IN VITRO ANTIBACTERIAL ACTIVITY OF THREE ROOT CANAL SEALERS AGAINST *ENTEROCOCCUS FAECALIS*

Mini thesis submitted in partial fulfilment of the requirements for the

Masters' degree in Restorative Dentistry

MSc Restorative Dentistry

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SUMMARY

The goal of root canal treatment is to eradicate microorganisms in the root canal system of the tooth. However; it has been found that no method of tooth preparation is efficient in eliminating all microorganisms present in root canals. Therefore, obturation materials with anti-microbial properties are advantageous, so that any residual microorganisms in the root system of the tooth can be eliminated.

Therefore, the aim of the study was to assess the antimicrobial effect of 3 endodontic sealers: Sealapex™, EndoREZ™ and Guttaflow bioseal™ against *Enterococcus faecalis.*

The Direct Contact test was used to assess the antibacterial effect of the 3 sealers against *E. Faecalis.* Sample size was n=50 per sealer. The survival of bacteria was assessed by culturing aliquots of 100 µL onto Tryptic Soy Agar plates after 10-fold serial dilutions. After incubation for 24 hours at 37ºC, colonies on the plates were counted, and the CFU/mL was calculated. The experiments were performed in triplicates. Testing after setting enabled the assessment of the antimicrobial activity of aged sealers after 7 days, 14 days, 21 days and 28 days.

All 3 sealers displayed evidence of antibacterial activity against *E. Faecalis* with various degrees of antibacterial activity at day 0, 7, 14, 21 and 28.

Antibacterial activity was displayed by all 3 sealers against *E. Faecalis* which will have an effect on entombed bacteria.

DECLARATION

I, Tafadzwa Fraderick Mukorera do hereby solemnly declare that the mini thesis "In vitro antibacterial activity of three root canal sealers against *Enterococcus faecalis*" which I do here submit electronically to the University of the Western Cape in partial fulfilment of the requirements for the degree MSc (Restorative Dentistry); is my original work and has neither been submitted for any academic award to this University nor to any other institution of higher learning.

SIGNATURE  Date: 09 Dec 2020

DEDICATION

This work I dedicate it to my late parents who went to be with the Lord in the course of my studies. May their souls rest in eternal peace.

Job 1 vs 21 "…...The Lord gave and the Lord has taken away. Just as it pleased the Lord, so has it been done. Blessed be the name of the Lord". Amen

I also dedicate it to my beautiful wife Melissa and my lovely energetic son Liam.

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LIST OF ABBREVIATIONS

DNA – Deoxyribonucleic acid

QMIX- solution containing chlorhexidine and EDTA

EDTA- ethylenediaminetetra-acetic acid

pH – potential of hydrogen

3D – three dimensional

µm – micrometer.

LAB – Lactic acid bacteria

º C – Degrees Celsius

VBNC – Viable but non cultivable

MTA – Mineral trioxide aggregate

ADT – Agar diffusion test.

DCT – Direct contact test

CFU/ml – colony forming units per millilitre

ATCC – American type culture collection

PBS – Phosphate buffered saline

RLU – Relative light units

CHAPTER 1: LITERATURE REVIEW

1. Root canal Treatment

The overall aim of endodontic treatment is to treat an infected tooth, restore form and function of the chewing apparatus and to promote oral health (Peters 2004). Root canal treatment involves the optimum shaping and debridement of the canal system to gain a tapered centered canal ensuring that there is no transportation of the apex. This allows for optimal adequate cleaning through irrigation and placement of intracanal dressing (Torabinejad & Walton 2009). Complete removal of microbes from the root canal system of the tooth remains the overall goal of endodontic treatment (Hasheminia *et al.* 2017). There is evidence, however, that no single method of root canal preparation is capable of completely eradicating the microbial population in root canal systems (Dalton *et al.* 1998). Thus, materials used for obturation which have antibacterial properties are advantageous so that any residual microbial population remaining in the root canal system can be destroyed (Wainstein *et al.* 2016).

1.1 Role of root canal preparation in eradicating microbes

Root canal preparation aims to mechanically debride the canals with the subsequent creation of a space for distribution of antimicrobial substances (Zehnder 2006). Root canal preparation consists of two intimately related procedures namely mechanical preparation and disinfection, yet, it remains an essential component of endodontic treatment (Darcey *et al.* 2015). Several methods and instruments have been developed for root canal preparation. Nickel titanium represents the latest metallurgy in endodontics for hand, rotary and reciprocating files. The number of files needed to complete endodontic treatment has been gradually reduced over time. Single file systems have also entered the market, theoretically making the possibility of completing root canal preparation with a single file. However, it is important for the clinician to select a system that one can use for effective shaping of the canal (Darcey *et al.* 2015). The success of endodontic treatment does not seem to be influenced by the method of instrumentation used; either hand or rotary (Ng *et al.* 2011).

While a significant portion of microorganisms in dentine are removed during instrumentation some areas within the canal remain untouched partly due to the complexity of the root canal system that encompasses lateral canals, fins, anastomoses and ramifications (Darcey *et al.* 2015). Accordingly, in one study up to 53% of the canal walls were untouched by instrumentation (Peters *et al.* 2001). Utilizing new instruments like Self Adjusting File (SAF), TRUshape and XP-endo, that can deal with irregular canal anatomy is often advisable. Studies have shown that SAF in oval canals leaves 6 – 35% un touched areas. Entombing bacteria in unprepared sites is not reliable and predisposes to poor treatment outcome (Siqueira *et al*. 2018). Therefore, the new order seems to suggest that mechanical preparation should shape the canal to facilitate irrigation of the canal (Hubscher *et al*. 2003).

Irrigants used in endodontics should be able to destroy micro-organisms, neutralize endotoxin and remove organic tissue components (Hubscher *et al.* 2003). A variety of substances have been used as irrigants including but not limited to chlorhexidine, sterilox, sodium hypochlorite, EDTA and QMIX. Sodium hypochlorite possesses properties of an ideal antimicrobial and is still regarded by the profession as the gold standard irrigant (Holliday & Alani 2014). The method of action of sodium hypochlorite is related to its high pH which denatures proteins and the hydroxyl ion which destroys the bacterial lipid membrane, DNA amongst other things (Darcey *et al.* 2016a). On the other hand, the chloride ion is responsible for dissolving proteins through breakage of peptide bonds. However, no difference between irrigants was found according to a Cochrane systematic review (Fedorowicz *et al.* 2012). Even though a significant number of microorganisms can be eradicated from the canal by irrigants alone or in combination with mechanical procedures, cultivable bacteria has been isolated in canals after root canal preparation before obturation (Haapasalo 2012). One such bacterial species that has been isolated following shaping and disinfection of the canals is *Enterococcus faecalis*. Research done by Haapasalo *et al.* (2012) found that 1% sodium hypochlorite could not kill *E. faecalis* in the presence of dentin. In a study by Bystrom and Sundqvist (1983), necrotic root canals could not be rendered free of bacteria using different concentrations of sodium hypochlorite and EDTA (Bystrom & Sundqvist 1983). Working with *E faecalis*, Rocas *et al.* (2004) found similar results. It is clear from these studies that even with irrigation, bacteria can be difficult to eradicate from the canals. These studies found similar results to the study by Dalton *et al.* (1998) which earlier postulated that no root canal preparation method is capable of eradicating all the bacteria (Rocas *et al.* 2004; Dalton *et al.* 1998).

1.2 Obturation

Following the complete debridement of the root canal system, obturation needs to be completed with non-toxic materials to ensure a full 3D obturation of the root canals (Kokorikos *et al.* 2009). 3D obturation should aim to provide a fluid tight seal that prevents reinfection of the canals (Darcey *et al.* 2016b). The fluid tight seal is composed of coronal seal, lateral seal and apical seal; with the apical seal terminating within 1 mm of the radiographic apex. A positive correlation has been found between a good root canal seal and a positive outcome of the endodontic treatment (Ng *et al.* 2008). To obtain a fluid tight seal, obturation is routinely performed with the combination of a solid core material and an endodontic sealer (Garcia-Molina *et al.* 2005). Solid gutta percha is usually the core used in endodontic obturation. Different obturation techniques have been advocated although cold lateral and warm vertical compaction are more common (Khalil *et al.* 2016). However, while the goal is to produce a fluid tight seal in obturation, micro leakage studies show evidence of leakage in all techniques (Wu & Wesselink 1993).

2. Endodontic Infections

Endodontic infections can broadly be categorized into intra radicular or extra radicular infections (Sakamoto *et al.* 2006). Microorganisms colonizing the root canal system cause an intra radicular infection, which is further subdivided into groupings according to the duration the microorganisms have entered the pulp chamber

• Primary or initial infection results when microorganisms enter and colonize non vital pulpal tissue (Siqueira & Rocas 2016).

• Secondary infection is when microorganisms that were not part of the primary infection are then introduced into the canals of the tooth during endodontic treatment (Siqueira & Rocas 2016).

• Recurrent persistent infection results when the microbial population in the primary or secondary infections resists intracanal procedures (manual and medicinal) and are able to survive in the treated root canal (Siqueira & Rocas 2016).

Extra radicular infection in turn is a result of the colonization by microbes of the periradicular tissues, which is usually as a consequence of intra radicular infection. Extra radicular infections

may be conditional on the intraradicular infection, or it can be completely independent thereof (Siqueira & Rocas 2016).

Primary intra radicular infection is as a result of necrotic pulp tissue infection. Untreated carious lesions are a predisposing factor to the development of apical periodontitis. Initial infections comprise of a multispecies community of bacteria dominated by anaerobes (Blome *et al.* 2008; Sundqvist 1990). The concentration and amount of bacterial species and cells determine the size of the apical periodontal lesion (Vianna *et al.* 2006). Prevalent bacterial species found in initial infections include: gram-negative bacteria (*Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Pyramidobacter, Campylobacter, Veillonella*) and gram-positive (*Parvimonas, Filifactor, Pseudoramibacter, Streptococcus, Propionibacterium*) (Gomes & Herrera 2018; Saito *et al.*2006). During different phases of root canal infection, certain species may dominate over other bacterial species. The change in the microbial population makeup is most likely due to changes in the environmental conditions, especially oxygen tension and the availability of nutrients. Facultative bacteria dominate in the initial infectious stage, and as there is depletion of oxygen within the root canal system obligate anaerobes start increasing (Gomes & Herrera 2018; Sakamoto *et al.*2006).

Survival of bacteria in the root canals depends on the nutrients supplied by:

- Necrotic pulpal tissue
- Proteins and glycoproteins obtained from tissue fluids and exudate that leach into the canal system via apical and lateral foramina
- Saliva and its components that penetrate the root canal coronally
- Metabolic products from other bacteria (Siqueira and Rocas 2016).

The purpose of root canal mechanical preparation is to remove all pulpal contents and the eradication of any infection present (Goldberg *et al*. 2008). Once debridement is completed, adequate obturation and coronal restoration placement is necessary to prevent any site of entry for bacteria as well as entombing any residual bacteria to prevent the proliferation of any remaining micro-organisms (Young *et al.* 2007). Apical periodontitis is caused by microbial infection of the pulp complex. Colonized root canals, including the non-vital pulp tissue acts as a harbor for the

microbes which grow in biofilms, accumulated masses and co-aggregates which are sealed off by the protective extracellular matrix (Nair *et al.* 2005).

The point of entry for microbes into the pulp is from the typical oral microbial population usually via the extension of a carious lesion from the tooth crown; dentinal tubules are opened enabling access to the bacterial population (Baumgartner & Falkler 1991). The dentino-pulp complex is usually a sterile environment, and invasion with microorganisms only occur when there is a breach. Examples of this may be due to caries, trauma and/or restorative treatment. During endodontic intervention, the potential for entry of microorganisms also exist (Sjogren *et al.* 1997).

2.1 Bacterial species in endodontically treated root canals

Persistent intra radicular colonization is a result of bacteria that resist cleaning and disinfection of the canal thereby continuing to survive in obturated canals. These bacteria can be the remaining population of primary or secondary infections (Waltimo *et al.* 2005). The growth of the microbial population in canals can occur due to root canals that were missed, dislodgement of coronal or intermediate restorations and inadequate isolation of working area. Certain microbial species can endure under harsh conditions, and lack of nutrition is one of these conditions (Figdor & Sundqvist 2008). During mechanical debridement, certain bacterial species may resist the biomechanical procedures. Coronal leakage (via intermediate or final restorations) allows microbes to re-enter the root canal system. As such, these microorganisms can survive and continue to multiply within the root canals. There is a distinct difference with the microbial population between initial and secondary infections. Bacterial genus of *Peptostreptococcus, Lactobacilli, Actinomyces,* and *Enterococci* are positively identified in secondary infected root canals (Gomes *et al.* 2004). One of the main causes of endodontic failure is attributed to secondary infections (Waltimo *et al.* 2005). Various studies have identified *E. faecalis* as one of the most frequently observed bacteria in obturated root canals with an incidence of up to 90% of cases (Sedgley *et al.* 2006; Pinheiro *et al.* 2003). Dentinal tubule diameter is measured at approximately 0.9µm which is compatible with the cell diameter for common bacteria (0.2 – 0.7µm). In non-vital teeth, bacterial invasion occurs at a swift rate, conceivably due to the absence of host defense mechanism (Pinheiro *et al.* 2003).

Both persistent and secondary infections display various clinical symptoms, including:

- recurrent exudation

- persistent symptoms
- inter-appointment pain and flare-ups
- endodontic treatment failure, which is demonstrated by post treatment apical periodontitis lesion (Siqueira & Rocas 2016).

The bacterial population in obturated canals with apical periodontitis display a decreased variation when compared to primary infection (Siqueira 2001). Several culture and molecular biology research projects concluded that *E. faecalis* is the most recurrent species in endodontically obturated teeth, with an incidence up to 90% of cases (Rocas *et al.* 2004; Pinheiro *et al.* 2003; Molander *et al.* 1998). *Enterococcus faecalis* in obturated canals can be thought to be a secondary invader capable of colonizing the canal and resisting treatment (Siren *et al.* 1997).

2.2 *Enterococcus faecalis*

Enterococcus faecalis belongs to the *Enterococcus* genus, which consist of catalase negative, gram positive, non-spore forming, facultative anaerobes. These microorganisms may present as cocci or chains (John *et al.* 2015). According to Health Protection Agency 2005, *enterococci* is in a class of microorganisms known as lactic acid bacteria (LAB), which yield bacteriocins. In culture *Enterococci* produce creamy whitish colonies (John *et al.* 2015).

The mechanism of action of *Enterococcus faecalis* infiltration is not known. However, it can survive in very severe conditions like extreme alkaline pH, and salt concentrations. They can propagate in the range 10 – 45 ºC (Flahaut *et al.* 1996). It exhibits a number of potent factors including:

- Aggregation substance (AS)
- Surface adhesins (SA)
- Sex pheromones
- Gelatinase
- Cytolysins (John *et al.* 2015).

However, the mechanism of action of *E. faecalis* infiltration is not known, the attachment of bacteria to the dentinal tubule walls happens early in the process (Love 2001). Adherence is facilitated by adhesins, which are specific bacterial cell surface receptors (Patti *et al.* 1994). Dentinal collagen type I is the prime substrate for precise attachment of *E. faecalis* to dentine. This

is achieved through Ace, a collagen binding protein and Spr, a serine protease (John *et al.* 2015; Van der Vyver *et al* 2014). Ace facilitates the binding of *E faecalis* to collagen type 1 (Hubble *et al.* 2003). After the initial attachment deeper penetration may seem to be a result of intra tubular cell growth rather than specific binding (Love & Jenkinson 2002). *Enterococcus faecalis* can colonize root canals in single infections and in obturated root canals exhibiting signs of persistent infection. *E faecalis* is isolated in 24 to 90 % of the positive cultures (Sonia 2013; Sundqvist *et al*. 1998). This may be due to the microorganism's ability to resist antimicrobial agents as well as the potential to adapt to a changing environment. This allows proliferation of the organism in the root canal system and may cause reinfection. *Enterococcus faecalis* binds to the dentine of root canals as described above resulting in formation of biofilms. The ecosystem of biofilms assists in resisting destruction by allowing the bacteria to become unaffected by phagocytosis, antibodies and antimicrobial measure. The antimicrobial resistance of this bacteria has been ascribed to the protective barrier provided by the extracellular polymeric matrix (Mallick *et al*. 2014). This bacterium penetrates dentinal tubules thus evading mechanical instrumentation and chemical irrigation during endodontic treatment (Siqueira & Rocas 2016). *Enterococcus faecalis* also has the ability to enter a viable but non-cultivable (VBNC) state. This survival mechanism is utilized by some bacterial species when exposed to negative environmental settings (Lleo *et al.* 2005).

3. Root canal sealers

Effective endodontic treatment requires a fluid tight seal of the tooth, which is achieved by successful and complete obturation (Garcia- Molina *et al.* 2005; Ingle *et al.* 2002). Currently, the known method of endodontic filling involves a solid or semi-solid core such as gutta percha and an endodontic sealer. The core like gutta percha has no sealing ability and antimicrobial activity, therefore, endodontic sealers are required to obtain a hermetic fluid tight seal in the root canal. This is achieved through obturation of the lateral, accessory canals, voids, spaces and anomalies between gutta-percha and root dentine wall (Khandelwal & Ballal 2016). Some root canal sealers have antibacterial properties and may help to entomb the remaining bacteria after endodontic preparation. Antibacterial activity is one of the prerequisites of an ideal endodontic sealer (Mali *et al.* 2016). Other requirements of Endodontic sealers are shown in table 1 below

Should exhibit tackiness when mixed to provide good adhesion to the canal wall when set	Should establish a hermetic seal
Should be radio-opaque	Should be prepared in very fine powder so it can mix with liquid easily
Should not shrink on setting	Should not stain tooth structure
Should be bacteriostatic or at least not encourage bacterial growth	Should exhibit a slow set
Should not be soluble in tissue fluids	Should not irritate periapical tissue
Should be soluble in a common solvent if it is necessary to remove it	

Table 1. Grossman ideal properties of a sealer (Darcey *et al.* 2016b).

Contemporary endodontic sealers are grouped according to their chemical composition:

- Zinc-oxide eugenol based
- Calcium-hydroxide endodontic sealers
- Glass-ionomer endodontic sealers
- Resin-based
- Calcium silicate-based
- Silicone-based (Tomson *et al.* 2014)

3.1 Zinc-oxide eugenol based sealers

Historically zinc-oxide eugenol based sealers group has been used effectively. They show decreased setting time, shrinkage on setting and solubility. Their greatest advantage is antimicrobial activity (Camilleri 2015). These sealers are usually packaged as powder-liquid formulation; for example, Roth™ root canal cement; or a two-paste preparation such as Tubliseal™. These zinc-oxide eugenol materials may be resorbed if there is accidental extrusion into the periapical tissues (Tomson *et al.* 2014).

3.2 Calcium-hydroxide endodontic sealers

Endodontic sealers based on calcium hydroxide were developed for therapeutic activity. Traditionally, it was believed these sealers had antibacterial activity. However, this belief is not entirely supported by studies (Camilleri 2015). Sealapex™ and Apexit™ are the current sealers in this group. They have antibacterial properties, though less than zinc oxide eugenol-based sealers (Tomson *et al.* 2014).

3.3 Glass-Ionomer endodontic sealers

Glass-ionomer sealers bond to dentine and are more soluble in oral fluids. They display less antibacterial activity than zinc oxide eugenol and calcium hydroxide-based sealers. Ketac Endo™ is an example of a glass-ionomer based sealer. The glass-ionomer sealers adhere to dentine and are difficult to remove during endodontic retreatment (Tomson *et al.* 2014).

3.4 Resin based sealers

These sealers can be epoxy resin based or methacrylate based. They provide adhesion and do not contain eugenol (Camilleri 2015).

3.4.1 Epoxy resin-based sealers

An epoxy resin–amine based system that is common in the market is AH Plus™ (two paste system). AH Plus™ was preceded by AH-26™ which suffered a major setback due to its release of formaldehyde during setting. Formaldehyde is toxic to tissues. AH Plus™ does not release formaldehyde (Camilleri 2015). AH Plus™ has antibacterial properties, excellent sealing ability due to its ability to adhere to dentine. However, it does not resorb easily if extruded through the periapical tissues. It has a working time of close to 4 hours (Tomson *et al.* 2014).

3.4.2 Methacrylate resin-based sealers

This group has seen four generations in commercial use. The first generation was Hydron™ which has since been removed from the market. EndoREZ™ is a second-generation bondable sealer which has hydrophilic properties, is non-etching and the use of dentine adhesive is not required. These sealers enable the formation of resin tags, which aids in retention and seal (once smear layer is removed). It does this by flowing into dentinal tubules and any lateral or accessory canals (Tomson *et al.* 2014). EndoREZ™ can effectively seal the canals even when applied to moist intraradicular dentin (Camilleri 2015). There are two ways of applying EndoREZ™, either with a

conventional gutta-percha cone or a specific EndoREZ™ points (resin-coated gutta-percha). Using EndoREZ™ points is recommended as they are compartible with the EndoREZ™ sealer. One study has recorded a 10-year endodontic outcome success rate of 92.1% after one visit endodontic therapy using gutta percha and EndoREZ™ sealer (Zmener & Pameijer 2012). The third generation of these sealers are self-etching and became popular after the introduction of Resilon™. RealSeal™ (SybronEndo), is a third generation methacrylate resin based sealer which comes as a single-bottle system incorporating self-etching primers (Camilleri 2015). The fourth generation are self-adhesive sealers which combine etchant, primer, and a sealer into an all-in-one self-etching, self-adhesive sealer. The advantage of this is that it may reduce application time and also decrease procedural errors that may occur during the bonding steps. MetaSEAL™ is the first fourth-generation self-adhesive dual-curable sealer to be introduced in the market (Lawson *et al.* 2008). Realseal SE™ is a fourth generation dual cure self-etching methacrylate based sealer that bond to Resilon core and dentin to produce a monoblock. However, the bond strength is dependent on the irrigants used during chemo mechanical preparation. (Kim *et al.* 2009).

3.5 Calcium silicate based sealers

These endodontic sealers are based on mineral trioxide aggregate (MTA) and have recently been commercially available. Calcium silicate endodontic material have excellent sealing properties and biocompatibility. Bio Root RCS™, iRoot SP™, Smartpaste bio™ and MTA Fillapex™ are good examples (Tomson *et al.* 2014). These sealers have a strong resemblance to Portland cement, with tricalcium silicate and dicalcium silicate powder also included in calcium silicate sealers. MTA is a hydraulically active powder that contains a tricalcium silicate core, dicalcium silicate and a radiopaque agent, bismuth oxide (Camilleri 2015). Tricalcium silicate cements/sealers reacts with water to form a highly alkaline (pH of about 12) mixture during the setting reaction. This mixture contains a firm composite of calcium silicate and calcium hydroxide. Fillapex™, iRoot SP™ aka Endosequence BC sealer™, Endo CPM Sealer™ and MTA Plus™ are four tricalcium silicate sealers currently available to clinicians (Wang 2015).

3.6 Silicone based sealers

An example in this group is RoekoSeal™, a polydimethylsiloxane-based root canal sealer. This material has a low viscosity that enables it to flow within the root canal system and is biocompatible. RoekoSeal™ endodontic material still requires independent research as little is

known about it (Tomson *et al*. 2014). Coltene/Whaledent introduced GuttaFlow™ and GuttaFlow2™ which are cold flowable matrices that should be triturated during use. They consist of gutta-percha particles of less than 30 µm added to RoekoSeal™. The most recent of these materials is Guttaflow Bioseal™ (Ruiz-Liganares *et al.* 2019).

Type of the sealer	Example	Pros	Cons
Zinc oxide and eugenol	Pulp Canal Sealer *EWT* *Roth's Sealer* *Tubli-Seal* *Wach's Sealer*	Long history of use Will absorb if extruded Slow setting time Antimicrobial effect Radio-opaque	Shrinkage on setting Soluble Can stain tooth structure May negatively affect bonding of core materials
Calcium hydroxide	*Apexit* *Apexit Plus* *Sealapex*	Antimicrobial effect (unproven) Radio-opaque	Soluble May weaken dentine
Glass ionomer	*Activ GP* *Ketac-Endo*	Dentine-bonding properties No antimicrobial properties	Hard to remove in retreatment Minimal antimicrobial effect
Resin	*AH-26* *AH Plus* *Diaket* *EndoREZ* *Epiphany* *RealSeal*	Long history of use Adhere to the wall Some can adhere to the core Do not contain eugenol Slow set	Some release formaldehyde when setting Chlorhexidine as an irrigant can reduce their bond strength May not bond any more effectively than conventional sealers
Silicone	*GuttaFlow* *RoekoSeal*	Triturated Long working time Fills canal irregularities with consistency Biocompatible	Expand slightly on setting Setting time is inconsistent Setting time gets delayed by sodium hypochlorite
Bioceramic	*SmartSeal* *SmartPaste Bio*	Hydrophilic Does not shrink on setting Biocompatible Antimicrobial properties	Minimal supporting clinical data Questions raised over ease of removal for retreatment

Table 2. Commercially available sealers (Darcey *et al.* 2016b).

As previously mentioned, an ideal root canal sealer should exhibit antimicrobial properties. Mechanical manipulation and disinfection of the root canals reduces the bulk of microorganisms but complete eradication is nearly impossible. Endodontic sealers with antimicrobial properties may have a positive effect on the outcome of endodontic treatment (Goldberg *et al.* 2008).

4. Methods of assessing antimicrobial activity of endodontic sealers

Investigation of the antibacterial effect of endodontic sealers has mostly been done using the Agar diffusion test (ADT) or the Direct contact test (DCT). Zhang *et al.* (2009) modified the direct

contact method to be able to test the bactericidal effect of root canal sealers which became known as the modified direct contact test (Zhang *et al* 2009).

4.1 Agar diffusion Test (ADT)

The Agar diffusion test involves inoculating agar plates with the test microorganism, then the endodontic sealer on filter paper discs (approximately 6mm diameter) are placed on the agar surface. After incubation, the method assumes that the endodontic sealer may diffuse into the inoculated medium and inhibits growth of the test microorganism. The antimicrobial activity is then evaluated by measuring the diameters of the inhibition growth zones (Balouiri *et al.* 2016). ADT has been used for a long time to evaluate the antibacterial activity of root canal sealers. Melek *et al.* (2016) used ADT to determine the antibacterial activity of root canal sealers. In their study they found Smartpaste Bio™ to have the widest inhibition zone at all time frames (Melek *et al.* 2016). However, the agar diffusion test is not without limitations as noted by Alshwaimi *et al.* (2016) when they compared the ADT and the DCT. Whilst performing the ADT, the examiner needs to be cognizant of the following factors: temperature of the medium, root canal sealer molecular weight together with the solubility of the sealer. The agar medium may have an influence on the results. If the agar medium encourages slower growth of microbial specimens, it may lead to false readings of antimicrobial effect (Alshwaimi *et al.* 2016). Due to the reasons cited above some researchers no longer recommend to use ADT as a method to test the antibacterial effect of root canal sealers. On the contrary, other researchers still use it. In a recent study Dalmia *et al.* (2018) used ADT to measure the antibacterial efficacy of AH Plus™, Tubliseal™, Sealapex™ and MTA Fillapex™. In that study Sealapex™ had the biggest zones of inhibition at 24, 48 and 72 hours resulting in the authors concluding that Sealapex had the highest antibacterial efficacy against *E. faecalis* (Dalmia *et al.* 2018).

4.2 Direct contact test (DCT)

The Direct Contact Test method represents another way of assessing the antibacterial effect of endodontic sealers. This method of investigation was first introduced by Weiss *et al.* (1996) for the assessment of the antibacterial effect of root canal sealers and root-end filling materials. Due to limitations of ADT, many studies advocate using the Direct Contact method. This method involves testing the antibacterial activity of the root canal sealer when there is a direct connection between the material under investigation and the specific bacterial organism. This method is able

to measure antimicrobial activity, independent whether the material is soluble or can diffuse through the medium. This is a quantitative and reproducible assay which allows for the investigation of insoluble materials resulting in a standardized assay. Thus, this method produces reliable results (Weiss *et al.* 1996).

Two methods of evaluating the results of the DCT have been used extensively in endodontic literature. Traditionally, colony forming units (CFU/ml) has been used to assess the results of the DCT, while recently the use of a spectrophotometer is being employed (Baer & Maki 2010). Zhang *et al.* (2009) used an adapted version of the direct contact test. The study involved testing seven endodontic sealers against *E. faecalis*. An adaptation of the test was required so that the antibacterial effect of the endodontic sealers could be investigated. When employing this method, they noticed Fresh iRoot SP™, AH Plus™ and EndoREZ™ eradicated *E. faecalis* successfully. Sealapex™ also displayed antimicrobial activity against *Enterococcus faecalis*, close to 7 days after mixing (Zhang *et al.* 2009). Although several studies have been done to investigate the antibacterial activity of endodontic sealers, no material has been able to completely eradicate the entire bacterial population in endodontics. Newer endodontic sealers are promising and with more research the development of an effective endodontic sealer may be possible, where it eradicates most if not all bacteria in the root canal. Although the use of ADT is no longer suggested as an investigative antibacterial test, it is still used. With the Direct Contact test, standardization of technique is required for reliable results (Zhang *et al* 2009).

4.2.1 Evaluating antibacterial activity using the Direct contact test

The evaluation of the antimicrobial activity using the DCT can be done by counting colonies and calculating colony forming units (CF/ml) or by measuring the wavelength of turbidity (Alshwaimi *et al.* 2016).

4.2.1.1 Colony forming Units

After DCT, serial dilution procedure is done which will then be cultured on the agar. The colonies are then counted and eventually the colony forming units (CF/ml) calculated (Zhang *et al.* 2009).

4.2.1.2 Spectrophotometer

This way of evaluating DCT is based on turbidity and is referred to as a turbidometric assay. After DCT, a microtiter plate is incubated at 37°C in the spectrophotometer to measure the optical

density in each well. The absorbance measurements are then plotted to provide the microbial growth curves for each well in the microplate. The linear segment of the logarithmic growth curve represents bacterial growth rate (Goldberg *et al.* 2008).

In the present study DCT will be used and evaluated by calculating CF/ml after counting colonies.

5. Antimicrobial studies of root canal sealers against *E. faecalis*

5.1 Sealapex™

Sealapex™ is one of the calcium hydroxide based endodontic sealers. It is one of the most studied endodontic sealers (Alshwaimi *et al* 2016). The release of hydroxide ions and creation of an alkaline pH is responsible for the antimicrobial activity of Sealapex™. As the setting reacting takes place, the pH decreases and the efficacy of the endodontic sealer decreases (Mickel *et al* 2003; Fuss *et al* 1997; Bystrom & Sundqvist 1983). In a DCT study, Fuss *et al.* (1997) tested 2 calcium hydroxide sealers including Sealapex™ and Zinc-oxide eugenol Roth™ cement against *E faecalis*. Roth™ cement was potent against the bacteria in the 1st hour and at 24 hours of aging while Sealapex™ showed better activity in 7 days of aging. In a study using ADT and DCT, Sealapex™ was found not to have antibacterial activity when ADT was used while DCT antimicrobial activity was found after 60 minutes of aging (Poggio *et al.* 2017). Regarding calcium hydroxide based sealers, included studies in a systematic review showed conflicting results with some showing antibacterial activity while others showed no antibacterial activity. Therefore, the review noted that there was conflicting evidence regarding calcium hydroxide based sealers (Alshwaimi *et al.* 2016). Regarding Sealapex™ in particular, another systematic review pointed out that there was no difference in antimicrobial efficacy of Sealapex™ and AH- Plus™ against *E. faecalis.* However, the review noted that the evidence was poor due to high risk of bias of the studies considered (Parolia *et al.* 2020).

5.2 EndoREZ™

EndoREZ™ is a urethane dimethacrylate resin based endodontic sealer. In a study by Eldeniz *et al*. (2006) using ADT and DCT, EndoREZ™ did not show any antimicrobial activity in 24 hours, 48 hours, 7 days, and 10 days. Later in a similar study Farmakis *et al.* (2012) found similar results as they noted that EndoREZ™ exhibited 0 mm exhibition zone using ADT while no antibacterial effect was also found with DCT. Heyder *et al.* (2013) also concluded the same for EndoREZ™. However, in an earlier study EndoREZ™ was found to be bactericidal against *Enterococcus*

faecalis at 3 and 7 days after mixing (Zhang *et al.* 2009). In a recent systematic review moderate evidence was found regarding methylmethacrylate resin sealers showing no antimicrobial activity against *E. faecalis* (Alshwaimi *et al.* 2016).

5.3 Guttaflow bioseal™

Guttaflow bioseal™ is the successor of silicone based root canal sealers Guttaflow™ and Guttaflow2™. These are derived from polydimethylsiloxane with powdered gutta-percha and microsilver particles (Kapralos *et al.* 2018). Guttaflow bioseal™ being the latest material of the series constitutes of polydimethylsiloxane, zirconium dioxide, gutta-percha powder, platinum catalyst, silver (preservative), bioactive glass ceramic and coloring. According to the manufacturer (Coltène/Whaledent, Altstatten, Switzerland) Guttaflow bioseal™ has improved biological properties including antibacterial activity compared to GuttaFlow™ and GuttaFlow 2™ (Ruiz-Linares *et al* 2019). Earlier studies involving Guttaflow™ and Guttaflow 2™ have indicated a lack of antibacterial activity. In a study comparing the antimicrobial activity against *E. faecalis* of Epiphany™, Guttaflow™ and AH-Plus™ endodontic sealers after 1 and 24 hours using ADT and DCT, Guttaflow™ was found to lack antimicrobial activity (Nawal *et al.* 2011). However, a study by Anumula *et al.* (2012) showed slight antibacterial activity of Gutta Flow™ for the first 3 hours after mixing which reduced drastically. In a recent study RoekoSeal™, Guttaflow 2™, TotalFill BC™ sealer, AH Plus™ were tested against planktonic and 24-hour old *Enterococcus faecalis* biofilms. The authors concluded that RoekoSeal™ and Guttaflow 2™ had no antibacterial activity against *E. faecalis* in both forms (Kapralos *et al.* 2018). A recent study using Guttaflow 2™, found that it had no antimicrobial activity against *E. faecalis* using both ADT and DCT testing methods (Huang *et al.* 2019). Studies involving Guttaflow bioseal™ and *E. faecalis* are still very few due to its recent introduction in the market. However, in a recent study it was found that the antibacterial efficiency of Guttaflow Bioseal™ improved till 4 weeks (Ruiz-Linares *et al.* 2019). The calcium silicate particles in Guttaflow Bioseal™ are thought to provide an alkaline environment after setting through release of calcium ions and this results in antimicrobial activity (Ruiz-Linares *et al.* 2019).

CHAPTER 2: AIM AND OBJECTIVES

2.1 Statement of the problem

Endodontic treatment failure may be due to persistent infection in the pulpal system of the tooth and *Enterococcus faecalis* is identified as the most common isolated organism. It would therefore be beneficial if endodontic sealers have antibacterial activity against *Enterococcus faecalis.*

2.2 Aim

The study seeks to assess the antimicrobial effect of 3 endodontic sealers: Sealapex™, EndoREZ™ and Guttaflow bioseal™ against *Enterococcus faecalis.*

2.3 Objectives

1. To determine the antibacterial effect of Sealapex™, Guttaflow bioseal™ and EndoREZ™ at 5 time points.
2. To determine which endodontic sealer has the greatest antibacterial effect against *Enterococcus faecalis*.

2.4 Null hypothesis

There is no difference in the antibacterial activity between the three endodontic sealers.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Study design

This is a laboratory-based comparative study testing the antimicrobial activity of three commonly used endodontic sealers against *Enterococcus faecalis.* The endodontic sealers were tested unset 20 minutes after mixing and after setting. Testing after setting enabled the assessment of the antimicrobial activity of aged sealers after 7 days, 14 days, 21 days and 28 days.

3.2 Sample size

The tested sealers were divided into 3 groups

Group 1 (EndoREZ™) n = 45 plates, n = 5 control plates

Group 2 (Guttaflow bioseal™) n = 45, n = 5 control plates

Group 3 (Sealapex™) n = 45, n = 5 control plates

A total of 150 plates were used for the study.

3.3 Materials

3.3.1 Microorganism

Enterococcus faecalis, American Type Cell Culture Collection (ATCC) 19434 was used as a test organism. The bacteria were cultured in air at 37 ⁰ C on Tryptic Soy Agar plates for the experiments. For each experiment a 24-hour culture was used. A suspension of bacteria in phosphate-buffered saline (PBS) was made and adjusted to 0.5 McFarland scale equivalent to 1.5 x 10^8 CFU.

Fig 1. *Enterococcus faecalis* strain used in the study.

3.3.2 Sealers

Three sealers were used for the study. Sealapex™ (Kerr) a calcium hydroxide based sealer, EndoRez™ (Ultradent, South Jordan, UT) and Roeko Guttaflow Bioseal™ (Coltene/ Whaledent, Switzerland).

Fig 2. Sealapex™

Fig 3. Guttaflow bioseal™.

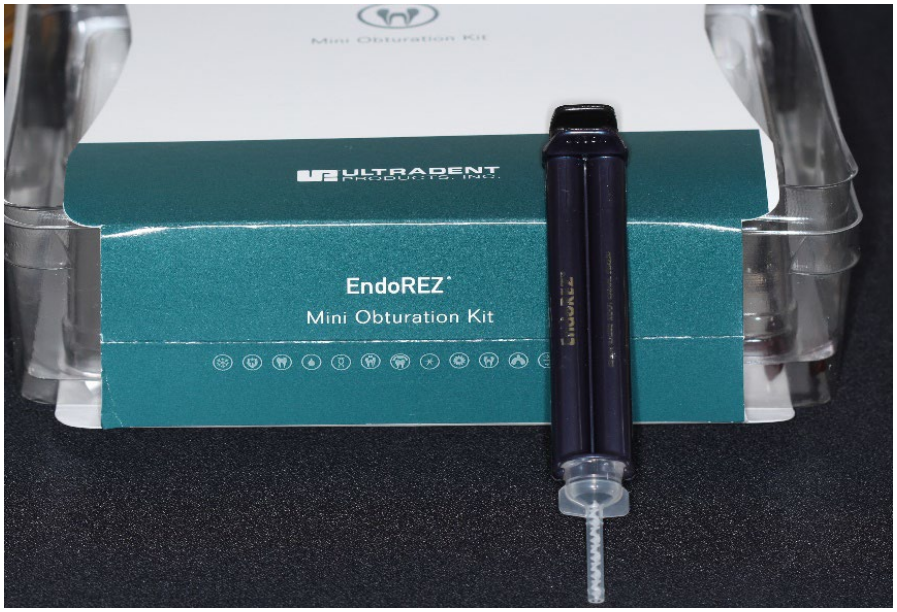

Fig 4. EndoREZ™

3.4 Direct contact test

The Direct contact test was introduced by Weiss *et al.* (1996). All sealers were prepared according to manufacturers' guidelines. A 96-well microtiter plate was held perpendicular to the floor, and an area on the side wall of the wells was coated with an equal amount of each material by using an appropriate dental instrument. The sealers tested at Day 0 (20 minutes after mixing) were regarded as fresh specimens while other specimens were allowed to set for 7,14,21 and 28 days in a humid atmosphere at 37 ºC before testing.

A 250µl of bacterial suspension was placed to be in contact with each sealer. Bacterial solution placed in the uncoated wells became the control. The incubation was done in 100% humidity at 37 ºC for 2, 5, 20, and 60 minutes. After gently mixing with a pipette for 30 seconds, the bacterial suspension from each well was transferred and serially diluted in Phosphate Buffered Saline (PBS).

The evaluation of the DCT was assessed by culturing aliquots of 100 µL onto TSA plates after 10-fold serial dilutions. After incubation for 24 hours at 37ºC, colonies on the plates were counted, and the CFU/mL was calculated. The experiments were performed in triplicates.

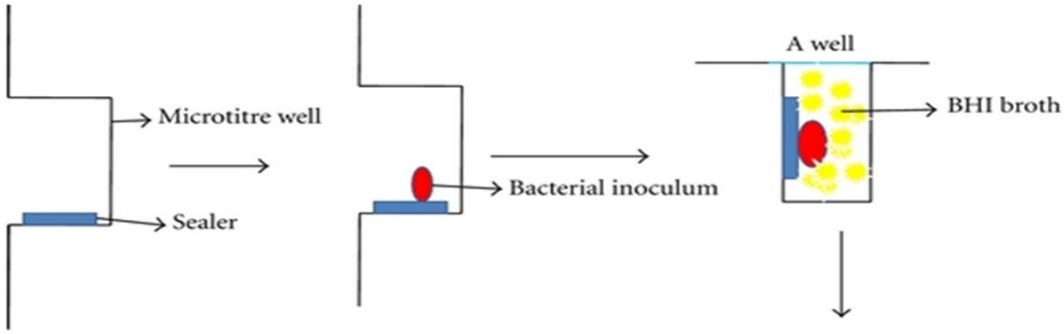

Fig 5. A schematic diagram showing Direct contact test (Anumula *et al.* 2012).

The calculation of colony forming units was based on the formula below

CFU/ml = **A** colonies (average) X **DF** (Dilution factor)

B volume plated (ml)

3.5 Statistical tests

All data was described with the mean and standard deviation. A one way mixed measures ANOVA test with Bonferroni correction was used to determine statistical significance between the three materials. All tests were deemed statistically significant at $p < 0.05$. All tests were conducted in Stata Corp.2017. Stata Statistical Software Release 15. College Station, TX: StataCorp LLC

CHAPTER 4: RESULTS & STATISTICAL ANALYSIS

4.1 Overview of results

The results of the activity of the sealers against *E. faecalis* show different patterns. All the materials exhibit some activity against the bacteria. The overall greatest antibacterial activity can be seen by Guttaflow bioseal™ (4.46, 0.01) on day 21, followed by Sealapex™ (5.12, 0.05) on day 7 and EndoREZ™ (6.37,0.08) on day 14. There was an overall difference between materials over time which showed Guttaflow bioseal™ vs EndoREZ™ had the biggest difference (-1.12;.0.14) followed by Sealapex™ vs EndoREZ™ (-0.805;0.14). Guttaflow bioseal™ and Sealapex™ did not show much difference in activity (0.32; 0.14).

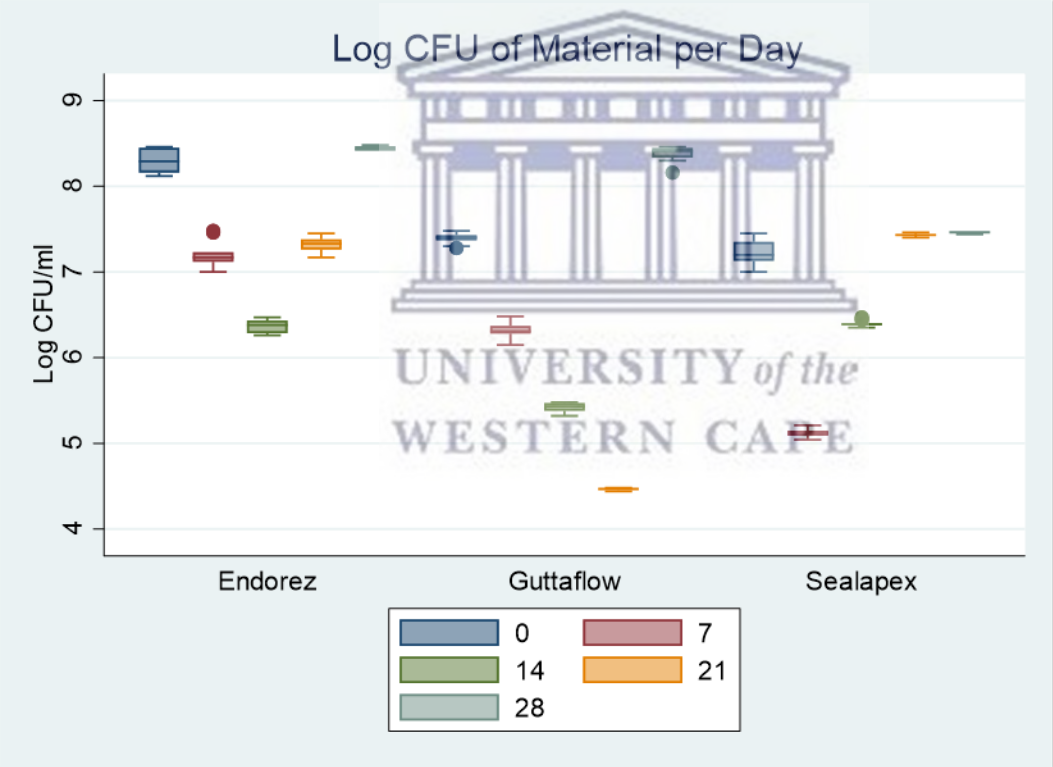

Fig 6: Boxplot of Antimicrobial activity of EndoREZ™, Guttaflow bioseal™ and Sealapex™ over 28 days.

4.2 Results per day

Day 0	Day 0 represents the activity of fresh samples of the materials. Sealapex™ has mean 7.39 (0.07), Guttaflow bioseal™ 7.22 (0.15) activity against *E. faecalis.* The difference between Sealapex™ and Guttaflow bioseal™ was -0.175 (95% C.I.: -0.33 to -0.25). The second greatest difference was seen between Sealapex™ and EndoREZ™ -1.08 (95% C.I.: -1.24 to -0.93). Finally, the smallest difference was seen between Guttaflow bioseal™ and EndoREZ™, -0.907 (95% C.I.: -1.06 to -0.755). The greatest activity was thus seen by Guttaflow bioseal™ compared to Sealapex™ at day 0.
Day 7	At 1 week of aging, all the materials showed an increased activity against *E. faecalis* as represented by their log CFU/ml EndoREZ™ 7.20 (0.166), Guttaflow bioseal™ 6.31(0.10) with Sealapex™ showing the greatest activity 5.11 (0.049). The greatest difference was seen between Sealapex™ and EndoREZ™ -2.08 (95% C.I: -2.234 to -1.913). This is followed by Guttaflow bioseal™ and Sealapex™ -1.19 (95% CI: -1.35 to -1.04) and lastly Guttaflow bioseal™ and EndoREZ™ -0.88 (95% CI:-1.03 to -0.73)
Day 14	The antibacterial activity of EndoREZ™ 6.37 (0.08) and Guttaflow bioseal™ 5.42 (0.06) continues to increase with EndoREZ™ reaching its peak of activity. However, Sealapex™ 6.40 (0.04) is starting to lose its activity. The difference between the materials Sealapex™ and Guttaflow bioseal™ 0.98 (95% CI: 0.82 to 1.12) is the greatest followed by EndoREZ™ and Guttaflow bioseal™ -0.95 (-1.11 to -0.79). EndoREZ™ and Sealapex™ do not show significant difference 0.025 (95% CI: -0.13 to 1.12) p value 1.
Day 21	Guttaflow bioseal™ 4.46 (0.014) has the greatest activity while EndoREZ™ 7.32 (0.09) and Sealapex™ 7.4 (0.019) are losing their activity. The difference between Sealapex™ and Guttaflow bioseal™ 2.95 (95% CI: 2.80 to 3.12) is the greatest, followed by EndoREZ™ and Guttaflow bioseal™ -2.86 (95% CI:

	-3.02 to -2.69).The difference between Sealapex™ and EndoREZ™ 0.098 (95% CI: -0.06 to 0.255) is not statistically significant with a p value of 1.
Day 28	The antibacterial activity of Guttaflow bioseal™ 8.37 (0.101) has decreased to approximate that of EndoREZ™ 8.44 (0.024). The activity of Sealapex™ 7.46 (0.101) has decreased a little as compared to its activity on day 21 (7.40; 0.019). The difference in activity between Sealapex™ and EndoREZ™ -0.98 (-1.12 to -0.825) is the greatest followed by that of Guttaflow bioseal™ and Sealapex™ -0.904 (-1.06 to -0.75). There is no difference in activity between Guttaflow bioseal™ and EndoREZ™ -0.072 (-0.23 to 0.85) p value 1.

Table 3: Results of root canal sealers antibacterial activity per each tested day.

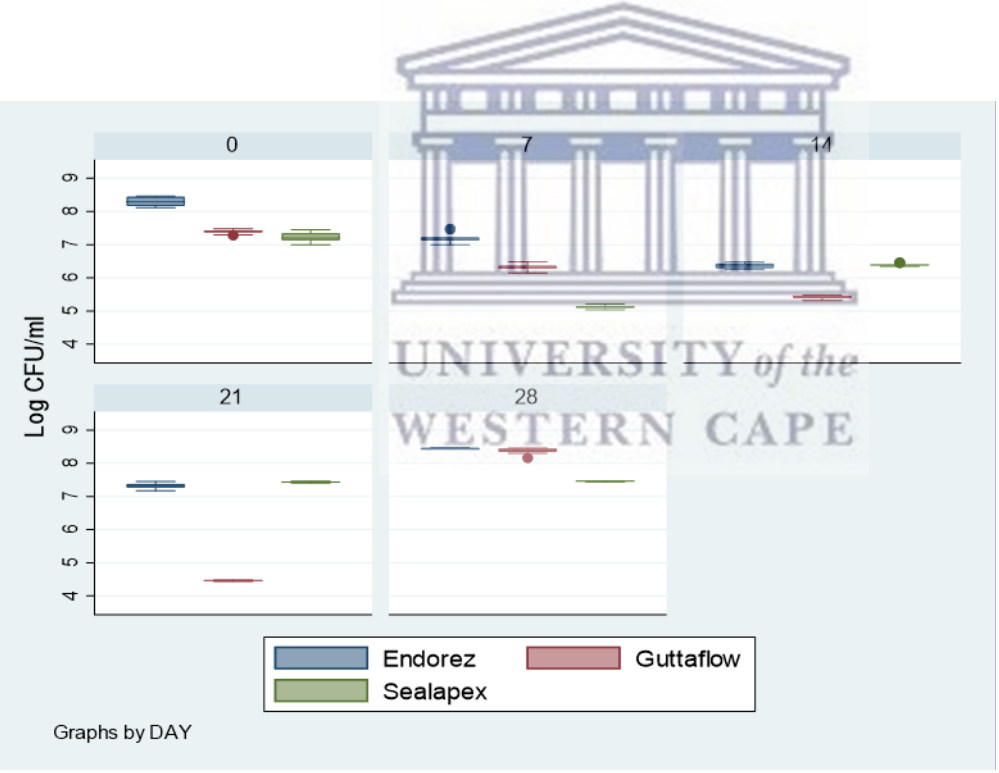

Fig 7: Comparison of activity of sealers per day.

4.3 Results per material

4.3.1 EndoREZ™

Table 4: Log CFU mean and SD of EndoREZ™, Guttaflow bioseal™ and Sealapex™

	Summary of Log CFU/ml					
	EndoREZ™		Guttaflow™		Sealapex™	
DAY	Mean (SD)	n	Mean (SD)	N	Mean (SD)	n
0	8.30 (0.15)	9	7.39 (0.07)	9	7.22 (0.15)	9
7	7.20 (0.17)	9	6.32 (0.10)	9	5.12 (0.05)	9
14	6.37 (0.08)	8	5.42 (0.06)	9	6.40 (0.04)	9
21	7.32 (0.09)	8	4.46 (0.01)	8	7.43 (0.02)	9
28	8.44 (0.02)	9	8.38 (0.10)	8	7.46 (0.01)	9
Total	7.56 (0.78)	43	6.39 (1.37)	43	6.73 (0.90)	45

The highest Log CFU count was 8.44 (0.02) at day 28 for EndoREZ™ (Table 4). The second highest Log CFU count was at day 0 (8.30; 0.15). However, the difference between the activity on day 0 and day 28 is not statistically significant ($p = 0.124$). The activity of EndoREZ™ gradually increases to reach its highest on day 14 (6.37; 0.08). Thereafter the activity gradually reduces to reach the highest Log CFU/ml on day 28.

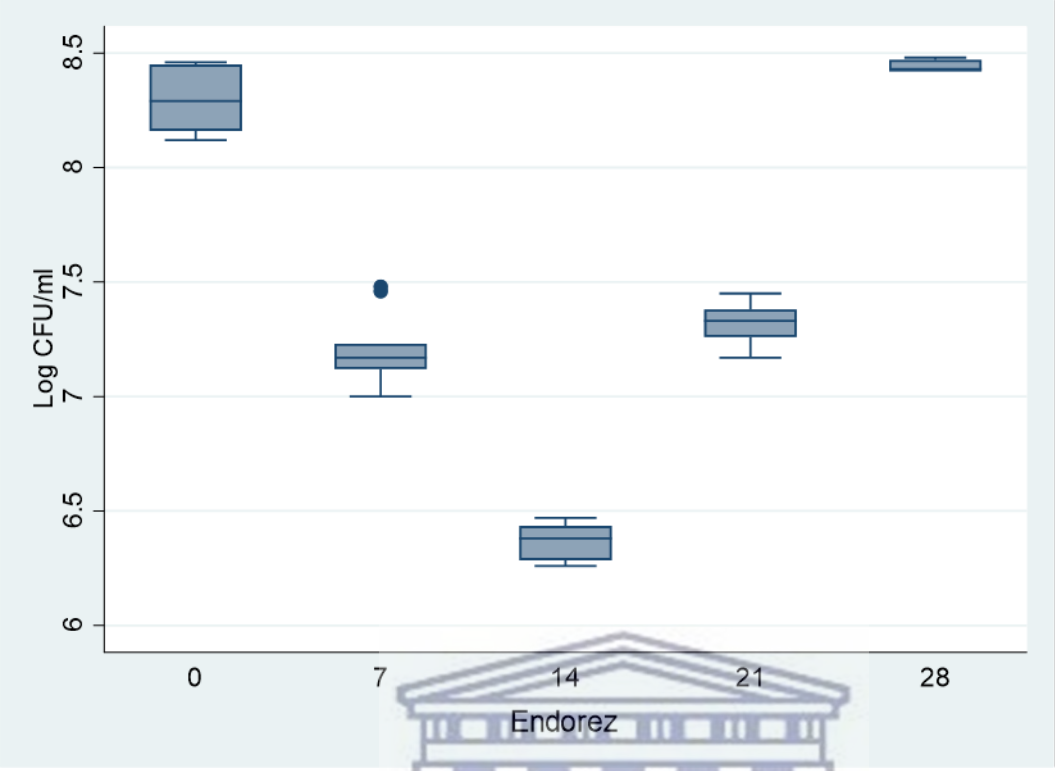

Fig 8: Activity of EndoREZ™.

4.3.2 Guttaflow bioseal™

The activity of Guttaflow bioseal™ increases from fresh samples to reach the maximum bactericidal property on day 21 (4.46, 0.01) as shown by the lowest log CFU/ml (Table 1). After day 21 the activity reduced dramatically (8.38, 0.10). The activity on Day 28 (8.38, 0.10) was much less than the activity at day 0 (7.39, 0.07), $p < 0.001$. The difference between Day 28 and day 0 for Guttaflow was 0.974 (95% C.I.: 0.817 to 1.142).

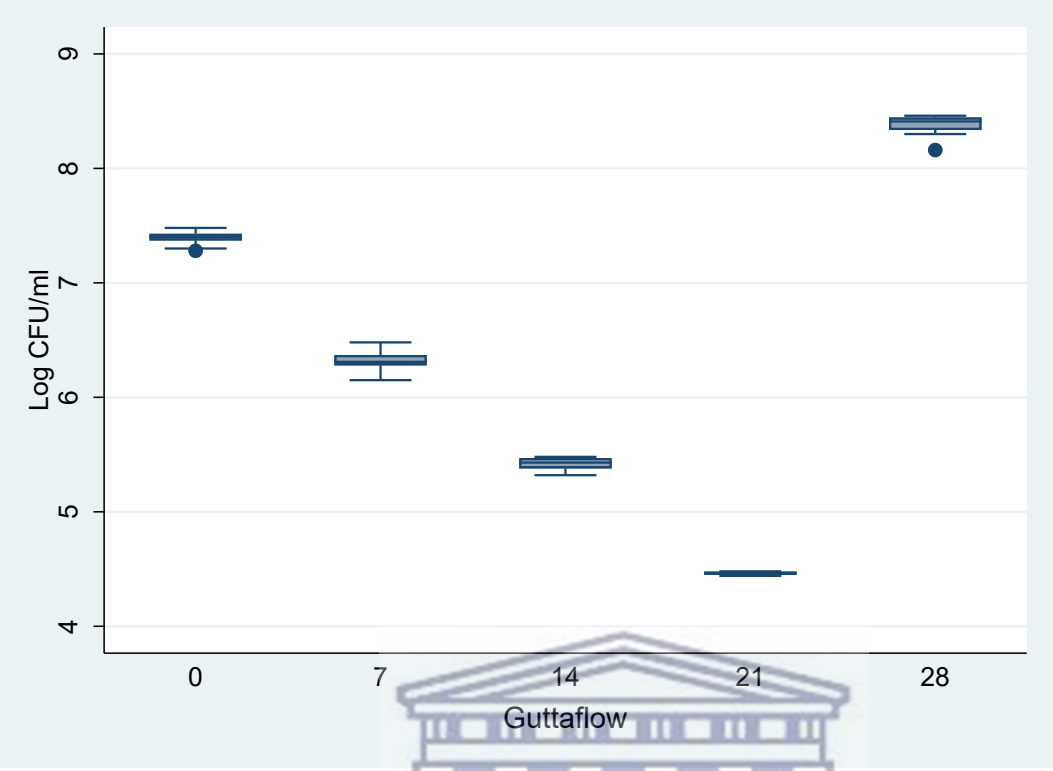

Fig 9: Activity of Guttaflow bioseal™.

4.3.3 Sealapex™

The activity of Sealapex™ increased and reached its peak on day 7 (5.12, 0.05), thereafter its potency dissipates (6.40, 0.04) on day14 (Table 1). On day 21 and 28 there is no difference in the activity of Sealapex as shown by the CI (-0.116 to 0.187), p =1.00. On comparing day 28 and Day 0, there is a difference in the activity (CI 0.095 to 0.398) with day 28 sample being less potent, p < 0.001.

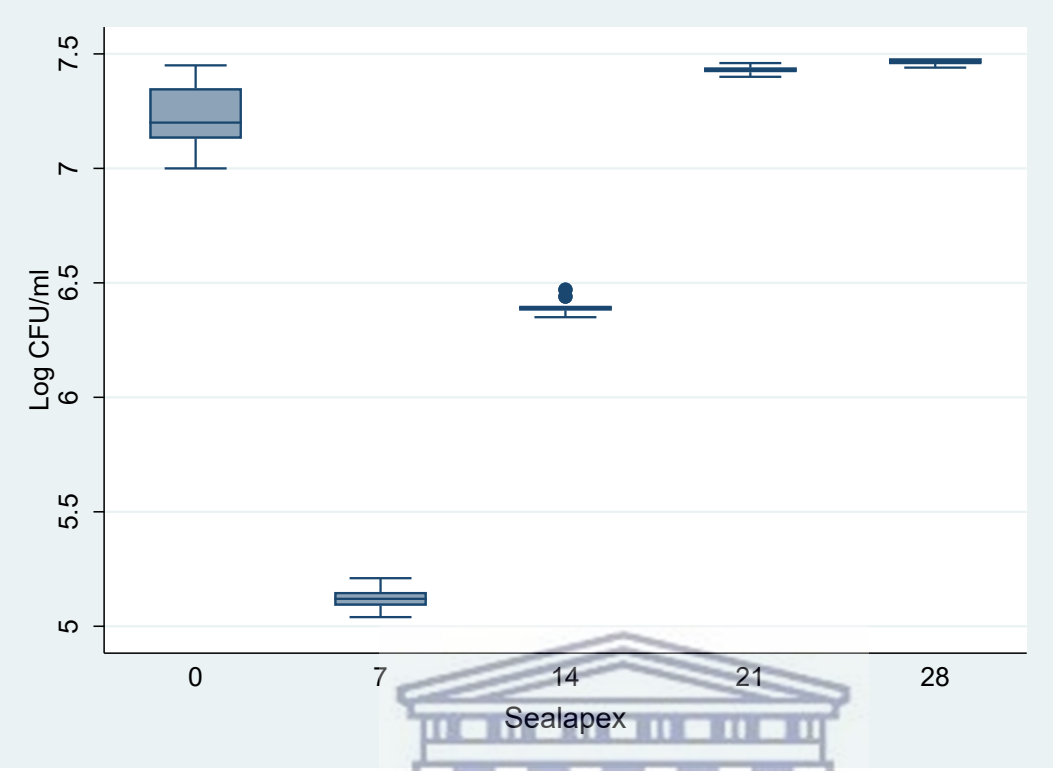

Fig 10: Activity of Sealapex™.

CHAPTER 5: DISCUSSION

The goal of endodontic obturation is to achieve a permanent fluid tight seal of the pulp chamber and roots of the tooth in order to eliminate the risks of infection or reinfection of the root canal system. To obtain a fluid tight seal, a sealer is usually employed (Garcia- Molina *et al.* 2005). Often failure of endodontic treatment is due to the spaces within the root canal as a result of not being obturated properly. The interaction between the oral environment and root canal spaces as well as residual bacteria in canals (from inadequate debridement) contributes to endodontic treatment failure. Thus, antibacterial activity of root canal sealers contributes to the success of endodontic treatment.

Antibacterial effect of root canal sealers may assist in the eradication of the remaining microbial organisms after root canal shaping and cleaning as it has been shown in other studies that no root canal preparation technique is capable of eliminating all the microorganisms from the root canal system (Zhang *et al.*2009). Many root canal sealers have claimed to be antimicrobial against the common endodontic microorganisms. In that regard, the purpose of this study was to evaluate the antimicrobial efficiency of root canal sealers Sealapex™, EndoREZ™ and the new improved Guttaflow Bioseal™ against *Enterococcus faecalis*.

Enterococcus faecalis was the selected test microorganism in this study due to its high prevalent isolation in cases of persistent apical periodontitis even after root canal treatment (Siqueira 2001). *E. faecalis* is known to survive, grow within dentinal tubules and reinfect canals (Love 2001). Bacterial survival in root canals may be ascribed to their ability to penetrate the dentinal tubules where biofilm formation can take place. This protects these microorganisms from disinfecting agents cleaning the root canal system. Other authors advocate that *Enterococcus faecalis* in obturated teeth with post-treatment disease continues being viable as it adheres to collagen in the presence of human serum and form resistant biofilms (Van der Vyver *et al.* 2014).

Traditionally, the Agar diffusion test (ADT) was commonly used to assess the antimicrobial activity of root canal sealers. However, the use of ADT is not advisable anymore due to limitations of the procedure. According to several authors, ADT has limitations of reliability, as results are affected by the solubility of the sealer being tested in the agar (Alshwaimi *et al.* 2016). In this study the Direct contact test was used, a method pioneered by Weiss *et al.* (1996) to evaluate the

antimicrobial activity of endodontic sealers. The DCT evaluates the efficacy of direct and close contact between the material and the tested bacteria on microbial viability. Therefore, it enables measurement of whether the bacteria are viable regardless of the ability to be soluble and to diffuse of the antibacterial components in the sealer (Alshwaimi *et al.* 2016). Standardization of root canal sealers antimicrobial testing protocols is lacking in literature. The DCT is a quantitative method which can be replicated to evaluate bacterial growth. However, its limitations are that it does not consider aspects such as anatomy and biochemical aspects of the tooth and biofilm.

Sealapex™ is a calcium hydroxide based endodontic sealer. In this study fresh samples of Sealapex™ at day 0 have a weak activity against E. faecalis. This is similar to the study by Fuss *et al.* (1997). A study by Poggio *et al.* (2017) noted that fresh samples of Sealapex did not have any activity against *E. faecalis*. It is important to realize that in the study by Poggio *et al.* (2017) the fresh samples were tested after 6 minutes. The antibacterial activity of Sealapex™ is derived from the release of OH ions. The study by Fuss *et al.* (1997) noted that fresh samples of Sealapex™ do not release OH ions in high concentrations hence explaining the weak activity against *E. faecalis* of these samples (Poggio *et al.* 2017; Fuss *et al.* 1997).

Regarding aged samples, in this study the activity of Sealapex™ increased to reach the maximum on day 7, there after the activity started to decrease. This is in agreement with earlier studies which also recorded maximum activity of Sealapex™ on day 7 (Poggio *et al.* 2017; Zhang *et al.* 2009; Fuss *et al.* 1997). Most studies limit the evaluation time to 7 days, which is different to this study as the activity of Sealapex™ was evaluated for 28 days. The reduced activity of Sealapex™ after day 7 may be explained by the reduced concentration of the hydroxide ions which are vital for antimicrobial activity. Fuss *et al.* (1997) noted that the set material had a limited amount of the availability of the hydroxide ions.

Earlier on Bystrom and Sundqvist (1983) postulated that for a calcium hydroxide sealer to maintain antimicrobial effectiveness the pH must be around 12.5, a position which was also advocated by Mickel *et al.* (2003). As the material sets the pH drops to around 9 causing it to lose its effectiveness (Mickel *et al.* 2003; Fuss *et al.* 1997; Bystrom & Sundqvist 1983). A recent systematic review noted that there is loss of antibacterial activity against *Enterococcus faecalis* in calcium hydroxide sealers that were allowed to age. However, the evidence provided by the review

is conflicting and may be due to the difference in methodologies of studies and time frames (Alshwaimi *et al.* 2016).

EndoREZ™ is a methacrylate sealer which sets by chemical cure or light cure and can penetrate dentinal tubules. In this study EndoREZ™ which sets by chemical cure was used. The fresh samples of EndoREZ™ showed weak antimicrobial activity against *E. faecalis.* The result agrees with the study by Eldeniz *et al.* (2006) which showed mild to no antibacterial activity for fresh samples though they used ADT in their study as opposed to the present study which used DCT. However, there is a contrast to the study by Zhang *et al.* (2009) which recorded an efficient killing of *E. faecalis* using the DCT method (Zhang *et al.* 2009; Eldeniz *et al.* 2006).

For aged samples of EndoREZ™, in this study the antimicrobial activity increased to reach the peak on day 14, thereafter the material started to lose its activity against *E. faecalis*. The antimicrobial effect of EndoREZ™ is thought to occur as a result of the inhibitory effect of the oxygen layer limiting the setting reaction of EndoREZ™. This results in a greater quantity of non-reacted monomers killing *E. faecalis* (Farmakis *et al.* 2012). This may help to explain the weak activity of EndoREZ™ at day 21 and 28 as the material was fully set so there was no free monomers to exert the antibacterial activity against *E. faecalis.* Heyder *et al.* (2013) in their study noted that the antibacterial activity of EndoREZ™ was inferior to that of Zinc-oxide eugenol sealers and Sealapex™. This is in agreement with the present study which noted that the activity of EndoREZ™ was weaker than that of Sealapex™ and Guttaflow Bioseal™.

Guttaflow Bioseal™ is a recent addition to the market of the silicone based polymethyl hydrogen siloxane endodontic sealers (Kapralos *et al.* 2018; Nawal *et al.* 2011). It is a successor to Guttaflow™ and Guttaflow 2™ and the manufacturer claims it has improved biological properties (Ruiz- Linares *et al.* 2019). Previous studies using either Guttaflow™ or Guttaflow 2™ showed that both materials had no activity against *E. faecalis* in fresh and aged samples (Kapralos *et al.* 2018; Anumula *et al.* 2012; Nawal *et al.* 2011). In this study the fresh samples had a weak antimicrobial activity against *E. faecalis*. Due to the recent introduction of Guttaflow Bioseal studies investigating its antimicrobial activity are few.

In this study aging the material resulted in increased antibacterial activity against *Enterococcus faecalis* reaching its peak on day 21 followed by sharp reduction of antimicrobial activity on day 28. This result partly agrees with the study by Ruiz- Linares *et al.* (2019) which showed increased

antimicrobial activity with respect to the control as the material ages. In that study the assessment of antimicrobial activity was performed after day 1, 1 week and 4 weeks. In contrast to their results, the present study showed a marked decrease in the antimicrobial activity against *E. faecalis* on day 28. This can be attributed to the difference in methods of counting viable cells of *E. faecalis* after the DCT. The present study used CFU/ml while the Ruiz-Linares *et al.* (2019) study used RLUs. Guttaflow bioseal™ is composed of a mixture of polydimethylsiloxane, platinum catalyzer, calcium silicate, gutta-percha powder and zirconium dioxide. It is postulated that the calcium silicate particles are responsible for providing an alkaline environment through constant release of calcium ions after setting. This high pH environment is responsible for the antimicrobial properties (Gandolfi *et al.* 2016). Guttaflow bioseal is a promising material in endodontics since its antimicrobial activity increases after setting, however further research is needed on this material.

CHAPTER 6: CONCLUSION & RECOMMENDATIONS

It is clear from the study that all the endodontic sealers exhibited some antimicrobial activity against *E. faecalis* with different behavior patterns at different times. It is difficult to pinpoint which endodontic sealer had the highest antimicrobial activity against *E. faecalis*. The null hypothesis is therefore rejected.

However, the use of root canal sealers with antibacterial activity should not be a replacement of chemo mechanical preparation of the root canal or of a substandard obturation technique leaving voids in the canal as it has been seen from the study that antimicrobial activity decreases with time.

The current study is not without limitations. The first limitation is that no tooth material was used and the study evaluated the effect of root canal sealers against planktonic bacteria which is rarely found in real life situations as endodontic bacteria frequently exists as biofilms. Related to that, the study used a single microorganism, a situation which is also rarely found in clinical cases of endodontic failure. However, the study results are valid as planktonic bacteria may be involved in the formation of biofilms and initiation of infections.

Therefore, the author recommends that studies to test antibacterial activity of root canal sealers should be conducted incorporating tooth tissues as a medium. This is important as in vitro studies should try to mimic oral or clinical situations. The dentin infection model is one such methodology that can be used. This model can yield better results regarding the antimicrobial activity of endodontic sealers as it enables the creation of biofilms in dentinal tubules.

Future research in this area should also explore the possibility of studying antimicrobial activity of sealers in polymicrobial states. This is important to understand as one bacterium may potentiate the activity of another bacterium especially in a biofilm environment.

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