Micro-CT assessment of the sealing ability of bio-ceramic root canal sealers

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Dedication

To my father, mother, brothers and sisters

My amazing nephews and nieces I hope to be the uncle to be proud of

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Abstract

Objectives:

The purpose of this study was to analyse the sealing ability of bio-ceramic sealers by measuring root canal void percentage using micro-computed tomography (micro-CT).

Material and Methods:

Forty single-root human premolars were selected and randomly allocated into four groups. Teeth were instrumented using #10 K and then Wave One Gold Primary files. Samples were obturated using matched taper Gutta-Percha cones with single cone technique and one of the four tested sealers (n=10) (MTA Fillapex, Bioroot, Endosequence and Wellroot). Following obturation, teeth were stored in Hank's balanced salt solution for 7 days. All samples were scanned pre-operatively and post-operatively using Micro-CT at a resolution of 13.65 µm. Voids percentages were calculated in the entire tooth length in addition to coronal, middle and apical thirds.

Results:

There was a significant difference ($P<0.05$) between Endosequence sealer and MTA Fillapex sealer as well as between Wellroot sealer and MTA Fillapex sealer in total voids percentage and coronal thirds voids percentage. Other comparisons showed no significant difference ($P>0.05$) in total voids percentage neither comparing middle and apical thirds of each sealer to another. Moreover, there was no significant difference comparing the three thirds of each sealer.

Volumetrically, voids percentage was highest in MTA Fillapex sealer followed by Bioroot sealer then Wellroot sealer and finally Endosequence sealer.

Conclusions:

None of the tested sealers showed void free results. Endosequence and Wellroot sealers showed higher sealing ability compared to MTA Fillapex sealer. Micro-CT is an excellent method to study the sealing ability of endodontic sealers by showing accurate quantitative results. The single cone technique is clinically acceptable when using bio-ceramic sealers.

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List of Abbreviations

NaOCl	Sodium hypochlorite
EDTA	Ethylenediamine tetraacetic acid
MB2	Mesiobuccal canal 2
ZOE	Zinc oxide eugenol
MTA	Mineral trioxide aggregate
CaOH	Calcium hydroxide
NiTi	Nickle Titanium
RCT	Root Canal Treatment
Micro-CT	Microscopic computed tomography
BC	Bio-ceramic
CEJ	Cemento-Enamel junction
ROI	Region of Interest
VOI	Volume of Interest
3-D	Three dimensional

Chapter I
Introduction

1.1 Introduction

Enamel, dentine, cementum and pulp are the four dental tissues that form the human tooth. Dental pulp is the only soft tissue component while the other three components are classified as hard tissues.

Enamel covers the entire anatomic crown of the tooth protecting the dentine and is mainly made of mineral hydroxyapatite which is a crystalline calcium phosphate (Staines et al., 1981). Indeed, enamel is considered as human body's hardest tissue since it contains almost no water. When abraded or damaged, tooth enamel does not regenerate (Chun et al., 2014).

Dentine consist of 70% minerals, 20% organic material and 10% of water (Ten Cate, 1998, p.150). Dentine is harder than bone but softer than enamel which is the reason why decay spreads faster and wider when it passes enamel (Vaderhobli, 2011). Dentine formation is initiated by odontoblasts which are located around the pulp chamber (Marshall et al., 1997). Following an S-shaped curvature, dentinal tubules pass through dentine and become wider and more numerous near the pulp (De Santis et al., 2002, pp.589-599).

Cementum is the dental tissue that covers the root. Periodontal ligament is a soft connective tissue interposed between the tooth and the surrounding bone. One of the main functions of cementum is to anchor the periodontal ligaments’ collagen fibres to root surface. Moreover, cementum also has adaptive and reparative functions (Bosshardt and Selvig, 1997).

Pulp is a unique tissue from many perspectives. It occupies the central part of tooth structure and is divided further into coronal pulp chamber and a radicular root canal (Goldberg and Lasfargues, 1995, De Santis et al., 2002, pp.589-599). It is a highly vascularized and innervated soft tissue. Arterioles of a diameter less than a 100 μm are responsible for tooth blood supply, entering the canal via apical foramen or foramina accompanied by nerve bundles responsible for sensation. Smaller vessels may also enter the canal through lateral or accessory canals. This blood supply is believed to be dominated by neuronal control (Aars et al., 1992, Berggreen and Heyeraas, 1999). The pulp undergoes inflammatory and immunologic reactions in response to microorganisms affecting the tooth. Those microorganisms and their products can reach the pulp from caries, open restoration margins, cracks and fractures through dentinal tubules. Dental caries is the most common aetiological agents of dental pulp injuries (Nanci, 2003, pp.192-239, Zero et al., 2011). Once the pulp is affected, it reacts in three different ways: Decreasing dentine permeability, forming a tertiary dentine layer and eliciting inflammatory reactions (Smith, 2002). Pulpal inflammatory reactions can be classified into reversible pulpitis, irreversible pulpitis and pulp necrosis (Berman et al., 2011, Johnson, 2010). Infections can spread from teeth into the periapical space causing periapical diseases such as apical periodontitis and acute and chronic apical abscess (Abbott, 2004). On the other hand, periodontal infections can sometimes extend to include the pulp causing a pulpal response which is known as an Endo-Perio lesion or Primary Periodontal Disease with Secondary Endodontic Involvement (Rotstein and Simon, 2004).

1.2 Endodontic treatment

Endodontology is defined as "*the branch of dentistry concerned with the morphology, physiology, and pathology of the human dental pulp and periapical tissues. Its study and practice encompass the basic clinical sciences including biology of the normal pulp; the etiology, diagnosis, prevention and treatment of diseases and injuries of the pulp; and associated periapical conditions*" (American Association of Endodontists., 2005).

Endodontic treatment -also known as root canal therapy- is a very common dental procedure. 14 million root canal treatments are performed every year in UK (Scottish dentistry, 2020). In the United States of America, it is 25 million per year (American Association of Endodontics, 2020).

The objectives of root canal therapy are the removal of the inflamed and necrotic tissue, elimination of root canal microbiota and the sealing of the root canal system to prevention of recurrence of infection (Torabinejad et al., 2014). If this can be achieved, dental function and appearance can be restored and maintained (Jensen et al., 2007).

Root canal treatment is essentially split into two phases; the chemo-mechanical preparation phase and the obturation phase. Chemo-mechanical preparation involves shaping of the root canal both to remove tissue and allow space for effective irrigation. Decontamination of the root canal system with effective cleaning and shaping can be a challenging task specially with root canal complexities such as narrow or curved canals (Peters, 2004, Paqué et al., 2005). Hand files and reamers have largely been replaced by nickel-titanium (NiTi) engine driven systems as they cause less canal transportation and give more accurate centred and tapered preparation (Peters, 2004, Guelzow et al., 2005; Taşdemir et al., 2005). An important conclusion from previous debridement studies suggested that complete cleaning of the root canal space cannot be achieved by hand instruments specially the apical region of curved canals (Wu et al., 2003, Peters, 2004). Even with the advancements in the endodontic armamentarium, preparation of the canal space is still less than optimal. Peters et al. reported that with the use of an engine driven NiTi instrumentation technique, around 35% of the dentine surface area remained untouched. These areas offer a great opportunity for microorganisms to recolonize causing reinfection and eventually root canal treatment failure (Peters et al., 2003). For this reason, thorough disinfection with an optimised irrigation protocol is very important in order to achieve successful treatment outcomes (Zehnder, 2006). Irrigation is an important step that plays a major role in debridement, lubrication, destruction of microbes and dissolution of tissues. Moreover, it flushes away tissue remnants and dentine debris preventing apical canal blockages (Ari et al., 2004). There are many irrigation solutions available for use. Ideally irrigants should have a broad antimicrobial spectrum and act effectively against anaerobic bacteria and facultative microorganisms, dissolve remaining necrotic pulp tissue, prevent smear layer formation or dissolve the layer if has been formed and inactivate microbial endotoxins (Zehnder, 2006). The most commonly used irrigants are sodium hypochlorite (NaOCl) (1%–5.25%), hydrogen peroxide (3%–30%), ethylenediamine tetraacetic acid (EDTA) (10%–17%) and chlorhexidine (0.2%–2%). Of all substances currently in use, the most efficacious solution appears to be NaOCl. It dissolves pulp tissue (Naenni et al., 2004), is strongly antimicrobial and can dissolve the extra-cellular matrix of biofilm present in root canals (Spratt et al., 2001) It is also capable of inactivating endotoxins, which are commonly linked with root canal treatment failure (Silva et al., 2004). EDTA is also commonly used, often as adjunct to NaOCl due to its properties as a chelating agent and for the removal of inorganic debris (Tartari et al., 2017). Even though irrigation looks like a simple task it has some challenges and difficulties specially when it comes to difficult-to-clean areas. Complex root canal anatomy is the main cause of difficulty in cleaning and disinfecting (Ricucci and Siqueira, 2010, Arnold et al., 2013, Ricucci et al., 2016). Presence of lateral canals in the root canal system can prevent effective irrigation, in turn leading to recolonization of the canal system and treatment failure (Paqué et al., 2011, Paqué et al., 2012). In maxillary molars -specially first molars- the root canal anatomy is usually complicated. The mesiobuccal roots of maxillary first molar have always presented a challenge due to the variation and the high prevalence of second canal MB2 (Von Arx, 2005, Cleghorn et al., 2006). In general, the MB2 is the most commonly untreated canal in all permanent teeth causing treatment failure and apical periodontitis (Karabucak et al., 2016). Regarding mandibular molars, extra canals can be found also such as middle mesial canal and second distal canal, these variations complicates the disinfecting step in general. The distal root in some cases has an asymmetrical oval shape or is divided into two canals after the middle third resulting in either untouched areas or poorly disinfected areas (Paqué et al., 2010, Filpo-Perez et al., 2015, Versiani et al., 2016).

The second phase of root canal treatment is obturation, which aims to seal the root canal system optimally to prevent recontamination via microleakage and to block the passage of any nutrients to microorganisms that may not have been removed during the chemo-mechanical phase (Schilder., 1967). One of the key determining factors of root canal treatment long-term success is the adequate filling of the prepared root canal space (Epley et al., 2006, Da Silva Neto et al., 2007, Ng et al., 2008). The main objective of obturation is to fully fill the canal space in all dimensions to create a fluid-tight seal which will prevent microorganisms and toxins from invading the canal space (Michaud et al., 2008; Özok et al., 2008) or escaping into the periapical tissues (James et al., 2007). The ideal root canal obturation should fill the entire canal space in all dimensions including irregularities and lateral canals, be well-adapted to canal walls and to form a homogeneous mass of gutta-percha and sealer (Schilder, 1967). To date, a large variety of endodontic filling materials have been produced.

Gutta-percha is the most commonly used material, in use for over a century, and considered the gold standard of core filling materials and been used with various obturation techniques (Imai and Komabayashi, 2003, Orstavik, 2005, Sly et al., 2007). Gutta-percha is composed of 20% gutta percha and 80% zinc oxide. For radiographic contrast and color, metal salts and a dye are added. Some manufacturers added antimicrobial agents to increase disinfecting ability such as calcium hydroxide (Lohbauer et al., 2005), chlorhexidine (Lui et al., 2004), iodoform (Chogle et al., 2005) (Orstavik, 2005).

Even though gutta-percha is the most frequently used root canal core material, it cannot fill the root canal space completely (Wu et al., 2003, Sevimay and Kalayci, 2005). To overcome this problem a large variety of sealers have been developed to fill in the multiple irregularities encountered in root canal systems. Even though sealers are considered as adjunctive materials, they influence the outcome of root canal treatment. Moreover, advanced properties of sealers impact the quality of final obturation (Sly et al., 2007, Zhou et al., 2013)

1.3 Endodontics Treatment Outcomes

When root canal treatment (RCT) is performed with highest standards, the success rate can be more than 90% (Benenati and Khajotia, 2002, Imura et al., 2007, NHS., 2019).

Microleakage has been suggested as a significant cause of endodontic treatment failure (Drukteinis et al., 2009, Liviu et al., 2010, Nair et al., 2011). A well-adapted obturation/sealer complex provides a tight seal that can prevent microleakage and escape of irritants such as bacteria, toxins and their flow from the oral cavity into the radicular tissue (Ozok et al., 2008, Hammad et al., 2009).

Substandard obturations will show voids within the filling, between gutta-percha and sealer or between filling and root canal walls. In some clinical studies this was suggested as the main reason of endodontics treatment failure (Er et al., 2006, Dadresanfar et al., 2008, Ingle et al., 2008, pp. 1053–1087).

1.4 Root Canal Sealers

The functions of an optimal root canal sealer should include

- (i) Provision of a tight seal in root canal space along with sealing accessory canals and multiple foramens
- (ii) Formation of a bond between obturation material and root canal wall
- (iii) lubricating that facilitates placement of the main core material
- (iv) Possession of an anti-microbial effect. (Kaur et al., 2015).

Therefore, sealers should help prevent leakage, reduce the risk of survived microbial invasion from canal into periapical tissues and assist healing of periapical lesions (Walton and Torabinejad, 2002, pp. 388-404, Pramudita et al., 2020).

It has been well established that a well-adapted and void-free obturation/sealer complex improves the clinical outcomes of root canal treatment (Epley et al., 2006, Ng et al., 2008).

1.5 Classification of Root Canal Sealers

According to their chemical constituents, root canal sealers can be categorized into: Epoxy resin-based, zinc oxide eugenol based, bio-ceramic sealers (calcium silicate based, calcium phosphate based & others), MTA based, CaOH based, silicone based and methacrylate resin sealers.

1.5.1 Epoxy resin-based Sealers

In endodontics, epoxy resin-based sealers were first introduced by Schroeder (Schroeder., 1981). Lots of modifications have been applied to the original formula and currently it is one of the most commonly used materials in root filling procedures (Lee et al., 2017). AH 26 and AH Plus are the two main commercial examples of Epoxy resin-based sealers. AH Plus was released to the market to overcome the two major disadvantages of AH 26 which were teeth staining after root canal treatment and release of formaldehyde during mixing which makes it toxic. In addition, it shows good sealing ability and favorable physicochemical and biological properties (Miletić et al., 2003, Schäfer et al., 2015; Silva et al., 2015, Silva et al., 2016). On the other hand, they are hydrophobic sealers that do not react with water and therefore have less dentinal tubule penetration when compared to hydrophilic sealers such as bio-ceramic sealers (Schäfer et al., 2015). Acroseal is another type of epoxy resin-based sealers which contains 28% calcium hydroxide added in order to increase its antimicrobial activity. Studies showed that it has a good action against *Enterococcus faecalis*, low toxicity and proper film thickness (Testarelli et al., 2003; Gambarini et al., 2003, Pinheiro et al., 2009)

1.5.2 Zinc Oxide Eugenol Sealers

Zinc oxide eugenol sealers have a long history of success. The are available as powder and liquid which are mixed together. The powder is composed mainly of zinc oxide while eugenol forms the liquid part. It was introduced to the field by Grossman in 1936. Various modifications have been applied to his original formula (Marín-Bauza et al., 2012). Tubliseal is a two-paste system sealer and the most commonly used under this category. The main advantages of ZOE sealers are their antimicrobial activity specially against *Enterococcus faecalis* (Mickel et al., 2003). Zinc oxide eugenol sealers do have some disadvantages including higher cytotoxicity rate than other common sealers (Huang et al., 2002, Kaur et al., 2015), due to the presence of eugenol it could cause periapical inflammatory reaction if extruded to the periapical area (Schwarze et al., 2002) and teeth discolouration has also been reported along with the use of ZOE root canal sealers (Lenherr et al., 2012)

1.5.3 Calcium Hydroxide Sealers

Calcium hydroxide was introduced to endodontics by Herman in 1920. It was first introduced due to its pulp repairing activity. It is mainly used for pulp capping procedures in addition to temporary intracanal medicament, apexification and as sealer in obturation (Desai and Chandler, 2009). There are two main reasons for using CaOH sealers: it maintains and promote periapical healing and due to its good antimicrobial activity (Desai and Chandler, 2009). Regarding disadvantages, CaOH sealers showed more leakage when compared to other used sealers which indicated inferior sealing ability among different sealers such as epoxy resin based and methacrylate resin based (Ersahan and Aydin, 2013). Moreover, minimal to moderate discoloration of teeth treated with the use of CaOH sealers were reported (Parsons et al., 2001, Davis et al., 2002)

1.5.4 Silicone Based Sealers

When it comes to silicon-based sealers, there are two types which are mainly used under this family of sealers. Roekoseal consists of ‘polydimethyl siloxane, paraffin-base oil, silicon oil, hexachloroplatinic acid as a catalyst and zirconium dioxide for radiopacity (Gençoglu et al., 2003). It has a great advantage that opposite to shrink it actually expands by 0.2% by volume which permits better flow into irregularities (Kala and Torvi, 2015).

The second type is Guttaflow which consists of gutta-percha added to roekoseal. The material is provided in capsules to be injected directly into the root canal space followed by insertion of a single master cone (Bouillaguet et al., 2008). The working time is 15 minutes and 25 to 30 minutes setting time. Working time could be extended if sodium hypochlorite was the irrigant of choice (Bouillaguet et al., 2006). Previous studies suggested that it fills the canal irregularities and accessory canals with consistency (Zielinski et al., 2008), it has good biocompatibility (Bouillaguet et al., 2006, Eldeniz et al., 2007). Sealing ability of silicon sealers appears to be comparable to other common sealers in few studies and inferior in another (Brackett et al., 2006, Kontakiotis et al., 2007; Monticelli et al., 2007, Ozok et al., 2008).

The main disadvantage limiting the use of such sealers is that they have no antibacterial activities specially against *Enterococcus faecalis* (Tyagi et al., 2013, Wainstein et al., 2016).

1.5.5. Methacrylate Resin Sealers

Before the advent of currently used methacrylate resin sealers designed specifically for endodontic purposes, there have been continued attempts at using low viscosity bonding agent and resin composite as root canal filling materials with promising *in vitro* results (Britto et al., 2002, Gogos et al., 2003, Bitter et al., 2004). This type of sealer has been widely recognised due to its highly desirable property of creating monoblocks within the root canal space (Tay and Pashley, 2007). The term monoblocks referred to when a gap-free, solid mass material fill the canal space with the advantage of improving the seal and fracture resistant of the filled canal (Teixeira et al., 2004, Schwartz, 2006).

To date, 4 generations of methacrylate resin–based sealers have been introduced.In 1970, the first generation (Hydron) appeared. Poly hydroxyethyl methacrylate was the main ingredient. It was an injectable sealer and commercialized to be easy to use, nonirritating, adaptable to canal wall and inhibit bacterial growth (Kim et al., 2010). Subsequent studies were disastrous as they proved it caused severe inflammatory reaction (Langeland et al., 1981), material absorption (Yesilsoy, 1984) and inferior sealing ability (Rhome et al., 1981). For these reasons it vanished and the second generation took its place.

The second generation was a non-etching and hydrophilic sealer that did not require the use of adjunctive dentine adhesive agent. It flows into dentinal tubules to form a tag for retention after removal of smear layer (De Munck et al., 2004, Tay et al., 2005, Zmener et al., 2008). EndoREZ was an example of the second generation, it is used either with conventional gutta-percha or specially designed EndoREZ points (Zmener et al., 2008). However, insufficient bond to dentinal walls has been reported (Jainaen et al., 2007).

To simplify bonding procedures, the third generation (self-etching) and fourth generation (self-adhesive) have been introduced. Self-etching primer and a dual-cured resin composite root canal sealer are the main components of the third generation type while in the fourth generation, acidic resin monomers -originally present in dentin adhesive primers- are now integrated into the resin-based sealer / composite to make them self-adhesive to dentin substrates (Radovic et al., 2008, Kim et al., 2010).

In comparison to other common sealers, cytotoxicity rate was higher for methacrylate resin sealers (Pinna et al., 2008, Al-Hiyasat et al., 2010). Previous studies were not decisive whether methacrylate resin sealers have superior, similar or inferior properties when it come to sealing ability and leakage prevention. There are studies mentioned it has better sealing ability compared to other conventional sealers (Bodrumlu et al., 2007; Verissimo et al., 2007; Wedding et al., 2007). Other studies showed similar properties to conventional sealers (Belli et al., 2008, Williamson et al., 2009; Lyons et al., 2009). On the other hand, inferior sealing ability was also reported (Jack and Goodell, 2008; De-Deus et al., 2008, Kokorikos et al., 2009)

1.5.6 Calcium Silicate Based Materials

Materials based on calcium and silicate in composition such as mineral trioxide aggregate (MTA) and Biodentine have many clinical applications due to their excellent sealing ability and biocompatibility, these materials are used frequently for primary and permanent teeth pulp capping, perforation repair, filling of root-end and apical plugs for teeth with open apices (Parirokh et al., 2018; Torabinejad et al., 2018). Based on the favorable characteristic of such cements, root canal sealers based on calcium silicates, so called ‘bio-ceramic’ sealers, have been introduced to the field (Donnermeyer et al., 2018). This rise corresponds to the increased use of bio-ceramic technology in the medical and dental field.

1.5.7 MTA Based Sealers

Mineral trioxide aggregate (MTA) is a biomaterial that has been used in different endodontic procedures since 1990 (Roberts et al., 2008). First, it was introduced to the field by Torabinejad to repair perforations (Torabinejad et al., 1993). Nowadays, it is being used in many different applications such as vital pulp therapy, repair of root resorption and apexification procedures (Menezes et al., 2004, Jacobovitz and De Lima, 2008). MTA products are widely accepted due to its excellent biocompatibility and high sealing ability (Scarparo et al., 2010). A highly desirable property of root canal sealers would be the ability to induce mineralised tissue formation and optimal biocompatibility in the event of extrusion. This demand led to the development of MTA based root canal sealers. MTA sealers consist of a powder of fine hydrophilic particles that in the presence of moisture form a colloidal gel similar to the original MTA (Orosco et al., 2008, Gomes-Filho et al., 2009). One of its greatest advantages is that MTA particles can penetrate and occlude dentinal tubules closing a common source of microorganisms and reinfection (Rawtiya et al., 2013). On the other hand, few disadvantages have been reported such as teeth discoloration due to ferrous ions release and improper handling properties (Rawtiya et al., 2013).

1.6 Bio-Ceramic Root Canal Sealers

1.6.1 Historical Development of Bio-Ceramic Sealers

Bio-ceramic sealers have only been used in the last thirty years. The first documented use was in 1984 when (Krell and Wefel, 1984) published a comparison between Grossman's sealer and experimental calcium phosphate cement in extracted human teeth. They found no significance difference between the two sealers regarding apical occlusion, dentinal tubule occlusion, adaptation, adhesion, cohesion or morphological appearance. On the other hand, apical sealing ability was impaired and not as effective as Grossman's sealer (Krell and Madison, 1985).

The first bio-ceramic endodontic sealer based on calcium silicate released to the market was iRoot SP on 2007 (Innovative Bioceramix, Vancouver, Canada) (Donnermeyer et al., 2018). Since then a large variety of sealers have been developed all with differing claimed benefits and further classification have been made to differentiate them from conventional sealers. Bio-ceramics are classified according to their interaction with the surrounding tissues into bioactive and bioinert materials (Best et al., 2008); bio-ceramic sealers interact with the surrounding tissue to enhance durable tissues growth (Koch and brave, 2009) and hence are classed as bioactive materials.

Bio-ceramic based sealers that usually contain calcium silicate and/or calcium phosphate have started to gain increased popularity as they have been suggested as the most suitable material to minimise voids and simplify the obturation technique (Al-Haddad and Aziz, 2016).

Indeed, in endodontics bio-ceramic sealers are considered to be one of the materials that have changed the face of the field (Raghavendra et al., 2017).

1.6.2 Characteristics of Bio-Ceramic Sealers

Bio-ceramic sealers are hydrophilic material that reacts with water present in dentinal tubule and dentine humidity to completely set (Al-Haddad and Aziz, 2016). In physical properties, lower contact angle means increased hydrophilicity which results in faster spread of the liquid to wet the surface (Extrand, 2004). Bio-ceramic hydrophilicity reduces the sealer's contact angle and facilitate penetration into fine areas of the canal system. This will enhance the antibacterial effectiveness of sealers (Zhang et al., 2009). Moreover, from a biological point of view, as a result of this reaction with water, calcium silicate form calcium hydroxide (Camilleri et al., 2014, Berzins, 2014, pp. 17–36). This reaction can be found in two different types:

"$2\ [3\ CaO.SiO_2] + 6H_2O \rightarrow 3CaO.2SiO_2.3H_2O + 3\ Ca(OH)_2$

OR

$2\ [2\ CaO.SiO_2] + 4H_2O \rightarrow 3CaO.2SiO_2.3H_2O + Ca(OH)_2$"

(Donnermeyer et al., 2018).

This is useful because as mentioned in Section 1.5.3, CaOH is very useful in promoting periapical healing and has a good antimicrobial activity.

Bio-ceramic sealers possess superior properties such as:

I. Great bioactivity and biocompatibility that prevent surrounding tissues from rejecting them or causing foreign body reactions (Koch and Brave, 2009, Zhang et al., 2010, Willershausen et al., 2011). Biocompatibility is defined as "*the ability of a material to achieve a proper and advantageous host response in specific applications*" (Williams , 1987). Lack of biocompatibility can result in an adverse reaction such as toxicity, irritation, inflammatory reaction, allergy or even carcinogenicity (Sun et al., 1997). This point carries a high importance in endodontics, for example in the case of accidental overfilling or during root repair procedure, as a bio-ceramic sealer will not cause an inflammatory response (Koch and brave, 2009). This biocompatibility is believed to be due to the presence of calcium-phosphate in bio-ceramic sealers which happens to be the main inorganic component of dental and bone tissues (Al-Haddad and Aziz, 2016). Furthermore, immersion of Bioroot RCS sealer –one type of bio-ceramic sealer- in phosphate buffered saline (PBS) resulted in surface deposition of calcium hydroxyapatite which is an evident indication of biocompatibility (Donnermeyer et al., 2018).

II. Contrary to shrinkage after setting it actually expands which indicates excellent flowability and stability (Zhou et al., 2013, Celikten et al., 2016). A sealer's flow indicates how effectively the sealer will fill the irregularities, spaces and accessory canals. On the other hand, very high flow may result in extrusion into the periapical space. According to ISO 6876/2012 recommendations, sealers flow rate should be no less than 17 mm. Endosequence bio-ceramic sealer and MTA Fillapex sealer have flow rates of (23.1 ± 0.69 and 24. 9 ± 0.54) mm respectively (Zhou et al., 2013).

III. Setting time ideally should permit adequate working time and flow. However, a delayed setting time can result in a tissue irritation as most root canal sealers show some degree of toxicity until they completely set (Al-Haddad and Aziz, 2016). According to manufacturers, bio-ceramic sealers have a setting time of less than 240 minutes. MTA on the other hand has a setting time of less than 130 minutes. Zhou et al., 2013 reported a setting time of bio-ceramic sealer less than 162 minutes and less than 120 minutes for MTA Fillapex sealer. This data for MTA Fillapex was confirmed in another study (Vitti et al., 2013). The major difference depends on type of delivery between premixed sealers that required an external water supply (body fluid) or conventional method that have aqueous solution during mixing (Donnermeyer et al., 2018).

IV. Due to their alkalinity, PH ranging between 10-12, and release of calcium ions (Desai and Chandler, 2009, Zhou et al., 2013), bio-ceramic sealers have excellent antimicrobial activity which act against any survived residual intraradicular infection or microbes invading through microleakage (Zhang et al., 2009, Chotvorrarak et al., 2017). Their antibacterial activity has been proved against *Enterococcus faecalis* which is the major cause of endodontics infection (Zhang et al., 2009, Morgental et al., 2011). Moreover, it shows a superior antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Lactobacillus* and antifungal activity against *Candida albicans* (Ozcan et al., 2013, Nirupama et al., 2014, Singh et al., 2016). The only disadvantage regarding its antibacterial activity is that it is reported to be less than AH Plus against *Candida albicans* and *S. aureus* (Nirupama et al., 2014)

In addition, lactic acid from osteoclast activity can be neutralized by high alkaline pH of sealers which plays a major role in preventing dissolution of mineralized components of teeth and healing process (Stock, 1985). Calcium ions on the other hand stimulates repair via mineralized tissue deposition (Okabe et al., 2006).

V. Removability. In case of endodontic treatment failure, old obturation must be removed to establish a bacterial free root canal space and healthy periapical tissue. During retreatment most of the remaining material is usually sealer (Wilcox et al., 1987). For this reason, complete removal of old sealer is mandatory to perform a successful endodontic retreatment. Removability of Endosequence BC sealer was noted to be comparable to AH Plus –an epoxy resin based sealer which is the most commonly used – (Ersev et al., 2012). On the other hand, Bioroot BC sealer had even better removability when was compared to AH Plus sealer (Donnermeyer et al., 2018). Moreover, MTA Fillapex removability was comparable to AH Plus by all aspects including material remaining, dentine removal and time required (Neelakantan et al., 2013).

VI. Adhesion defined as "*the capacity to adhere to the root canal dentine and promote gutta-percha cone adhesion to each other and the dentine*" (Sousa-Neto et al., 2005). Bio-ceramic sealers have the ability to bond to both core filling material and dentine (Koch and brave, 2009). Several studies have proved that bio-ceramic sealers have high bond strength and able to resist de-bonding and dislodgement (Nagas et al., 2012, Ozcan et al., 2012, Shokouhinejad et al., 2013). Sealers that can adhere well to root canal dentine surface enhance the strength of remaining tooth structure hence long term success can be attained (Schafer et al., 2007, Onay et al., 2009)

VII. Post-endodontics treatment fracture resistant. Endodontically treated teeth are weak and more susceptible to fracture compared to normal vital teeth. Sealers nature and characteristics plays a role in strength and fracture resistance. Several studies proved that bio-ceramic sealers possess superior fracture resistant ability when compared to different sealers. Teeth obturated with bio-ceramic sealer were more fracture resistant when compared to AH Plus sealer (Ulusoy et al., 2011, Sagsen et al., 2012, Topcuoglu et al., 2013), glass-ionomer based sealer (Ghoneim et al., 2011) and calcium hydroxide based sealer (Patil et al., 2017).

VIII. A number of bio-ceramic sealers exist in the marketplace, all with differing claimed benefits.

Previous studies on MTA based sealers, a sister family to bio-ceramics, reported reduced apical leakage over time (Weller et al., 2008, Gandolfi and Prati, 2010, Gandolfi et al., 2013). Studies have also found that bio-ceramic and MTA cements occlude dentinal tubules which are the key source of reinfection. Studies on certain brands of bio-ceramic sealers found dentinal tubules penetration as deep as 2 mm (Donnermeyer et al., 2018). This advantage is believed to be due to the remineralising properties of forming apatite precipitates (Gandolfi et al., 2008, Goldberg et al., 2009, Gandolfi et al., 2012) and the hydrophilic nature of bio-ceramic sealers. Although some evidence is available on bio-ceramic sealers, there is a lack of long-term data and robust studies on the suitability of these sealers (Al-Haddad and Aziz, 2016).

Therefore, robust evaluation of these new materials is essential to validate their suitability for clinical practice and to provide clinicians with strong evidence to help them select appropriate materials for better treatment outcomes. Ideally, conducting randomised clinical trials would provide clinicians with excellent strong clinical evidence that may change their practice. However, due to their time-consuming nature and the significant associated costs, robust well planned *in-vitro* studies could provide good suitable evidence on the properties of these materials and their suitability for proposed clinical applications.

1.7 Obturation Techniques

Several obturation techniques have been developed in an attempt to reduce void formation during root canal filling. The most common obturation techniques to fill the canal space include cold lateral compaction, single cone, warm vertical compaction and carrier-based techniques. However, existing obturation strategies offer little difference in their long-term outcome results and to date there is no technique that prevents leakage (Aqrabawi, 2006, Ng et al., 2007).

Lateral compaction of gutta-percha cones with a root canal sealer remains the most popular root canal filling technique (Alicia et al., 2007, Johnson, 2010, pp. 349–388) and continues to be the main method taught in UK dental schools. On the other hand, it has some disadvantages including that it is time consuming, with a lack of adaption of gutta-percha to root canal wall, a reduced ability to fill the complex root canal system or irregularities and difficulty of achieving a uniform density (Johnson, 2010, pp. 349–388).

The single cone technique became more popular after the use of rotary NiTi instrument and matched taper gutta-percha cones. This technique permits better adaptation in 3-dimensional preparation (Cavenago et al., 2012) and it is a time saving technique compared to lateral condensation technique (Tasdemir et al., 2009). According to several studies, the single cone technique is highly recommended to be used with bio-ceramic sealers due to its excellent dimensional stability and flowability (Celikten et al., 2016). iRoot SP sealer –one type of bio-ceramic sealers- was reported to have high push out bond strength when used with single cone obturation technique, this strength even increased over a period of time (Yap et al., 2017).

1.8 Microcomputed Tomography

Traditional CT technology cannot be used effectively in dental studies due to its limited vertical resolution capacity. Moreover, low resolution is absolutely insufficient for the reconstruction of small objects such as human teeth (Tachibana and Matsumoto 1990, Nielsen et al. 1995).

In the early 1980s, microscopic computed tomography (micro-CT) was first developed (Elliott and Dover, 1982, Flannery et al., 1987; Sasov, 1987). The X-ray cone beam is used in bench-top systems to magnify the X-ray beam. Thus, in the later 1980s, the development of a cone-beam reconstruction algorithm by (Feldkamp et al. 1984) has greatly facilitated a bench-top micro-CT.

Microcomputed tomography (micro-CT) is a non-destructive 3D imaging technique used to evaluate the microarchitecture, morphology and density of mineralized tissues and the internal structure and porosity of biomaterials.

Micro-CT technologies are basically similar but differ in details regarding image quality and resolution as well as 3D volume that can be imaged in a given duration.

(Ritman, 2011) has described its mechanism of action " *3D X-ray imaging method that involves obtaining X-ray projection images at many angles of view around an axis through an object and then applying a tomographic reconstruction algorithm to generate a stack of thin tomographic images of contiguous transaxial slices through the object. The transaxial images are made up of voxels. A specimen is usually scanned* *by rotating it around a vertical axis within a system comprising a stationary X-ray source and an X-ray imaging array.*"

The term micro-CT is mainly used for scans with submillimeter voxel resolution. Currently, further development has resulted in two main advantages;

1) Radiation exposure reduction and/or speed up the scanning procedure.

2) Reduction in the need for X-ray detector arrays to cover the transaxial extent of the body entirely.

In research, the use of micro-CT has progressively increased from 1982 until now. In 2009 alone, there were around 1000 publications based on the use of micro-CT technique (Ritman, 2011).

Vertical resolution was increased to reach 100-200 µm (Nielsen et al. 1995, Bjorndal et al. 1999), then furtherly improved to reach 81 µm (Rhodes et al. 1999). further improvements increased resolution values between 34 and 68 µm (Dowker et al. 1997, Peters et al. 2000, Gluskin et al. 2001) to 25 µm (Verdonschot et al. 2001) and 15 µm (Verna et al. 2002). Currently, resolutions less than 10 µm are possible.

In endodontics, micro-CT has been widely used as a research tool to evaluate root canal anatomy, geometry and shape (Peters et al., 2000), to analyse spatial details after root canal instrumentation (Peters et al. 2003, Metzger et al. 2010, Markvart et al. 2012) and to study retreatments (Huumonen et al. 2006, Barletta et al. 2008). More related to this study, it has been frequently used to evaluate the porosities within the root canal fillings using different endodontic sealers and various filling techniques (Jung et al., 2005, Zaslansky et al., 2011, El-Ma'aita et al., 2012, Gandolfi et al., 2013). Moreover, quantitative data can be obtained via 3D assessment.

Chapter 2
Aims and Objectives

The aim of this study was to investigate the sealing ability of four different bio-ceramic root canal sealers used in a single cone technique.

Specific objectives included:

- Developing a method using micro-CT scanning to analyse void volumes within root canal fillings.
- Comparing void volumes within the root systems of teeth filled with one of four types of bio-ceramic sealer.
- Comparing void volumes in apical, middle and coronal sections of teeth for each sealer. Along with comparing all the similar thirds of the four sealers.

Chapter 3
Materials and Methods

3.1 Teeth selection

Forty human single rooted premolars were selected from the School of Dentistry Tissue Bank (Tissue Bank application number 270919/SA/285, School of Dentistry, University of Leeds). All collected samples were allowed to be used for researches purposes after informed consent from patients. Teeth samples were examined visually and radiographically prior to their selection. Pre-operative radiographs were taken in both mesio-distal and bucco-lingual direction to evaluate the anatomy of the teeth. The inclusion criteria were single rooted teeth with fully developed root apices, single apical foramen and no root caries, fractures or resorption.

3.2 Testing groups

Teeth were randomly divided into four groups according to the type of sealer which will be used for obturation as shown in Table 1.

Table 1- Testing groups

Group	Sealer Type
Group A	MTA Fillapex sealer (Angelus – Brussels, Belgium)
Group B	Bioroot RCS sealer (Septodont GmbH – Germany)
Group C	Endosequence BC sealer (Innovative BioCeramix Inc, Vancouver, Canada)
Group D	Wellroot ST sealer (Vericom – Korea)

Table 2- Composition of Sealers

Composition of MTA Fillapex sealer	
Paste A	Methyl Salicylate, Butylene Glycol, Colophony, Bismuth Trioxide and Fumed Silicon Dioxide
Paste B	Fumed Silicon Dioxide, Titanium Dioxide, Tricalcium silicate, Dicalcium Silicate, Calcium Oxide, Tricalcium Aluminate, Pentaerythritol Rosinate and P - Toluenesolfonamide
Composition of Bioroot RCS sealer	
powder	Tricalcium Silicate, Zirconium Oxide and Povidone
Aqueous solution	Calcium Chloride and Polycarboxylate
Composition of Endosequence BC sealer	
Zirconium Oxide, Tricalcium Silicates, Dicalcium Silicates, calcium Phosphate and Calcium Hydroxide	
Composition Wellroot ST sealer	
Calcium Aluminosilicate Compound, Zirconium Oxide, Filler and Thickening Agent.	

3.3 Pilot study

A pilot study was carried out on three single rooted teeth (selected in a similar manner to the main sample criteria). These teeth were obturated with Bioroot RCS sealer. Post-preparation and post-obturation CT scanning was carried out. Voids percentage of the 3 teeth was compared to 3 teeth obturated by an endodontics consultant in the department and both results showed similar average voids percentage. The main reason of the pilot teeth study was to ensure the selected criteria fitted this study including micro-CT scanning, analysis parameters and any bias due to operator experience.

3.4 Root canal preparation

As tooth preparation is a technique sensitive procedure and to avoid self-improvement effects on the final result, teeth were prepared as follow: A1, B1, C1, D1, A2, B2, C2, D2…etc

An access cavity was performed with carefully such that burs did not pass the CEJ to ensure root canal preparation beyond CEJ was completed exclusively with the use of initial K-file and wave one gold rotary system.

Root canal preparation started with scouting of root canal using #10 K-file. The K-file was inserted into the root canal to the radiographic apex. The final working length was then determined by subtracting 0.5 mm from this length.

Once the proper glide path was obtained, reciprocating preparation with Wave One Gold Primary file in line with the manufacturer's procedure recommendation was undertaken. Canals were irrigated throughout the preparation phase with a total of 6ml of 5.25% NaOCl with a 27 mm gauge side vented syringe as follows: after K-file use, after preparing of every 3 mm of the canal to avoid blockage and after recapitulation. One file was used for each tooth then discarded and To avoid changing the concentration of NaOCl one bottle of 5.25% NaoCl was used for every 5 teeth prepared in one session. Final irrigation was done using 17% EDTA (Ethylenediamine tetraacetic acid) for 5 minutes then each canal was dried with paper points.

As teeth needed to be micro-CT scanned prior to obturation, teeth were stored in Hanks balanced salt solution which is the gold standard of storage media that produce conditions similar to the socket environment (Poi et al.,2013). All samples were prepared and obturated by a single operator.

3.5 Teeth obturation

Teeth were randomly divided into 4 groups of 10 teeth each. All canals were obturated using matched taper gutta-percha cones with a single cone technique. Each root canal sealer was prepared and used in accordance with the manufacturer's recommendation. Obturation was done in an order A1, B1, C1, D1 and so on as what was applied on preparation phase. Group A was filled with MTA Fillapex sealer that was mixed on a glass slide using a restorative plastic instrument then inserted into the root canal using a size 15 K-file and spread onto the surface of the root canal wall. Gutta-percha cones were then coated with a thin layer of the sealer and inserted into the root canal space. The coronal excess was severed with a hot excavator and compacted vertically to the level of CEJ with a plugger. Group B was filled with Bioroot RCS sealer that used in a similar manner to group A. Group C filled with Endosequence BC sealer that was injected directly into the root canal space using a sealer syringe tip inserted into the canal at the end of coronal one-third then a size 15 K-file used to spread the existing sealer into the canal walls. A gutta-percha cone coated with a thin layer of sealer was then inserted slowly into the root canal space. Group D was filled with Wellroot ST sealer that was used in a similar manner to group C. Intermediate peri-apical radiographs were taken to ensure the quality and full length obturation before final vertical compaction.

As teeth were to be stored in Hanks solution. A coronal seal was achieved by flowable composite restoration. Another composite restoration was applied to the apex to avoid any leakage from there.

After the obturation, teeth were incubated at 37 °C in 100% humidity to be scanned in a period of 7 days after obturation.

3.6 3D Micro-CT scanning

Scanning was performed using a high resolution micro-CT (Skyscan 1172 Bruker, Belgium). Micro-CT scanning and analysis was conducted at three time points; before preparation, immediately following preparation to measure the intra canal volume which will be considered as the predefined volume of the unfilled canal. This was used as a base line for comparison to measure the percentage of voids/unfilled spaces following root canal obturation and 7 days after obturation to allow full setting of the sealer

Scanning parameters were kept constant for each scan as follow: 13.65 µm resolution, medium camera pixel 2k x 1k, 100 kV source voltage, 100 µA source current, 180˚ of rotation around its vertical axis using 0.51˚ of rotation step, 0.5 mm of Al filter and the total scanning time was around 1 hour. Each tooth was positioned centrally on the specimen platform with buccal surface facing the operator. Micro-CT scanning using these parameters provided 378 TIFF images (1220 x 1332 pixels).

Scanning parameters were selected to match a previous department study and along with the manufacturer's recommendation. The suitability of the samples was confirmed with the pilot study.

3.7 Micro-CT analysis

NRecon software (version 1.7.4.6 Skyscan, Bruker, Belgium) was used to perform image reconstruction to obtain 2D axial 1012 x 1012 pixel images. Reconstruction parameters were set at ring artifice correction = 3, beam hardening correction of 30% and contrast limits following manufacturers recommendations. On average 1,286 2-dimensional slices of the root from CEJ to radiographic apex cross sectional images were reconstructed per tooth.

This reconstruction was applied twice, the first reconstruction was applied on post-preparation CT scan to obtain empty canal volume images while the second reconstruction applied on post-obturation CT scan to obtain canal filling/void volume images.

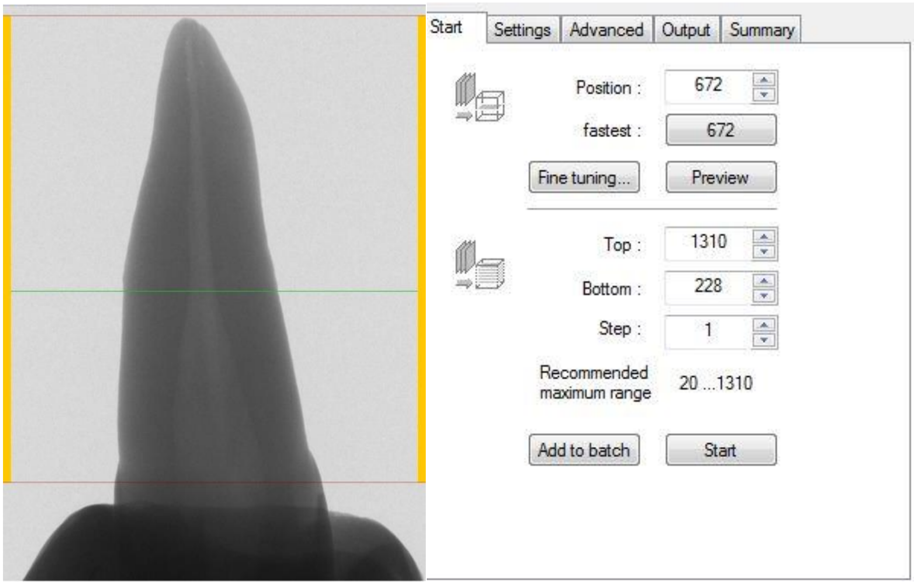

Figure 1- NRecon reconstruction of the entire tooth, bottom line placed near the CEJ while top line should be placed at the end of radiographic apex.

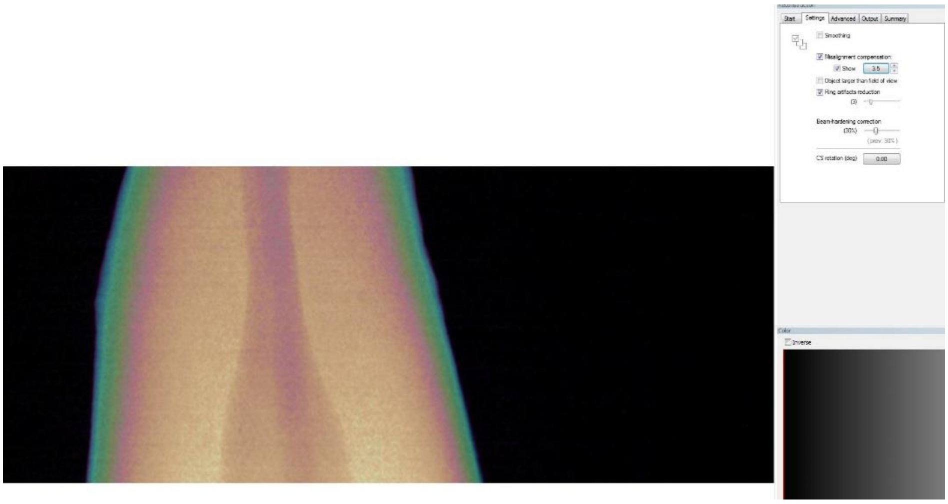

Figure 2- NRecon parameters selection and preview image of the root to ensure no rings or mishaps will be shown in the root slices image.

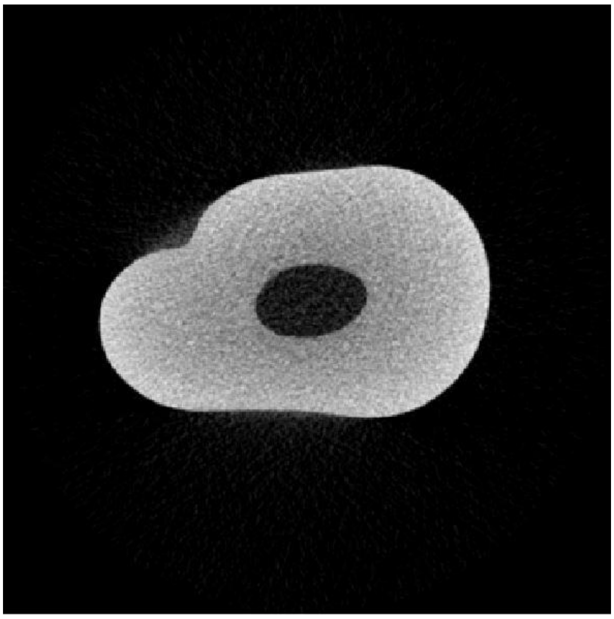

Figure 3- 2-D slice of the post-preparation root image showing the canal shape and borders accurately.

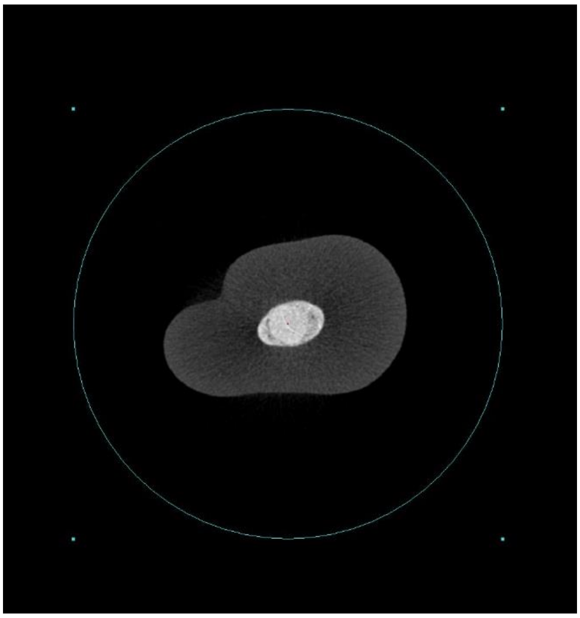

Figure 4- 2-D slice of the post-obturation root image showing the filled canal.

After reconstruction, Data Viewer software (version 1.5.4.0 Skyscan, Bruker, Belgium) was used for post-preparation post-obturation scan superimposition and 3-dimensional registration. This step resembles taking the root canal filling from the filled root and situate it in the empty canal of the prepared root in order to allocate the voids and gaps. The superimposition takes place in vertical, horizontal and axial axis. This step plays the main and most important role in accurate positioning of the two canals over each other for the matter of quantitative analysis in following steps. Two sets of images resulted from 3-D registration, reference and target images. 'Reference' image stands for the post-prepared canal while 'target' image stands for the post-obturated image.

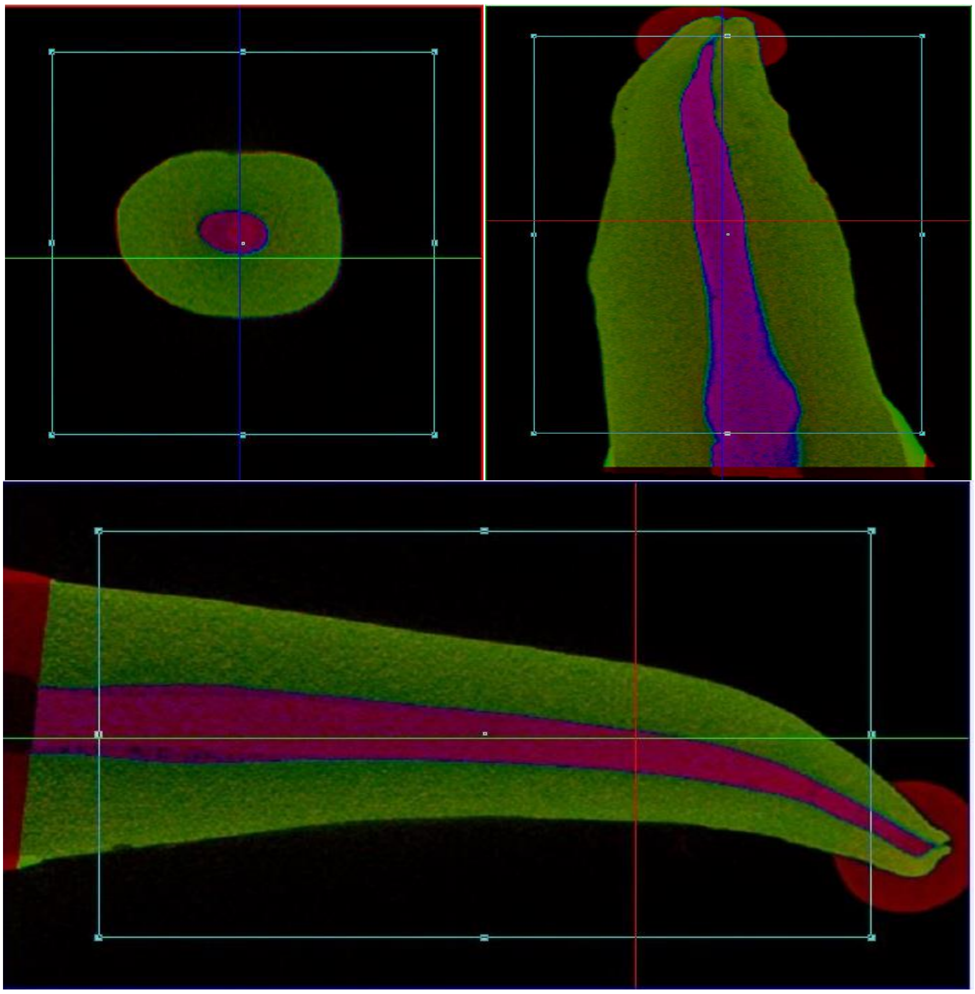

Figure 5- 3-dimensional registration of post-prepared post-obturated scans. Red color represents area that have not been covered e.g. composite apical block placed only after obturation. Root canal wall colored dark blue after superimposition for accuracy matter

The CTAn software (version 1.17.7.2 Skyscan, Bruker, Belgium) was used for 3-D visualization, analysis and volumetric measurements of root canal.

After 3-D registration was performed by data viewer software, new cross sectional images perpendicular to the long axis of the root were obtained with an average number of 320 images.

Steps for void measurements were as follow;

- A proper grey threshold level was applied on the reference image that changed slides into black/white canal/dentine images. 73 grey level was chosen for all samples as it was the most appropriate level that resemble canal geometry accurately without including any parts of the dentine to avoid misreading of dentine as unfilled canal space.

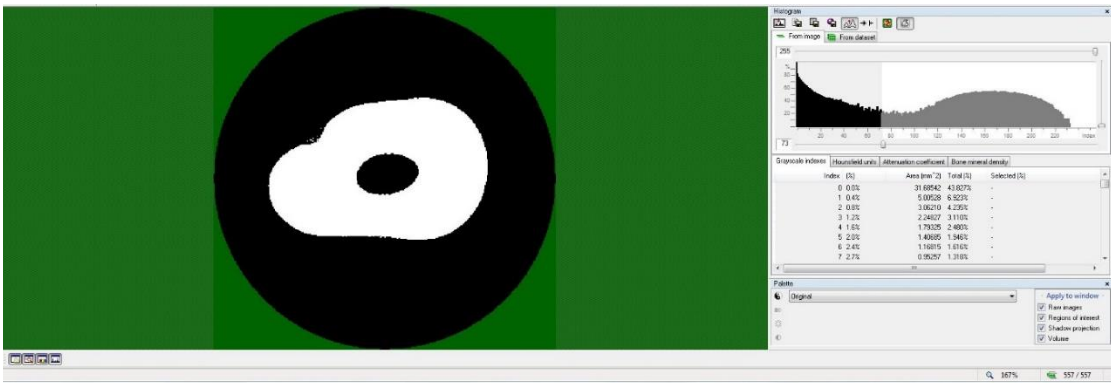

Figure 6- Choosing 73 grey threshold level to change slides into black/white canal/dentine images.

- Root canal space alone was extracted by copying the Reference image into a temporary region of interest (ROI) then removing the root canal space from Reference image by the command “remove pores” and finally “image = image subtraction ROI” and root canal space alone will be resulted. Despeckling was performed to remove any white spots less than 10 pixels that may appear laterally to the canal space. Empty root canal space then saved as a ROI.

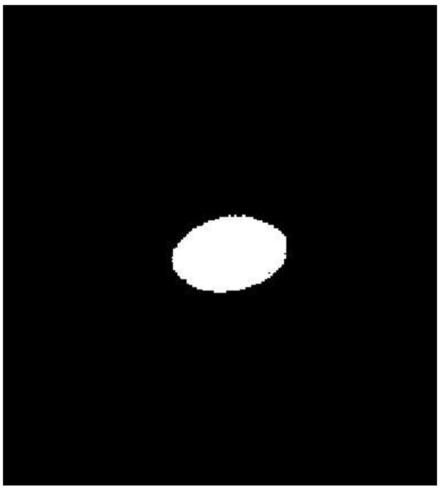

Figure 7- Extraction of unfilled root canal space

- ROI was uploaded into target image resulted from 3-D registration that represent the filled canal. Matching accuracy was checked by ensuring ROI strictly following the canal space anatomy over the filled canal. This superimposition saved as volume of interest (VOI).

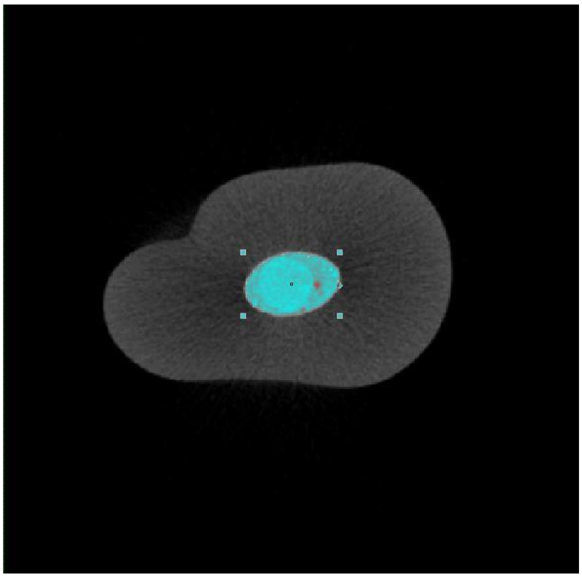

Figure 8- ROI uploaded into target image resulted from 3-D registration that represent the filled canal. This represents obturation superimposed the canal space.

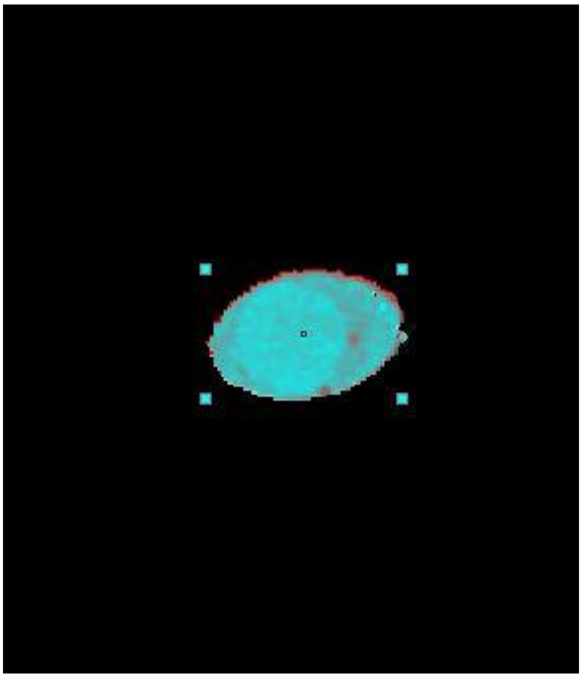

Figure 9- VOI that represent root canal filling superimposed the canal space.

- VOI was uploaded and the proper grey threshold level selected again. At this moment slide was shown as white density represent the obturation and black points represents the voids.

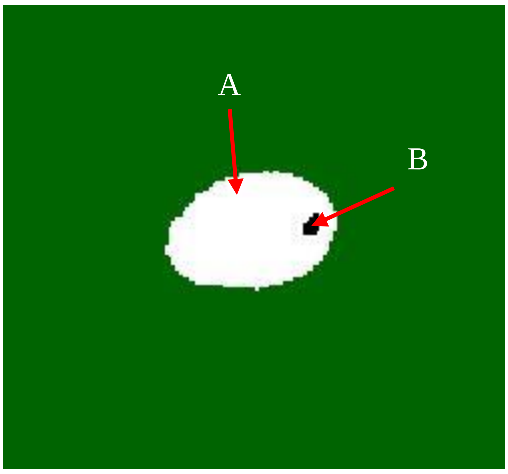

Figure 10- Grey threshold level selecting for VOI. a. obturation b. voids.

Finally for the purpose of voids percentage analysis the formula image = ROI subtract image was applied to show voids only as WHITE spots.

Figure 11- Voids only are shown as white spots for voids percentage analysis

- Finally, 3-D analysis was applied four times, entire root measurements that starts from CEJ until the apex, coronal third measurements, middle third measurements, and apical third measurements to obtain the following volumes;
    - Canal volume (the volume of empty root canal space), voids volume and voids percentage.
    - Filling volume -the volume of obturation within root canal space- (sum of the volume of the gutta-percha and sealer) found by subtracting voids volume from canal volume.
    - Filling percentage found by the equation
    - Filling percentage = (filling volume / canal volume) x 100

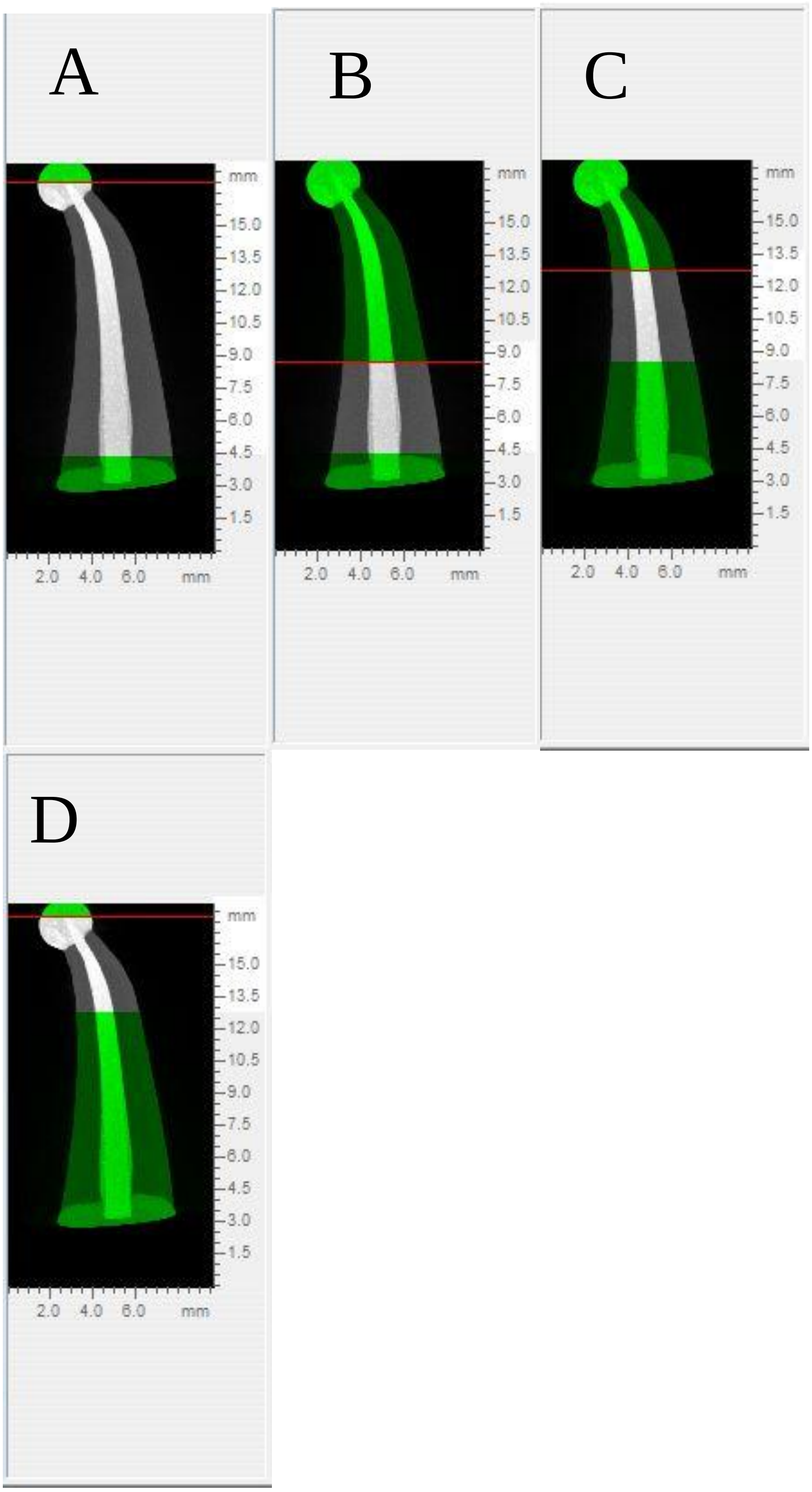

Figure 12- 3-D analysis of a. full root length. b. Coronal third. c. Middle third. d. Apical third.

All software parameters were kept standard for all samples.
Scanning and analysis performed by one examiner. To ensure procedure accuracy, analysis of the pilot teeth were repeated until the same volumes resulted at each time for each sample.

3.8 Statistical analysis

The results were analysed using SPSS statistical software (Version 26, Chicago, USA). Kruskall-Wallis H and Dunn-Bonferoni post-hoc testing was used to compare between and within groups. A *P* value of less than 0.05 was considered to indicate statistical significance.

3.9 Normality test

An assessment of the normality of data is a prerequisite for many statistical tests because normal data is an underlying assumption in parametric testing. A normality test therefore guides the researcher to either parametric or non-parametric statistical tests to look for differences between groups. Table 3 below, generated using SPSS software, displays the results for the data in this study from two well-known tests of normality, namely the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test, displayed in Tables 3-1 to 3-8 for each material. The Shapiro-Wilk Test is more appropriate for small sample sizes (< 50 samples), and for this reason, this study uses the Shapiro-Wilk test to assess normality.

Chapter 4
Results

4.1 Normality test

The significance value was less than 0.05 for 3 out of the four groups which indicates data are not normally distributed apart from for the Bioroot group. Relevant histograms are presented in (Appendix).

For this reason, statistical analysis was done using the Kruskal–Wallis H test. This can be regarded as the non-parametric alternative to the one-way ANOVA and is used to allow the comparison of more than two independent groups. Just like the ANOVA test, the Kruskal-Wallis H test cannot tell you which *specific* groups are statistically significantly different from each other; it only tells you that at least two groups were different. Accordingly, it may be necessary to carry out a post hoc test to identify between which groups differences occur.

Table 3-1 To 3-8- Samples normality test of total voids percentage and every third void percentage

Tests of Normality (MTA Fillapex)						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Canal Volume	.143	10	.200*	.946	10	.624
Void Volume	.219	10	.191	.852	10	.061
Void Percentage	.280	10	.025	.835	10	.038
Filling Volume	.159	10	.200*	.944	10	.596
Filling Percentage	.280	10	.025	.835	10	.038
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Tests of Normality (Bioroot)						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Canal Volume	.185	10	.200*	.875	10	.114
Void Volume	.201	10	.200*	.878	10	.125
Void Percentage	.244	10	.094	.878	10	.124
Filling Volume	.215	10	.200*	.831	10	.034
Filling Percentage	.244	10	.094	.878	10	.124
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Tests of Normality (Endosequence)						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Canal Volume	.169	10	.200*	.894	10	.189
Void Volume	.232	10	.137	.790	10	.011
Void Percentage	.271	10	.036	.753	10	.004
Filling Volume	.231	10	.140	.864	10	.086
Filling Percentage	.271	10	.036	.753	10	.004
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Tests of Normality (Wellroot)						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Canal Volume	.130	10	.200*	.948	10	.650
Void Volume	.185	10	.200*	.866	10	.090
Void Percentage	.262	10	.050	.796	10	.013
Filling Volume	.127	10	.200*	.939	10	.538
Filling Percentge	.262	10	.050	.796	10	.013
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Tests of Normality (MTA Fillapex) Coronal Third						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MTA Fillapex	.274	10	.033	.818	10	.024
a. Lilliefors Significance Correction						

Middle Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MTA Fillapex	.279	10	.026	.823	10	.028
a. Lilliefors Significance Correction						

Apical Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MTA Fillapex	.353	10	.001	.660	10	.000
a. Lilliefors Significance Correction						

Tests of Normality (Bioroot) Coronal Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Bioroot	.276	10	.029	.802	10	.015
a. Lilliefors Significance Correction						

Middle Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Bioroot	.416	10	.000	.478	10	.000
a. Lilliefors Significance Correction						

Apical Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Bioroot	.389	10	.000	.580	10	.000
a. Lilliefors Significance Correction						

Tests of Normality (Endosequence) Coronal Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Endosequence	.233	10	.131	.798	10	.014
a. Lilliefors Significance Correction						

Middle Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Endosequence	.333	10	.002	.675	10	.000
a. Lilliefors Significance Correction						

Apical Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Endosequence	.385	10	.000	.703	10	.001
a. Lilliefors Significance Correction						

Tests of Normality (Wellroot) Coronal Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wellroot	.362	10	.001	.689	10	.001
a. Lilliefors Significance Correction						

Middle Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wellroot	.242	10	.098	.811	10	.020
a. Lilliefors Significance Correction						

Apical Third						
	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wellroot	.243	10	.098	.885	10	.148
a. Lilliefors Significance Correction						

4.2 MTA Fillapex sealer's voids percentage

Voids percentages of samples obturated with MTA Fillapex sealer are shown in table 4. The mean voids percentage was 12.07 % with minimum sample reading of 1.49% and maximum reading of 30.68% as shown in table 5.

Table 4- Voids percentage of teeth obturated with MTA Fillapex root canal sealer.

MTA Fillapex sealer					
Samples	**Canal volume**	**Voids volume**	**Voids percentage**	**Filling Volume**	**Filling percentage**
A1	13.76	0.57	4.17	13.19	95.83
A2	17.45	1.56	8.92	15.89	91.08
A3	10.76	2.81	26.12	7.95	73.88
A4	14.75	4.53	30.68	10.23	69.32
A5	9.36	0.57	6.06	8.79	93.94
A6	9.17	0.14	1.49	9.03	98.51
A7	15.49	0.79	5.12	14.69	94.88
A8	12.20	1.22	9.99	10.98	90.01
A9	7.86	0.48	6.08	7.38	93.92
A10	16.37	3.62	22.10	12.75	77.90
Mean	12.72	1.63	12.07	11.09	87.93

Table 5- Minimum and maximum voids, mean and SD of MTA Fillapex sealer samples

MTA Fillapex voids Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
voids percentage	10	1.49	30.68	12.07	10.29

4.3 MTA Fillapex sealer's voids percentage per part

Dividing the tooth into three thirds (coronal, middle and apical) resulted in most voids percentage in the coronal third (mean= 15%) followed by the middle third (mean= 6.60%) and least voids was in the apical third (mean= 5.08%). Table 6 shows the voids percentage of each sample.

Minimum voids of coronal third was 1.95% while the maximum was 37.74%. In the middle third 0.44% was the minimum voids while 16.76% was the maximum. Apical third recorded minimum voids of 0.60% and maximum of 22.66% as shown in table 7. Figure 13 shows mean voids percentage and error bar of MTA Fillapex samples per third.

Table 6- Voids percentage per part of samples obturated with MTA Fillapex sealer.

MTA Fillapex voids percentage per third			
Samples	**Coronal Third**	**Middle Third**	**Apical Third**
A1	5.28	1.58	0.87
A2	12.51	2.68	1.28
A3	31.44	16.74	3.53
A4	37.74	16.76	22.66
A5	3.70	11.53	9.63
A6	1.95	0.44	0.60
A7	5.92	3.59	2.53
A8	11.87	6.85	2.99
A9	7.57	2.11	4.18
A10	32.05	3.71	2.47
Mean	14.99	6.60	5.08

Table 7- Minimum and maximum voids, mean and SD of coronal, middle and apical third of MTA Fillapex sealer samples

MTA Fillapex Coronal Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	1.95	37.74	14.99	13.44
MTA Fillapex Middle Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.44	16.76	6.60	6.21
MTA Fillapex Apical Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.60	22.66	5.08	6.69

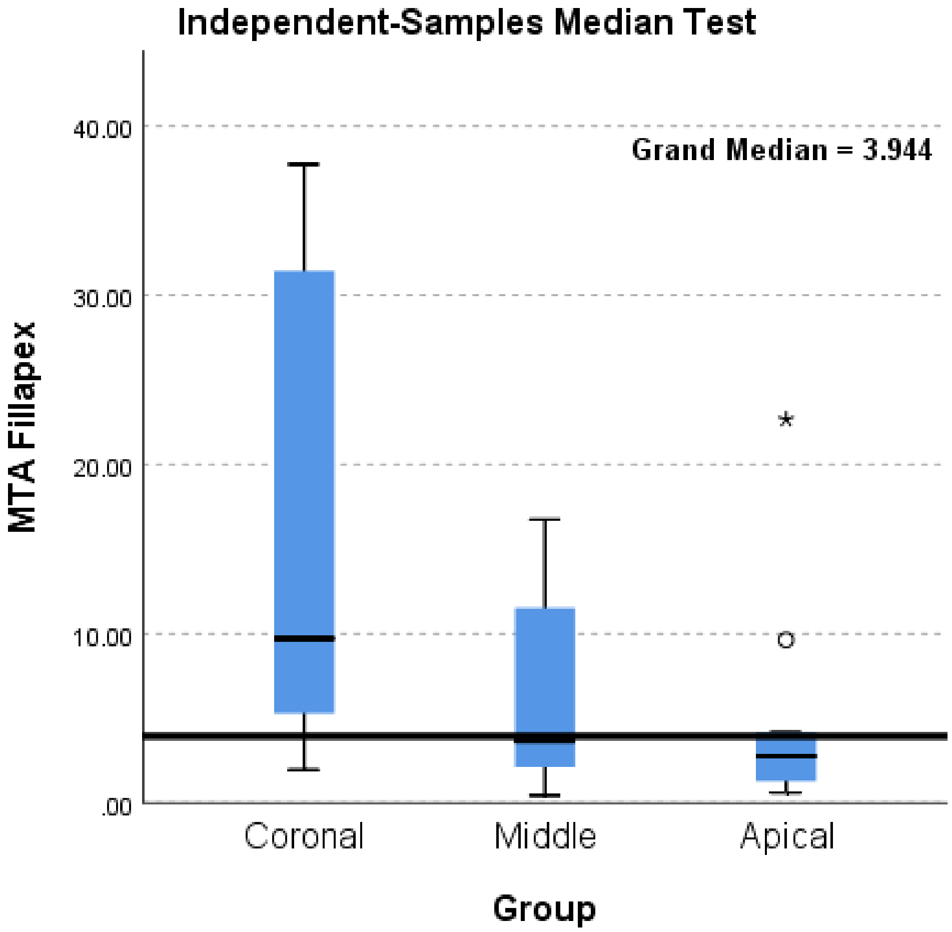

Figure 13- Mean voids percentage and error bar of MTA Fillapex samples per third.

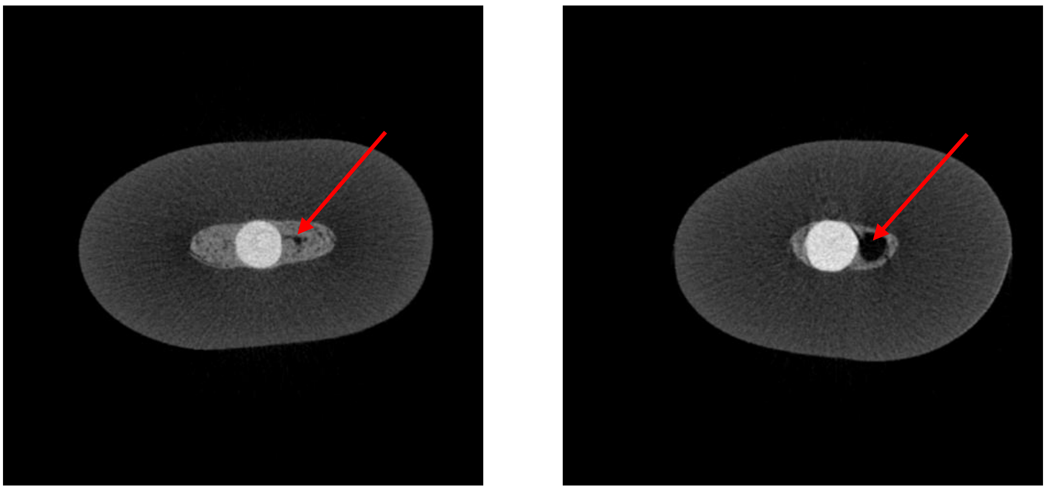

Figure 14- Two coronal third's slides (high and low voids volumes) of samples obturated with MTA Fillapex sealer, red arrows point for voids within obturation

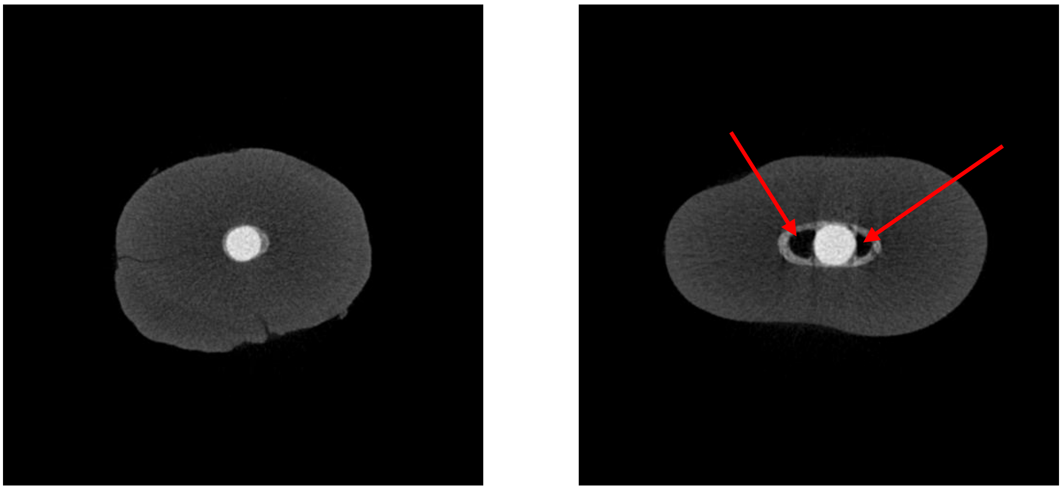

Figure 15- Two middle third's slides (high and low voids volumes) of samples obturated with MTA Fillapex sealer, red arrows point for voids within obturation

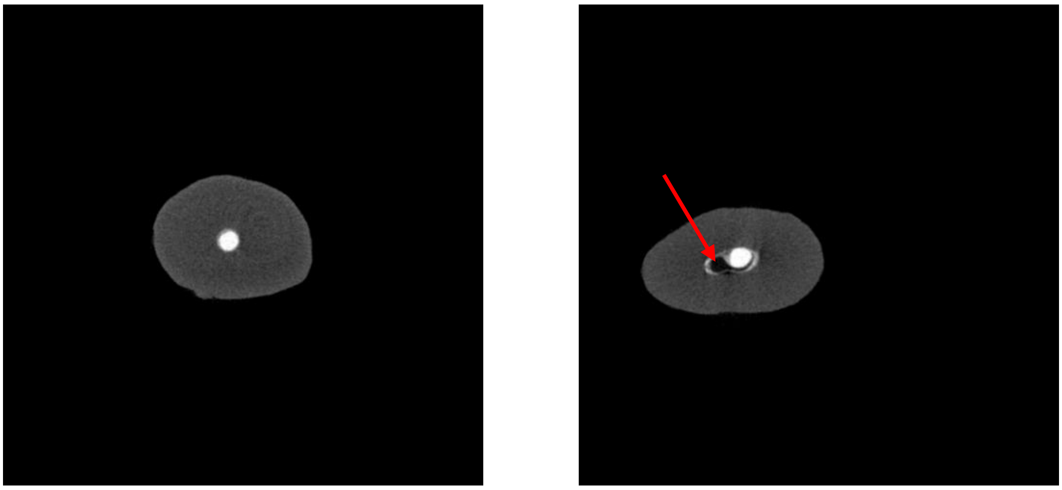

Figure 16- Two apical third's slides (high and low voids volumes) of samples obturated with MTA Fillapex sealer, red arrows point for voids within obturation

4.4 Comparison between voids percentage per third of MTA Fillapex sealer's samples.

Table 8 shows comparison between the void percentage per part of the tooth. Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no statistically significant difference between the three thirds (P>0.05) when using MTA Fillapex sealer. volumetrically, the coronal third showed the most voids and the apical third showed the least voids.

Table 8- comparison of voids percentage per part of samples obturated with MTA Fillapex sealer

MTA Fillapex Thirds Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of MTA Fillapex are the same across categories of Group.	Independent-Samples Median Test	.061	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

4.5 Bioroot sealer's voids percentage

Voids percentages of samples obturated with Bioroot sealer are shown in table 9.
The mean voids percentage was 10.53% with minimum sample reading of 1.01% and maximum reading of 27.27% as shown in table 10.

Table 9- Voids percentage of teeth obturated with Bioroot root canal sealer.

Bioroot sealer					
Samples	**Canal volume**	**Voids volume**	**Voids percentage**	**Filling Volume**	**Filling percentage**
B1	11.88	0.84	7.04	11.05	92.96
B2	14.95	2.24	15.01	12.71	84.99
B3	27.32	1.16	4.24	26.16	95.76
B4	14.81	0.46	3.12	14.35	96.88
B5	12.02	2.05	17.03	9.98	82.97
B6	12.63	3.45	27.27	9.19	72.73
B7	18.17	4.21	23.18	13.96	76.82
B8	10.84	0.11	1.01	10.73	98.99
B9	8.24	0.49	5.93	7.75	94.07
B10	16.62	0.24	1.43	16.38	98.57
Mean	14.75	1.52	10.53	13.22	88.46

Table 10- Minimum and maximum voids, mean and SD of Bioroot sealer samples.

Bioroot voids Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	1.01	27.27	10.53	9.45

4.6 Bioroot sealer's voids percentage per part

Most voids percentage was observed in the coronal third (mean= 13.28%) followed by the apical third (mean= 5.14%) and least voids was in the middle third (mean= 5.08%). Table 11 shows the voids percentage of each sample.

Minimum voids of coronal third was 0.70% while the maximum was 37.58%. In the middle third 0.14% was the minimum voids while 35.72% was the maximum. Apical third recorded minimum voids of 0.02% and maximum of 28.76% as shown in table 12.

Figure 17 shows mean voids percentage and error bar of Bioroot samples per third.

Table 11- Voids percentage per part of samples obturated with Bioroot sealer.

Bioroot voids percentage per third			
Samples	**Coronal Third**	**Middle Third**	**Apical Third**
B1	10.53	2.46	0.35
B2	1.63	35.72	28.76
B3	5.26	1.45	5.21
B4	4.51	0.38	2.43
B5	25.79	1.78	2.90
B6	37.59	4.55	2.06
B7	35.53	2.68	5.37
B8	0.70	0.14	4.05
B9	9.15	0.31	0.27
B10	2.09	0.33	0.02
Mean	13.28	4.98	5.14

Table 12- Minimum and maximum voids, mean and SD of coronal, middle and apical third of Bioroot sealer samples

Bioroot Coronal Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.70	37.58	13.28	14.25
Bioroot Middle Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.14	35.72	4.98	10.89
Bioroot Apical Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.02	28.76	5.14	8.52

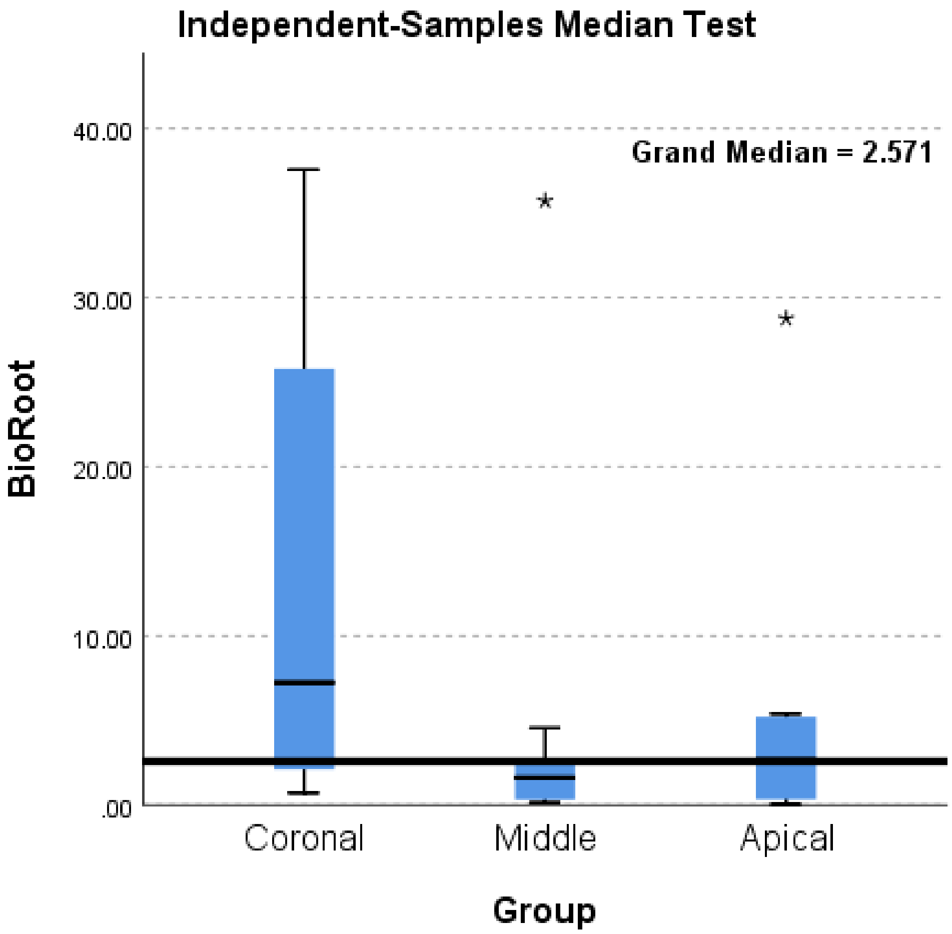

Figure 17- **Mean voids percentage and error bar of Bioroot samples per third.**

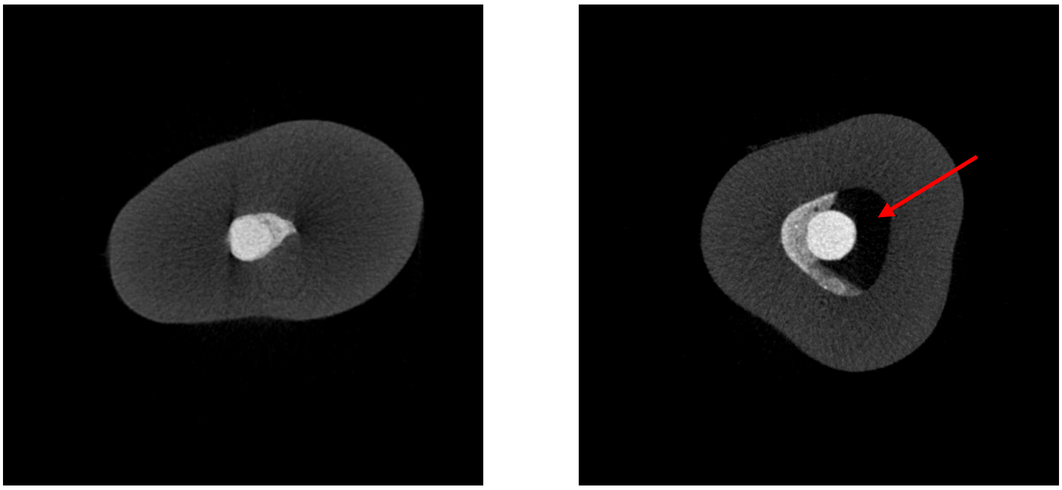

Figure 18- Two coronal third's slides (high and low voids volumes) of samples obturated with Bioroot sealer, red arrows point for voids within obturation

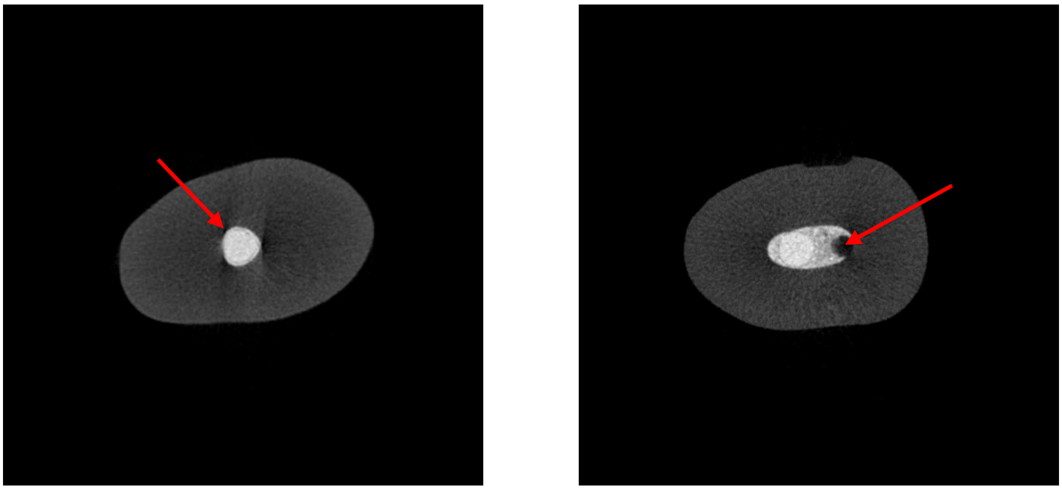

Figure 19- Two middle third's slides (high and low voids volumes) of samples obturated with Bioroot sealer, red arrows point for voids within obturation

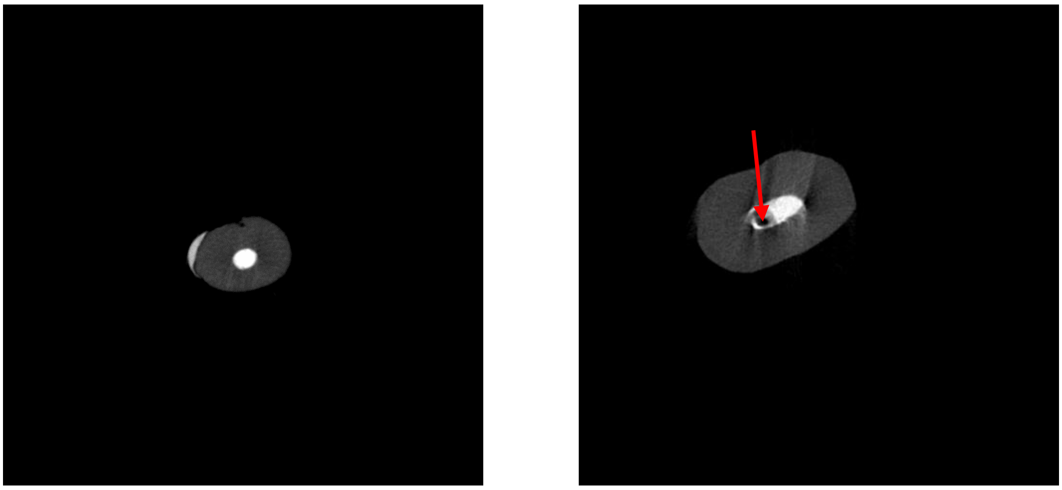

Figure 20- Two apical third's slides (high and low voids volumes) of samples obturated with Bioroot sealer, red arrows point for voids within obturation

4.7 Comparison between voids percentage per third of Bioroot sealer's samples.

Table 13 shows comparison between the void percentage per part of the tooth. Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no statistically significant difference between the three thirds (P>0.05) when using Bioroot sealer. Volumetrically, the coronal third showed the most voids and the middle third showed the least voids.

Table 13- Comparison of voids percentage per part of samples obturated with Bioroot sealer

Bioroot Thirds Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of Bioroot are the same across categories of Group.	Independent-Samples Median Test	.202	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

4.8 Endosequence sealer's voids percentage

Voids percentages of samples obturated with Endosequence sealer are shown in table 14. The mean voids percentage was 2.91 % with minimum sample reading of 0.31% and maximum reading of 11.65% as shown in table 15.

Table 14- Voids percentage of teeth obturated with Endosequence root canal sealer.

Endosequence sealer					
Samples	**Canal volume**	**Voids volume**	**Voids percentage**	**Filling Volume**	**Filling percentage**
C1	20.44	0.10	0.50	20.34	99.50
C2	15.98	1.86	11.65	14.12	88.35
C3	20.88	0.13	0.63	20.75	99.37
C4	14.22	0.32	2.27	13.89	97.73
C5	10.29	0.04	0.38	10.25	99.62
C6	10.28	0.03	0.31	10.25	99.69
C7	10.35	0.13	1.29	10.22	98.71
C8	23.03	0.73	3.17	22.30	96.83
C9	13.93	0.86	6.21	13.06	93.79
C10	24.66	0.66	2.69	23.99	97.31
Mean	16.40	0.49	2.91	15.92	97.09

Table 15- Minimum and maximum voids, mean and SD of Endosequence sealer samples.

Endosequence voids Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.31	11.65	2.91	3.57

4.9 Endosequence sealer's voids percentage per part

Most voids percentage was observed in the apical third (mean= 7.57%) followed by the coronal third (mean= 2.65%) and least voids was in the middle third (mean= 1.21%). Table 16 shows the voids percentage of each sample.

Minimum voids of coronal third was 0.05% while the maximum was 9.97%. In the middle third 0.00% was the minimum voids while 5.65% was the maximum. Apical third recorded minimum voids of 0.00% and maximum of 26.10% as shown in table 17.

Figure 21 shows mean voids percentage and error bar of Endosequence samples per third.

Table 16- Voids percentage per part of samples obturated with Endosequence sealer.

Endosequence voids percentage per third			
Samples	**Coronal Third**	**Middle Third**	**Apical Third**
C1	0.61	0.09	0.59
C2	9.97	5.65	26.10
C3	0.71	0.51	0.46
C4	2.89	0.94	1.47
C5	0.05	0.42	2.28
C6	0.21	0.51	0.59
C7	1.89	0.00	0.00
C8	2.62	1.07	19.14
C9	3.68	2.23	22.54
C10	3.92	0.68	2.48
Mean	2.65	1.21	7.57

Table 17- Minimum and maximum voids, mean and SD of coronal, middle and apical third of Endosequence sealer samples.

Endosequence Coronal Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.05	9.97	2.65	2.94
Endosequence Middle Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.00	5.65	1.21	1.68
Endosequence Apical Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.00	26.10	7.57	10.53

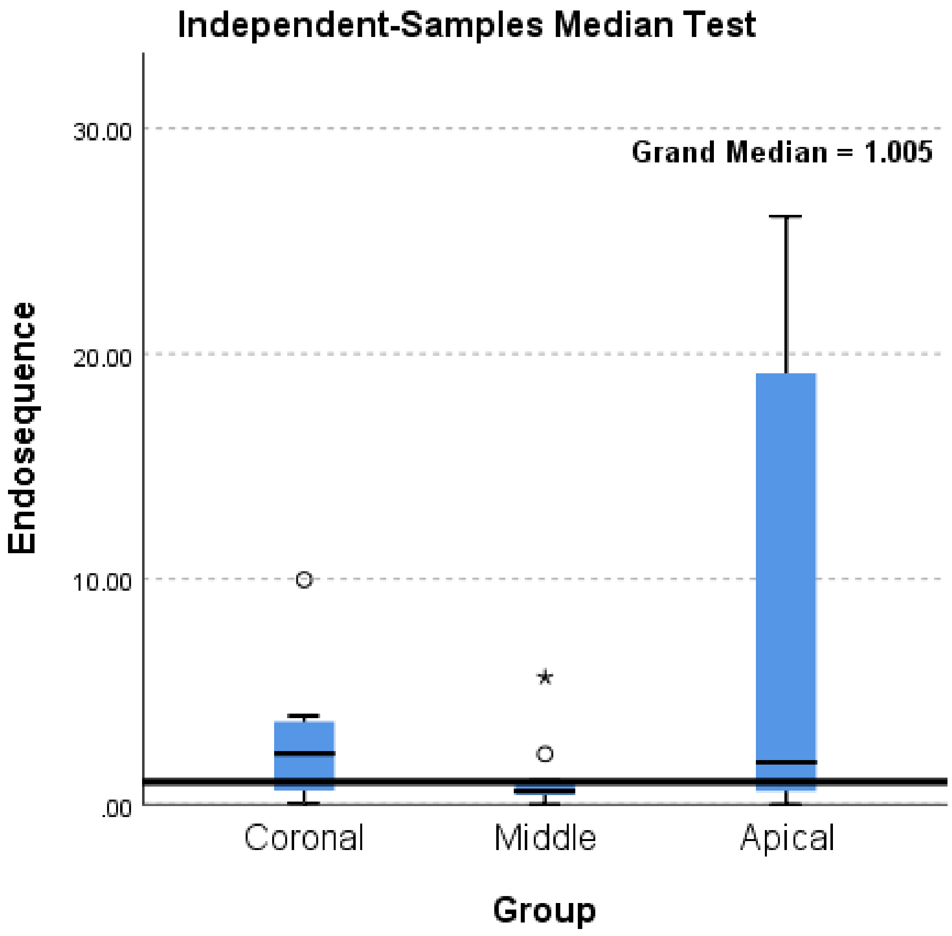

Figure 21- Mean voids percentage and error bar of Endosequence samples per third.

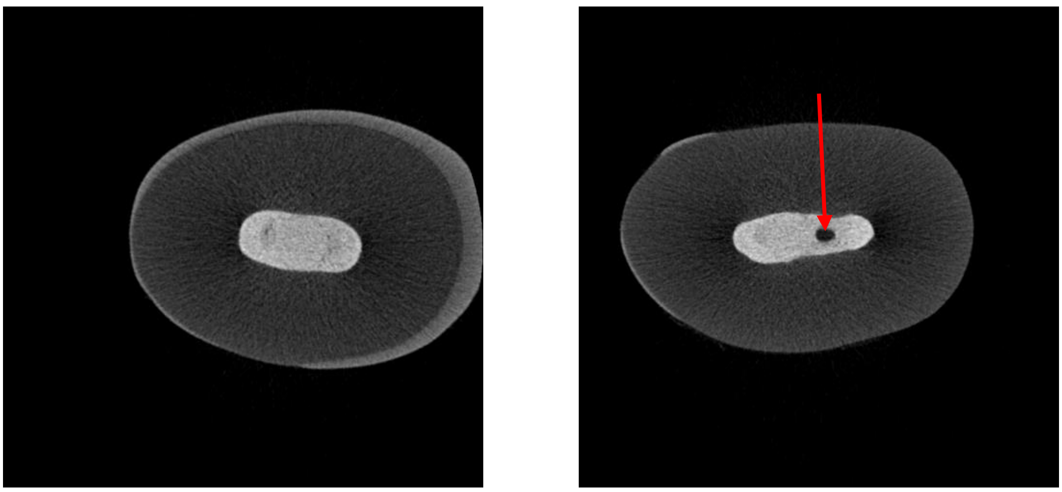

Figure 22- Two coronal third's slides (high and low voids volumes) of samples obturated with Endosequence sealer, red arrows point for voids within obturation

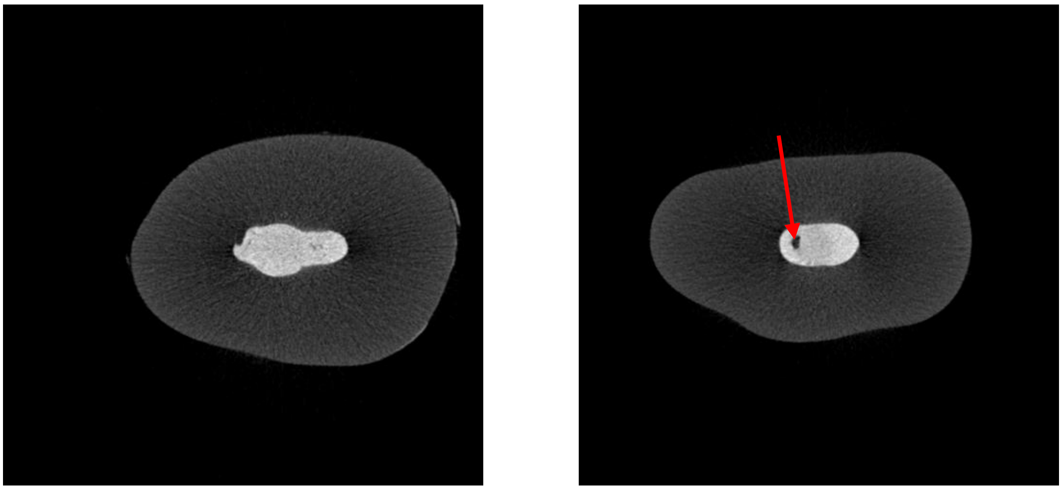

Figure 23- Two middle third's slides (high and low voids volumes) of samples obturated with Endosequence sealer, red arrows point for voids within obturation

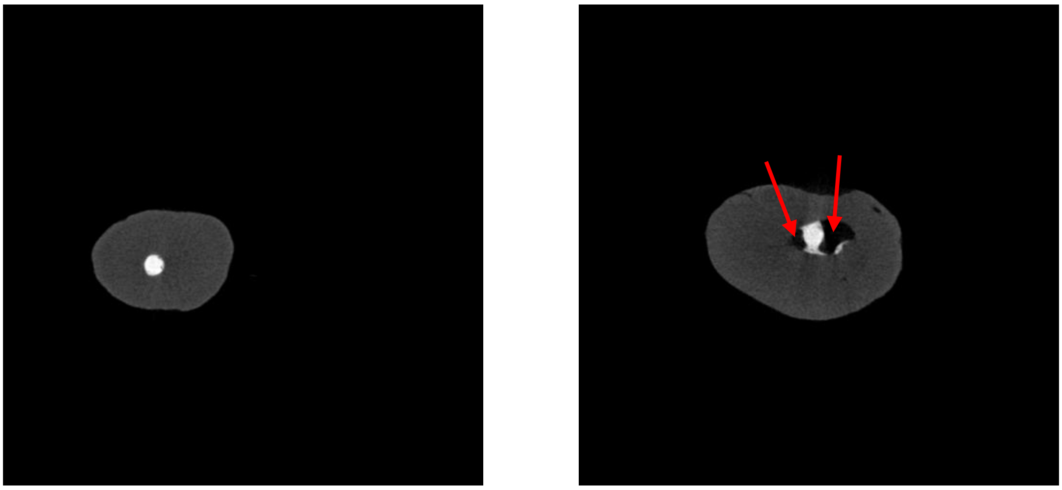

Figure 24- Two apical third's slides (high and low voids volumes) of samples obturated with Endosequence sealer, red arrows point for voids within obturation

4.10 Comparison between voids percentage per third of Endosequence sealer's samples.

Table 18 shows comparison between the voids percentage per part of the tooth. Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no statistically significant difference between the three thirds (P>0.05) when using Endosequence sealer. Volumetrically, the apical third showed the most voids and the middle third showed the least voids.

Table 18- Comparison of voids percentage per part of samples obturated with Endosequence sealer.

Endosequence Thirds Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of Endosequence are the same across categories of Group.	Independent-Samples Median Test	.301	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050				

4.11 Wellroot sealer's voids percentage

Voids percentages of samples obturated with Wellroot sealer are shown in table 19. The mean voids percentage was 4.10 % with minimum sample reading of 0.91% and maximum reading of 12.69% as shown in table 20.

Table 19- Voids percentage of teeth obturated with Wellroot root canal sealer.

Wellroot sealer					
Samples	**Canal volume**	**Voids volume**	**Voids percentage**	**Filling Volume**	**Filling percentage**
D1	26.31	1.16	4.42	25.15	95.58
D2	23.88	0.86	3.59	23.02	96.41
D3	19.90	2.53	12.69	17.37	87.31
D4	34.23	0.83	2.43	33.40	97.57
D5	20.78	1.31	6.30	19.47	93.70
D6	18.03	0.64	3.55	17.39	96.45
D7	9.65	0.16	1.65	9.49	98.35
D8	14.47	0.58	4.04	13.89	95.96
D9	11.05	0.10	0.91	10.95	99.09
D10	9.05	0.13	1.39	8.93	98.60
Mean	18.73	0.83	4.10	17.90	95.90

Table 20- Minimum and maximum voids, mean and SD of Wellroot sealer samples.

Wellroot voids Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.91	12.69	4.10	3.43

4.12 Wellroot sealer's voids percentage per part

Most voids percentage was observed in the coronal third (mean= 4.80%) followed by the apical third (mean= 3.77%) and least voids was in the middle third (mean= 1.92%). Table 21 shows the voids percentage of each sample.

Minimum voids of coronal third was 1.21% while the maximum was 18.03%. In the middle third 0.00% was the minimum voids while 7.40% was the maximum. Apical third recorded minimum voids of 0.00% and maximum of 9.59% as shown in table 22. Figure 25 shows mean voids percentage and error bar of Wellroot samples per third.

Table 21- Voids percentage per part of samples obturated with Wellroot sealer.

Wellroot voids percentage per third			
Samples	**Coronal Third**	**Middle Third**	**Apical Third**
D1	3.79	3.98	7.62
D2	1.23	7.40	7.48
D3	18.03	1.00	2.54
D4	3.33	0.25	1.97
D5	8.39	2.25	1.63
D6	4.01	2.20	4.48
D7	2.18	0.03	1.89
D8	3.86	1.88	9.59
D9	1.21	0.00	0.46
D10	1.96	0.18	0.00
Mean	4.80	1.92	3.77

Table 22- Minimum and maximum voids, mean and SD of coronal, middle and apical third of Wellroot sealer samples.

Wellroot Coronal Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	1.21	18.03	4.80	5.09
Wellroot Middle Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.00	7.40	1.92	2.32
Wellroot apical Third Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Voids percentage	10	0.00	9.59	3.77	3.35

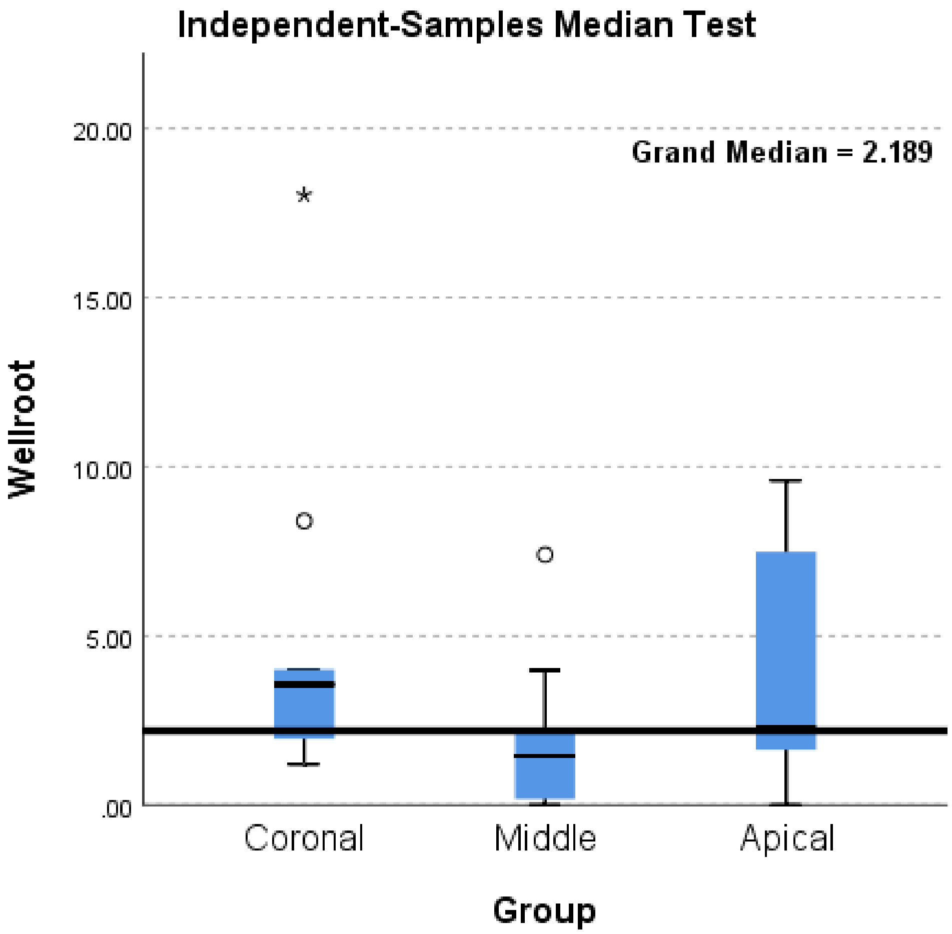

Figure 25- Mean voids percentage and error bar of Wellroot samples per third.

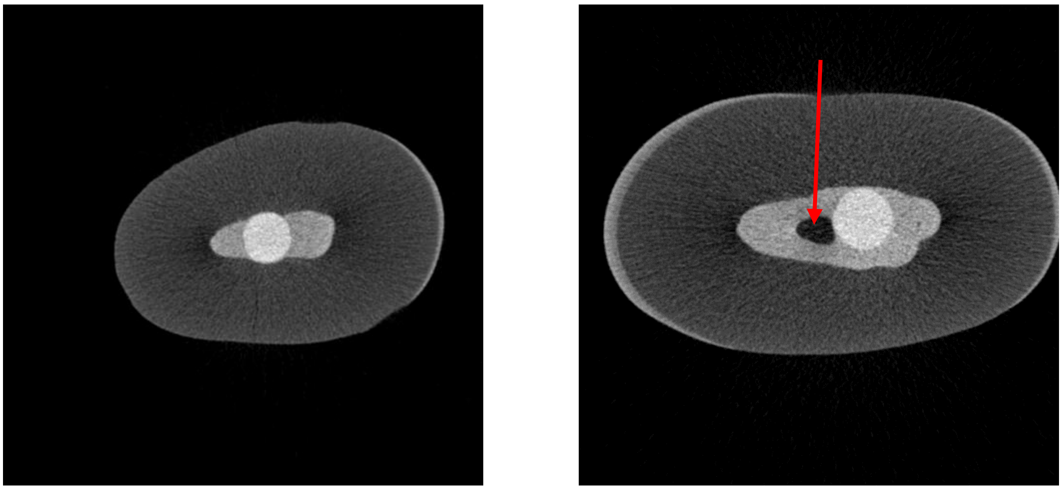

Figure 26- Two coronal third's slides (high and low voids volumes) of samples obturated with Wellroot sealer, red arrows point for voids within obturation

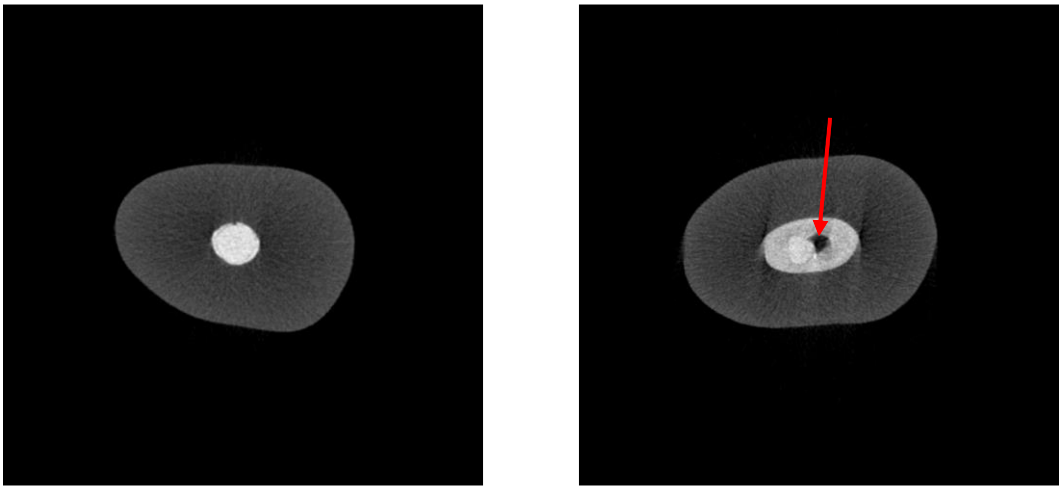

Figure 27- Two middle third's slides (high and low voids volumes) of samples obturated with Wellroot sealer, red arrows point for voids within obturation

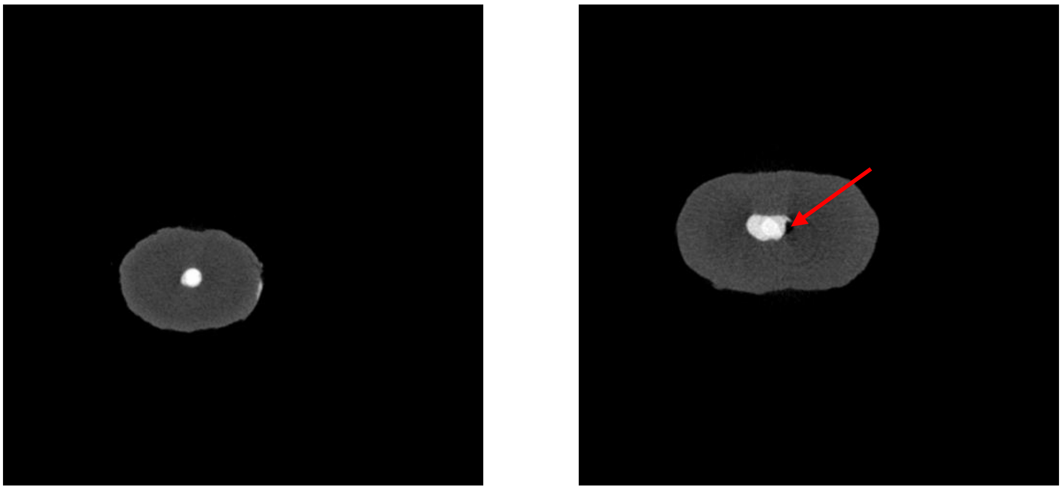

Figure 28- Two apical third's slides (high and low voids volumes) of samples obturated with Wellroot sealer, red arrows point for voids within obturation

4.13 Comparison between voids percentage per third of Wellroot sealer's samples.

Table 23 shows comparison between the voids percentage per part of the tooth. Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no statistically significant difference between the three thirds (P>0.05) when using Wellroot sealer. Volumetrically, the coronal third showed the most voids and the middle third showed the least voids.

Table 23- Comparison of voids percentage per part of samples obturated with Wellroot sealer.

Wellroot Thirds Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of Wellroot are the same across categories of Group.	Independent-Samples Median Test	.670	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

4.14 Comparison between voids percentage of each sealer.

Regarding the sealing ability of each sealer, total voids percentage found to be highest in MTA Fillapex sealer (mean= 12.07 %) followed by Bioroot sealer (mean = 10.53 %) then Wellroot sealer (mean= 4.10 %) and least voids was found on Endosequence sealer (mean= 2.91 %).

Table 24 shows the voids percentage per sample per sealer.

Figure 29 shows mean voids percentage and error bar of each sealer.

Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was a significance difference ($P<0.05$) between Endosequence and MTA Fillapex sealers and between Wellroot and MTA Fillapex sealers in total voids percentage. On the other hand, other comparisons showed no significance difference ($P>0.05$) as shown in table 25 and 26.

Table 24- voids percentage of each sample for all sealers.

Sealer	MTA Fillapex	Bioroot	Endosequence	Wellroot
Sample	**Total Voids percentage**			
1	4.17	7.04	0.50	4.42
2	8.92	15.01	11.65	3.59
3	26.12	4.24	0.63	12.69
4	30.68	3.12	2.27	2.43
5	6.06	17.03	0.38	6.30
6	1.49	27.27	0.31	3.55
7	5.12	23.18	1.29	1.65
8	9.99	1.01	3.17	4.04
9	6.08	5.93	6.21	0.91
10	22.10	1.43	2.69	1.39
Mean	12.07	10.53	2.91	4.10

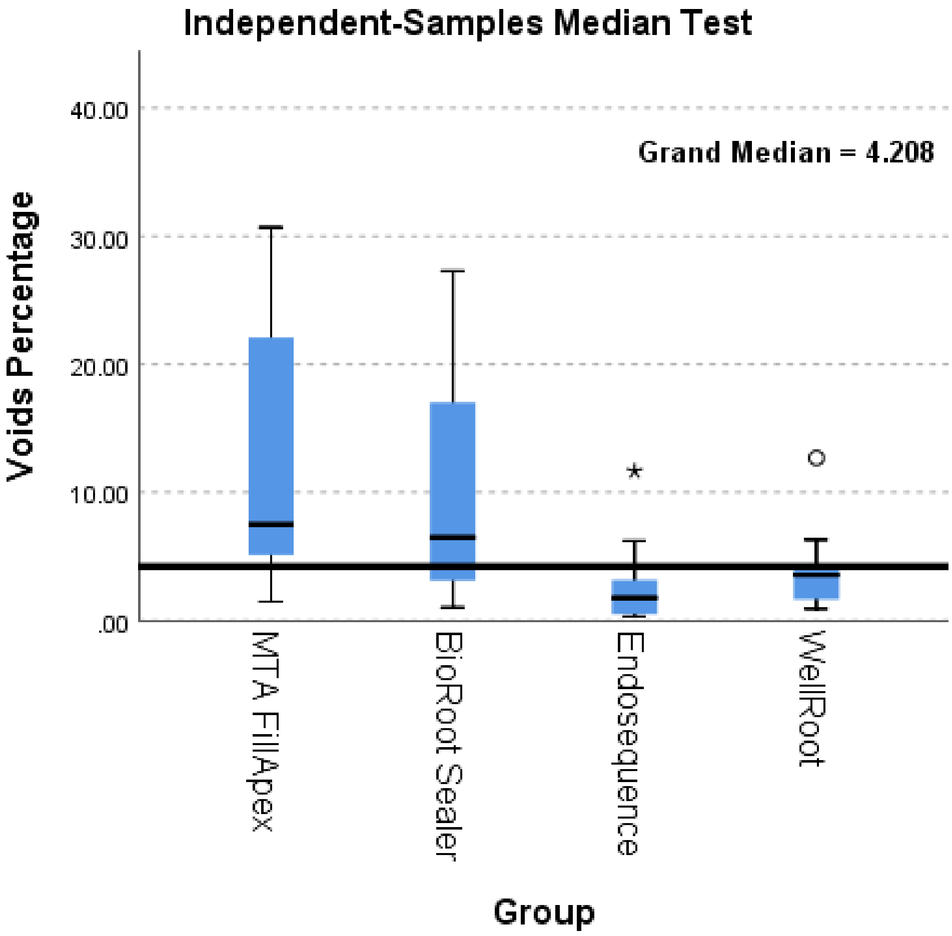

Figure 29- Mean voids percentage and error bar of each sealer.

Table 25-26 Comparison of total voids percentage of the four sealers.

All Groups Total Voids Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of voids percentage are the same across categories of Group.	Independent-Samples Median Test	.015	Reject the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

Pairwise Comparisons of Groups	
Sample 1-Sample 2	**Sig. a**
Endosequence - Wellroot	1.000
Endosequence - Bioroot Sealer	.442
Endosequence - MTA Fillapex	.044
Wellroot - Bioroot Sealer	.442
Wellroot - MTA Fillapex	.044
Bioroot Sealer - MTA Fillapex	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

4.15 Comparison between coronal thirds' voids percentage of each sealer.

Focusing on the coronal third only, each sealer showed different voids percentages. The least voids was recorded on samples obturated with Endosequence sealer (mean= 2.65%) followed by Wellroot sealer that showed mean voids of 4.80% then Bioroot sealer (mean= 13.28 %) while the most voids were found on MTA Fillapex sealer samples (mean= 15%) as shown in table 27 .

Figure 30 shows mean voids percentage and error bar of each sealer.

Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was a significance difference ($P<0.05$) between Endosequence and MTA Fillapex sealers and between Wellroot and MTA Fillapex sealers in coronal third's voids percentage. On the other hand, other comparisons showed no significance difference ($P>0.05$) as shown in table 28 and 29.

Table 27- Coronal thirds' voids percentage of all sealers.

Sealer	MTA Fillapex	Bioroot	Endosequence	Wellroot
Mean Coronal Thirds' Voids percentage	14.99	13.28	2.65	4.80

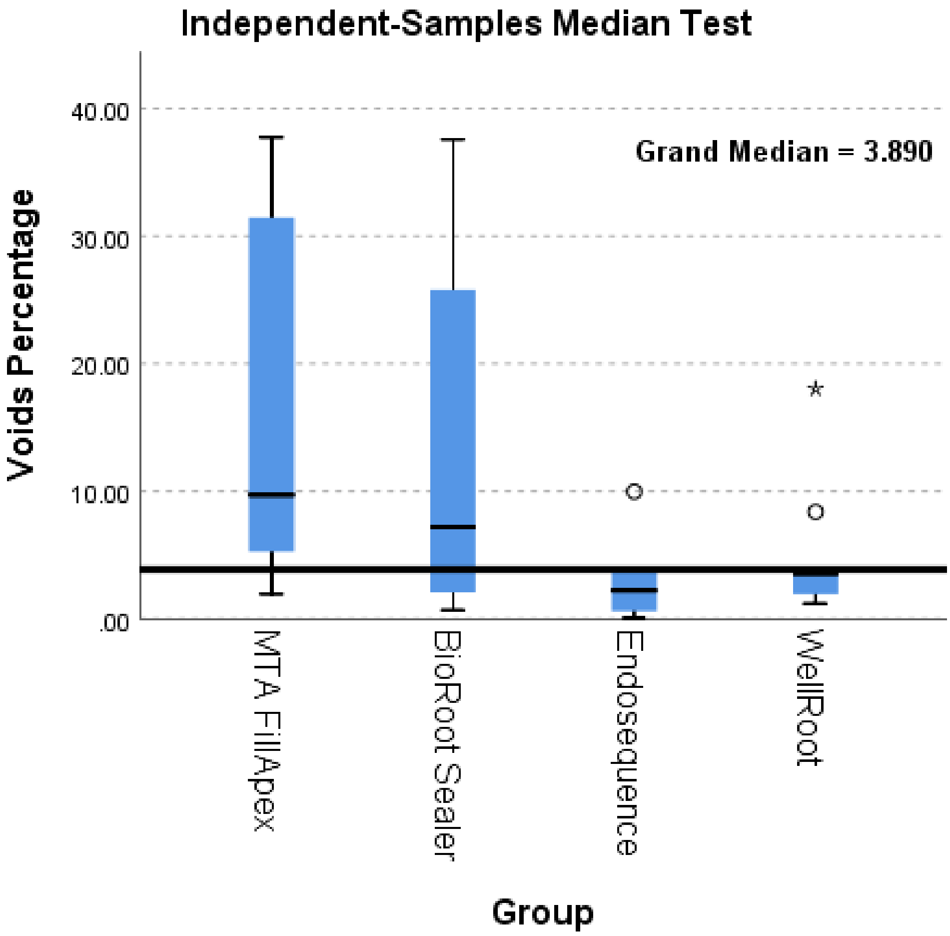

Figure 30- Mean voids percentage and error bar of coronal thirds' voids of each sealer.

Table 28-29 Comparison of coronal thirds' voids percentage of each sealer.

All Groups Coronal Thirds' Voids Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of voids percentage are the same across categories of Group.	Independent-Samples Median Test	.015	Reject the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

Pairwise Comparisons of Groups	
Sample 1-Sample 2	**Sig. a**
Endosequence - Wellroot	1.000
Endosequence - Bioroot Sealer	.442
Endosequence - MTA Fillapex	.044
Wellroot - Bioroot Sealer	.442
Wellroot - MTA Fillapex	.044
Bioroot Sealer - MTA Fillapex	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

4.16 Comparison between middle thirds' voids percentage of each sealer.

Focusing on the middle third only, different voids percentage has been obtained. The least voids was recorded on samples obturated with Endosequence sealer (mean= 1.21%) followed by Wellroot sealer that showed mean voids of 1.92% then Bioroot sealer (mean= 4.98 %) while the most voids were found on MTA Fillapex sealer samples (mean= 6.60 %) as shown in table 30 .

Figure 31 shows mean voids percentage and error bar of each sealer.

Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no significant difference (P>0.05) between any of the sealers regarding sealing ability of the middle third as shown in table 31.

Table 30- Middle thirds' voids percentage of each sample for all sealers.

Sealer	MTA Fillapex	Bioroot	Endosequence	Wellroot
Mean Middle Thirds' Voids percentage	6.60	4.98	1.21	1.92

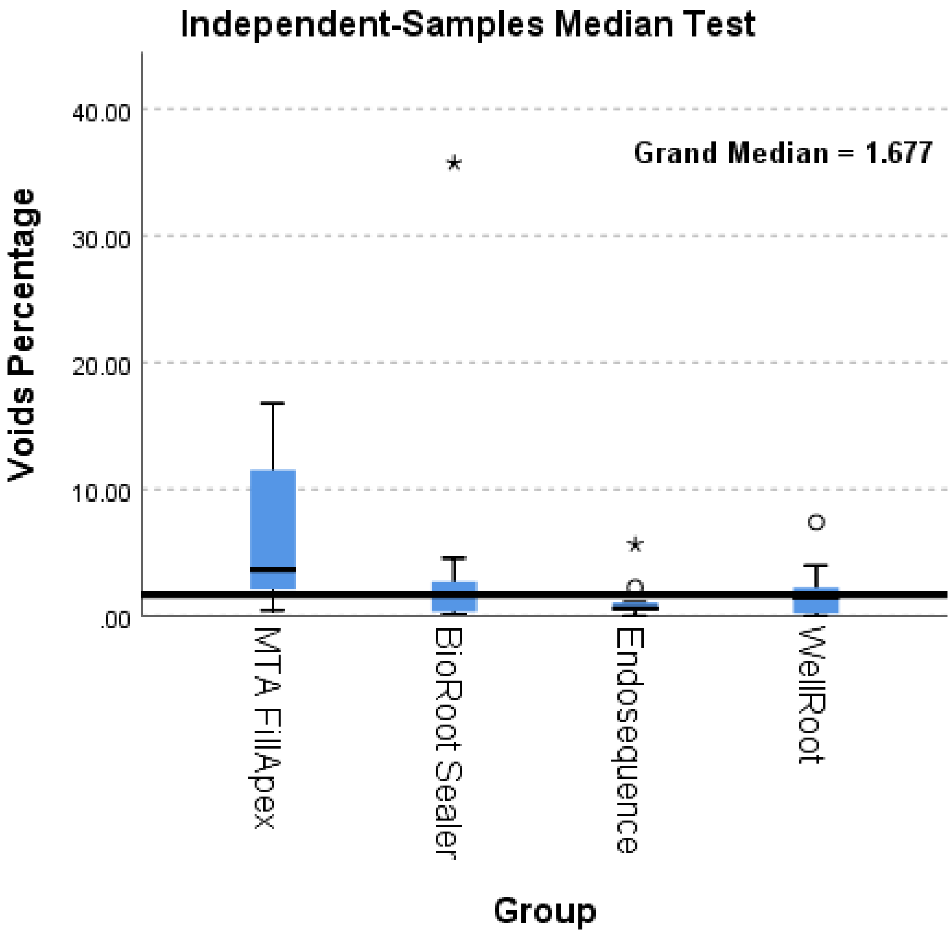

Figure 31- Mean voids percentage and error bar of middle thirds' voids of each sealer

Table 31- Comparison of middle thirds' voids percentage of each sealer.

All Groups Middle Thirds' Voids Comparison (Hypothesis Test Summary)				
	Null Hypothesis	Test	Sig.	Decision
1	The medians of voids percentage are the same across categories of Group.	Independent-Samples Median Test	.066	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

4.17 Comparison between apical thirds' voids percentage of each sealer.

Focusing on the apical third only, different voids percentage has been obtained. The least voids was recorded on samples obturated with Wellroot sealer (mean= 3.77%) followed by MTA Fillapex sealer that showed mean voids of 5.08% then Bioroot sealer which was very close by mean voids percentage to MTA Fillapex (mean= 5.14 %) while the most voids were found on Endosequence sealer samples (mean= 7.57 %) as shown in table 32.

Figure 32 shows mean voids percentage and error bar of each sealer.

Statistical analysis by Kruskall-Wallis H and Dunn-Bonferoni post-hoc tests (Appendix) showed that there was no significant difference (P>0.05) between any of the sealers regarding sealing ability of the apical third as shown in table 33.

Table 32- Apical thirds' voids percentage of each sample for all sealers.

Sealer	MTA Fillapex	Bioroot	Endosequence	Wellroot
Mean Apical Thirds' Voids percentage	5.08	5.14	7.57	3.77

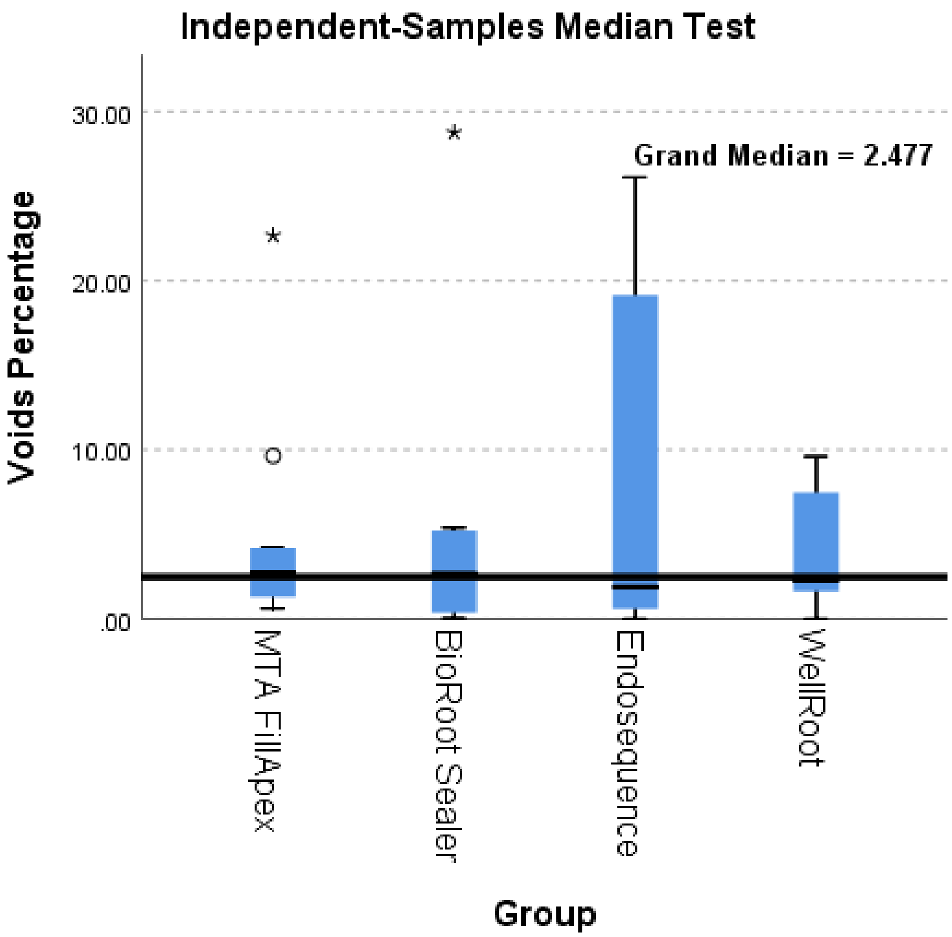

Figure 32- Mean voids percentage and error bar of apical thirds' voids of each sealer.

Table 33- Comparison of apical thirds' voids percentage of each sealer.

All Groups Apical Thirds' Voids Comparison (Hypothesis Test Summary)				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The medians of voids percentage are the same across categories of Group.	Independent-Samples Median Test	.849	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				

Chapter 5
Discussion

The objectives of root canal therapy are the removal of the inflamed and necrotic tissue, elimination of root canal microbiota and the sealing of the root canal system to prevention of recurrence of infection (Torabinejad et al., 2014). If this can be achieved, dental function and appearance can be restored and maintained (Jensen et al., 2007).

Root canal treatment is essentially split into two phases; the chemo-mechanical preparation phase and the obturation phase. Chemo-mechanical preparation involves shaping of the root canal both to remove tissue and allow space for effective irrigation. Obturation aims to seal the root canal system optimally to prevent recontamination via microleakage and to block the passage of any nutrients to micro-organisms that may not have been removed during the chemo-mechanical phase (Schilder, 1967). One of the key determining factors of root canal treatment outcome is the adequate filling of the prepared root canal space (Epley et al., 2006, Da Silva Neto et al., 2007, Ng et al., 2008). Even though sealers are considered as adjunctive materials, they influence the outcome of root-canal treatment with the advanced properties of sealers impact the quality of final obturation (Sly et al., 2007, Zhou et al., 2013). It has been well established that a well-adapted and void-free obturation/sealer complex improves the clinical outcomes of root canal treatment (Epley et al., 2006, Ng et al., 2008).

Bio-ceramic based sealers that usually contain calcium silicate and/or calcium phosphate have started to gain increased popularity as they have been suggested as the most suitable material to minimise voids and simplify the obturation technique (Al-Haddad and Aziz, 2016).

The single cone technique became more popular following the development of rotary NiTi instrument with matched taper gutta-percha cones. This technique permits better adaptation in 3-dimensional preparation (Cavenago et al., 2012) and it is a time saving compared to lateral condensation technique (Tasdemir et al., 2009). According to several studies, the single cone technique is highly recommended to be used with bio-ceramic sealers due to its excellent dimensional stability and flowability (Celikten et al., 2016).

Several methods have historically been used to evaluate root canal filling quality and sealing ability. These include dye penetration, bacterial leakage and fluid filtration. These conventional methods have some disadvantages including time taken to undertake studies and lack of standardization. More importantly, dye penetration studies do not translate well to the clinical situation and bacterial leakage tests do not allow quantification analysis (Siqueira et al., 2000, Pommel and Camps, 2001, Verissimo and Do Vale, 2006).

Recently, micro-CT has been widely used as a research tool to evaluate root canal anatomy, geometry and shape (Peters et al., 2000), to analyse spatial details after root canal instrumentation (Peters et al. 2003, Metzger et al. 2010, Markvart et al. 2012) and to study retreatments (Huumonen et al. 2006, Barletta et al. 2008). Related to the current study, micro-CT has frequently been used to evaluate the porosities within root canal fillings using different endodontic sealers and a variety of filling techniques (Jung et al., 2005, Zaslansky et al., 2011, El-Ma'aita et al., 2012, Gandolfi et al., 2013). Moreover, quantitative data can be obtained via 3D assessment. Based on a previous study (Peters et al. 2000), a resolution between 34 and 68 is considered sufficient for quantitative assessment of obturation quality. In the current study, a resolution as high as 13.65 µm voxel size was employed to ensure accurate detection of voids. 73/255 grey threshold level was chosen for all samples as it was the most appropriate level to record canal geometry accurately without including any parts of the dentine to avoid misreading of dentine as unfilled canal space.

In the current study, micro-CT was used to compare the sealing ability of four contemporary bio-ceramic sealers by measuring the percentage of voids in the filled root canal space. To date and based on an extensive literature search, there are few studies that used similar methods to study the sealing ability of bio-ceramic sealers. Wellroot sealer was introduced recently to the field of endodontics. There are no published studies regarding its sealing ability. Most of the previous studies compared Endosequence sealer as one of the first introduced bio-ceramic sealers, to AH plus sealer which consider a well-researched and most commonly used root canal sealers.

Our research is a novel study as bio-ceramic sealers are expected to dominate in the future, this study can offer an evidence based knowledge of different types of bio-ceramic sealers and which is better than the other to be used clinically in order to expect superior results. In addition to that, higher resolution micro-CT can be used for robust studies to provide accurate qualitative and quantitative results that provide excellent prove of sealing ability. Storing the teeth in Hank’s solution as well as incubate them in 37 °C in 100% humidity is one of the best methods to resemble the oral environment and reduce the gap between in vitro and clinical studies.

A sample size of 40 teeth was chosen based on previous studies power calculation that indicated 9 teeth per group is sufficient for detection of differences in root canal fillings. Thus, this study was conducted using 40 extracted teeth (Celikten et al., 2016).

This study demonstrated that the lowest voids percentage was seen in Endosequence sealer (2.69%) followed by Wellroot sealer (4.10%) then Bioroot sealer (10.53%) with the highest void percentage with MTA Fillapex sealer (12.07%). Statistically there was a significance difference ($P<0.05$) between Endosequence and MTA Fillapex sealers and between Wellroot and MTA Fillapex sealers regarding total voids percentage. Moreover, there was no significance difference ($P>0.05$) in other sealers comparisons.
Dividing the root into three thirds (coronal, middle and apical), Endosequence sealer showed most voids in the apical third while the other three sealers showed most voids in the coronal third. Statistically, there was a significance difference ($P<0.05$) in terms of coronal thirds' voids percentage between Endosequence and MTA Fillapex sealers and between Wellroot and MTA Fillapex sealers. On the other hand, there was no significance difference ($P>0.05$) comparing the voids percentage of each third for each sealer alone or middle and apical thirds of all sealers. However, the outcome could translate to a significant clinical difference.
Single cone obturation technique have been reported to have more voids at the coronal third when compared to other obturation techniques such as lateral compaction and carrier-based obturation (Celikten et al., 2015). The use of single cone obturation may results in more sealer deposited in the coronal region as this technique contains less amount of gutta percha coronally when compared to other techniques that use accessory cones or injectable gutta perch, which may undergo dimensional change during setting and/or dissolution which may be the cause of more voids in that part of the canal space (Keles et al., 2014). This could explain why in the present study coronal third showed the most voids in three sealers.
Conversely, Endosequence sealer showed most voids in the apical third. Related to that, Ferrari et al. (2000) reported that sealers' adhesion to the dentine walls depends mostly on the dentine's cleanliness and intermolecular surface energy, as on the surface tension and wetting ability of the sealers. Different parts of the root show different degrees of the surface energy and cleanliness. Cleanliness could be difficult to achieve in the apical region as it is more complicated to remove smear layer entirely. Smear layer blocks sealers entry into dentinal tubules.
Endosequence and Wellroot sealers are both premixed injectable sealers. Premixed sealers were introduced to improve both convenience and delivery by eliminating the heterogenous consistency during mixing (Jain, 2016). This is a potential explanation why in the present study these two sealers showed the least voids compared to MTA

filaapex and Bioroot sealers. On the other hand, as the syringe tip should be inserted into the middle third, sealer flow may not fill the apical third entirely which could explain why Endosequence sealer showed most voids at the apical third and Wellroot sealer showed more voids in the apical third compared to the middle third. Regarding that, further investigations are needed focusing on the apical third canal geometry effects.

Celikten et al. (2016) studied the filling quality of four different sealers (Endosequence, Smartpaste bio, ActiV GP and AH plus) by means of voids. All the four sealers were comparable to each other. Endosequence sealer showed more voids in the coronal third followed by the middle third then the apical third, with a statistically significant difference between the coronal and the apical thirds. In this study, outcomes were different as the apical third showed more voids followed by the coronal third then the middle third with no significant difference. Contrary to our study, their methodology mentioned teeth were decoronated and adjusted so that all teeth are approximately 12 mm in length. This adjustment may eliminate the wide part of the coronal third at the orifice level which may increase the voids volume while we preferred to mimic the natural clinical scenarios as much as possible. In addition to that, in that study teeth were obturated using 40.06 gutta-percha while wave one gold primary matched taper gutta-percha cones 25.07 were used in our study as primary file was the file of choice that fitted all samples sizes. These differences between the two methods may explain the results differences.

Huang et al. (2018) compared the sealing ability between Endosequence sealer and AH Plus sealer by means of open and closed pores rather than void percentage. Endosequence sealer in that study showed a higher volume of pores at the coronal third followed by middle then apical thirds with significant difference between coronal and apical thirds. As with Celikten study, Haung's study decoronated and adjusted the roots to be approximately 12 mm in length. Moreover, size 40 lentulo spiral was used for sealer's distribution in the whole canal space which may overcome sealer flow into the apical third that we faced in few of our samples that resulted in apical third's voids more than coronal.

Milanovic et al. (2020) compared porosities of four sealers (Endosequence, Bioroot, MTA Fillapex and AH Plus sealers) by means of open and closed pores. Ignoring AH plus sealer, there was no significant difference between the total pores of the three sealers. Volumetrically, MTA Fillapex showed more pores compared to Endosequence and Bioroot sealers that showed almost similar results. MTA Fillapex showed more pores at the coronal third followed by middle then apical third. There were significantly greater pores in the coronal region when compared to middle and apical regions. In addition to that, MTA Fillapex coronal pores were significantly greater compared to Endosequence coronal pores. Bioroot sealer showed more pores at the coronal third followed by apical then middle third. There was statistically significant difference comparing the coronal third to the middle and apical third. Endosequence sealer showed greater pores at the coronal third while middle and apical thirds showed almost similar pores with no significant difference comparing the three thirds.

A major difference between Milanovic's methodology and this study was that their sample size is 4 maxillary central incisors for each sealer group. In addition to that roots were decoronated to similar length of all samples. Central incisors canal space are generally considered to be wider than premolars, while we prepared the canals with wave one gold primary files, they used TF files to #50.04 size.

Voids of MTA Fillapex sealer was volumetrically the greatest in both our study and Milanovic study with significance difference in ours. In addition to that, coronal third voids showed significance difference in both studies. Its noteworthy that high voids percentage maybe due to the presence of bismuth oxide as a radiopacifier agent which increase solubility of the sealer that decreases its mechanical stability (Torres et al., 2018).

Endosequence sealer showed great sealing ability in our study and the previously mentioned studies. Al-Haddad et al. (2015) reported that Endosequence sealer composed of nanoparticles (about 2 µm in diameter) and viscosity level that facilitate penetrating into dentinal tubules providing better sealing ability and less voids formation.

Chapter 6
Conclusion

Within the limitation of this study, the results showed that;

- Micro-CT is an excellent method to study the sealing ability of endodontics sealers by showing accurate quantitative results.
- The single cone technique is accepted while using bio-ceramic sealers.
- None of the tested sealers showed voids free results.
- There was a significance difference between Endosequence and MTA Fillapex and Wellroot and MTA Fillapex sealers regarding the total voids percentage along with comparing the coronal thirds' voids percentage.
- There was no significance difference comparing the other sealers.
- There was no significant difference comparing the three thirds of each sealer neither comparing middle and apical thirds of all sealers.

Clinical Implications

- Volumetrically, Endosequence sealer showed the least voids formation followed by Wellroot, Bioroot, then MTA Fillapex sealers.
- Endosequence sealer showed most voids at the apical third while all other three sealers showed most voids at the coronal third.
- Premixed injectable sealers (Endosequence and Wellroot sealers) showed fewer voids than conventional sealers.

Chapter 7
Limitation of This Study

As with any *in vitro* study, caution must be taken when extrapolating the results to the clinical situation. The present study was designed with the clinical situation in mind, but it was difficult to replicate the clinical situation completely as any lab study.
Some of the limitations include:

- since the study was an *in vitro* study, even though samples were stored in Hanks balanced solution in 37 °C and 100% humidity, all the intraoral variables cannot be simulated entirely.
- whether the results will differ in real clinical situations or not due to other variables such as oral hygiene, between appointment effects, surrounding tissues and final restorations application should be investigated.
- This study conducted using single rooted premolars, studying of sealing ability of complex canal anatomy did not take place.
- Due to unfortunate situation of covid-19 that affected the entire world, 6 months evaluation of the sealing ability improvement was not possible.

Chapter 8
Future Research

- Using the same protocol to assess the sealing ability using different preparation systems and obturation techniques specially to overcome the high voids percentage in the coronal third.
- Include other brands of bio-ceramic sealers in the comparison.
- Repeat the present study using instruments that assist sealer distribution into the canal space such as lentulo spiral to investigate if it can reduce voids formation or not.
- Studying the sealing ability of bio-ceramic sealers of complex root canal systems.
- Increase the sample size.
- Investigate the sealing ability improvement over a period of time.
- *In vivo* research to see how in vitro findings are applicable in real clinical scenarios.

Chapter 9
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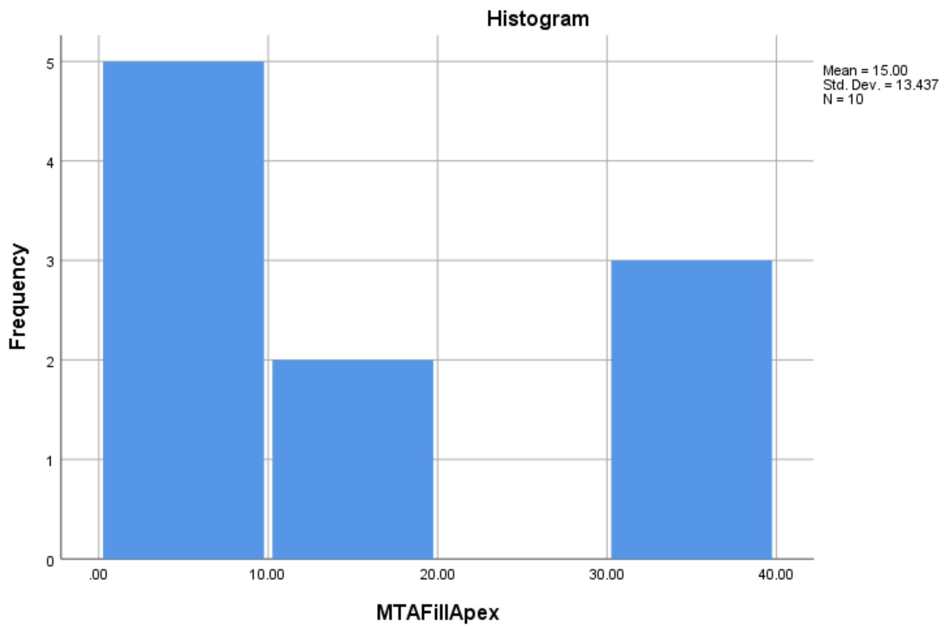
Chapter 10
Appendix

Kruskal–Wallis and Bonferroni tests to compare the voids percentage per third of MTA Fillapex sealer's samples.

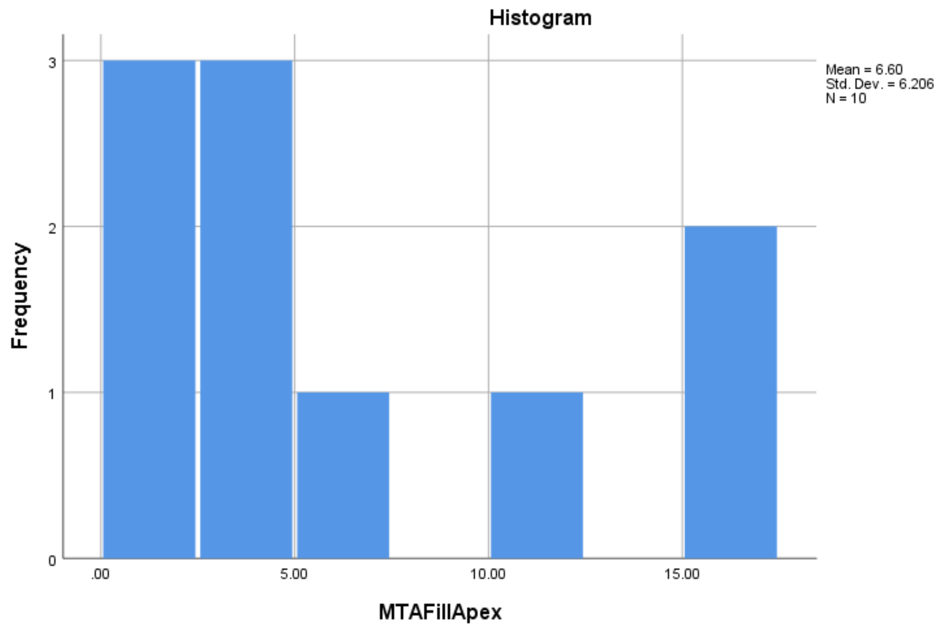
Kruskal-Wallis Test			
	Group	N	Mean Rank
MTA Fillapex	Coronal	10	20.90
	Middle	10	14.30
	Apical	10	11.30
	Total	30	

Test Statistics[a,b]	
	MTA Fillapex
Kruskal-Wallis H	6.225
df	2
Asymp. Sig.	.045
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

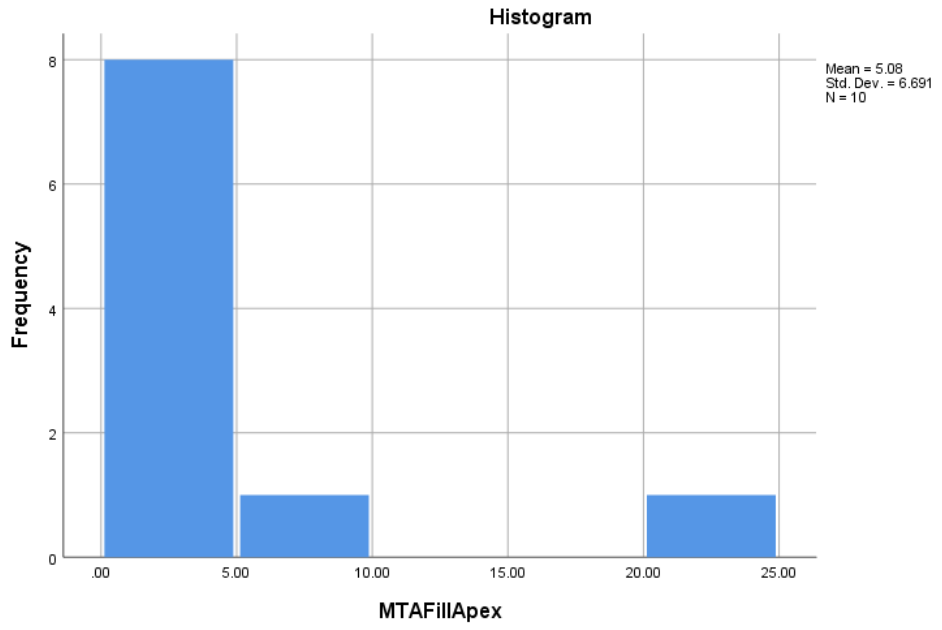
MTA Fillapex sealer coronal third normality test



MTA Fillapex sealer middle third normality test



MTA Fillapex sealer apical third normality test

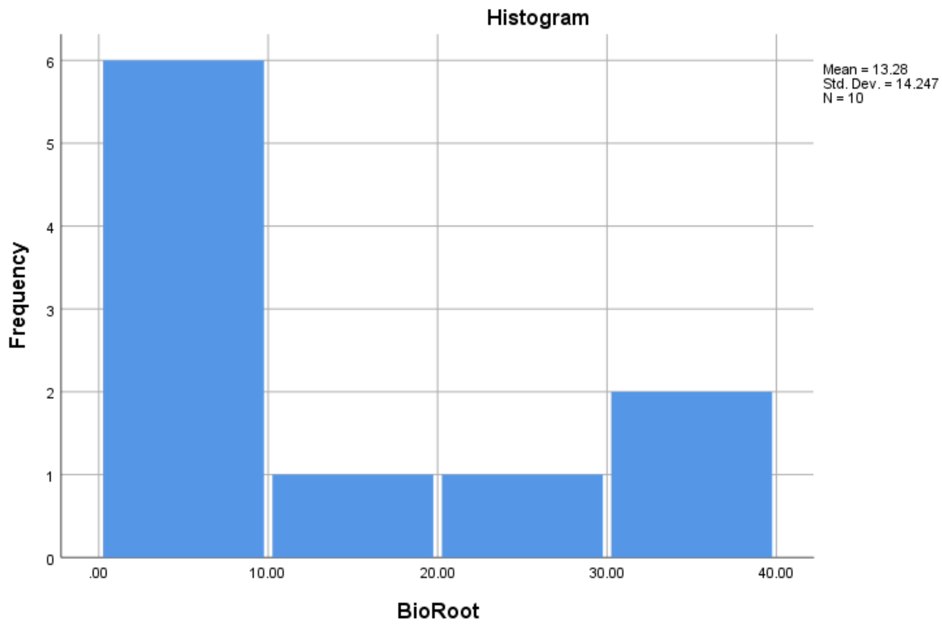


Kruskal–Wallis and Bonferroni tests to compare the voids percentage per third of Bioroot sealer's samples.

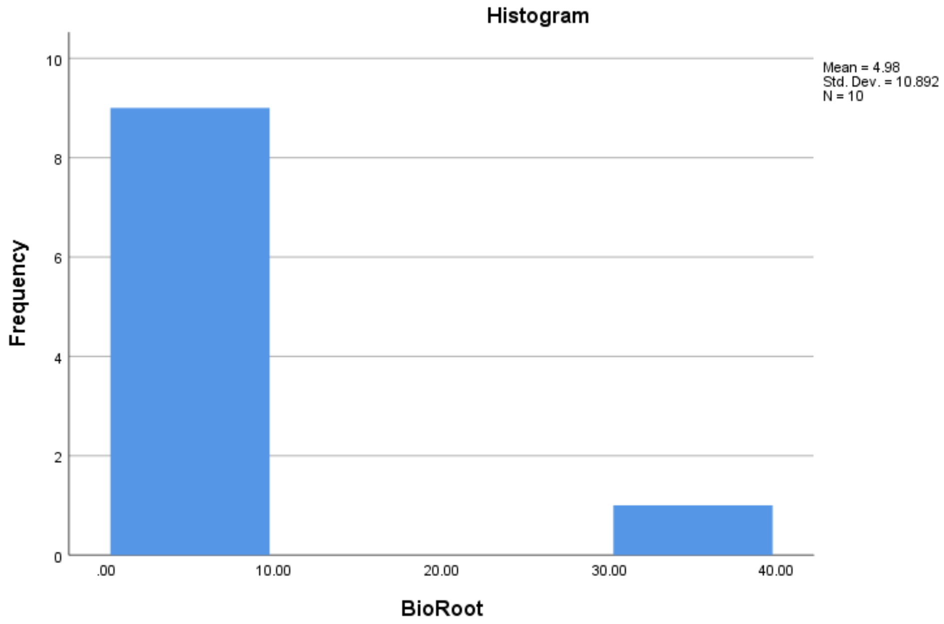
Kruskal-Wallis Test			
	Group	N	Mean Rank
Bioroot	Coronal	10	20.50
	Middle	10	11.80
	Apical	10	14.20
	Total	30	

Test Statistics[a,b]	
	Bioroot
Kruskal-Wallis H	5.210
df	2
Asymp. Sig.	.074
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

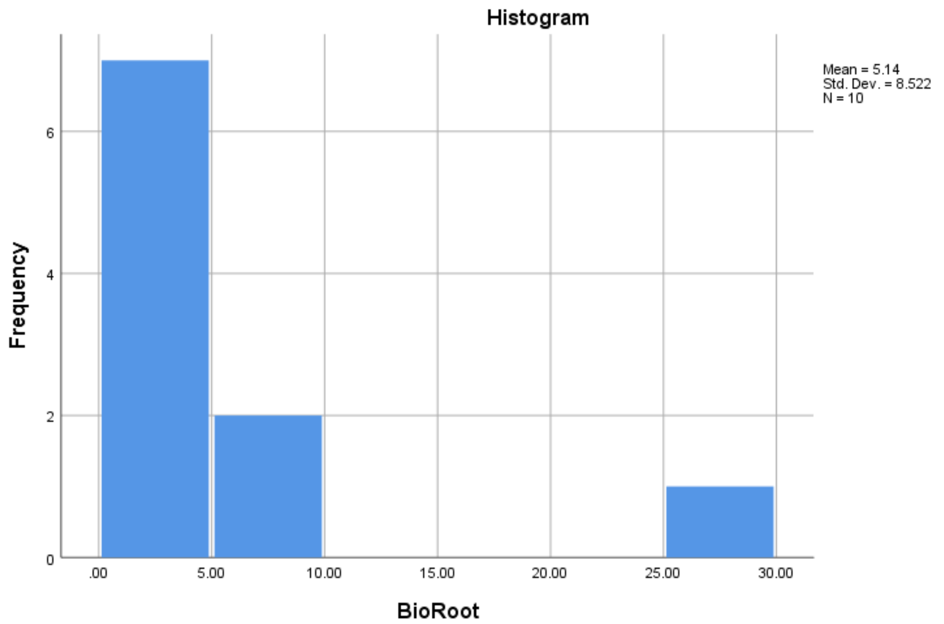
Bioroot sealer coronal third normality test



Bioroot sealer middle third normality test



Bioroot sealer middle third normality test

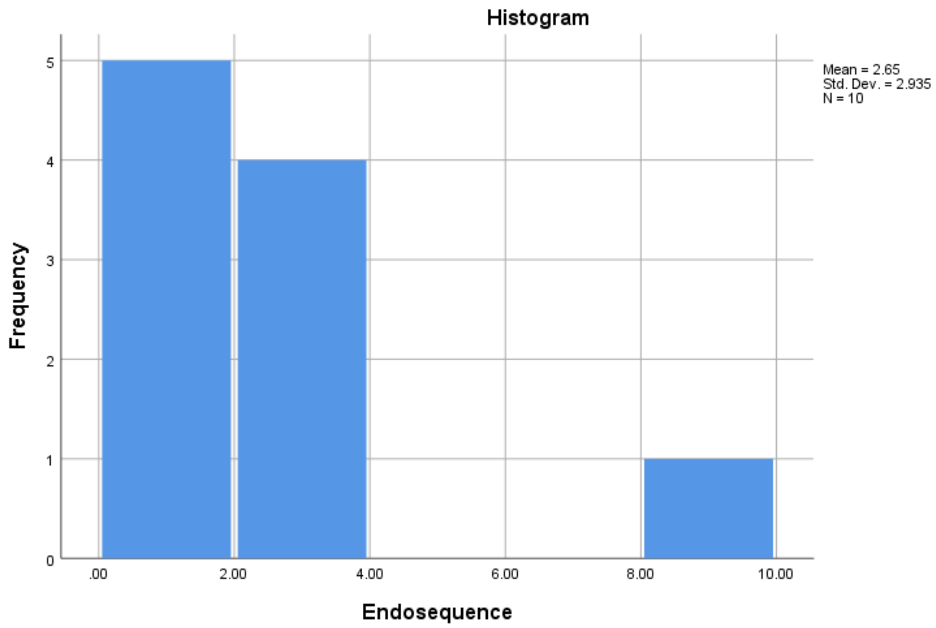


Kruskal–Wallis and Bonferroni tests to compare the voids percentage per third of Endosequence sealer's samples.

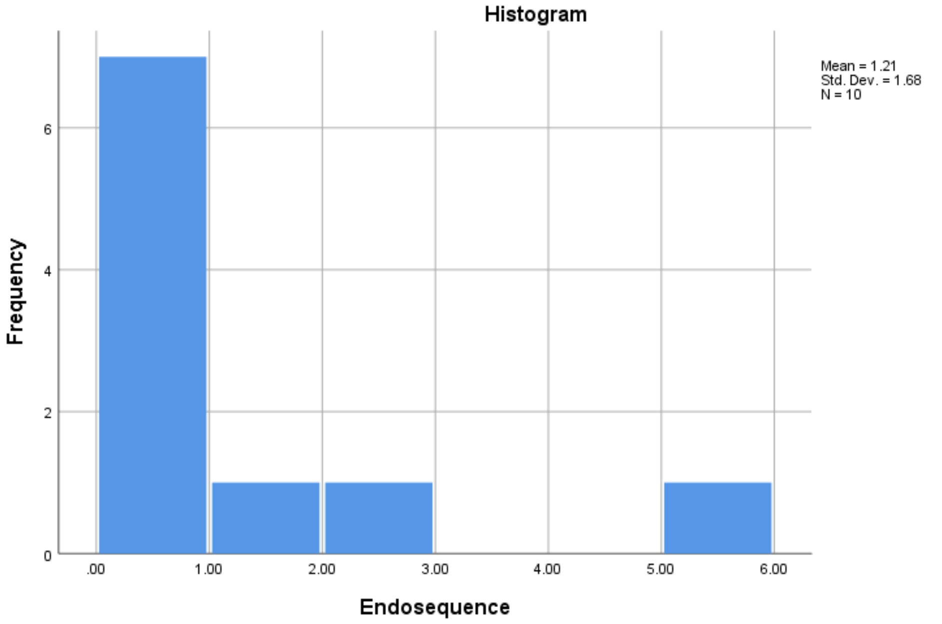
Kruskal-Wallis Test			
	Group	N	Mean Rank
Endosequence	Coronal	10	17.30
	Middle	10	11.80
	Apical	10	17.40
	Total	30	

Test Statistics[a,b]	
	Endosequence
Kruskal-Wallis H	2.650
df	2
Asymp. Sig.	.266
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

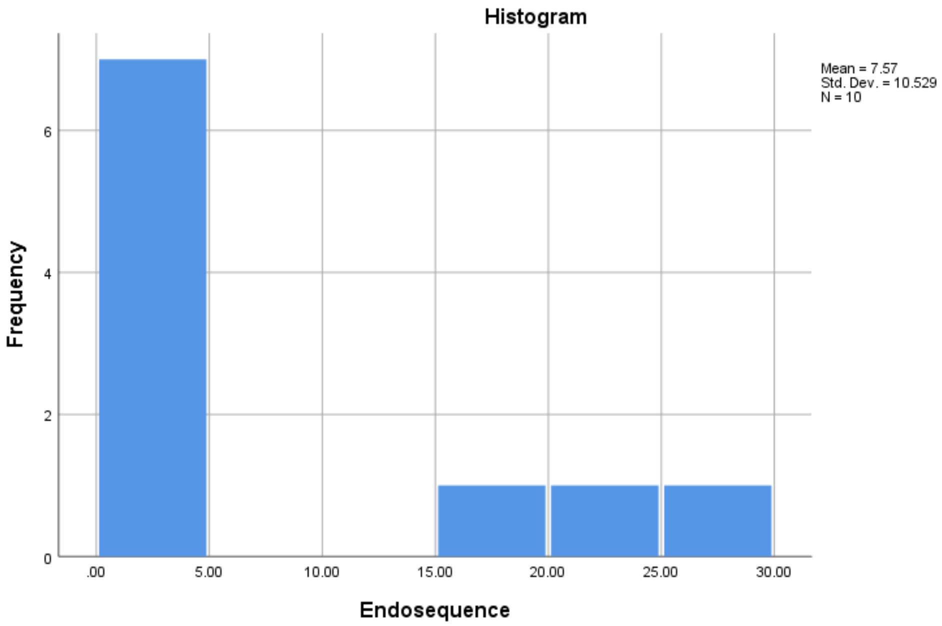
Endosequence sealer coronal third normality test



Endosequence sealer middle third normality test



Endosequence sealer apical third normality test

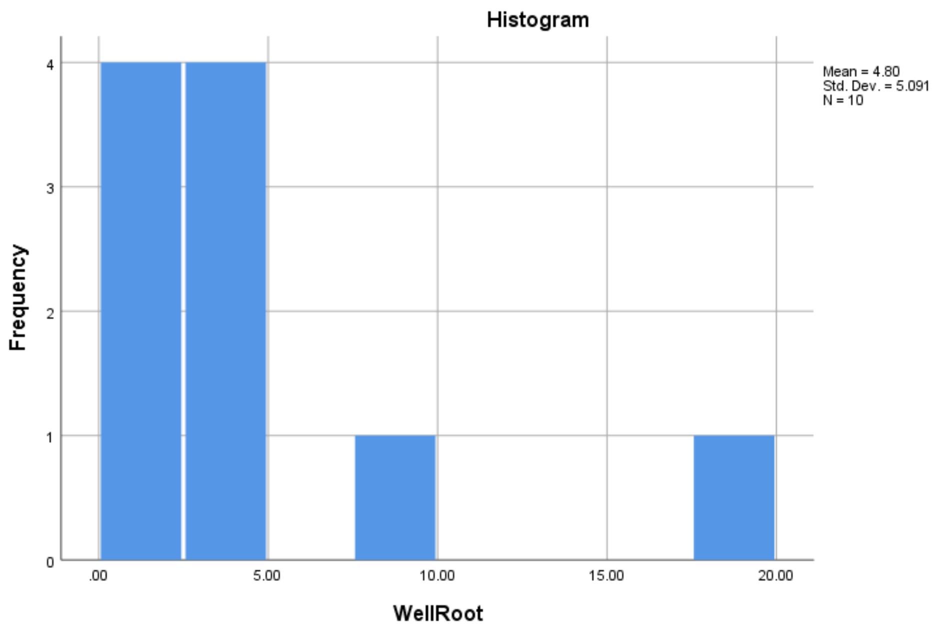


Kruskal–Wallis and Bonferroni tests to compare the voids percentage per third of Wellroot sealer's samples.

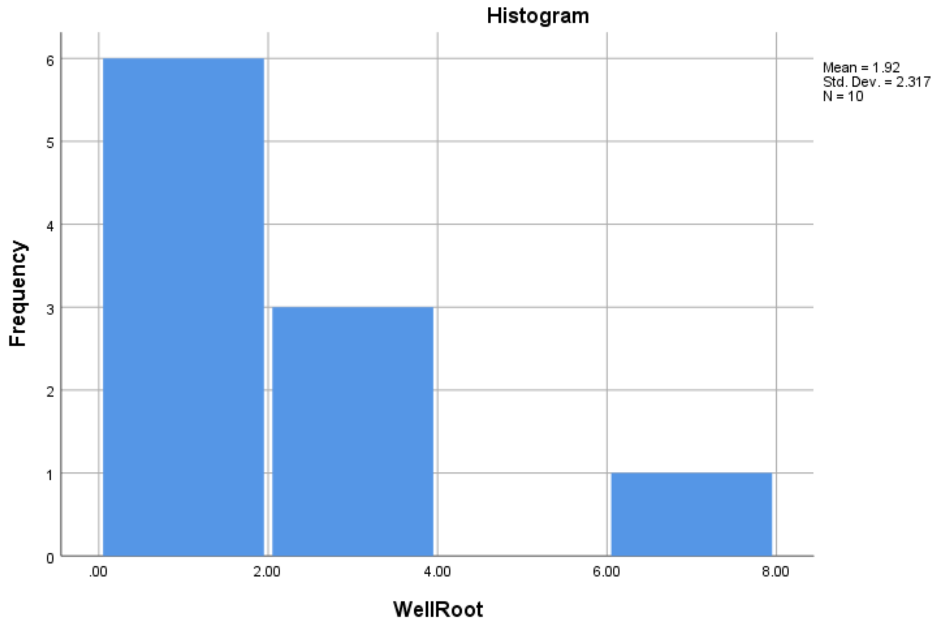
Kruskal-Wallis Test			
	Group	N	Mean Rank
Wellroot	Coronal	10	18.60
	Middle	10	11.15
	Apical	10	16.75
	Total	30	

Test Statistics[a,b]	
	Wellroot
Kruskal-Wallis H	3.884
df	2
Asymp. Sig.	.143
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

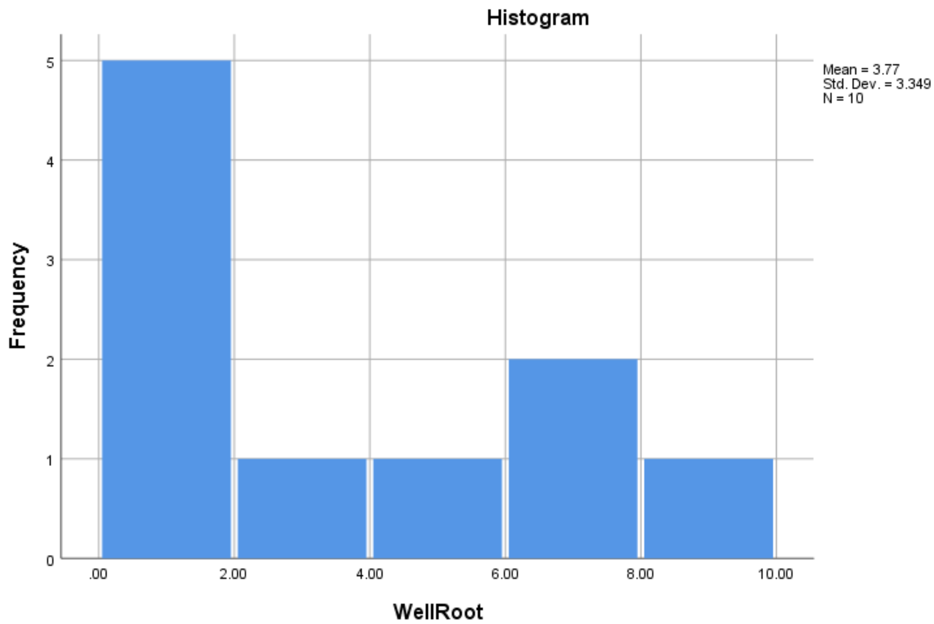
Wellroot sealer coronal third normality test



Wellroot sealer middle third normality test



Wellroot sealer apical third normality test



Kruskal–Wallis and Bonferroni tests to compare the voids percentage "in total" of each sealer.

Kruskal-Wallis Test

	Group	N	Mean Rank
Voids Percent age	MTA Fillapex	10	27.90
	Bioroot Sealer	10	24.90
	Endosequence	10	11.80
	Wellroot	10	17.40
	Total	40	

Test Statistics[a,b]

	Voids Percent age
Kruskal-Wallis H	11.665
df	3
Asymp. Sig.	.009

a. Kruskal Wallis Test

b. Grouping Variable: Group

Kruskal–Wallis and Bonferroni tests to compare coronal thirds' voids percentage of each sealer.

Kruskal–Wallis Test			
	Group	N	Mean Rank
Voids Percent age	MTA Fillapex	10	28.30
	Bioroot Sealer	10	24.30
	Endosequence	10	11.80
	Wellroot	10	17.60
	Total	40	

Test Statistics[a,b]	
	Voids Percent age
Kruskal-Wallis H	11.662
df	3
Asymp. Sig.	.009
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

Kruskal-Wallis and Bonferroni tests to compare middle thirds' voids percentage of each sealer.

Kruskal–Wallis Test			
	Group	N	Mean Rank
Voids Percentage	MTA Fillapex	10	29.30
	Bioroot Sealer	10	20.10
	Endosequence	10	15.20
	Wellroot	10	17.40
	Total	40	

Test Statistics[a,b]	
	Voids Percentage
Kruskal-Wallis H	8.437
df	3
Asymp. Sig.	.038
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

Kruskal–Wallis and Bonferroni tests to compare apical thirds' voids percentage of each sealer.

Kruskal–Wallis Test			
	Group	N	Mean Rank
Voids Percent age	MTA Fillapex	10	22.70
	Bioroot Sealer	10	20.00
	Endosequence	10	18.95
	Wellroot	10	20.35
	Total	40	

Test Statistics[a,b]	
	Voids Percent age
Kruskal-Wallis H	.550
df	3
Asymp. Sig.	.908
a. Kruskal Wallis Test	
b. Grouping Variable: Group	