

Researchers differ with regards to an optimal irrigation regimen (with regards to sodium hypochlorite and EDTA), i.e. ideal sequence, volume and concentration. Clinicians use sodium hypochlorite in endodontics to keep the canal flooded with irrigant during debridement. The chelator (e.g. EDTA) can be used as final rinse to facilitate smear layer removal. An alternating regimen with EDTA and sodium hypochlorite is favoured, with sodium hypochlorite as a final rinse (Marending *et al.* 2007b). Studies have suggested that when EDTA is used as the final solution in root canals, the collagen matrix remains on the surface of the root canal. As a result bacteria may adhere to the collagen matrix and promote canal reinfection. Therefore the use of sodium hypochlorite as a final rinse is encouraged; in order to restore surface characteristics of dentine by removing the exposed collagen matrix (deproteination). Sodium hypchlorite use after the chelating action of EDTA dissolves proteins and prepares the dentine surfaces for the adhesion materials (sealers) used during obturation (Tartari *et al.* 2013).

8. NICKEL-TITANIUM ROTARY INSTRUMENTATION

With the advent of the first nickel-titanium rotary instruments endodontics in dental practices has been revolutionized; with a shift from hand instrumentation to rotary instrumentation (Young *et al.* 2007). The properties of nickel-titanium alloy allow the nickel-titanium rotary instruments to have shape memory, be highly flexible and super-elastic and have resistance to torsional separation; which allows for the safe preparation of root canal systems as compared to stainless steel instruments (Larsen, Watanabe, Glickman, 2009; Young *et al.* 2007).

8.1 Metallurgy of Nickel-titanium alloy

The nickel-titanium alloy used in the manufacture of endodontic instruments consists of 56% nickel and 44% titanium. The super elasticity of the alloy is related to a stress induced phase conversion in the crystalline structure of the material (Young *et al.* 2007). The alloy exists in two crystal formations (austenite and martensite); and the conversion from one crystal structure to the other allows the alloy to have super elastic and flexible properties as well as retaining shape memory (Necchi *et al.* 2008; Roa, 2009). With stress, the austensitic phase converts into the martensitic phase, which requires light force. As soon as the stress is released, the martensitic phase returns to the austensistic phase and the file returns to its original form (Vaudt, Bitter and Kielbassa, 2007; Young *et al.* 2007). Nickel-titanium instruments allow for up to 8 percent strain to be fully recoverable, compared to a peak of less than 1 percent with stainless steel instruments (Young *et al.* 2007). Nickel-titanium instruments have two times higher elastic flexibility in bending and torsion, including resistance to corrosion; when compared to stainless steel instruments (Vaudt *et al*. 2007).

8.2 Nickel-Titanium instrument design and features

Nickel-titanium rotary systems are available with various functional features. These features include taper, size, cutting flute depth, cross-sectional shape, helical angle and radial lands (Di Fiore, 2007; Kim *et al.* 2008). Instruments that are greater in size are highly susceptible to fatigue failure. As the cross-section of the instrument increases, it becomes less resistant to cyclic fatigue. With an increase in size and taper of the instrument, torque created during rotation increases and fracture time decreases (Di Fiore, 2007). Nickel-titanium instruments with progressively larger tapers, display a greater percentage of fractures. Nickel-titanium instruments with progressive tapers display fractures, while instruments with consistent taper display unwinding deformation (Shen *et al*. 2011). Nickel-titanium instruments with deep cutting flutes have a changing cross-section along the instrument shaft, and this may result in higher torque levels which can increase incidence of metal fatigue and fracture (Di Fiore, 2007).

8.3 Rotation of Nickel-Titanium instruments

Due to the superelasticity of the nickel-titanium alloy, rotary instruments can be used in a continuous rotation in root canals to produce a centred, tapered root canal shape (Kim *et al.* 2008). The rotational speed at which nickel-titanium instruments are used is variable. Instruments should be utilised at a rate that keeps the incidence of fracture to a minimum during canal preparation (Lopes *et al*. 2009).

8.4 Bending properties of Nickel-Titanium instruments

The success of canal debridement using nickel-titanium rotary instruments is influenced by the ability of the instrument to resist bending. Increased flexibility in instruments will result with less negative changes in the shape of canals, compared to instruments that have a greater ability to resist bending stresses (Schäfer, Dzepina and Danesh, 2003).The increased features of rotary instruments are achieved by the use of nickel-titanium alloys and different design features (Kim *et al.* 2008). Resistance to bending of instruments is determined by the metallurgic properties and their geometrical design. Low bending ability of instruments indicates the extreme measure of flexibility of the instruments, which is a desirable property in the clinical scenario. Due to this flexibility, the load on the cutting edges in canals is reduced. This also reduces instrument stress and risk of instrument fracture (Schäfer *et al.* 2003).

8.5 Shaping ability of Nickel-Titanium instruments

Retaining the original shape of the root canal is an important feature of nickel-titanium instruments. Various studies have indicated nickel-titanium instruments have a greater ability to preserve original root curvatures when compared to stainless steel instruments (Young *et al.* 2007; Vaudt *et al*. 2007).

8.6 Centring ability of Nickel-Titanium instruments

Centring ability of an instrument refers to the direction of canal transportation during canal preparation. If the root canal is displaced to the outer curvature of the root or the furcation area, the risk of perforation is increased. Nickel-titanium instruments display good centring ability, with little deviation from the main root canal. Thus nickel-titanium instruments have excellent centring ability compared to stainless steel instruments (Vaudt *et al*. 2007). The nickel-titanium alloy has a lower modulus of elasticity when compared to stainless steel files and this together with nickel-titanium flexibility and specific design features contributes to the centring ability of nickel-titanium rotary instruments (Lopes *et al.* 2009).

8.7 Nickel-Titanium instrument fracture

Fracture of nickel-titanium rotary instruments may occur due to fatigue (rotational bending), or shear fracture (when the instrument tip is caught in dentine). Instrument fatigue is the greater cause of instrument fracture (Kim *et al*. 2012; Shen *et al*. 2011). Cyclic fatigue occurs when a material is exposed to repeated cycles of tension and compression that causes structural breakdown and ultimately fracture (Larsen *et al.* 2009). Fracture due to fatigue is usually due to crack initiation at the surface and it progresses intergranularly as the instrument rotates. Flexural stress is dependent on the root canal anatomy and on its angle and radius of curvature, and is independent of the clinician. Canal curvature is thought to be the predominant risk factor for instrument separation (Pasqualini *et al.* 2008). When nickel-titanium instruments are used in a continuous rotation, the potential still exists for the nickel-titanium file to thread within the canal. Certain nickel-titanium rotary instruments are designed to prevent threading by having flat radial land areas, which helps to reduce threading of the instrument in the canal. However, radial-landed instruments generate higher stresses in the canal (Diemer *et al*. 2013). Three design features that influence instrument fracture are: instrument size and taper, and cutting flute depths (Jonker and Van der Vyver, 2013).

9. DENTINAL DAMAGE AFTER NICKEL-TITANIUM INSTRUMENTATION

9.1 Overview of etiology of dentinal damage

The objective of root canal shaping is to create a root canal shape with continuous taper, with a small diameter at the apical foramen and the greatest at the coronal orifice. This is to maintain the original canal shape and curvature and also to allow for adequate irrigation and completion of obturation (Peters 2004). Stainless steel instruments are rigid and the instruments cannot engage curvatures in the canal system and this can lead to the increase in incidence of canal irregularities such as ledges, zips and perforations. The new generation of nickel titanium rotary instruments overcome these obstacles by the specific design features of these instruments, i.e. varying tapers, non-cutting tips, various lengths of the cutting blades. Greater taper in instruments were included in nickel-titanium designs: to increase cutting efficacy, decrease instrument failure and to maintain canal shape (Yang *et al.* 2006).

During canal debridement with nickel-titanium rotary instruments, rotational forces are applied to the root canal walls. Although nickel titanium preparation allows for reduction of instrumentation time, centred canals and standardised preparations, research has shown that an adverse consequence of canal preparation with nickel titanium instrumentation may be damage to dentine and the creation of defects in root canal walls. These defects can vary from craze lines, microcracks to incomplete and complete fractures in the root canal dentine (Adorno *et al.* 2013, Arias *et al.* 2014; Çiçek *et al.* 2015; El-Sayed *et al.* 2015, Pop *et al*. 2015; Shemesh et al. 2011, Yoldas *et al.* 2012). The formation of dentinal defects and damage may be caused by instrumentation and obturation of canals, the effect of sodium hypochlorite on dentine structure, tooth anatomy and morphology, and post space preparation and cementation (Shemesh *et al.* 2009).

With overzealous biomechanical preparation of the root canal, the root canal dentine is potentially damaged and the adverse consequence is dentinal microcrack formation, minute fractures or vertical root fractures. The greater the amount of dentine removed the greater the chance of dentinal damage (Bier *et al.* 2009; Priya *et al.* 2014). Rotary instrumentation in root canals may result in increased friction, which may increase dentinal defects and microcrack formation when compared to hand instrumentation (Shori *et al.* 2015). The volume of dentine removed during preparation may influence the development of craze lines in dentine. The use of rotary instrumentation causes a greater incidence of craze lines compared to hand instrumentation (Çiçek *et al.* 2015). Occlusal loading and masticatory forces can result in the amplification of forces at the tip of a defect and this can initiate or further propagate cracks into fractures (Kansal *et al*. 2014). The dentinal damage is of a continuous cumulative nature, as preparation alone results in less dentinal damage than that of preparation and obturation. Preparation, obturation and as well as retreatment result in an even greater percentage of dentinal defects. Defects on root canal walls are evident after both root canal preparation and filling (Shemesh *et al.* 2011).

9.2 Classification of dentinal damage:

Damage to dentine can be classified as follows:

- No defect- no lines/cracks present in dentine.
- Incomplete crack-presence of a line from canal wall into the dentine but not extending to the outer surface of root.
- Complete crack- presence of a line extending from root canal wall to outer surface of the root.
- Craze lines- presence of lines that do not reach any surface of the root/ outer surface of dentine but do not reach canal wall (Bürklein, Tsotsis and Schäfer, 2013).

9.3 Factors contributing to dentinal damage and defects

9.3.1 Dentine removal during preparation

The greater the amount of dentine removed, the higher the risk of causing dentine damage during instrumentation. Greater friction is observed between nickel titanium rotary files and root canal walls compared to hand instrumentation (Bier *et al.* 2009). Less aggressive movements during hand instrumentation is evident compared to engine operated instruments and this may contribute to decreased dentinal damage with hand instrumentation (Hin *et al.* 2013). The total volume of dentine removed is greater with rotary instrumentation than with hand instrumentation. The amount of dentine removed during preparation may influence the development of craze lines and microcracks in dentine. The use of rotary instrumentation causes a greater incidence of craze lines compared to hand instrumentation (Arias *et al.* 2014; Ashwinkumar *et al.* 2014; Al-Zaka, 2012; Çiçek *et al*. 2015).

9.3.2 Action of nickel titanium preparation on root canal dentine as a contributing factor to dentinal damage or defects

Rotary nickel titanium instrumentation can create additional stress within the root canal. With increased rotations (as required with rotary instrumentation), a rotational force is applied to the root canal walls which may contribute to dentinal damage and dentine microcrack formation (Al-Zaka, 2012; Milani *et al.* 2012). Transitory stress concentrations, where the rotary instruments and the root canal dentine make contact, may cause dentinal defects. These stress concentrations may be transmitted through the root to the outer surface where the dentinal bonds are destroyed (Adl, Sedigh-Shams and Majd, 2015; El Nasr and El Kader, 2014; Kansal *et al.* 2014; Pop *et al.* 2015). Mechanical preparation of root canals may induce compressive stresses in the dentinal walls that may propagate the crack formation, when these compressive stresses exceed the tensile strength of the dentine collagen matrix (Bürklein *et al.* 2013; Pop *et al.* 2015). Lam, Palamara and Messer (2005) stated that dentinal cracks occurred when the tensile stress in the root canal wall exceeds that of the tensile stress of dentine. Friction produced by rotary instrumentation can lead to high temperatures in the root canal and this can be observed as damage to the tooth structure. The tooth surface structure shows signs of thermal and mechanical damage in the form of dentinal microcracks (Muñoz *et al.* 2012).

9.3.3 Nickel-titanium rotary instrumentation design features' influence on dentinal damage and defects

Various design concepts and new techniques for canal preparation have emerged since nickel-titanium rotary instrumentation was first introduced to endodontics. The superelastic nature of the nickel-titanium alloy allows for faster canal preparation, decreases in apical extrusion of debri and allows for maintenance of canal shape by continuous rotation in canals (Adl *et al*. 2015). Certain rotary instruments have been shown to cause a greater incidence of dentinal cracks (Milani *et al.* 2012). Specific features of rotary instrumentation and shaping procedures may influence dentine damage and dentine defect formation. These include tip design, design of cutting blade, cross-sectional geometry, tip configuration, taper (constant vs progressive), pitch (constant vs variable) and flute form (Al-Zaka, 2012; Çiçek *et al.* 2015; Hin *et al.* 2013; Priya *et al*. 2014; Ustun *et al.* 2015; Yoldas *et al.* 2012). The incidence of dentinal defects may also be associated with nickel-titanium rotary preparation techniques (reciprocating motion, single instruments, multiple instruments and combination of different techniques) (Bürklein *et al.* 2013). File design may affect the force that is applied to root dentine, which may contribute to fracture potential (Lam *et al.* 2005). File designs that are rigid generate greater stress concentrations and this may increase the risk of dentinal defects (Adl *et al.* 2015). The stiffness of instruments is related to the cross-section, size, taper, manufacturing method and material of instrumentation (El Nasr and El Kader, 2014).

9.3.4. Cutting blade of nickel-titanium rotary instruments

The design of the cutting blade of nickel-titanium rotary instruments may be responsible for increased friction and stress within the root canal. Greater friction is observed between rotary files and canal walls. Although fractures do not ensue immediately after canal preparation, dentinal microcracks and craze lines may occur (Bier *et al.* 2009). The design of the cutting blades may increase friction and stress concentration in canals (Çiçek *et al.* 2015). The presence of radial lands (blade support) or convex cutting blades with a rake angle (positive or negative) may affect the apical dentine and can cause varying degrees of damage (Pop *et al.* 2015).

9.3.5 Taper of nickel-titanium instruments

Greater taper in instruments were included in nickel-titanium designs to increase cutting efficacy, decrease instrument failure and to maintain canal shape (Yang *et al.* 2006). The taper of a canal preparation and rotary nickel-titanium files also contributes to the occurence of dentinal damage and dentine defects, and this accentuates the evidence of the greater amount of dentine removed; the greater the possibility of dentinal defects (Karatas *et al.* 2016). Rotary instrument shape and the depth of penetration in the canal determine the amount of dentine that will be removed; therefore tapered files would increase the stress on dentinal walls (Bier *et al.* 2009; El Nasr and El Kader, 2014). Rotary instruments with large tapers may cause more complete and incomplete dentinal cracks (Milani *et al.* 2012; Priya *et al.* 2014). Decreasing the taper sequence of finishing files, results in increased strength of the files as well as increased stiffness at instrument tip. Larger and greater tapered instruments should be used with caution. As larger instruments are stiffer, this can cause an increase of lateral forces in canals. In coronal aspects of canals, larger tapered instruments are able to flare the coronal orifice and form a better taper.

Theoretically, larger apical tapers can cause more dentinal cracks, specifically in small weak roots (El-Sayed *et al.* 2015). High taper designs together with the number of rotations of the NiTi instrument in the root canal have found to be a contributing factor in dentine damage (Capar *et al.* 2014b).In the apical area, constant smaller taper may be better to maintain curvature (Yang *et al.* 2006). Rotary instrumentation resulted in significantly increased stress on dentine and this may be due to increased torque and increased taper in some rotary systems (Ashwinkumar *et al*. 2014). Rotary nickel-titanium files with greater tapers can thus cause increased friction and stresses within the canals, compared to hand instrumentation with 0.02 taper (Topçuoglu *et al.* 2014). In a study by Hin *et al.* (2013) ProTaper Universal, Mtwo and Self adjusting file (SAF) were compared. SAF caused less dentinal microcracks compared to Mtwo and ProTaper Universal. This could be attributed to the taper of the instruments, as SAF has no taper, Mtwo has a taper up to 0.06; while Protaper Universal reaches a taper of 0.09 (Hin *et al.* 2013).

9.3.6 Nickel-titanium material of manufacture

Newer nickel-titanium rotary instrumentation systems on the market are constructed of M-wire, which has greater flexibility than conventional nickel-titanium. The greater flexibility of M-Wire creates less stress on root canal walls and subsequently less pressure is required during instrumentation (El Nasr and El Kader, 2014; Kansal *et al.* 2014). In the study by El Nasr and El Kader, (2014) where Wave One rotary instrumentation (reciprocating motion with M-wire) is compared to F2 ProTaper Universal instrumentation (rotational motion with conventional nickel-titanium), the decrease in dentinal microcracks with Wave One can be attributed to the flexibility of M-wire as compare to the stiffer ProTaper Universal with conventional nickel-titanium. The stiffer files may result in greater stress in the canal, which increases the risk of dentinal microcracks (El Nasr and El Kader, 2014).

9.3.7 Cross section design of nickel-titanium rotary instrumentation

Cross section design and taper influences the behaviour of nickel-titanium rotary files. In a study conducted by Capar *et al*. (2014a), nickel-titanium rotary systems which varied in cross-section design, and taper as well as nickel titanium material were investigated. These systems included nickel-titanium rotary instrumentation systems that had a convex triangular cross-section with a variable taper (ProTaper Universal), off-centred rectangular design with a progressive and regressive taper (ProTaper NEXT) and symmetrical cross-section design and constant taper (Hyflex). All three systems had a variable pitch and non-cutting tips. The difference in cross-section design may influence the incidence of dentinal microcracks in these systems. The off centre rectangular design in the ProTaper NEXT system, has a swaggering motion, decreases the screw effect, taper lock and torque on files.This decreases the contact between the rotary files and root canal dentine. This design feature could be responsible for the decreased amount of dentinal microcracks seen with ProTaper NEXT.

ProTaper Universal was made with conventional nickel-titanium and has a greater taper than both ProTaper NEXT and Hyflex. The ProTaper NEXT system is made with M-wire, while the Hyflex system is made with controlled memory nickel-titanium wire. This may be responsible for the decrease in dentinal microcracks observed with ProTaper NEXT and Hyflex use, compared to ProTaper Universal. The ProTaper Universal system requires a greater number of instruments for canal debridement compared with the ProTaper NEXT and Hyflex systems, which may also increase the incidence of dentinal microcracks (Capar *et al.* 2014a). In a study by Bürklein *et al.* (2013) Wave One rotary instruments were investigated alongside Reciproc, ProTaper Universal and Mtwo systems. Both Mtwo and Reciproc have an s-shaped cross-section design, while ProTaper and Wave One have triangular and modified triangular cross-sections, respectively. The results indicated that greater incidence of dentinal microcracks was observed with the Reciproc system (Bürklein *et al.* 2013).

9.3.8 Rotational force and torque during root canal debridement

Continuous rotational force and constant torque by nickel-titanium rotary instruments on canal walls may result in dentine microcrack formation (Ashwinkumar *et al.* 2014). No defects in dentine are found in stainless steel hand instruments during canal debridement, due to the absence of rotational motion in canals that is present with nickel-titanium rotary instrumentation (Çiçek *et al.* 2015). Single file nickel-titanium rotary systems like Wave One system requires less time to complete canal preparation, thus it may induce less dentinal microcracks as there are fewer rotations of the rotary instruments. Reciprocation motion in Wave One system and the balanced force used during hand instrumentation may result in decreased dentinal microcrack formation. In a study by Ashwinkumar *et al.* (2014) all rotary instrumentation caused dentinal microcracks compared to hand instrumentation. This finding may be attributed to the total volume of dentine removed during rotary instrumentation, compared to that in hand instrumentation (which was less).

9.3.8.1 Number of rotations in root canals during nickel-titanium canal preparation

More rotations are required in canals during rotary instrumentation when compared with hand instrumentation. This increased number of rotations can contribute to dentinal defects (Al-Zaka, 2012; Baretto *et al.* 2012). During nickel-titanium instrumentation, the total amount of dentine removal from root canals is greater than that with hand instrumentation, and this factor may also detrimentally affect the prognosis of the tooth. In a study by Yoldas *et al.* (2012), 25% to 60% of roots examined after preparation presented with dentinal microcracks. The researchers found that repeated instrumentation of these roots may increase the percentage of defects. The effectiveness of nickel-titanium rotary instruments during debridement is influenced by the rotations of the instrument in the canal; thus the greater the number of rotations in the canals, the greater the chance of dentinal defects. The use of single file systems results in fewer rotations and less contact with the dentine and therefore less dentinal damage (Bier *et al*. 2009; El Nasr and El Kader, 2014).

9.3.8.2 Rotational speed

An increase in rotational speed is associated with increased cutting efficacy. In the study by Capar *et al.* (2014a), the Hyflex rotary system that was observed used a rotational speed of 500 rpm, which reflected in the results of this particular study where Hyflex had less incidence of dentinal microcracks compared to ProTaper Universal which were operated at a rotational speed of 250rpm. Rotation type and speed may affect incidence of dentinal microcracks (Kansal *et al.* 2014).

9.3.8.3 Torque of instrument

By increasing the torque, instruments may lock in dentine and separate. Torsional stress occurs when the instrument tip or any other part thereof is wedged in root dentine, and the shaft of the instrument continues to rotate. In curved or narrow canals where removal of dentine is difficult, excessive torque may be generated if the clinician continues working after an instrument has lodged in dentine (Dane *et al.* 2016; Ceyhanli *et al*. 2015). A study by Dane *et al.* (2016) found decreased amount of dentinal microcracks with a lower torque setting. The greater dentine damage may be attributed to the greater stress concentration on root dentine.

9.3.9 Single file nickel-titanium rotary system and dentine damage

With advances in the field of nickel-titanium manufacturing; endodontics has been transformed by decreasing preparation time, operator fatigue and minimizing procedural errors. Single reciprocating nickel-titanium systems were introduced, amongst other reasons, to relieve stress on the instruments (single file reciprocating motion), although single file systems may use reciprocal or rotational movement according to manufacturer specification. This movement reduces the risk of cyclic fatigue (Kansal *et al.* 2014; Priya *et al.* 2014). In a study by Liu *et al.* (2013) where three single file systems were compared to a multiple file system, the single file systems caused less damage. This could be due to the greater handling in canals with multiple file systems compared to single file systems (where there is less manipulation in canals).

9.3.10 Rotational vs reciprocal motion during nickel-titanium debridement

In a study by Çeçik *et al.* (2015) both reciprocal motion and rotational motion of the nickel-titanium instrumentation caused microcracks. According to these investigators this can be attributed to the contact of the instruments with root canal dentine. Reciprocating motion during nickel-titanium canal preparation decreases stress on the instrument by counter clockwise (cutting action) and clockwise (release of the instrument). This movement is assumed to decrease the chance of cyclic fatigue that is caused by tension and compression. Reciprocal motion reduces torsional and flexural stresses and reduces canal transportation. Thus the resistance to cyclic fatigue is increased compared to conventional rotary systems with continuous rotations (Bürklein *et al*. 2013; De-Deus *et al*. 2014. Liu *et al*. 2013).

Conflicting results have been obtained with regards to the effect of reciprocating motion on the development of dentinal damage and dentinal microcracks. Various studies conducted on reciprocating motion suggested, that by using a single large tapered instrument with reciprocating motion increases the probability of dentinal damage. The fact that a considerable amount of dentine is removed by the single instrument in a short period of time contributes to this deduction.

Conventional full sequence preparation involves a more progressive and slower canal preparation (De-Deus *et al*. 2014). In a study by Çeçik *et al.* (2015) both reciprocal motion and rotational motion of the nickel-titanium instrumentation caused microcracks. This can be attributed to the contact of the instruments with root canal dentine. Reciprocal motion showed an increased resistance to cyclical fatigue and caused fewer microcracks in the study by Çeçik *et al.* (2015). This result could be due to the cross section design of the instrument. Reciprocation can prevent continuous rotational force and constant torque in a canal and therefore result in less damage on the dentinal wall.

Berutti *et al.* (2012b) showed that reciprocating motion of certain nickel-titanium systems may assist with stress release before the file progresses further in the root canal as well as time required for preparation. This could be beneficial when preparing root canals with curved anatomy. The same study by Berutti *et al*. (2012b) found that reciprocating motion was advantageous as the root canal preparation stayed centred, and preparation was less invasive, which may be of importance when preparing root canals with lower levels of dentine thickness (Berutti *et al.* 2012b).

In a study by Bürklein *et al*. (2013) reciprocal motion (by using Reciproc system) induced a greater amount of dentinal microcracks compared to full sequence rotary systems. Reciprocal movement may enhance debri transportation to the apex and this may contribute to the increase of apical microcracks.

In a study by El Nasr and El Kader, (2014) Wave One rotary instrumentation (reciprocating motion) was compared to F2 ProTaper file (rotational motion); and it was found that the reciprocal motion caused less dentinal microcracks (El Nasr and El Kader, 2014). In a study by Kansal *et al.* (2014), less dentinal microcracks were evident with single file Wave One instrumentation with reciprocating motion compared with conventional full sequence ProTaper instrumentation. Kansal *et al*. (2014) postulated that reciprocating motion resulted in less dentinal damage.

Active rotation in root canals may result in higher levels of stress in the root dentine, and this may contribute to crack formation. During reciprocating motion, there is also less torsional and flexural stress on root canal dentine. Bürklein *et al.* (2013) found that reciprocating motion did not cause less dentinal damage when compared to conventional rotational instrumentation. The reciprocal motion produced a significant amount of microcracks in the apical root area, compared to rotary instrumentation. The difference in results could be due to the taper not being standardized as in the study by Kansal *et al.* (2014).

<u>9.3.11 Cyclic loading and dentinal damage and defects</u>

Mechanical cycling investigates the fatigue of an instrument and can simulate masticatory function which can cause structural fractures after repeated loading. The progression or development of microscopic cracks from areas of force or stress concentrations may lead to the development of fractures (Baretto *et al*. 2012). The authors concluded that cyclic loading alone did not increase the incidence of dentinal defects. This study by Baretto *et al.* (2012) also concluded that dentinal microcracks together with clinical procedures (canal preparation and obturation without apical pressure) can result in an increase of defects and consequently increase in fracture susceptibility.

<u>9.3.12 Canal length preparation and apical stress and strain</u>

Root stresses created from within the root canals are greater in the apical region and along the canal wall than an external surface. The stress distribution patterns in the apical areas may lead to crack propagation in apical areas and future fracture development (Adorno, Yoshioka and Suda, 2009). The researchers found that preparing canals to full working length had a greater incidence of crack formation than preparing canals at working length at root length minus 1mm. Thus the conservation of dentine adjacent to the apical root canal is important to maintain the strength and fracture resistance of roots. Studies have indicated that preserving canal size and creating a smooth round preparation without irregularities can reduce crack or fracture susceptibility. Sufficient tooth structure around the apical tip in the study by Adorno *et al.* (2009) could be responsible for resisting crack formation.

The apical area of the root canal is considered to be an important area to be instrumented adequately. No consensus has been reached with regards to optimal apical size. Difference in opinion exists between a minimally tapered canal (by aggressive apical instrumentation) and increasing the size of canal by 6-8 file sizes after the first binding file. By maintaining canal size, the fracture susceptibility of canals is decreased; whereas a larger apical preparation may be beneficial in removing debri and bacteria. A difference of opinion of terminating instrumentation from the apex exists. A study by Adorno, Yoshioka and Suda (2011) found that nickel-titanium instrumentation could cause dentinal damage and cracks in the apical root area and instrumentation short of the apical foramen had a lower incidence of crack initiation, regardless of the nickel-titanium file design. When the working length is calculated to the apical foramen, there is a greater chance of producing apical cracks than when the working length is calculated at 1mm short of the apical foramen. Preparation of the canals through the apex (1mm) may also cause an increase in apical external surface microcracks (Adorno *et al.* 2011; Pop *et al*. 2015). The file design may also have an effect on the apical stresses and strain and this may also contribute the development of dentinal microcracks (Ashwinkumar *et al.* 2014; Kim *et al*. 2010). Kim *et al.* (2010) compared the apical stresses generated by 3 types of nickel-titanium instrumentation; ProTaper Universal generated increased tensile and compressive stresses in dentine. This could also contribute to the development of dentinal defects. Kim *et al.* (2010) hypothesised that the design of nickel-titanium instrumentation would affect the incidence of apical microcracks.

<u>9.3.13 Instrumentation used in the agitation of sodium hypochlorite and its effect on dentine</u>

In preparations where ultrasonics is used to agitate sodium hypochlorite, the root canal walls may be roughened and this may also lead to defects after canal preparation (Bier *et al.* 2009).

9.4 Consequences of dentinal damage

Resistance to tooth fracture is a very important aspect during canal preparation, as any dentinal defect or root fracture may decrease the longevity of the affected tooth (Al-Zaka, 2012; Yoldas *et al.* 2012). Teeth that initially present with craze lines or microcracks have the potential to develop fractures after being exposed to repeated stress from endodontic and/or restorative procedures. Forces exerted on the roots during mastication and occlusal loading may also cause the propagation of microcracks (Shemesh *et al*. 2009). With repeated stress application by occlusal forces, dentinal microcracks or craze lines may progress into vertical root fractures during root canal preparation and obturation procedures (Çiçek *et al.* 2015). Evidence suggests that fractures may form due to the propagation of smaller defects and is not always related to the force used during canal preparation or obturation. These defects have the potential to develop into fractures due to longterm occlusal loading and functional stresses; like masticatory forces (Bier *et al.* 2009).

10. VERTICAL ROOT FRACTURES

A negative consequence of canal shaping is the possible weakening of roots and ensuing crack formation due to exposure to stress from the clinical procedure (Jamleh *et al.* 2014; Hin *et al*. 2013). When incomplete cracks in the dentine are exposed to external forces, these cracks may become high stress concentration areas, from which they may propagate to the root surface and develop into full or incomplete fractures (Capar *et al*. 2014b). Vertical root fractures are frustrating complications of root canal treatment, which decreases the longevity of the tooth and results in tooth extraction (Tsesis *et al.* 2010).

10.1 Definition of vertical root fractures

"A vertical root fracture is a longitudinally oriented fracture of the root that originates from the apex and propagates to the coronal part" (Khasnis *et al*. 2014). Vertical root fractures initiate on the internal canal wall and propagate through the root dentine to the external canal surface (Khasnis *et al.* 2014; Mireku *et al*. 2010).

10.2 Overview of etiology of vertical root fractures

Certain etiological factors of vertical root fractures such as: root morphology, canal shape and size, and initial amount of dentine structure; are beyond the control of the clinician. However, there are certain factors that can be controlled during treatment so that the susceptibility to fracture can be reduced. These factors comprises of eventual root canal shape, the extent of root canal enlargement, and reduction of irregularities that may form stress concentration areas (Versluis, Messer and Pintado, 2006). Although vertical root fractures are frequently as a result of loading due to canal obturation or post- space preparation; fractures may also initiate from stress concentrations in root canal dentine. These stress concentrations can arise during shaping and cleaning of the canal system (Kim *et al.* 2010). Dentine defects can act as a starting point for vertical root fractures and may influence the longevity of an endodontically treated tooth (De-Deus *et al.* 2015).

10.3 Predisposing factors of vertical root fractures

10.3.1 Stress concentrations in root canal dentine

Stress concentrations can arise during shaping and cleaning of the canal system. During shaping of the canals, contact between the instruments and root canal dentine is made (Lertchirakarn, Palamara and Messer, 2003). The contact areas create many transitory stress concentrations in root canal dentine. This may result in these stress concentrations creating dentinal defects from which vertical root fractures can propagate. With increased stress within the root canal during instrumentation, there will be a possible increase in dentinal defects and in turn the risk of vertical root fractures would be greater (Kim *et al*. 2010). The preparation of root canals with nickel-titanium rotary instruments allows for a rounder canal shape, with greater control over the taper and apical preparation than with hand files. The preparation of a round smooth root canal shape throughout the canal length will result in greater uniform stress distribution and decrease in overall stress. Oval shaped root canals have an added difficulty by virtue of the narrow radius of curvature of the buccal and lingual areas. These areas serve as stress concentration areas, and any canal preparation that eliminates these stress areas will result in uniform distribution of stress within the root canal. Additionally this will lead to a decrease of fracture susceptibility (Versluis *et al.* 2006). By reducing stress concentrations, the possibility of vertical root fractures is reduced (Kim *et al*. 2010). Stress values and the distribution pattern depend on the root anatomy and loading forces exerted on roots (Sathorn *et al*. 2005; Versluis *et al.* 2006). In a study by Versluis *et al.* (2006) stress distribution during simulated compaction was investigated and it was found that when round canals were subjected to an internal load, the stress distribution was low and uniform from the apical to coronal area; as well as circumferentially around the canal wall. Oval canals exhibited increased stresses and an irregular stress distribution circumferentially and from apical to coronal area. Stresses were especially focused in the buccal and lingual canal extensions. In areas where the sharp areas in an oval canal was evened out, the stress concentrations were decreased or eliminated. By creating a round preparation, stress concentrations within the canal were reduced. Decreased fracture susceptibility with nickel-titanium instruments allows for the attainment of round canal profiles and smooth canal taper, and this decreases the fracture potential (Sathorn *et al.* 2005; Versluis *et al.* 2006).

10.3.2 Tensile strength and tensile stresses of root canal dentine

When an applied load creates stress that exceeds the absolute strength of a material, structural failure or fracture will occur. Tensile stresses are used in stress analysis studies to investigate fracture predilection, because the stresses that predispose dentine to fracture are tensile (in a circumferential direction around canal wall). The crack propagation direction is usually perpendicular to the stress direction. Tensile strength can be affected by structural defects, cracks or canal irregularities; which are present in any material investigated (Sathorn *et al.* 2005).

10.3.3 Rotary vs hand instrumentation

New design concepts and techniques during endodontic debridement have allowed nickel-titanium instruments to increase canal cleanliness and decrease canal straightening, as well as decrease perforations and transportation of apices. These features can be attributed to the flexibility and shape memory of nickel-titanium instruments. Specific geometric design features also increases these positive features by maintaining the natural canal curvature. The design of nickel-titanium instruments may affect the shaping forces on root canal dentine (Kim *et al*. 2010). Canal preparation with rotary nickel-titanium instrumentation showed an increase in dentinal defects compared to stainless steel hand instrumentation. This can be attributed to the number of rotations of the file within the canal; which is markedly higher during rotary preparation (Bier *et al.* 2009).

10.3.4 Mechanical behaviour of rotary instrumentation

Mechanical behaviour of rotary instrumentation influences the stress concentration within the root canal dentine. The design features of rotary instrumentation, such as cross section and longitudinal design influence mechanical behaviour. In this particular study, Kim *et al.* (2010) investigated the effect of different rotary designs on root canal dentine; specifically studying the effect of taper of the rotary instrument. Instruments with constant taper, increasing taper and no taper were compared and it was found that a higher incidence of dentinal damage was reported with rotary instrumentation with increasing taper.

10.3.5 Dentine thickness

Excessive canal shaping, especially in teeth with curved roots or oval canals can contribute to a higher incidence of vertical root fractures. The preparation of a round root canal shape in an oval canal reduces the remaining dentine and places the root at risk of fracture (Khasnis *et al.* 2014). Thinner dentine thickness may increase the likelihood of fracture (Sathorn *et al.* 2005). Excessive removal of root dentine during preparation leads to an overall weakening of the tooth and can result in a higher incidence of vertical root fractures. The degree of canal enlargement and the presence of irregularities or defects formed during clinical procedures may also influence the development of vertical root fractures (Lynch & Burke, 2002; Shemesh *et al.* 2008;). A study by Wilcox, Roskelley and Sutton (1997) found that where there was a great degree of canal enlargement, the likelihood of fracture was greater. The stresses generated from within the root canal are conveyed through the inner root to the external root surface where they undo the bonds that keep dentine together. This could explain why stresses are released at a specific surface (where the fracture occurs) and is not initiated at other surfaces once a fracture has manifested.

10.3.6 Root canal anatomy

Intrinsic factors related to root and canal morphology (like volume of dentine thickness, root canal shape and size and external root morphology) has a great influence over fracture susceptibility (Sathorn *et al.* 2005). A study by these authors found that the increase in fracture susceptibility is influenced by dentine thickness, curvature of the external root surface, canal size and shape. The combination of these factors influence the fracture susceptibilityas well as the pattern of fracture, and any variable may dominate. The risk of fracture may increase when there is a reduced curvature radius of roots in the buccal and lingual areas. The ratio of the canal width to the width of the total root may also increase the risk of root fracture (Khasnis *et al*. 2014). A low radius of canal curvature can lead to increased stress area concentrations and increase the likelihood of fracture. The direction of root fracture can be determined by the external root morphology. The evidence of vertical root fracture is found mostly in the bucco-lingual direction. A study by Hassan *et al.* (2009) found that fractures were more common in the buccolingual direction (67.5%) than mesiodistal fractures (32.5%). If the degree of curvature within the root canal is less, the fracture susceptibility would be reduced. The preparation of a smooth ovoid shaped canal would result in a decreased degree of curvature in the canal; and thus less fracture susceptibility (Sathorn *et al.* 2005). Studies conducted by Tamse *et al*. (1999) found that more vertical root fractures were observed in the mesial roots of lower molars, and this could be attributed to the anatomy of lower molars with narrow mesio-distal diameters. Premolars and mesial roots of mandibular molars are more susceptible to vertical root fractures according to studies by Fuss *et al.* (2001).

10.3.7 External root morphology

The direction of root fracture can be determined by the external root morphology. The location and direction of vertical root fractures involves the root canal size and shape, external root morphology and dentine thickness (Lertchirakarn *et al.* 2003). Most vertical root fractures occur in the bucco-lingual direction, where the dentine thickness is more than in the mesio-distal direction. The reduction of proximal dentine thickness results in an increased bucco-lingual stress concentration, and this may predispose roots to fracture in the bucco-lingual direction. This can be attributed to the stress area concentrations being high where the canal curvature is the greatest (in the bucco-lingual direction). When round canals are prepared, the highest stress concentration area depends on external root morphology (Lam, Palamara, Messer, 2005, Lertchirakarn *et al.* 2003). When an applied load creates stress that exceeds the absolute strength of a material, structural failure or fracture will occur. Tensile stresses are used in studies to investigate fracture predilection. This is because the stresses predisposing to fracture are tensile (in a circumferential direction around canal wall). The crack propagation direction is usually perpendicular to the stress direction. Tensile strength can be affected by structural defects, cracks or canal irregularities; which are present in any material investigated (Sathorn *et al.* 2005). In a study by Lertchirakaran *et al.* (2003) crack initiation started on the buccal or lingual canal surface and would propagate outwards to the outer root surface. All three factors (dentine thickness, canal shape and root shape) affect the tensile stress distribution and often there is an interaction between all three factors. Canal shape is notably a serious factor, as a reduced radius of curvature greatly influences stress concentrations. An oval root shape has greater susceptibility to higher stress than a circular/round shape. A round/circular shape decreases the localised stress concentrations and stresses will be evenly distributed (Fuss *et al.* 2001; Lertchirakarn *et al.* 2003).

10.3.8 Obturation and post-space preparation forces

The use of excessive pressure during lateral and vertical compaction of filling materials may also contribute to the development of vertical root fractures (Lam *et al.* 2005; Shemesh *et al*. 2008). During obturation, the wedging effect of the spreader during lateral condensation and excessive mechanical force during condensation may produce apical strain and increase the risk of vertical root fractures (Khasnis *et al.* 2014; Lynch & Burke, 2002; Mireku *et al.* 2010). The decrease of dentine during post-space preparation and excessive pressure during post cementation may contribute to fracture susceptibility (Mireku *et al*. 2010). Lertchiakarn *et al*. (1999) found a decrease of applied forces during canal filling and post-space prep, resulted in a decrease in stress and consequently a decrease in risk of fractures.

10.3.9 Changes in the dentine

Dentine of endodontically treated teeth displays greater plastic deformation than vital teeth. With dehydration of dentine, the Young's modulus of elasticity is increased and dentine stiffness increases. This may increase the risk of fracture (Khasnis *et al.* 2014).

10.3.10 Age –related etiology

The age of the patient and tooth can also be linked with vertical root fractures. The age-related changes of dentine can reduce the ability of dentine to resist damage. With age, the strength of dentine under static and cyclic loading as well as fracture toughness decreases. The decrease of resistance to vertical root fractures with age is due to the alteration in dentine microstructure (sclerosis) as well as lower resistance to damage under cyclic loading (Mireku *et al.* 2010).

10.3.11 Canal enlargement

As the design features of nickel-titanium instrumentation have been developed, it has allowed for different canal shapes to be attained. These canal shapes can vary from the taper of the canals to apical enlargement size. The degree of canal enlargement and the presence of irregularities or defects formed during clinical procedures may also influence the development of vertical root fractures (Kim *et al*. 2010, Lam *et al*. 2005). The effect of instrument taper (constant, increasing or no taper) on root canal dentine was compared by Kim *et al*. (2010), and it was found that a higher incidence of dentinal damage was reported with rotary instrumentation with increasing taper

10.3.12 Reciprocating action of nickel-titanium instrumentation

Conventional nickel-titanium instrumentation (without reciprocating action) may lead to the expansion of forces at the tip or close to the instrument tip. These forces can result in strain on the root canal wall and the end result may be crack formation and fracture propagation (Jamleh *et al.* 2014). The use of nickel-titanium instruments with reciprocating action consists of counterclock wise-cutting direction and clockwise-release of instrument motion, and the angle of the counterclockwise cutting direction is greater than the clockwise direction (Bürklein *et al*. 2012). The reciprocating motion results in successful shaping and less canal modifications. Jamleh *et al*. (2014) found that when simulating the canal shaping technique, the surrounding tensile stresses were concentrated on the buccal and lingual walls of the root canal. This implies that the teeth are weaker buccal-lingually, and therefore microcracks may be generated bucco-lingually. Canal shaping caused microcracks in the apical root walls, although less with reciprocating motions (Jamleh *et al*. 2014).

10.4 Diagnosis of vertical root fractures

Diagnosis of vertical root fractures is not usually made during endodontic treatment, but years later; when it manifests as bone loss, malfunction of the involved tooth and pain (Shemesh *et al.* 2008).

CHAPTER 2:

2.1 AIM

The aim of this *in-vitro* study was to investigate the influence of four different nickel-titanium rotary instruments and stainless steel hand files on the appearance of dentinal defects on root canal dentine.

2.2 OBJECTIVE

The expansive objectives of this study were to:

i) Decoronate molars of extracted mandibular molars; so that the remaining root measured approximately 11mm.

ii) Perform root canal treatment on the root segments using the various nickel-titanium rotary systems, as well stainless steel hand instruments.

iii) Sectioning of each root segment at 3mm, 6mm and 9mm from the apex; using Stueres Minitom diskcutter.

iv) Examine the root segment under stereomicroscope and digital image by 2 observers; for the presence of dentine damage.

2.3 NULL HYPOTHESIS

The null hypothesis is that there is no difference in the effect that the four different nickel-titanium rotary instruments and stainless steel hand files have on the root canal dentine.

CHAPTER 3:

3. MATERIALS AND METHODS

The methodology used in this study is similar to those proposed by Liu *et al.* (2013) and Bier *et al.* (2009), where teeth were decoronated and roots were prepared with the different NiTi rotary instrumentation systems. The root segments were then segmented at 3mm, 6mm and 9mm from the apex. These segments were observed under stereomicroscope and digital camera for the appearance of dentine damage.

3.1 COLLECTION OF MATERIAL

One hundred and eighty permanent human mandibular mesial roots were selected for the study. Mandibular molars were obtained from the oral surgery and service rendering clinics of the Faculty of Dentistry, University of the Western Cape. The teeth collected for the purposes of this study were extracted for reasons unrelated to the objectives of this study. Prior to commencement of this study, ethical clearance was obtained from the Research Committee of the Faculty of Dentistry, University of the Western Cape (Project number: 13/10/72). Every aspect of this study was conducted in accordance with the ethical and safety guidelines for handling human tissues and conducting laboratory studies, as prescribed by South African law: The Health Professions Act 56 of 1974 (Health Professions Council of South Africa, 2008).

3.2 SELECTION, EXCLUSION AND STORAGE

Teeth were stored in distilled water after extraction, and were not stored for longer than two weeks. The crowns of teeth were removed at the cement-enamel junction (CEJ) using a diamond disk cutter (Struers Minitom) using a 350 µm blade thickness. The distal roots of the teeth were removed so that the remaining mesial roots had an approximate length of 11mm. All roots were inspected with transmitted light under a stereo microscope (Nikon SMZ 10) at 12X magnification to detect any pre-existing defects, fractures, craze lines or cracks. These teeth were excluded and replaced.

A polyvinyl silicone impression material (President Putty Coltene/Whaledent) was used to coat the cemental surface of the roots to simulate the periodontal ligament space and to mimic the mechanism of stress distribution during debridement.

The total samples were randomly divided into six groups, where one group (n=30) was left unprepared to serve as the control group. The remaining five groups were randomly assigned to a nickel-titanium rotary instrumentation system as well as a stainless steel hand file group. The canal patency for all roots was gauged with a no. 10 stainless steel K-file (FKG DENTAIRE™).

Figure 3.1: Struers Minitom (Denmark) and blade

Figure 3.2a: FKG (Dentaire Switzerland) hand files (no. 8, 10 & 15) for glide path preparation

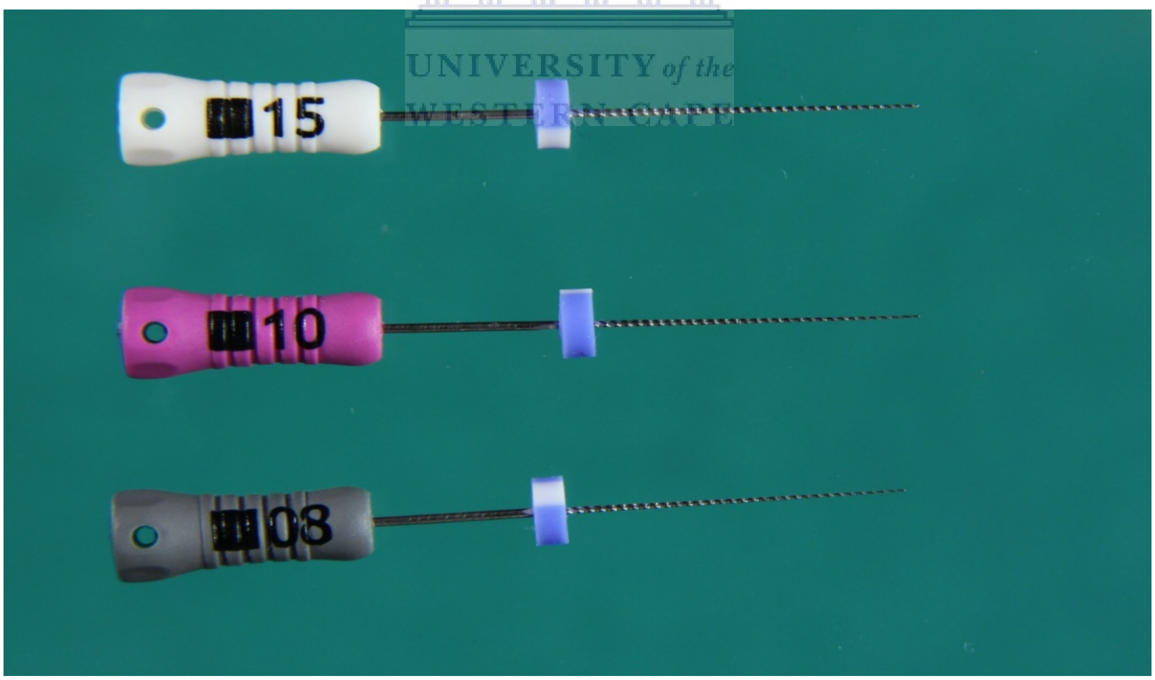

Figure.3.2b: FKG (Dentaire Switzerland) hand files (no. 8, 10 & 15) for glide path preparation

3.3 ROOT SEGMENT PREPARATION

The detailed debridement procedure for each system was as follows:

For all groups: Canal patency was established with a no. 10 stainless steel K-file (FKG DENTAIRE™). The no. 10 stainless steel K-file was introduced into the canal until it exits the apical foramen. The working length was set 1mm short of that length. The glide path was established with hand files: numbers 8, 10 and 15 stainless steel K files (FKG DENTAIRE™). The canals were irrigated with 5.25% sodium hypochlorite (Protea Chemicals) during glide path establishment. 17% EDTA (Glyde File Prep™, Dentsply Maillefer) was used as a lubricant during glide path establishment. After the glide path was established for each sample group, the allocated rotary nickel-titanium system was used for canal debridement. 5.25% Sodium hypochlorite and 17%EDTA (Glyde File Prep™, Dentsply Maillefer) was used as an irrigant and chelator during debridement. Sim *et al.* 2001 noted that the greater efficacy of sodium hypochlorite at greater concentrations has influenced clinicians to use higher concentrations of sodium hypochlorite during root canal preparation. Approximately 12 ml sodium hypochlorite solution was used per canal. Canals were irrigated with sodium hypochlorite after each file change, and instrument flutes were cleaned on cotton gauze after insertion in canal. EDTA (Glyde File Prep™, Dentsply Maillefer) was used after sodium hypochlorite rinse in the canals approximately twice). Patency was confirmed between each file. Sodium hypochlorite was used as the final rinse. The Wave One endo motor and handpiece (Dentsply Maillefer) was used for all canal preparation. All the NiTi rotary sample groups were prepared in rotational motion, except for the Wave One group which was prepared in reciprocal motion. One set of rotary instruments was used for two canals (mesial roots), as ideally one set of rotary instruments should be used per tooth during endodontic treatment. The speed and torque was programmed for each NiTi rotary system according to the manufacturers' recommendations to standardise the study. All procedures were carried out by a single operator.

Figure 3.3: Wave One Endo motor and handpiece (Dentsply Maillefer Switzerland)

Figure 3.4: Glyde ™ File prep (EDTA) (Dentsply Maillefer Switzerland)

Figure 3.5: Sodium hypochlorite 5,25 % (Protea Chemicals, Milnerton, South Africa)

GROUP 1: ProTaper NEXT™ (Dentsply Maillefer) (n=30)

The ProTaper NEXT™ X1 Dentsply Maillefer (017/04) was used for initial debridement till working length was achieved. With EDTA, files were brushed and followed down glide path. This was followed by ProTaper NEXT™ X2 (025/06) till working length was achieved. After each ProTaper NEXT™ instrument, recapitulation with a no 15 K file was done. 5.25% sodium hypochlorite and 17%EDTA was used during debridement.

Recommended speed: 300 rpm

Torque: 2 Ncm

Figure 3.6a (i): ProTaper NEXT (Dentsply Maillefer Switzerland) NiTi instruments

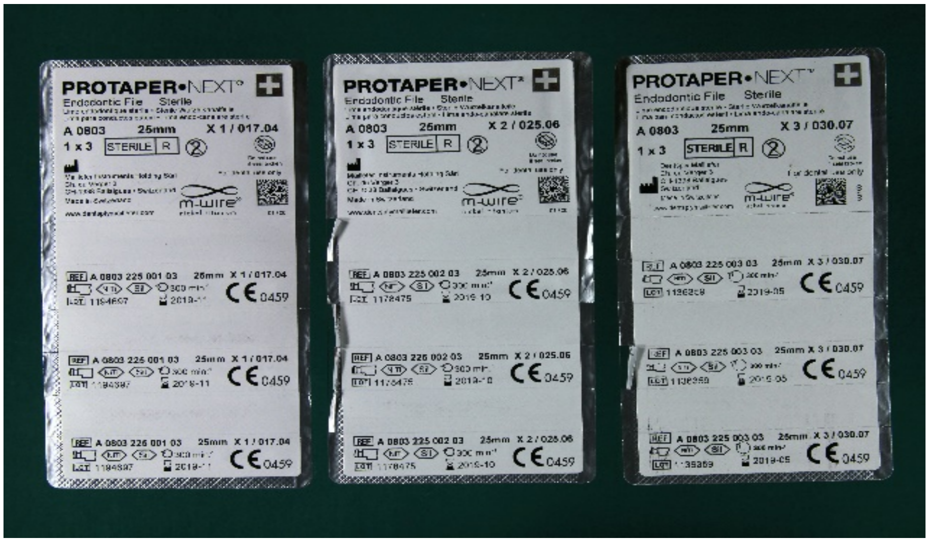

Figure 3.6a (ii): ProTaper NEXT (Dentsply Maillefer Switzerland) NiTi instruments

Figure 3.6b: ProTaper NEXT (Dentsply Maillefer Switzerland) NiTi instruments(X1, X2 & X3)

<u>*GROUP 2: Wave One™ (Dentsply Maillefer) (n=30)*</u>

The single file for Wave One™ (Dentsply Maillefer) was selected accordingly and used to debride till working length was achieved. If a no. 10 K-file moved to length easily, is loose or very loose, a WaveOne™ Primary file (25/08) was used. The files were used in up and down movement no more than three to four times, with little force. 5.25% sodium hypochlorite and 17%EDTA was used during debridement. Recapitulation was done with a no 15 K-file.

Recommended speed: 300 rpm; Torque: 2 Ncm

Figure 3.7a(i): Wave One (Dentsply Maillefer Switzerland) NiTi instruments

Figure 3.7a(ii): Wave One (Dentsply Maillefer Switzerland) NiTi instruments

Figure 3.7b: Wave One (Dentsply Maillefer Switzerland) NiTi instruments (small, primary and medium)

<u>*GROUP 3: BT Race FKG Dentaire (n=30)*</u>

BT1 (10/.06) was used for finalisation of glide path and conservative enlargement of the coronal third.

BT2 (35) was used for preparation of the apical third till full working length.

BT3 (35/.04) was used for final shaping of canals till full working length. 5.25% sodium hypochlorite and 17%EDTA was used during debridement. Instruments were used with long and gentle pecking motion (3-4 back and forth strokes). Recapitulation was done with a no 15 K-file.

Recommended speed: 800 rpm

Torque: 1.5 Ncm

Figure 3.8a: BT-Race NiTi instruments (FKG Dentaire Switzerland)

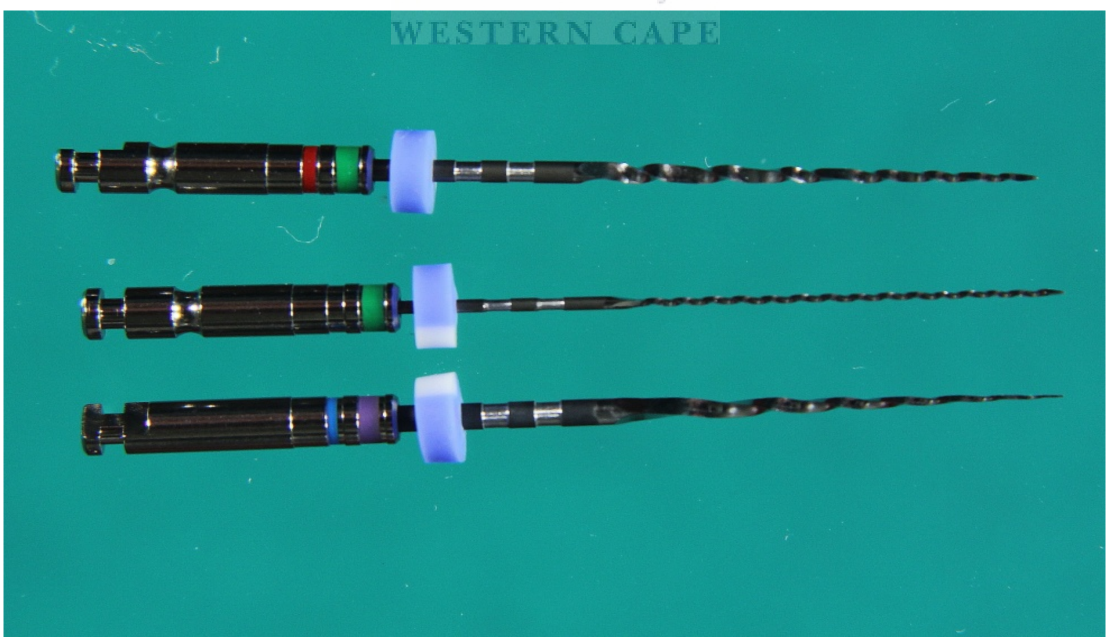

Figure 3.8b: BT-Race NiTi instruments (BT1, BT2 & BT3) (FKG Dentaire Switzerland)

<u>GROUP 4: iRaCe™ (FKG Dentaire) (n=30)</u>

R1 file (15/06) was used till working length. Thereafter R2 file (25/04) was used till working length to continue shaping. 5.25% sodium hypochlorite and 17%EDTA was used during debridement. Long back-and-forth strokes with the instrument was used, with a light touch. Recapitulation was done with a no 15 K-file.

Recommended Speed: 600 rpm

Torque: 1.5 Ncm

Figure 3.9a: iRaCe NiTi instruments (FKG Dentaire Switzerland)

Figure 3.9b: iRaCe NiTi instruments (R1, R2 & R3) (FKG Dentaire Switzerland)

<u>*GROUP 5: Stainless steel hand instruments (Dentsply Maillefer) (n=30)*</u>

All canals were prepared using stainless steel instruments till working length, and recapitulating between each file. Canals were prepared till a no. 25 stainless steel K-file (Dentsply Maillefer). Once the instrument went to working length, recapitulation was done, followed by the consecutive instrument. 5.25% sodium hypochlorite and 17%EDTA was used during debridement.

Figure 3.10: Stainless steel hand files (Dentsply Maillefer Switzerland)

<u>GROUP 6: Control group (n=30)</u>

No preparation was carried out in this sample group.

3.4 ROOT SECTIONING

The roots in all six groups were sectioned at 3, 6, 9mm from the apex, using a diamond disc cutter (350 µm blade thickness) under water cooling attached to Stueres Minitom. Root segments were then observed under stereomicroscope (Nikon SMZ 10) under 12X magnification and digital images of each section was captured at 40x using a digital camera (Leica DFC 290). The root segments were kept wet at all times and observation under stereo microscope was done immediately after sectioning of roots.

The root segments were evaluated for any defect in the dentine at any level in the segment slice. Defects were categorised as: ‘no defect’ and ‘all other defects’. ‘No defect’ will be defined as dentine devoid of any lines or cracks where both the external root surface and the internal root canal wall had no defects. Other defects will include all lines that will be observed from the root canal wall to the outer root surface (fractures); as well as any lines that do not reach either the canal lumen or the outer root surface (Shemesh *et al* 2009).

The images were observed by the author and an impartial second observer.

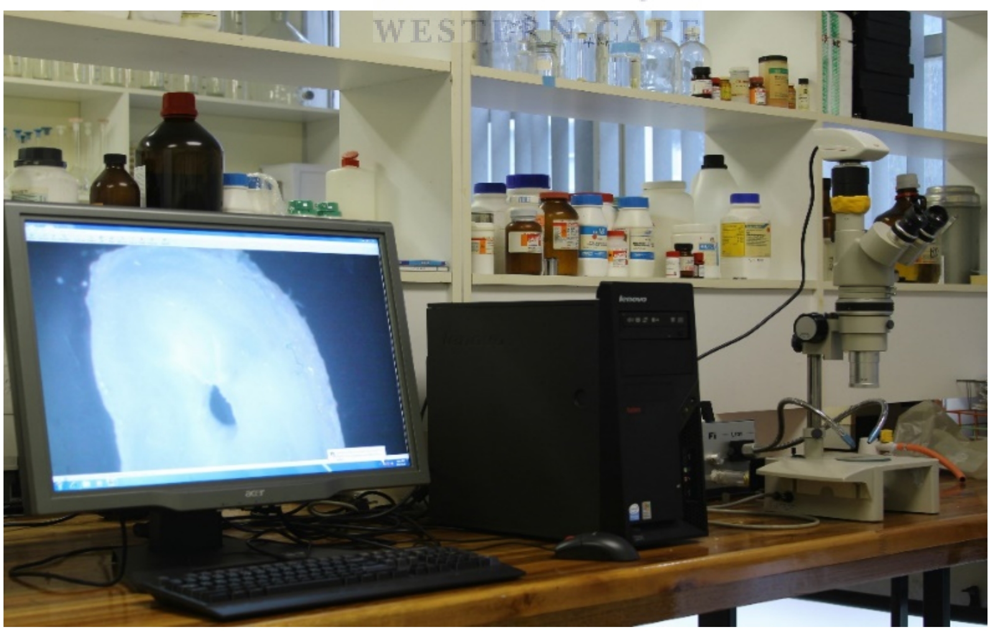

Figure 3.11a: Stereomicroscope (Nikon SMZ 10) and digital camera (Leica DFC 290)

Figure 3.11b: Stereomicroscope (Nikon SMZ 10) and digital camera (Leica DFC 290)

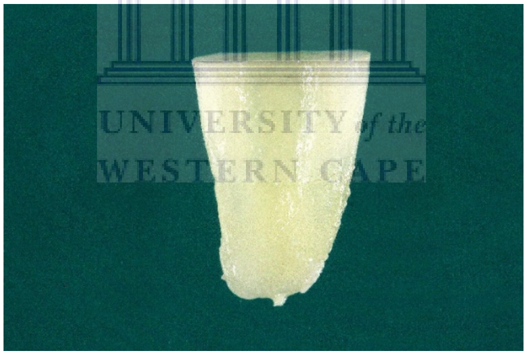

Figure 3.12a: Root segment after decoronation

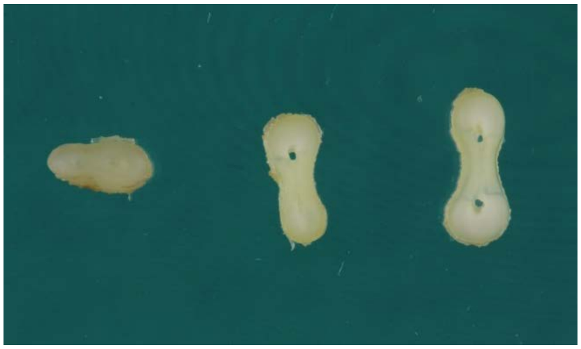

Figure 3.12 b: Root segments at 3mm, 6mm and 9mm

CHAPTER 4: RESULTS AND STATISTICS

4.1 DESCRIPTIVE STATISTICS

The study had a total sample size of 180, which was divided into 6 groups and each group had 30 samples. Each sample was investigated at 3 segments; namely 3mm, 6mm and 9mm from the apex. In table 1, the proportion of times (out of full sample of 30) that the damage/defect was present is described. In table 1 the following is noted:

The control group (n=30) had zero (0) events and the stainless steel group (n=30) also had zero (0) events. The BT-Race group (n=30) had 17 events out of the total sample, giving a 56,67% incidence of dentine damage/defects. The iRaCe group (n=30) had 18 events out of the total sample, giving a 60% incidence of dentine damage/defects. The ProTaper NEXT group (n=30) had 18 events out of the total sample, giving a 60% incidence of dentine damage/defects. The WaveOne group (n=30) had 17 events out of the total sample, giving a 56,67% incidence of dentine damage/defects. There are no significant differences between the groups: WaveOne, ProTaper NEXT, iRaCe and BT-Race. However, there is a significant difference between the four groups and the stainless steel group ($p<0.0001$).

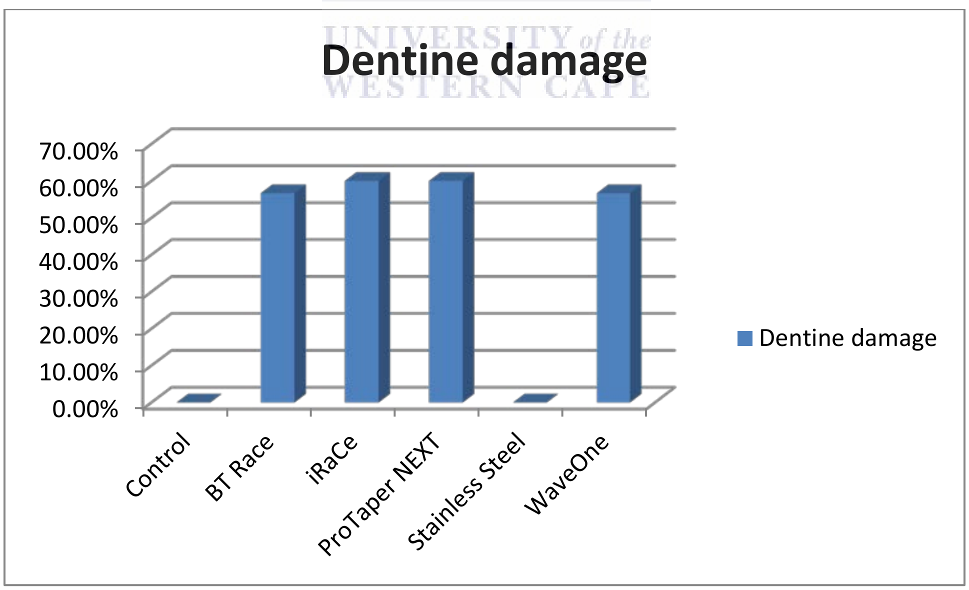

Figure 4.1: Dentine damage between all 6 groups

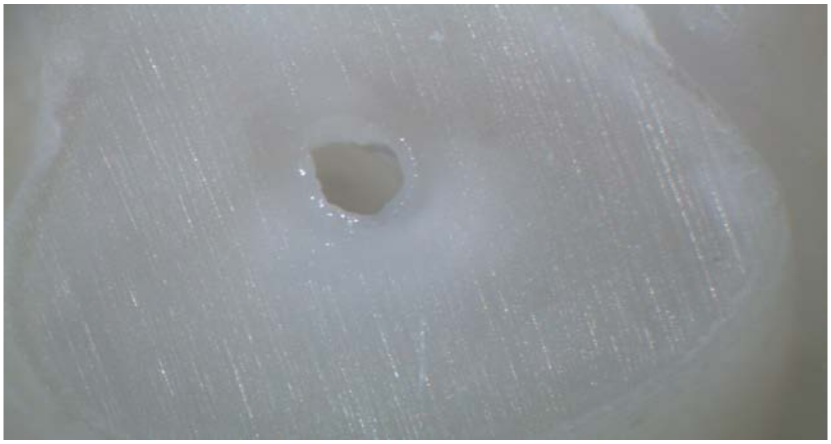

Figure 4.2: Control segment

Figure 4.3: Preparation with stainless steel hand files

4.2 FREQUENCY ANALYSIS OF 3mm

This table describes the variance between the control groups and each of the groups. At condition/event at 3mm level the following was noted:

The control group and the stainless steel group had zero (0) events or incidence at 3mm. The BT-Race group had 6,67% (2 events) incidence of dentine damage at 3mm. The iRaCe group had 23,33% (7 events) incidence of dentine damage at 3mm. The ProTaper NEXT group had 13.33% (4 events) incidence of dentine damage at 3mm. The WaveOne group had 20% (6 events) incidence of dentine damage at 3mm.

Fisher’s exact test

Fisher’s exact test is a statistical significance test used when there are two nominal variables.

Fisher’s exact test for 3mm

P value describes the probability and for the 3mm the P value is 0.0025 and this means that there is a statistically significant difference (of dentine damage events) between the groups.

Based on exact logistic regression; there is a statistically significant difference (of dentine damage events) between the groups. Specifically the iRaCe group and the WaveOne group had significantly more damage events than the stainless group (or the control group) with p=0.0105 and 0.0237 respectively.

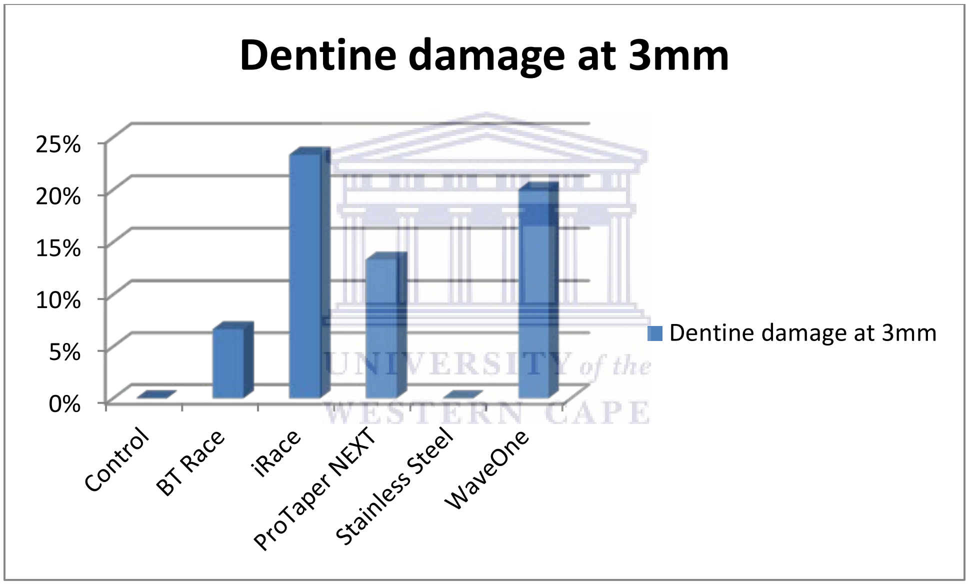

Figure 4.4: Dentine damage at 3mm segmentation

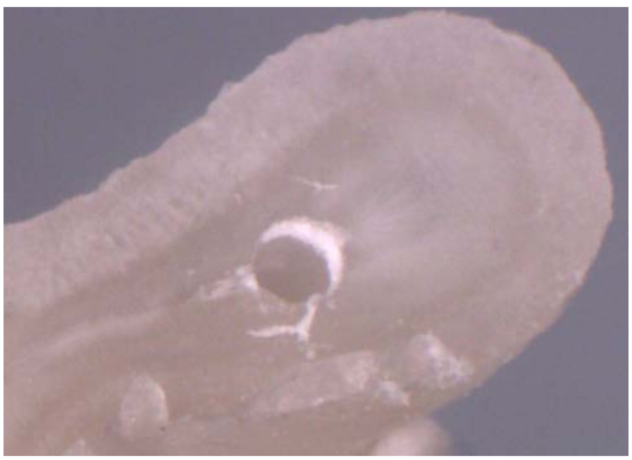

Figure 4.5: ProTaper NEXT segment with dentine damage/defect

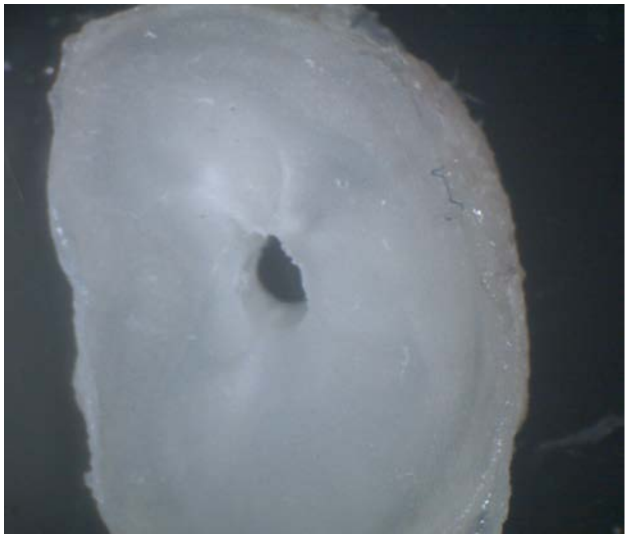

Figure 4.6: BT-Race segment with dentine damage

4.3 FREQUENCY ANALYSIS OF 6mm

This table describes the variance between the control groups and each of the groups. At condition/event at 6mm level the following was noted:

The control group and the stainless steel group had zero (0) events or incidence at 6mm. The BT-race group had 23.33% (7 events) incidence of dentine damage at 6mm. The iRaCe group had 23,33% (7 events) incidence of dentine damage at 6mm. The ProTaper NEXT group had 20% (6 events) incidence of dentine damage at 6mm. The WaveOne group had 20% (6 events) incidence of dentine damage at 6mm.

<u>Fisher's exact test for 6mm</u>

P value describes the probability and for the 6mm the P value is 0.0011 and this means that there is a statistically significant difference (of dentine damage events) between the groups.

Based on exact logistic regression, there is a statistically significant difference (of dentine damage events) between the groups. Specifically all four groups had significantly more damage events than the stainless group (or the control group) with p-values of 0.0105 or 0.0237.

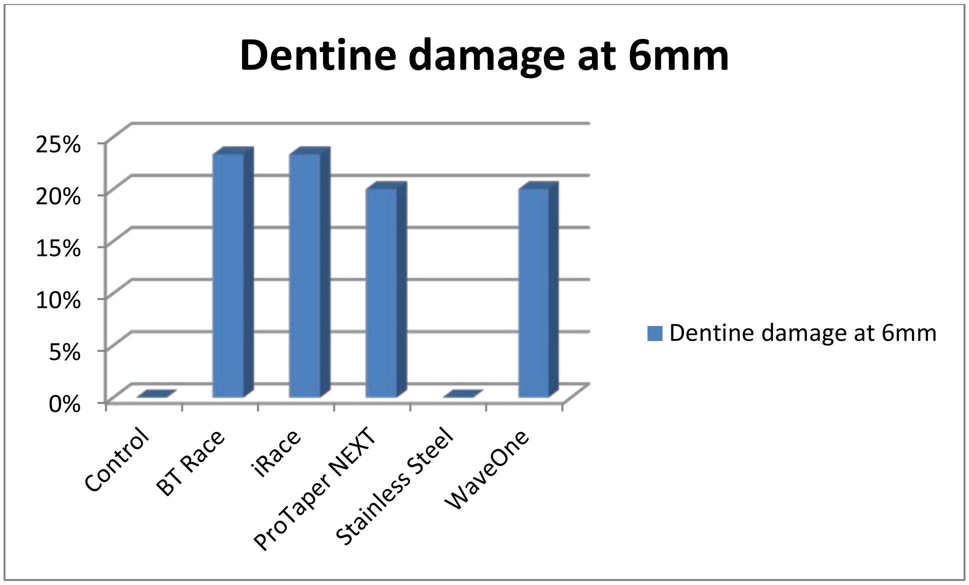

Figure 4.7: Dentine damage at 6mm segmentation

Figure 4.8: Wave One preparation with no dentine damage

Figure 4.9: Wave One preparation with dentine damage

4.4 FREQUENCY ANALYSIS OF 9mm

This table describes the variance between the control groups and each of the groups. At condition/event at 9mm level the following was noted:

The control group and the stainless steel group had zero (0) events or incidence at 9mm. The BT-Race group had 26.67% (8 events) incidence of dentine damage at 6mm. The iRaCe group had 13,33% (4 events) incidence of dentine damage at 9mm. The ProTaper NEXT group had 26.67% (8 events) incidence of dentine damage at 9mm. The WaveOne group had 16.67% (5 events) incidence of dentine damage at 9mm.

Fisher's exact test for 9mm

P value describes the probability and for the 9mm the P value is 0.0005 and this means that there is a statistically significant difference (of dentine damage events) between the groups.

Based on exact logistic regression, there is a statistically significant difference (of dentine damage events) between the groups. Specifically the BT-Race group and the ProTaper NEXTgroup had significantly more damage events than the stainless group (or the control group) with p=0.0046 for both.

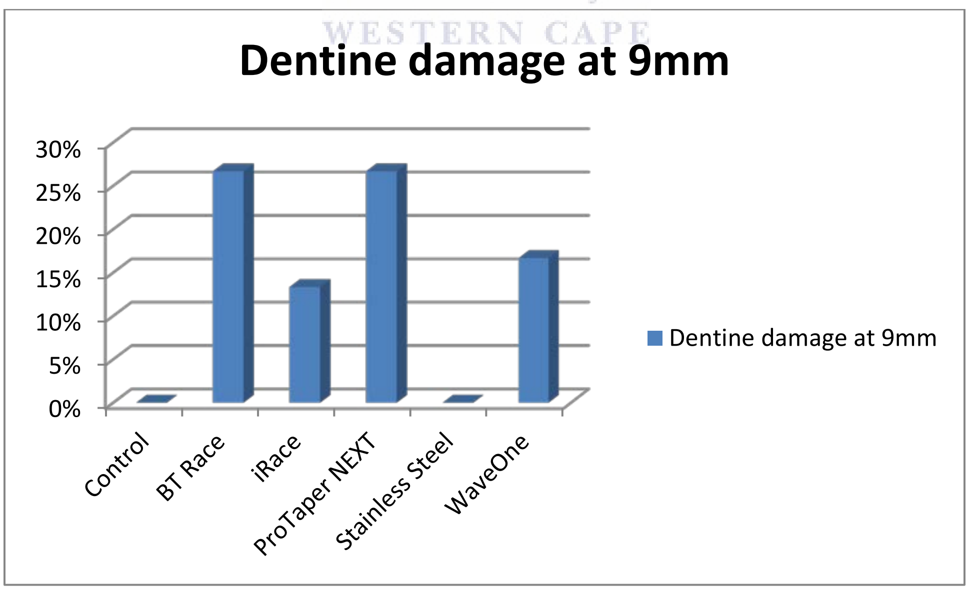

Figure 4.10: Dentine damage at 9mm segmentation

Figure 4.11:.iRaCe segment with dentine damage

CHAPTER 5: DISCUSSION

The purpose of this in vitro study was to investigate the effect of nickel titanium rotary instrumentation on root dentine, by recording the appearance of dentine damage, whether it be a defect, microcrack, crack, craze line or fracture. Six groups were studied, where four nickel titanium rotary systems, a stainless steel hand instrument group and a control group (which was left unprepared) were investigated to determine the effect on root dentine. The four nickel titanium rotary groups were: WaveOne, ProTaper NEXT, iRaCe and BT-Race.

The dentine damage observed in this study may be caused by the interaction of four actions on root canal dentine; namely, the mechanical preparation and shaping of canals by the different NiTi rotary systems (each with their own specific features and differences), the chemical attack of the irrigants and chelating agents on the root dentine (in this case 5,25% sodium hypochlorite and 17% EDTA), the sectioning method of the root segments and the inherent anatomy of the extracted roots.

These aspects are discussed as follows:

A greater number of rotations within the canal are necessary to complete preparation with nickel titanium rotary files as compared to hand instruments as noted by Pasqualini *et al.* (2008). The increased rotations by rotary instrumentation, compared to stainless steel hand files, in root canals may be responsible for the dentine damage. This would explain the lack of dentine damage seen in the stainless steel hand instrument sample group. The present study is in agreement with Bier *et al.* (2009) and Shemesh *et al.* (2009), where no dentine damage was observed in the stainless steel hand file group. These results could be due to the less aggressive nature of hand instrumentation as compared to nickel titanium rotary instrumentation. This is in accordance with results obtained by Priya *et al*. (2014) as well; where no dentine damage was noted with hand instrumentation. The lack of dentine damage in the stainless steel hand file group could be attributed to both the lack of the continuous rotational motion as well as the 0.02 taper of hand files, when compared to the taper of the NiTi rotary instruments in the present study. This plausible explanation is in agreement with additional previous studies, which also reported no defects with hand instrumentation (Yoldas *et al.* 2012, Ashwinkumar *et al.* 2014).

The effectiveness of nickel titanium rotary instruments during debridement is influenced by the rotations of the instrument in the canal; thus the greater the number of rotations in the canals, the greater the chance of dentinal defects (El Nasr & El Kader, 2014; Bier *et al.* 2009). As all NiTi rotary instrument groups in this study presented with dentine damage, rotational force during preparation conceivably contributed to dentine damage.

In the present study, both rotary and reciprocal motion nickel titanium instruments resulted in significantly more dentine damage compared to hand instrumentation ($p<0.0001$). This result could be related to the contact areas between the rotary instrument and the root dentine. Nickel titanium rotary instrumentation can create additional stress within the canal; and with increased rotations (as required with rotary instrumentation) the rotational force that is applied to the root canal walls may contribute to dentine damage (Milani *et al.* 2012, Al-Zaka, 2012). These contacts result in momentary stress foci which could cause dentine damage on the root dentine surface. Greater contact stress levels are present in the root canal during NiTi instrumentation, which are influenced by the mechanical behaviour of files. As a result thinner dentine may weaken the root structure, and there will subsequently be an increased risk of fracture of roots (Kim *et al.* 2010). According to Cicek *et al*. (2015) and Priya *et al.* (2014), greater volume of dentine is removed during canal preparation with nickel titanium rotary instruments due to rotational forces, and this may have an effect on the incidence of dentine damage. The results of the present study maintained the conclusion that nickel titanium rotary instrumentation damages the root dentine as determined by Shemesh *et al.* (2009).

As determined by Burklein *et al.* (2013) the incidence of dentine damage could possibly be connected with nickel titanium rotary preparation techniques (reciprocating motion, single instruments, multiple instruments and combination of different techniques). Reciprocating motion allows for a more centred preparation compared to rational motion (Berutti *et al.* 2012b). Berutti *et al.* (2012b) reported the reciprocating motion of WaveOne aids with stress release as the file progresses down the canal. NiTi systems with both reciprocal and rotational motion were investigated in this study; and both motions resulted in dentine damage, although there was no statistical significance noted between the reciprocal and rotational systems when looking at the total number of roots with dentine damage.

Burklein *et al.* (2013) found that reciprocating motion produced more incomplete cracks in the apical area of roots, which is not consistent with the findings of the present study. Conversely, Ashwinkumar *et al.* (2014) found that canal preparation with rotational systems resulted in more dentine damage than reciprocating systems; and Liu *et al.* (2013) found that the multiple file system resulted in more damage in the form of apical cracks, when compared to a single file system, and a reciprocating system. In the present study there was not a statistical significance between reciprocal motion and rotational motion.

With increased rotational speed, greater cutting efficiency is achieved. Studies by Capar *et al.* (2014a) showed that increased revolutions per minute (rpm), where 500rpm was compared to 250 rpm, less dentine damage was found with increased speed (rpm). In the current study, no statistical difference was noted between the rotary instrumentation using higher rpm (BT-Race™ and iRaCe™ at 800 and 600 rpm respectively) compared to the lower rpm (both WaveOne™ and ProTaper NEXT™ at 300rpm) when investigating the total amount of dentine damage. Differences were found at the root segmentation levels (3mm,6mm,9mm); and this will be discussed later in this chapter.

Dane *et al.* (2016) found that rotary instrumentation with a lower torque caused less dentine microcracks than higher torque settings. In the present study BT-Race and iRaCe both have a torque of 1,5 Ncm; compared to ProTaper NEXT and Wave One which both have a torque of 2 Ncm. The difference in this study when looking at total amount of dentine damage at root segmentation levels could be attributed to this.

The extent of dentine damage may be influenced by the tip design, cross-section geometry, constant or progressive taper type, constant or variable pitch, and flute form (Yoldas *et al.* 2012). Nickel-titanium instruments' properties and behaviour differ with each other in different rotary systems according to torsional and bending behaviour, cyclic fatigue and flexibility; thus all nickel-titanium rotary instruments do not have the same mechanical behaviour (Yoldas *et al.* 2012). As nickel-titanium rotary instruments have various design and functional features, the effect that these features could have on dentine varies between different manufacturer's systems (Kim *et al.* 2008). Topçuoglu *et al.* (2014) stated that nickel titanium rotary files with greater tapers can thus cause increased friction and stresses within the canals, compared to hand instrumentation with 0.02 taper. The differences between the various nickel titanium instruments resulting in the incidence of dentine damage may be due to the preparation technique and the cross-sectional design of the instruments (Burklein *et al.* 2013). Consequently, the design and taper of the instruments and the motion of instrumentation were the variables in the present study.

All four groups had dentine damage in the coronal area, and this is in agreement with previous studies by Priya *et al.* (2014); where dentine damage was greater in the coronal area. In the coronal area (9mm from apex), WaveOne had 16,67% incidence of dentine damage, ProTaper NEXT had 26,67% dentine damage and iRaCe and BT-Race had 13,33% and 26,67% respectively.

In the present study, the amount of dentine damage caused by ProTaper NEXT™ decreases from the coronal third to the apical third; this could be attributed to the variable taper of the instrument. The dentine damage seen in the ProTaper NEXT nickel-titanium system was 26,67% in the coronal third, 20% in the middle third and 13,33% in the apical third. The ProTaper NEXT files have an off-centred rectangular cross-section and promotes a swaggering motion in the canal. It decreases taper lock and screw effect. At any given time only two points of the file's cross section will make contact with the root canal wall (Capar *et al.* 2014a). This may be the reason for decreasing damage in root canal from the coronal third to the apical third.

In the BT-Race™ group 6,67% of dentine damage was noted in the apical third. This could be attributed to the BT2 instrument having no taper, while BT3 has a taper of 4%. ProTaper NEXT™ has a variable taper (X1 has 0.04 taper, X2 has 0.06 taper and X3 has 0.07taper), iRaCe™ has a 4% taper and WaveOne™ has a taper of 8%, and this may have contributed to the difference of dentine damage in the apical third between the different rotary systems.

Rotary instruments with large tapers may cause more complete and incomplete dentinal cracks (Priya *et al.* 2014, Milani *et al.* 2012). Decreasing the taper sequence of finishing files, increases the strength of the files but also increases the stiffness at the instruments tip and larger and greater tapered instruments should be used with caution (Yang 2006). The taper of rotary files could be a contributing factor in the generation of cracks due to increased stress concentration on root canal walls caused by the tapered files (Yoldas *et al.* 2012; Adorno *et al.* 2009). As stainless steel files have 0, 02 taper and no rotational force, the lack of dentine damage in the stainless steel group compared to NiTi rotary instrumentation groups could be attributed to this.

As greater amounts of dentine are removed, the risk of dentine damage and root fracture becomes greater. Hin *et al.* (2013) found that a non-tapered instrument, showed no dentine damage on the root wall. As a greater tapered instrument is used, the incidence of dentine damage could be increased and larger files could possibly result in more pressure and damage to root canal walls (Hin *et al.* 2013).

The stiffness of a file is related to cross-section, size, taper, method of manufacture, and material of the instrument (El Nasr & El Kader 2014). The differences between the instruments tested regarding the incidence of dentinal damage may be due to the cross-sectional design of the instruments. ProTaper NEXT has a modified rectangular cross-section, WaveOne has a modified convex triangular cross-section and both BT-Race and iRaCe have a triangular cross-section. The cross-section influences the behaviour of the files in canal, which may result in dentine damage as contact with root dentine occurs.

Both ProTaper NEXT and WaveOne are manufactured with M-wire to increase strength and resist cyclic fatigue. Any difference in dentine damage between ProTaper NEXT and WaveOne compared to BT-Race and iRaCe at the different segmentation levels could be attributed to the flexibility of the M-wire when compared to nickel titanium.

As WaveOne is a single file system and less time is used during preparation (Ashwinkumar *et al.* 2014), the difference at segmentation levels could be attributed to a single file used for canal shaping compared to multiple files used for canal shaping in ProTaper NEXT, BT-Race and iRaCe preparations. Preparation with WaveOne on thinner dentine would be useful (Berutti *et al.* 2012b), so any root sample that had thin canal walls would have a positive outcome in the Wave One sample group.

BT-Race features a booster tip which is designed to allow files to follow curvature in the root without undue stress on instruments and root canal walls, while keeping files centred in the root canal. This allows canals to be instrumented to the correct biological size. The booster tip may be a plausible factor to explain the difference in apical dentine damage compared to the other sample groups. iRaCe features a rounded safety tip which allows for canal centering and guidance in the root canal, which may aid in the explanation of differences between sample groups.

The ability of dentine to resist fracture is an important structural characteristic (Imbeni *et al.* 2003), as dentine forms the barrier to prevent crack propagation from enamel (Giannini *et al.* 2004, Imbeni *et al.* 2003). Thus, any potential factors that could result in changes to dentine structure will potentially result in the incidence of dentinal damage. The use of root canal irrigants and chelating agents in combination with nickel titanium rotary instrumentation therefore has a potential effect on root dentine. In this study, sodium hypochlorite solution at a concentration of 5,25% and 17% EDTA (Glyde™) was used during root canal debridement and shaping. Studies have shown that sodium hypochlorite alters the mechanical properties of root canal dentine during canal preparation. A higher concentration of sodium hypochlorite solution significantly reduces the modulus of elasticity and flexural strength of root dentine (Adorno *et al.* 2013, Kansal *et al.* 2014).

The use of sodium hypochlorite as a root canal irrigant is the most common choice during root canal treatment, but the concentration of the sodium hypochlorite varies due to the clinician's preference (Siqueira *et al.* 2000). The bactericidal effect of sodium hypochlorite is the positive factor in determining the choice of irrigant used during root canal treatment (Wong and Cheung, 2014). However, irrigants like sodium hypochlorite can alter the surface characteristics of dentine (Karunakaran *et al.* 2012). The concentration and contact time of sodium hypochlorite on dentine microhardness is a factor to consider when looking at the effect on dentine, especially in terms of dentinal damage. Greater efficacy of sodium hypochlorite is observed at higher concentrations of sodium hypochlorite, and thus it is tempting in the clinical situation to use a higher concentration of sodium hypochlorite. Slutzky-Goldberg *et al.* (2004) found that a higher concentration of sodium hypochlorite resulted in a greater decrease in dentine microhardness compared to a lower concentration (Slutzky-Goldberg *et al.* 2004). The contact time of sodium hypochlorite (and especially at higher concentrations) and dentine, will have a decalcifying effect on the dentine microhardness (Butt and Talwar, (2013), Zaparolli, (2012).

Butt and Talwar, (2013) and Aslantas *et al.* (2014), found a decrease in dentine microhardness at sodium hypochlorite concentrations of 5,25% and 6% respectively. When investigating flexural strength, it has to be kept in mind that a decrease in flexural strength could be produced by exposure to sodium hypochlorite, and at higher concentrations greater decrease of flexural strength is observed (Marending *et al.* 2007a). A 3-point bending test is used to determine changes to the modulus of elasticity, and a study by John *et al.* (2013) found that higher concentrations of sodium hypochlorite resulted in a decrease in the modulus of elasticity (John *et al.* 2013). The potential for a decreased modulus of elasticity in the present study also exists; although it was not investigated in this study. Pop *et al.* (2015) used 1% sodium hypochlorite and this limited the effect of sodium hypochlorite on dentine, so the dentine damage could be ascribed mostly to the mechanical shaping only. It can be deduced that an increased concentration of sodium hypochlorite may have an effect on dentine structure and consequently induce dentine damage.

In this study sodium hypochlorite was used at a concentration of 5, 25% and thus the modulus of elasticity, mineral composition, flexural strength and microhardness of dentine could have been influenced by the irrigant and chelating agent used. The high concentration of sodium hypochlorite used could have contributed to the dentine damage observed.

Sodium hypochlorite reduces microhardness (Adl *et al.* (2015); Ari *et al*. (2004)), as well as the elastic modulus and flexural strength (Sim *et al.* 2001) of human root dentine. Although many studies have been conducted to evaluate the dentine damage caused by various instruments, very few studies have studied the effect of different irrigation solutions on dentine damage. Further studies are required to evaluate whether different endodontic irrigation solutions are associated with an increased incidence of dentine damage.

Once root canal debridement is started, a smear layer forms on the root canal wall and the removal of this smear layer contributes to the success of root canal treatment (Kalluru *et al.* 2014). An irrigation regimen of EDTA and sodium hypochlorite is recommended for complete removal of the smear layer so that both the inorganic and organic components are removed (Marending *et al.* 2007b, Tartari *et al*. 2013). EDTA is used as a chelator to remove the inorganic component of the smear layer, while sodium hypochlorite removes the organic component. The concentration of EDTA is preferred at 17% (Aranda-Garcia *et al.* 2013). Time of exposure with EDTA also has an effect on the dentine decalcification, so increased exposure will result in greater decalcification of dentine (Pérez-Heredia et al. 2008). As chelating agents change the calcium and phosphorous ratio, it may have an effect on the dentine microstructure (Tuncer *et al.* 2015). With the combination of EDTA and sodium hypochlorite, a decrease in dentine microhardness is observed, and Taneja *et al.* (2014) found that exposure of dentine to a combination of 5,25% sodium hypochlorite and 17% EDTA caused a reduction in dentine microhardness. Belli *et al*. (2014) found that 17% EDTA also resulted in increased stresses in root dentine which may contribute to risk of fracture. Although dentine microhardness was not investigated in this study, the same combination and concentration of sodium hypochlorite and EDTA was used, and this could potentially have had an influence on the dentine microhardness which could contribute to the confirmation of dentine damage observed.

Shortcomings of the study

Negative control/ sectioning method: All roots were inspected under a stereomicroscope for the presence of dentine damage (cracks and fractures), in order to eliminate any roots that may have had dentine damage before the start of the experiment. However, internal damage may have been present and these would not have been visible on the outer surfaces of the roots. Forces during extraction or during root sectioning procedures may contribute to observation of dentine damage. In this study, dentine damage was observed in all nickel titanium rotary instrumentation groups (WaveOne™, ProTaper NEXT™, iRaCe™, BT-Race™) and not in the stainless steel group and the control group, which was not instrumented. This would imply that the sectioning method did not induce dentine damage, so it may be concluded that the dentine damage was likely as a result of the different rotary preparation procedures alone. At present, no nickel titanium rotary preparation method is able to completely avoid dentine damage. The absence of dentine damage in the control group in this study is consistent with the findings of Priya *et al.* (2014), Bier *et al*. (2009) as well as Shemesh *et al.* (2009).

Variation in root canal anatomy: The cross-sectional anatomy of roots varies, and this may influence fracture predisposition of roots. The removal of dentine does not always result in increased fracture susceptibility, which may be inherent to the root and canal morphology and is often not within the control of the clinician (Sathorn *et al*. 2005). When interpreting the results, the observations by Sathorn *et al.* (2005) should be taken into account. It remains unclear whether dentine damage in the form of craze lines, or incomplete cracks may progress to complete fractures (Burklein *et al.* 2013).

Replication of clinical scenario: Although it is attempted to replicate the clinical scenario in the laboratory when conducting the study, it is impossible to completely replicate the clinical conditions. This may result in lack of correlation between the results obtained in these studies and the clinical situation (Burklein *et al.* 2013). Even though this in vitro study did not reproduce the complete clinical scenario, we can determine that NiTi rotary instrumentation does induce dentine damage during root canal preparation.

Storage method of dentine: The storage method of dentine specimens may also have a bearing on outcomes, particularly when the mechanical properties of dentine are studied (Burklein *et al.* 2013). All specimens were stored in distilled water and kept hydrated during experimental procedures in order to avoid any artefacts.

Simulation of the periodontal ligament (PDL): Poly vinyl siloxane putty was used around root segments to simulate the periodontal ligament to assist in dispelling some vertical applied force during canal preparation; to mimic the clinical situation as far as possible. This was in agreement with previous study methodologies, namely Hin *et al.* (2013) and Milani *et al.* (2012). Arias *et al.* (2014) indicated that the lack of periodontal ligament and bone around the teeth could change the internal forces affected on root structure during shaping procedures. Also, the clinical scenario during the extraction of teeth could impact results as forces used during extraction are an unknown entity. Possible differences in results between this study and previous studies could be related to the lack of periodontal ligament imitation.

However, to replicate the periodontal ligament is very difficult and it stands to reason that by attempting to replicate the periodontal ligament; may result in simulated force distributions as well. The clinical picture would be very different as the periodontal ligament would further distribute forces (Shemesh *et al.* 2009).

CHAPTER 6: CONCLUSIONS

Within the limitations of the present study, the following can be concluded:

1. The null hypothesis was rejected.
2. All NiTi rotary instrumentation systems studied resulted in dentine damage in varying degrees.
3. Stainless steel instrumentation did not result in dentine damage.
4. Differences were found between NiTi rotary systems w.r.t the amount of dentine damage and also the specific third of the root (apical, middle or coronal).

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ADDENDUM A

Office of the Deputy Dean
Postgraduate Studies and Research
Faculty of Dentistry & WHO Collaborating Centre for Oral Health

UNIVERSITY OF THE WESTERN CAPE
Private Bag X1, Tygerberg 7505
Cape Town
SOUTH AFRICA

Date: 8th November 2013

For Attention: Dr S Ahmed
Restorative Dentistry

Dear Dr Ahmed

STUDY PROJECT: Evaluation of dentine damage following NiTi rotary canal preparation

PROJECT REGISTRATION NUMBER: 13/10/72

ETHICS: **Approved**

At a meeting of the Senate Research Committee held on Friday 8th November 2013 the above project was approved. This project is therefore now registered and you can proceed with the study. Please quote the above-mentioned project title and registration number in all further correspondence. Please carefully read the Standards and Guidance for Researchers below before carrying out your study.
Patients participating in a research project at the Tygerberg and Mitchells Plain Oral Health Centres will not be treated free of charge as the Provincial Administration of the Western Cape does not support research financially.

Due to the heavy workload auxiliary staff of the Oral Health Centres cannot offer assistance with research projects.

Yours sincerely

Professor Sudeshni Naidoo

Tel -27-21-937 3148 (w); Fax -27-21-931 2287 e-mail: suenaidoo@uwc.ac.za

ADDENDUM B

ORIGINALITY REPORT

%15	%9	%15	%3
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	crobm.iadrjournals.org Internet Source	%1
2	www.ada.org.au Internet Source	%1
3	www.jcd.org.in Internet Source	%1
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