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An *in vitro,* SEM and micro-CT comparison of dentinal tubule penetration and void formation by four bioceramic sealers in premolars

By

Dr Lotive Shabalala

Dissertation submitted in fulfilment of the Degree of MSc (Dent) at the Department of Odontology, School of Dentistry, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

Contact details:

Tel: 012 319 2231

E-mail: lotive.shabalala@up.ac.za

Supervisor: Dr Samantha Arnold, samantha.arnold@up.ac.za

Co-Supervisor: Dr Glynn Dale Buchanan

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DECLARATION AND CONFLICT OF INTEREST

I, Lotive Shabalala, declare that this dissertation entitled **"An in vitro, SEM and micro-CT comparison of dentinal tubule penetration and void formation by four bioceramic sealers in premolars"**, which I herewith submit to the University of Pretoria in fulfilment for the degree MSc (Dent), is my own original work.

Acknowledgement of all the resources I have used and/or quoted in this dissertation have been given, either in the Acknowledgements section of this manuscript or in complete references that can be found at the end of this manuscript.

This work has never been submitted to any other institution of higher education.

Furthermore, I declare that I have no financial, commercial or any other associated interests that represent a conflict of interest with regards to the research undertaken and all other aspects related to the content of this manuscript.

Lotive Shabalala

November 2023

DEDICATION

- My husband, Dr Nhlakanipho Shabalala, for your unwavering support in anything I set out to do – always encouraging me and comforting me when things get tough. You have always encouraged me to run after my dreams and for that I will always be grateful.

- My children, Simphiwe and Nosipho, for your patience with me. It is truly an art to be a working mother, but the love and understanding from you and the many hugs and kisses made this journey so much easier.

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ABBREVIATIONS/ACRONYMS/SYMBOLS

%	-	percentage
3D	-	three-dimensional
2D	-	two-dimensional
ANOVA	-	Analysis of Variance
BC	-	bioceramic
CSB	-	calcium silicate-based
CLSM	-	confocal laser scanning microscopy
EDTA	-	ethylenediaminetetraacetic acid
IQR	-	Interquartile range
Micro-CT	-	microcomputed tomography
mm	-	millimetre/s
mm^3	-	cubic millimetre/s
ml	-	millilitre/s
NaOCl	-	sodium hypochlorite
NECSA	-	South African Nuclear Energy Corporation
nm	-	nanometre/s
ROI	-	region of interest
sec	-	second/s
SEM	-	scanning electron microscope/microscopy
SD	-	Standard Deviation
µm	-	micron
WL	-	working length

ZOE	-	zinc-oxide eugenol

SUMMARY

Introduction: Bioceramic sealers are receiving substantial attention because of their favourable physicobiological properties. Studies have shown that the use of materials containing calcium-silicates results in the formation of a crystalline structure similar to tooth and bone apatite, which is favourable in the healing of endodontic lesions.

Aim: The aim of this study was to compare the sealing ability of four bioceramic sealer materials, available commercially in South Africa, by measuring their penetration depth into root dentin tubules and the formation of voids at three levels (coronal, middle and apical).

Materials and Methods: In this study, four different bioceramic sealers (BioRoot RCS, CeraSeal, TotalFill, and AH Plus Bioceramic [BC]) were compared. Single rooted premolars were included. Coronectomies were performed on each tooth and the root canals were prepared using reciprocating instruments. The canal was then obturated using a single gutta-percha point and one of the four specified root canal sealers. The sealers were allowed to set, after which the obturations were analysed and compared using microcomputed tomography (micro-CT) to evaluate void formation and scanning electron microscopy (SEM) to determine dentin tubule penetration depth.

Results: For the overall average of all three positions (coronal, middle, and apical), the mean surface area of voids of the four sealers did not differ significantly ($p>0.05$). The overall median surface area of voids for AH Plus BC and BioRoot differed significantly ($p=0.049$).

For the overall average of coronal, middle and apical root thirds, the mean and median penetration depths of AH Plus BC and BioRoot did not differ significantly ($p>0.05$). Likewise, the mean and median penetration depths of CeraSeal and TotalFill did not differ significantly ($p>0.05$).

Conclusion: None of the four tested bioceramic sealers demonstrated void-free fillings and voids were found in all samples to varying degrees.

BioRoot showed significantly higher penetration depth values than the other three bioceramic sealers (mean and median = $p<0.001$) with a mean penetration depth of 38.82 µm. The lowest sealer penetration depth was found for TotalFill at 13.32 µm, which was not significantly different from Ceraseal, with a mean depth of 16.40 µm.

KEY WORDS

Bioceramic

Calcium silicate

Dentin tubules

Penetrability

Void formation

Single cone

Surface area

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The success of endodontic treatment relies, in part, on adequate and complete filling of the root canal system during obturation.[1, 2] One of the major causes of endodontic failure is microleakage, which may occur either due to insufficient adaptation of the sealer between the gutta-percha and the dentin, or through the formation of voids within the sealer itself.[3-5]

Due to microleakage, microbial contamination of the entire length of root canal could occur within as little as 30 days of obturation.[3] As a result of the complicated networks found inside root canal systems, microorganisms and debris can remain in areas where irrigation solutions and endodontic files cannot easily access, resulting in their survival within the root canal, and subsequent growth and spread to the peri-radicular areas between the sealer and dentin.[6] Coronal seal is equally as important as the apical seal following obturation.[7] When coronal leakage occurs, or the root canal remains open, oral bacteria can access the periapical tissues, causing reinfection.[5, 8] For the above-mentioned reasons, high-level sealing of the root canal system is of primary importance to ensure long-term treatment outcomes in endodontics.

In modern-day dentistry, a variety of endodontic sealers are available for use in clinical practice. Bioceramic sealers are calcium silicate-based sealers and have been shown to possess excellent physiochemical properties.[9, 10] iRoot SP (Innovative BioCeramix Inc, Vancouver, British Columbia, Canada) was the first material in this group, introduced as an ideal premixed and injectable material, exhibiting the following properties: radiopacity, little to zero-shrinkage, insolubility in oral fluids, and hydrophilicity, which aided the initiation and completion of the setting reaction of the material.[11-13] Since the introduction of iRoot SP in 2007, many other bioceramic sealers have been added to the market.[14] Studies comparing these different bioceramic sealers to each other are, however, limited.[15]

Dentinal tubule penetration and evaluation of the dentin-sealer interface has previously been evaluated using stereomicroscopy, confocal laser microscopy, scanning electron microscopy (SEM), leakage tests, and digital imaging.[2, 16, 17] Microcomputed tomography (micro-CT) is an advanced, non-destructive imaging modality, which may be used to scan and reconstruct obturated root canals three-dimensionally (3D) and has previously been used for the assessment of voids in dental materials.[16, 18]

The present study aimed to compare and quantitatively evaluate the depth of penetration of four different bioceramic sealers into the dentin tubules (at apical, middle, and coronal thirds of the root), as well as evaluate void formation using micro-CT and SEM analysis, respectively. The null hypothesis was that no differences in sealer penetration depth would be observed between the four tested root canal sealants and that the surface area and number of voids would be the same for all sealers.

1.2. Literature Review

1.2.1. The rationale for obturation

The root canal systems of human teeth are complicated networks.[2] Root canal systems demonstrate a main canal that extends into the following tributaries: microcanals (*i.e.* lateral canals), an apical delta, and intra-canal rays and fins.[2] When obturating a root canal system, root canal sealers are intended to seal these tributaries, as well as any irregularities that are located along the dentinal root canal walls, by penetrating into the dentinal tubules.[19, 20] The primary objective of root canal treatment is to minimise the amount of microorganisms from the infected root canal system to levels that will support healing and also to stimulate the regeneration of the dental- and surrounding periodontal tissues.[21] Root canal filling prevents the diffusion of microorganisms and their by-products, and has been subject to various modifications, from the use of solid material to gutta-percha cones in association with root canal sealers.[5] Sealing ability is an important property of an endodontic sealer, as one of the goals of root canal therapy is to obtain a bacteria-tight seal of the canals,[22] because voids may serve as breeding sites for the proliferation of the microorganisms that resisted the chemo-mechanical preparation or were able to reach the root canal system secondarily.[23]

In combination with gutta-percha cones, root canal sealers have been shown to provide the best possible seal of the root canal system, after extirpation of the pulp and shaping of the root canal with files, together with lubrication and copious irrigation.[20, 24] The sealers fill the gaps and interact with dentin, leading to a bond between the sealer and the dentin wall. The nature of the bond depends on the composition of the sealer used. For most sealer types, the bond is usually mechanical and results from sealer tags penetrating the dentinal tubules.[21]

The development of novel root canal sealers and root canal obturation materials have been shown to be at the forefront of attempting to improve root canal treatment in efficiently eliminating inflammatory and infectious components, and also preventing recontamination of the root canal system.[20, 24, 25] The use of root canal sealers to perform root canal filling in obturation procedures is essential in preventing treatment failure.[4]

The complete filling and sealing of instrumented root canals are important steps that can affect the long-term success of root canal treatment.[2] The properties of an ideal sealer include the following:[26]

- Ease of placement into root canals.
- Absence of shrinking after obturation/setting.
- Bactericidal and bacteriostatic attributes.
- Ease of removal in the event of need for retreatment or for post- and core-preparations.
- Good mono-block seal upon setting.
- Resistance to fluid-solubility.

Root canal sealers occupy the space between the gutta-percha cone and the dentinal wall of a prepared root canal.[18] The single-cone technique is frequently used for obturating root canals, because of its simplicity and speed, and also because it is less operator-dependent.[27, 28] Sealers also fill the irregularities present in the complex microanatomy of root canal systems.[25, 29] Furthermore, these materials can penetrate into the orifices of the dentinal tubules of the root canal wall to form an impenetrable barrier and also adhere the gutta-percha to the dentinal walls.[29, 30] The penetration of root canal sealers into dentinal tubule orifices and micro-irregularities have been shown to form a physical barrier[31] to prevent bacterial microleakage and recontamination of the root canal system.[18, 30] Additionally, root canal sealers that penetrate into dentinal tubules can maintain their bactericidal effect, which is favourable for the healing of the periapical lesion.[20, 30]

Various methods have been used to achieve a three-dimensional root canal filling. Examples of these include cold lateral condensation, warm vertical condensation, continuous wave of condensation, carrier-based obturation and single-cone obturation techniques, all of which use gutta-percha as the core material and a flowable cement-like material as the sealer.[32] The single-cone technique is an easy and low-cost obturation method that uses one gutta-percha cone of the same size and taper as the root canal preparation.[2, 33] The technique, however, does display some limitations, e.g., there are larger areas of flattening in the coronal third of premolars (as well as other teeth), favouring the presence of voids.[33, 34] Thus, appropriate flow of root canal sealers is essential for root canal filling when using the single-cone technique.[33, 35]

The penetrability of endodontic sealers into dentin tubules and complex anatomical areas is directly related to their flow property.[36] The materials placed inside the root canal should be dimensionally stable, biocompatible, and present adequate handling properties.[37] Endodontic sealers with a high level of flowability may extrude through the apical and/or secondary foramina to a variable extent during root canal treatment and into the surrounding supporting tissues.[37, 38]

Different materials have been used as root canal sealers in endodontic therapy.[39] Traditionally, endodontic sealers consisted of materials such as zinc-oxide eugenol (ZOE), calcium hydroxide, glass ionomer or resin.[1, 40] The more recently developed bioceramic sealers are reportedly comparable to the resin-based sealer, AH Plus (Dentsply, DeTrey, Germany), in terms of sealing ability.[39, 41]

Bioceramic is a term introduced for an important subset of biomaterials and includes materials that can be classified as bioinert, bioactive, or biodegradable according to the interaction with surrounding tissues.[42] In the present study, the class of bioceramic sealers that will be discussed are the calcium silicate-based (CSB) sealers.

1.2.2. History of bioceramic sealers

The first bioceramic sealer that was introduced in 2007 was called iRoot SP (Innovative Bioceramix, Vancouver, Canada). This sealer was the first that was associated with the attribute "bioceramic".[42] iRoot SP is mainly composed of di- and tricalcium silicates and, therefore, it can be classified as a true bioceramic.[42] Today, CSB sealers are available in a wide range of products varying in composition, properties, and consistency.

1.2.3. Composition of bioceramic sealers

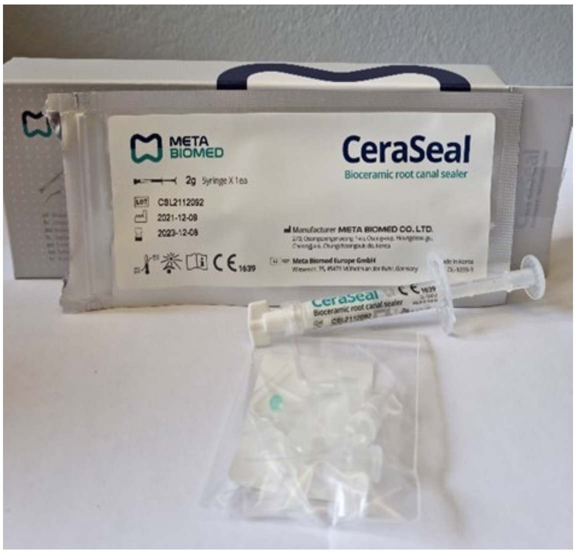
The bioceramic sealers used in the present study are water-based sealers composed of calcium silicate and zirconium oxide.[43] Calcium silicates mainly belong to bioactive bioceramic materials, as these durable materials can undergo interfacial interactions with surrounding tissue.[42] They release high levels of calcium ions and also form a calcium phosphate phase that induces secretion of angiogenic and osteogenic growth factors.[43] This enhances the setting properties of bioceramics, resulting in a chemical composition and crystalline structure similar to tooth and bone apatite materials, thereby improving sealer-to-root dentin bonding.[14] The following bioceramic sealers were compared in the present study:

COMPOSITION

Powder: Tricalcium silicate, zirconium oxide, and excipients.

Aqueous solution: Calcium chloride and excipients.

Figure 1-A. BioRoot Root Canal Sealer (Septodont, Saint-Maur-des-Fosses, France)



COMPOSITION

Calcium silicates, zirconium oxide, thickening agent.

Figure 1-B. CeraSeal Bioceramic Root Canal Sealer (Meta Biomed Co., Cheongju, Korea)

COMPOSITION

Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler, thickening agents.

Figure 1-C. TotalFill Bioceramic Root Canal Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland)

COMPOSITION

Zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, thickening agents.

Figure 1-D. AH Plus Bioceramic Sealer (Dentsply Sirona, Charlotte, USA)

Figure 1. Images of the materials used in this study.

1.2.4. Presentational forms

Bioceramic sealers consist mainly of calcium silicates, calcium phosphates, and calcium hydroxide. They are presented in two forms: pre-mixed ready-to-use syringes or in powder/liquid format.[44] The main difference between these presentations is how the material obtains the water necessary for its hydration reaction and setting.[45-48] In hand-mixed powder/liquid sealers, the hydration is initiated prior to their insertion into the root canal. In pre-mixed ready-to-use sealers, the residual moisture inside the root canal, along with dentin humidity, provide the water necessary for hydration of the material.[14, 45]

TotalFill, CeraSeal and AH Plus Bioceramic (BC) sealers are premixed calcium silicate-based materials presented in a ready-to-use syringe format. BioRoot RCS is also a calcium silicate-based sealer, but it is presented in powder/liquid form, requiring it to be mixed by hand prior to its use.[44]

1.2.5. Setting reaction of bioceramic sealers

Calcium silicate-based sealers are hydraulic and hygroscopic with a particular setting process, and their setting is conditioned by the presence of humidity.[5] The setting reaction of calcium silicates results in the precipitation of calcium phosphate. This can encourage bioactivity and tissue growth after contact with dentin tubules,[42] and water sorption of CSB sealers which causes slight expansion and promotes sealing of the materials into the dentin tubules.[42]

The first step of the setting reaction of bioceramic sealers is a hydration reaction, which can be two different types/ratios, depending on the composition of the powder (A and B).[49]

$$2[3CaO \cdot SiO2] + 6H2O \rightarrow 3CaO \cdot 2SiO2 \cdot 3H2O + 3Ca\ (OH)2 (A)$$

$$2[2CaO \cdot SiO2] + 4H2O \rightarrow 3CaO \cdot 2SiO2 \cdot 3H2O + Ca\ (OH)2 (B)$$

The hydration reaction is followed by a reaction of the resulting calcium hydroxide with calcium phosphate, which precipitates (step two) in the form of hydroxyapatite.[49] Water is formed as a by-product of the precipitation reaction.

$$7Ca(OH)_2 + 3Ca(H_2PO_4)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12H_2O$$

Bioceramic sealers show unique bioactivity, as they set and harden in the presence of moisture, finally forming hydroxyapatite at the interface and creating a chemical bond to dentin.[28] This solubility of CSB sealers has been shown to be higher than that of epoxy resin-based sealers.[50]

1.2.6. Adhesion of sealer to tooth structure

The exact mechanism of bioceramic-based sealer bonding to root dentin is unknown. The following mechanisms have been suggested for CSB sealers:[14]

1. Tubular diffusion – mechanical interlocking by sealer particle penetration and bonding into dentin tubules.
2. The development of a mineral infiltration zone, because of the denaturing of collagen fibres by the alkaline sealer and consequent diffusion of the sealer’s mineral content into the inter-tubular dentin.
3. The formation of hydroxyapatite next to the mineral infiltration zone. Calcium silicates react with moisture from dentin to produce calcium silicate hydrogel and calcium hydroxide, which then react with the phosphate in tooth structure.

Calcium silicates produce a tag-like structure at the calcium silicate/dentin interface, so-called "mineral infiltration zone", which is a hybrid zone where hydroxyapatite recrystallisation occurs when calcium silicate is applied in dentin.[50] The mineral infiltration zone is the ion-exchange layer that appears at the interface between dentin and the CSB sealer, and is attributed to a dual effect of the calcium-hydroxide-releasing sealer – an alkaline corrosive etching followed by mineral diffusion.[21]

Dentin tubular penetration of CSB sealers is advantageous in that it represents a physical barrier for microorganisms and increases the retention of the sealer.[44] From a histological perspective, a proper three-dimensional seal is largely based on the penetration of the materials placed inside the root canal into the dentinal tubules.[15] A higher depth of penetration increases the contact surface between the dentin substrate and the filling material, leading to a greater sealing ability, which can potentially prevent the penetration of new microorganisms and trap any remaining ones.[15]

1.2.7. Advantages of bioceramic sealers

The advantages of bioceramic endodontic sealers include good physicochemical and biological properties,[20, 32] such as tissue biocompatibility, low-toxicity, low expansion with no shrinking, antibacterial properties, stability within the biological environment, and the promotion of periapical healing through stimulation of osteogenesis.[20, 32] Furthermore, bioceramic sealers are not moisture sensitive,[31] expand slightly upon setting and, once set, are dimensionally stable.[31]

In the presence of tissue fluids, bioactivity of calcium silicate occurs resulting in the deposition of hydroxyapatite on the material surface. This bioactivity induces hard tissue formation and healing of soft tissue.[21]

The use of bioceramic materials as root canal sealers therefore improves biocompatibility as it prevents rejection of the sealer by the surrounding tissues.[14, 34, 43]

1.2.8. Disadvantages of bioceramic sealers

The main disadvantage of bioceramic sealers is the difficulty in removing the material from the root canal once they are set for possible retreatment or post-core preparation.[14] Although the effects of bioceramic sealers are ideal from an endodontic point of view, this kind of bond and the tubule penetration reached by these materials might interfere in the future restoration of the teeth.[51]

Kirmali *et al.,*[52] Utneja *et al.,*[53] Vilas-Boas *et al.*[54] and Peña Bengoa *et al.*[55] reported a reduction of the bond strength of fibre posts in teeth filled with bioceramic sealers. They attributed these lower values to the high tubule penetration and the difficulty of removing this type of material from the canal walls.[51, 54, 55]

1.2.9. Comparative studies of bioceramic sealers

Existing literature that compares different bioceramic sealers to other sealers and to each other with regards to biocompatibility, sealing ability and sealer penetration, void formation, and re-treatability of the root canal is briefly discussed below.

1.2.9.1. Biocompatibility

Biocompatibility is the most important feature of root canal sealers since they come into contact with peri-radicular tissues.[31, 41] The high pH (12.8) during the initial 24 hours of the setting process makes bioceramic sealers strongly antibacterial.[56]

Regarding the biocompatibility of bioceramic sealers, Giacomino *et al.*[57] found EndoSeqence BC Sealer and ProRoot ES to demonstrate higher bioactivity than AH Plus (resin sealer) and a ZOE cement.[57] These findings were supported by Lopez-Garcia *et al.*,[58] who found Bio-C Sealer and TotalFill to demonstrate higher cytocompatibility than AH Plus,[58] and Donnermeyer *et al*.,[59] who found that good biocompatibility was reported for AH Plus BC and TotalFill sealers.[59] BioRoot was demonstrated to show good biocompatibility with periodontal ligament cells.[60, 61]

AH Plus BC sealer and EndoSequence BC sealer showed significantly positive results in the cytocompatibility assays (cell viability, migration/proliferation, attachment, and morphology) compared with AH Plus resin sealer, which showed significant negative results.[62]

1.2.9.2. Sealing ability and sealer penetration

Parameters such as the percentage, depth, and area of dentinal tubule penetration, along with antimicrobial properties, are relevant when assessing the adequacy of endodontic sealers for clinical use.[15] The expansion, chemical and micromechanical bonding collectively increase the bonding of the sealer to root canal walls.[56]

Asawaworarit *et al*.[63] found that bioceramic sealers have better apical sealing ability compared to resin-based sealers,[63] and Akhtar *et al.*[64] compared bioceramic sealers with calcium hydroxide sealers.[64] These finding were supported by other researchers.[56, 65, 66] The interface between bioceramic sealers (CeraSeal and BioRoot) with the dentin wall showed very good sealing ability in all parts of the root canal (coronally, middle, and apically), compared to a ZOE, as well as acceptable adhesion in the presence of a gap in all parts of the root canals.[41]

1.2.9.3. Void formation

Overall, it has been reported that no filling technique produced void-free fillings and the presence of voids was associated with spreader tracts, quality of canal preparation, operator expertise, filling technique, consistency of the sealer, volume, and polymerization shrinkage of sealers.[16, 67, 68]

Celikten *et al*.[34] and Pedulla *et al*.[68] conducted *in vitro* studies using micro-CT to evaluate the quality of root canal sealer filling using a single-cone technique in oval-shaped root canals. They reported a decrease in void formation of the sealer in the apical third of root canals.[34, 68]

Huang *et al.*[69] compared three endodontic sealers using micro-CT and nano-CT and found bioceramic sealers (Total BC sealer and Sure Seal Root) to demonstrate a lower incidence and volume of voids as compared to a resin-based sealer (AH Plus).[69]

1.2.9.4. Retreatability

The aim of the root canal retreatment is to disinfect the root canal space adequately to facilitate peri-radicular healing.[70] Independent of the technique used to remove root canal filling materials, studies have reported that complete removal of filling material cannot be achieved.[70-72] Lower values of remnant filling material were found for bioceramic sealers compared to AH Plus.[73] Romeiro *et al.*[74] found that retreatment of canals filled with bioceramic sealers may be more time consuming.[74]

CHAPTER 2: AIMS AND OBJECTIVES

2.1. Aims

The aims of this study were to compare the penetrability of four different bioceramic sealers into the dentinal tubules of roots using single cone obturation and to evaluate the formation of voids when using the materials.

2.2. Objectives

The objectives of this research study were:

1. To determine and compare void formation of these products using microcomputed tomography (micro-CT).
2. To determine and compare the sealing ability of four bioceramic sealers by evaluating the depth of penetration of these materials into dentin tubules using scanning electron microscopy (SEM).

2.3. Null hypothesis

The null hypothesis was that no significant differences would be observed in either the sealer penetrability or the surface area of voids formed between the four tested materials.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study design

The present study was an *in vitro*, prospective, comparative, quantitative study.

3.2. Study settings

The study was conducted at three different venues:

1. The Oral and Dental Hospital, School of Dentistry at the University of Pretoria.
2. The Department of Material Sciences and Metallurgical Engineering, University of Pretoria.
3. The Micro-focus X-ray Radiography/Tomography Facility (MIXRAD) of the South African Nuclear Energy Corporation (NECSA), Pelindaba.

3.3. Research sample selection

This was an investigational comparative study of four bioceramic sealers, namely BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France), CeraSeal Bioceramic Root Canal Sealer (MetaBiomed Co., Cheongju, Korea), TotalFill Bioceramic Root Canal Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and AH Plus Bioceramic (BC) Sealer (Dentsply Sirona, Charlotte, USA). The extracted teeth used in the present study included sixty premolars, with a sample size of 15 teeth per sealer group, approved by the statistician as adequate for the study

Teeth were randomised in a 1:1:1:1 ratio to the four sealers in accordance with a predetermined blocked randomisation plan drawn up by an experienced statistician. Blocks consisted of 15 teeth each, i.e., for every consecutive 15 teeth included in the study, five teeth were randomised to each sealer.

3.4. Measurements

3.4.1. Selection of specimens

Sixty single-rooted, single canal, human premolar teeth, mandibular and maxillary, with closed apices were selected from freshly extracted teeth.[20] The extracted teeth were collected from the Maxillofacial and Oral Surgery Department at the University of Pretoria Oral Health Centre (UPOHC). All teeth were cleaned in ultrasonic baths, rinsed in 3.5% sodium hypochlorite, and stored in 10% formalin at room temperature (37°C) until testing commenced.[75]

During the initial screening of samples, all premolars with roots measuring 10.0 ± 1.5 mm from the cemento-enamel junction to the anatomical root apex were added for possible inclusion.[20] The pre-screened premolars were subjected to a radiographic analysis (Kodak 2100 intraoral x-ray system, Carestream Dental, USA).[20] Radiographs of the potential samples were taken from both a buccolingual and mesiodistal direction to verify the presence of a single canal, that the canal was non-sclerotic, and the absence of any previous root canal treatment.[20] Teeth presenting with more than one root and/or more than one canal, any anatomical aberrations, previous endodontic treatment, or signs of complete or incomplete root fracture were excluded from this study.[20]

3.4.2. Root canal preparation and obturation

Precautions pertaining to the handling of specimen immersed in formalin included the wearing of gloves, a mask, a visor/protective eyewear, a lab coat and operating in a well-ventilated research lab (Room 5-1.1) in the Department of Odontology.[75]

Each specimen was removed from storage bottles containing 10% formalin, rinsed with running tap water, then placed in 3.5% sodium hypochlorite (NaOCl, Jik, Elandsfontein, South Africa) for two hours to allow for surface disinfection, and then placed in bottles filled with distilled water at 37°C until testing was performed.[75] Coronectomies were thereafter performed on each tooth, using a diamond friction grip super coarse bur (Dentsply Sirona, Midwest, USA) at the level of the cemento-enamel junction and adjusted so that the roots were approximately equal in length, measuring 10.0 ± 1.5 mm.[20]

A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to verify apical patency by observing the protrusion of the file past the apex.[20] The size 10 K-file was thereafter watch-wound at full working length until it demonstrated a loose fit within the canal. A ProGlider (Dentsply Sirona, Charlotte, USA) rotary file was then used to create a glide path large enough for the introduction of the root canal preparation files.[20] All canals were then shaped with the Primary file of the Wave One Gold (25 / 07) reciprocating file system (Dentsply Sirona, Charlotte, USA) and each file used in three canals before discarding, in accordance with previously described methodology.[76, 77] The size of the Wave one Gold files was chosen to accommodate small canals, ensure minimal destruction of the dentin and prevent apical transportation.[77, 78] Files were controlled by using three gentle in-and-out pecking motions, with short amplitude strokes.[76, 77] Each file was coated with 17% ethylenediaminetetraacetic acid (EDTA) (RC-Prep, Premier, USA) before introduction into the root canal to keep the debris in suspension, dissolve the inorganic component of the smear layer, and aid in disinfection.[20] Each canal was irrigated using 5 ml 3.5% NaOCl during instrumentation and then was thoroughly rinsed after completion of instrumentation, using 3 ml EDTA (17%) for 1 minute to remove the smear layer followed by 3 ml NaOCl,[66] as NaOCl is anti-bacterial, anti-fungal, anti-viral, and capable of dissolving the biofilm and organic matter.[20] The final irrigant may affect the properties of the root canal sealer used during obturation, particularly with regards to calcium silicate-based sealers, which interact with dentin.[21] Using NaOCl for the final irrigation created an alkaline environment that was suitable for calcium silicate cement hydration and improved the sealing ability of calcium silicate-based sealers.[50]

Each bioceramic sealer material was introduced into the canal according to manufacturer's instructions. BioRoot powder and liquid were mixed according to the manufacturer's instruction. The remaining sealers were gently placed in the canals using the proprietary syringes. A fully coated Primary Wave One Gold gutta-percha cone was thereafter firmly placed into the canal to length and burnt off at the level of the cemento-enamel junction, then allowed to set. The same procedure was followed for the obturation of all four groups. Among a variety of obturation techniques, the single-cone obturation technique is generally recommended with calcium silicate-based sealers, as they do not shrink compared to other types of sealers, and the application of heat may affect their properties.[18]

Table 1. Delivery method of bioceramic root canal sealers used in the present study.

Trade name	Manufacturer	Delivery method	Setting Time
BioRoot RCS	Septodont, Saint-Maur-des-Fosses, France	Two bottle system: Powder (1 scoop) and liquid (5 drops),	4 hours

		introduced into root canal using a paper point or gutta-percha point.	
CeraSeal Bioceramic Root Canal Sealer	MetaBiomed Co., Cheongju, Korea	Premixed Syringed directly into the root	3.5 hours
TotalFill Bioceramic Root Canal Sealer	FKG Dentaire, La Chaux-de-Fonds, Switzerland	Premixed Syringed directly into the root	2-4 hours
AH Plus Bioceramic Sealer	Dentsply Sirona, Charlotte, USA	Premixed Syringed directly into the root	2-4 hours

Following obturation, the teeth were stored in an incubator (Binder ED23, Tuttlingen, Germany) (Figure 2 below) at 37°C and 100% humidity for five days to facilitate complete setting of the sealers, prior to microcomputed tomography (micro-CT) scanning and scanning electron microscope (SEM) analysis.[79]

Figure 2. Binder ED23 Incubator

3.4.3. Micro-CT analysis for void formation calculation

Micro-CT is a non-destructive three-dimensional (3D) imaging technique used to evaluate the microarchitecture, morphology and density of mineralised tissues and the internal structure and porosity of biomaterials and scaffolds.[80]

Samples were scanned using the XTH 225kV micro-focus X-ray/CT system (Nikon Metrology, Leuven, Belgium) (Figure 3), situated at the Micro-focus X-ray Radiography/Tomography Facility (MIXRAD) of the South African Nuclear Energy Corporation (NECSA).[81]

Figure 3. XTH 225 ST micro-focus X-ray tomography system (Nikon) at the MIXRAD Facility

Each of the four functional units of the system consist of a lead-lined cabinet containing an X-ray tube, a sample manipulator, a flat panel detector, an external chiller, an external control module, and computers with software for the recovery- and 3D reconstruction of images.[81]

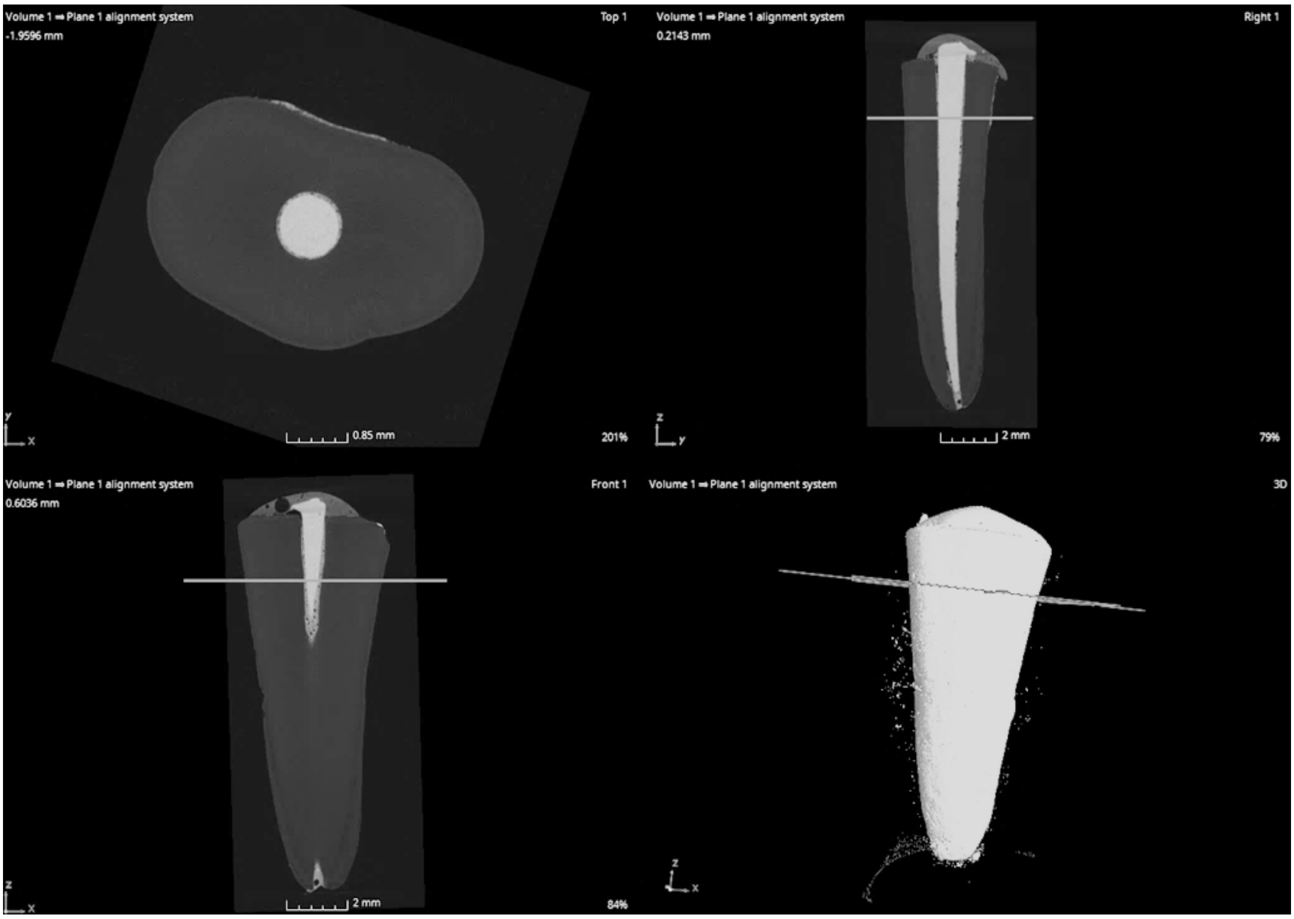


Figure 4. Image showing 3D reconstruction of samples using VG StudioMax software.

Two-dimensional (2D) projections were converted into 3D volumes using CT-Pro reconstruction software (Nikon XT software, USA). The CT-Pro 3D raw volume files were then imported into VG StudioMax software (High-End Industrial CT Software, Heidelberg, Germany).[81]Each sample was rotated 360° within an integration time of 5 min under the following scanning conditions: 100 kVp, 100-mA beam current, 0.2 mm pixel size. Thus, the scans were recovered and reconstructed into a pinpoint sharp 3D virtual image (10 µm as the voxel size). This facilitated the visualisation and analysis of multiple images, making it possible to assess the internal surfaces, material densities and other key physical characteristics of the materials.[82] The volumetric fraction and size distribution for any specific specimen could also be determined using this software.[81] Each specimen was measured from the determined level plane coronally and then subdivided into three parts, namely the apical third, middle third, and a coronal third. Within each third, a region of interest (ROI) was established at an exact halfway point location and used to determine surface area of voids.

The software programme (Avizo 7.0, VG StudioMax 3.0) was manipulated to establish a straight vertical axis through the teeth and to distinguish the images of tooth structure from the sealer material and gutta-percha.[81]

To the researcher's knowledge this was the first study that evaluated surface area of calcium silicate based (CSB) sealers. The surface area of the canal at a specific region of interest **(VC)** and the surface area of gutta-percha **(VGP)** were measured in μm^2 and then the difference, which was the surface area of the sealer material **(VS)**, was calculated by deducting the VGP from the VC.

$$\textbf{VS}\ \mu m^2 = \textbf{VC}\ \mu m^2 - \textbf{VGP}\ \mu m^2$$

Within VS, the software programme calculated the surface area of the voids and color-coded them individually.

3.4.4. SEM analysis

After micro-CT testing, the samples were embedded in self-curing transparent acrylic[51] and sectioned perpendicular to the long axis of the tooth, using an ISOMET™ low-speed precision cutter (Buehler, Illinois, USA) (Figure 5), in preparation for SEM analysis of dentin tubule penetration depth of the sealers. The samples were submerged in EDTA (17%) for two minutes, followed by distilled water for 15 seconds each, and ethanol for 3 minutes to remove any residues produced by the cutting disc.[51, 83] Three representative slices from coronal, middle and apical thirds of each tooth were selected, resulting in 225 slices in total.[51]

Figure 5. ISOMET™ low-speed precision cutter

The scanning electron microscope (SEM) is a large precision instrument used for high-resolution micro-area analysis.[84] The machine used in this study was SEM (JEOL JSM IT300, JEOL Ltd., Akishima Tokyo, Japan) (Figure 6) with EDS Detector (Oxford X-max 50). The fragments were observed at an operating voltage of 15 kV, over a range of magnifications. The computer made use of Oxford Aztec software (Oxford Instruments, United Kingdom) to analyse the SEM images. Images were taken of each slice at 350× magnification. In the present study, using a ruler tool on the laser scanning microscopy (LSM) image browser software, in each of the images obtained, the most representative zone of the sealer-dentin interface was selected,[51] and the depth of sealer penetration was measured and recorded at three random representative points on each section, due to the destructive nature of the technique. The measured readings were averaged to obtain a single mean value for each section. The deepest penetration was measured from the canal wall to the point of maximum sealer penetration using the measuring tool.[18]

Figure 6. SEM Facility at University of Pretoria, Department of Metallurgical Engineering

3.5. Ethical considerations

Approval for this study was obtained from the University of Pretoria's Faculty of Health Sciences' Research Ethics Committee (Protocol number: 627/2021, Appendix A) and from the Research Committee of the School of Dentistry (RESCOM). The Declaration of Helsinki was adhered to during this study.

All patients at the Maxillofacial and Oral Surgery Department at the University of Pretoria signed a patient information leaflet and consented to treatment, which included permission for the use of extracted teeth for educational and research purposes. The identities of the patients whose extracted teeth were used in this study were unknown, and no tooth was linked to a particular patient. Individual consent was waivered for the purposes of this study, and a letter requesting that consent be waivered was also submitted to the Ethics Committee.

Safety and ethical guidelines for handling human teeth were consistently adhered to and, on completion of the study, these teeth were disposed of as part of medical waste, according to the guidelines of the Health Professions Council of South Africa (HPCSA) for good practice in the healthcare professions: Booklet 16.

3.6. Statistical considerations

3.6.1. Sample size

Sixty premolars were included in the study, with a sample size of 15 teeth per sealer. Teeth were randomised in a 1:1:1:1 ratio to the four sealers in accordance with a predetermined blocked Randomisation Plan drawn up by the statistician. Blocks consisted of 15 teeth each, i.e., for every consecutive 15 teeth included in the study, five teeth were randomised to each sealer.

3.6.2. Data capturing

The study data was captured using a Microsoft Excel (Microsoft Corporation, Santa Rosa, California) spread sheet and then imported into statistical analysis software (SAS Institute Inc., North Carolina, USA) for statistical analysis.

3.6.3. Statistical analysis

Penetrability of the root dentinal tubules by the four different bioceramic sealers was compared by statistical analyses. Mean values were compared by a One-way Analysis of

Variance (ANOVA). When a statistically significant p-value was found for the ANOVA test, pair-wise comparisons of the sealers were performed with associated p-values.

Surface area of voids of the four different sealers were also compared by ANOVA.

All the statistical procedures were performed using SAS (SAS Institute Inc., Carey, North Carolina, USA), and p-values ≤0.05 were considered significant.

CHAPTER 4: RESULTS

4.1. Introduction

The results of this study are presented as follows.

Mean values for the four sealers (AH Plus Bioceramic, BioRoot, CeraSeal and TotalFill) were compared by parametric as well as nonparametric Analyses of Variance (ANOVAs). Two assumptions are needed for a parametric ANOVA, namely normality and equal variances of the distributions of the variables being compared. The recorded study data for the four sealers were evaluated for compliance with the assumptions but did not always comply. The sample sizes for the sealers were also small, which was not conducive to normality testing. Consequently, the nonparametric Kruskal-Wallis ANOVA, which is based on median values, and which does not depend on the assumptions, was also applied. Pairwise comparisons of the sealers were performed and the p-values from both ANOVAs are reported in the tables below. With only a few exceptions, the p-values from both ANOVAs agree in the sense that both p-values were significant and both p-values were not significant. The agreement found between the p-values contribute to the credibility of the conclusions.

The four sealers were compared in respect of void formation and dentin tubule penetration depth.

4.2. Void Formation

The images obtained of the samples under microcomputed tomography (micro-CT) scanning showed the sizes and the surface area (μm^2) of voids in each predetermined region of interest (ROI) (Figures 7-A, -B, -C, and -D.).

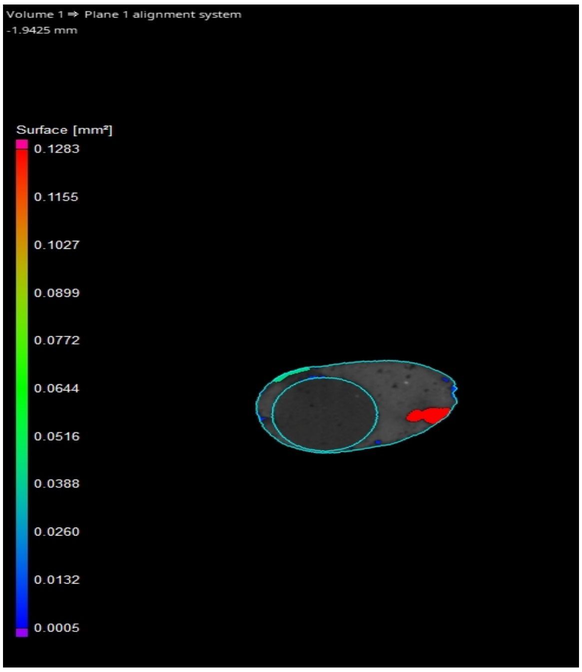


Figure 7-A. AH Plus BC.

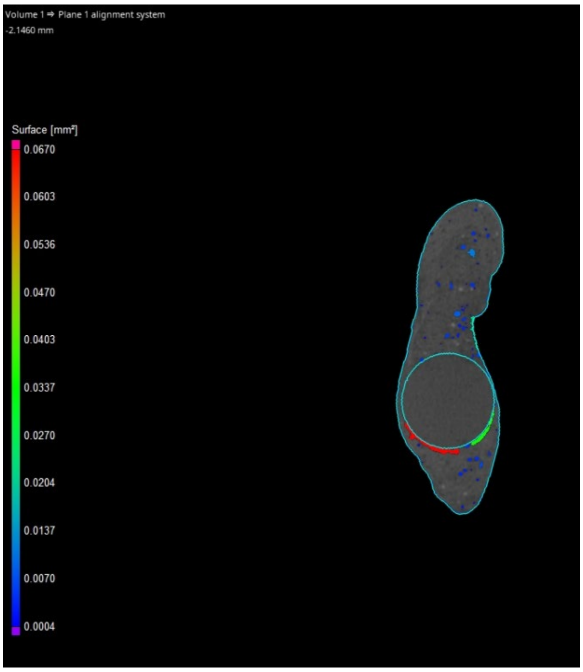


Figure 7-B. TotalFill.

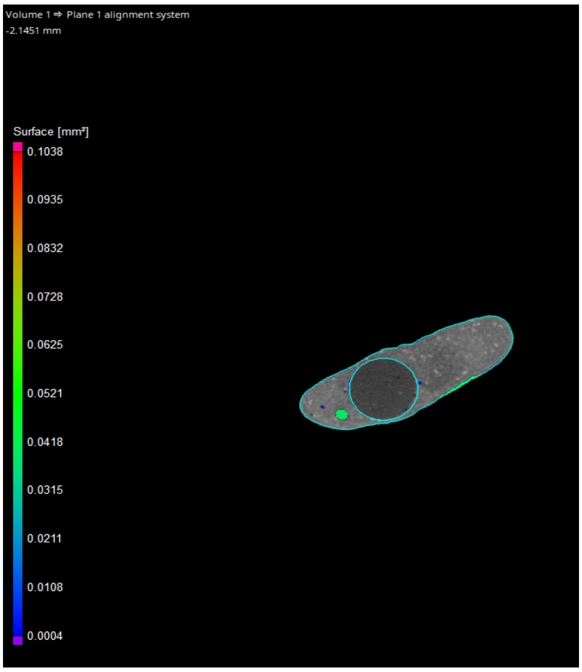


Figure 7-C. CeraSeal.

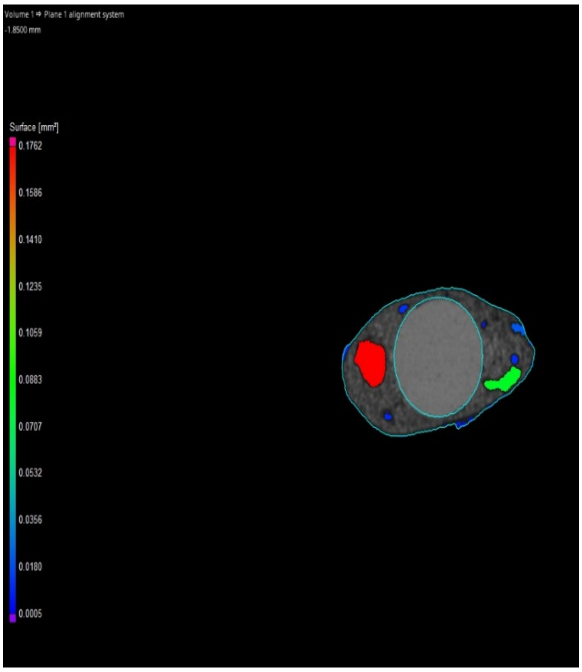


Figure 7-D. BioRoot.

Figure 7. Images obtained on micro-CT scanning showing the size and surface area (µm²) of voids at each region of interest for all sealers.

The mean and median surface area of voids (µm²) at all three positions (coronal, middle, and apical) of all four sealers is demonstrated in Table 2.

The number of samples analysed for each sealer group within each root third at times deviated from the expected number (n=15), due to the sealer not reaching that part of the root. That meant if the ROI fell within that part of the root, it could not be analysed, leading to a decreased sample size in the apical third for some materials.

Table 2. Surface area of voids *(μm^2)*

Sealer	n	Mean (SD)	Median (IQR)	p-values for: means */ medians**	
Position 1 – Coronal root third (μm^2)					
AH Plus BC	15	0.232 (0.129)	0.230 (0.126 - 0.350)	AH Plus BC vs BioRoot	0.426 / 0.178
BioRoot	15	0.362 (0.841)	0.104 (0.080 - 0.234)	AH Plus BC vs CeraSeal	0.943 / 0.820
CeraSeal	15	0.220 (0.169)	0.219 (0.063 - 0.313)	AH Plus BC vs TotalFill	0.767 / 0.520
TotalFill	15	0.280 (0.178)	0.244 (0.107 - 0.402)	BioRoot vs CeraSeal	0.386 / 0.520
				BioRoot vs TotalFill	0.616 / 0.065
				CeraSeal vs TotalFill	0.714 / 0.310
Position 2 – Middle root third (μm^2)					
AH Plus BC	15	0.122 (0.084)	0.111 (0.063 - 0.178)	AH Plus BC vs BioRoot	0.191 **/ 0.049**
BioRoot	15	0.075 (0.071)	0.054 (0.033 - 0.088)	AH Plus BC vs CeraSeal	0.311 / 0.576
CeraSeal	15	0.159 (0.144)	0.119 (0.058 - 0.210)	AH Plus BC vs TotalFill	0.976 / 0.756
TotalFill	15	0.124 (0.074)	0.132 (0.052 - 0.196)	BioRoot vs CeraSeal	**0.023 / 0.014**
				BioRoot vs TotalFill	0.182 / 0.078
				CeraSeal vs TotalFill	0.325 / 0.663
Position 3 – Apical root third (μm^2)					
AH Plus BC	15	0.092 (0.082)	0.071 (0.044 - 0.109)	AH Plus BC vs BioRoot	0.067 / 0.118
BioRoot	12	0.049 (0.025)	0.041 (0.032 - 0.067)	AH Plus BC vs CeraSeal	0.385 / 0.861
CeraSeal	14	0.072 (0.044)	0.067 (0.050 - 0.095)	AH Plus BC vs TotalFill	0.242 / 0.174
TotalFill	13	0.065 (0.066)	0.042 (0.028 - 0.068)	BioRoot vs CeraSeal	0.314 / 0.150
				BioRoot vs TotalFill	0.491 / 0.828
				CeraSeal vs TotalFill	0.752 / 0.286
Overall average number of voids at all three positions					
AH Plus BC	15	0.149 (0.067)	0.143 (0.085 - 0.219)	AH Plus BC vs BioRoot	0.775 **/ 0.049**
BioRoot	15	0.166 (0.287)	0.064 (0.048 - 0.128)	AH Plus BC vs CeraSeal	0.884 / 0.917
CeraSeal	15	0.158 (0.100)	0.128 (0.085 - 0.227)	AH Plus BC vs TotalFill	0.734 / 0.756
TotalFill	15	0.169 (0.109)	0.166 (0.066 - 0.221)	BioRoot vs CeraSeal	0.889 / 0.097
				BioRoot vs TotalFill	0.958 / 0.120
				CeraSeal vs TotalFill	0.847 / 0.820

* Two-sample t test

* Wilcoxon rank sum test

- Except for the AH Plus BC versus BioRoot pairwise comparisons in (i) Position 2, and in (ii) the Overall average surface area of all three positions, the p-values from the parametric and the nonparametric ANOVAs agree in the sense that for every pairwise comparison both p-values are significant or both p-values are not significant.

- In **Position 1 (Coronal root third)** no significant differences (p>0.05) were found between the mean void levels or the median void levels of the four sealers.

- In **Position 2 (Middle root third)** the median void levels for AH Plus BC compared to BioRoot differed significantly (p=0.049). The mean and the median void levels of BioRoot compared to CeraSeal differed significantly (p=0.023 and p=0.014 respectively).

- In **Position 3 (Apical third)** no significant differences were found between the mean void levels or the median void levels of the four sealers (p > 0.05).

- For the **Overall average of all three positions** the mean surface area of voids of the four sealers did not differ significantly. The median surface area of voids for AH Plus BC and BioRoot differed significantly (p=0.049).

Figure 8. below shows the mean and median surface area of voids for all four sealers.

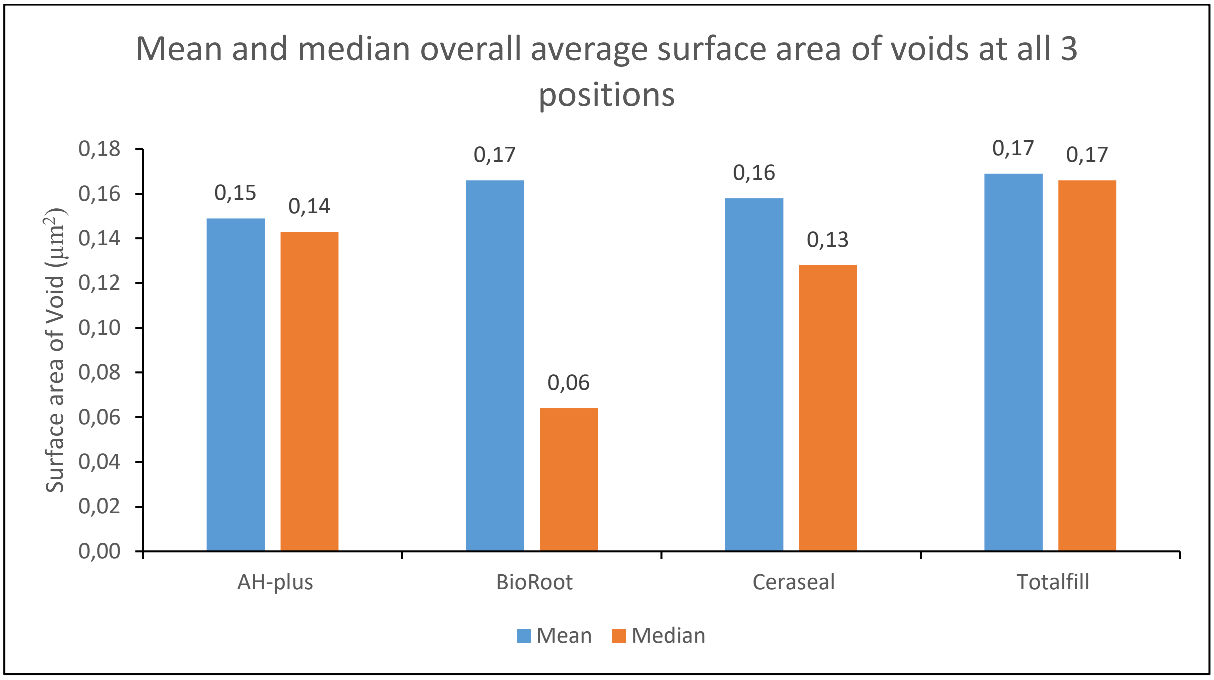


Figure 8. The mean and median average surface area of voids

4.3. Penetration depth

The images obtained of the samples under scanning electron microscopy (SEM) showed complete smear layer removal produced by the final irrigation. The dentinal walls were smooth with open dentinal tubules, as can be seen, as well as sealer material present within the dentin tubules. (Figure 9-A, B, C, D.)

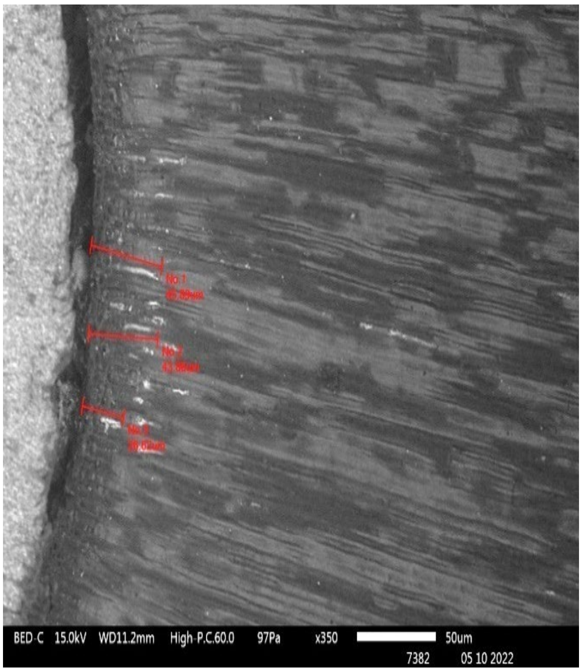


Figure 9-A. AH Plus BC.

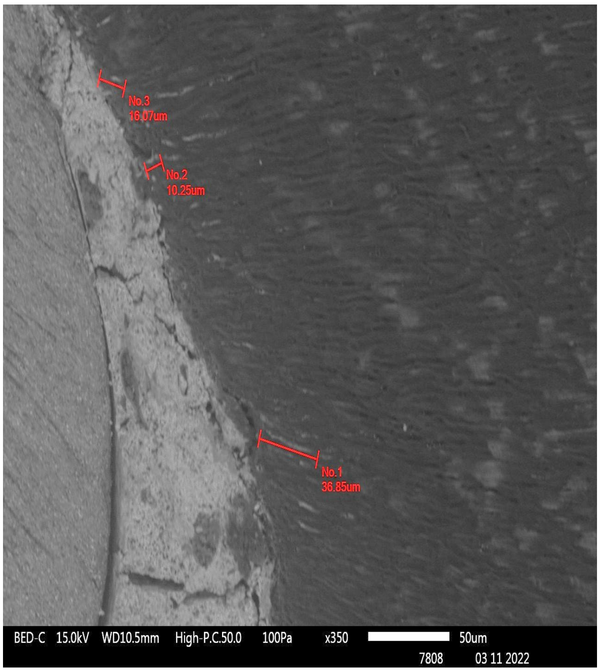


Figure 9-B. TotalFill.

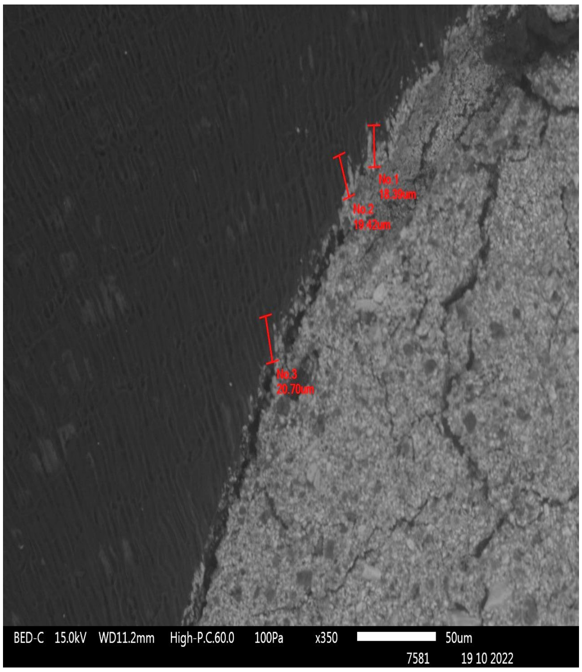


Figure 9-C. CeraSeal.

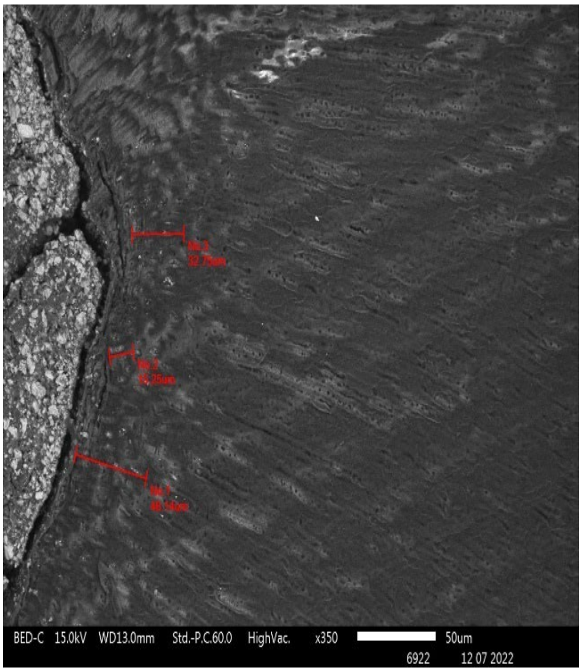


Figure 9-D. BioRoot.

Figure 9. Images obtained under SEM magnification (350x) of the tubular penetration of all sealers.

The results obtained from the analysis of the SEM images showed that BioRoot presented greater tubule penetration compared to AH Plus BC, CeraSeal and TotalFill. The penetration depth of the four sealers in the dentin tubules of the coronal, middle, and apical third of the root canal walls has been summarised in Table 3.

The number of slices analysed for measurement of sealer depth within the representative samples selected sometimes deviated from the expected number (n=15) due to reasons such as:

- Sclerotic dentin – there were no dentin tubules present for penetration of the sealer and, therefore, could not be viewed on the scan.

- The dentin at the sealer-dentin interface was damaged and could, therefore, not be measured.
- Following slicing of the samples, the sealer was dislodged from the canal and could not be measured.

Table 3. Penetration depths (µm) of the four sealers

Sealer	**n**	**Mean (SD)**	**Median (IQR)**	**p-values for: means */ medians****	
Coronal					
AH Plus BC	14	32.94 (11.84)	33.50 (22.54 - 39.28)	AH Plus BC vs BioRoot	0.187 / 0.594
BioRoot	13	39.40 (20.94)	31.38 (24.70 - 47.80)	AH Plus BC vs CeraSeal	0.002 **/ <0.001**
				AH Plus BC vs TotalFill	**<0.001 / <0.001**
CeraSeal	13	17.12 (6.97)	15.65 (12.54 - 19.50)	BioRoot vs CeraSeal	**<0.001 / <0.001**
TotalFill	14	14.32 (4.07)	13.93 (11.14 - 16.34)	BioRoot vs TotalFill	<**0.001 / <0.001**
				CeraSeal vs TotalFill	0.565 / 0.308
Middle					
AH Plus BC	10	26.04 (10.95)	30.11 (18.94 - 31.35)	AH Plus BC vs BioRoot	0.008 / 0.078
BioRoot	11	41.44 (21.36)	39.44 (24.20 - 51.48)	AH Plus BC vs CeraSeal	0.141 / 0.096
				AH Plus BC vs TotalFill	0.013 **/ 0.006**
CeraSeal	10	17.52 (8.55)	14.76 (11.78 - 25.01)	BioRoot vs CeraSeal	**<0.001 / 0.006**
TotalFill	14	12.45 (4.82)	11.51 (8.95 - 13.78)	BioRoot vs TotalFill	**<0.001 / <0.001**
				CeraSeal vs TotalFill	0.341 / 0.143
Apical					
AH Plus BC	5	27.90 (9.45)	30.92 (20.98 - 34.26)	AH Plus BC vs BioRoot	0.218 / 0.540
BioRoot	10	34.65 (15.22)	30.55 (23.63 - 37.23)	AH Plus BC vs CeraSeal	0.028 / 0.020
				AH Plus BC vs TotalFill	0.013 / 0.020
CeraSeal	10	15.49 (5.01)	15.53 (12.18 - 20.46)	BioRoot vs CeraSeal	**<0.001 / 0.001**
TotalFill	9	13.44 (5.13)	11.48 (9.22 - 17.90)	BioRoot vs TotalFill	**<0.001 / <0.001**
				CeraSeal vs TotalFill	0.651 / 0.514
Overall average of coronal, middle and apical					
AH Plus BC	15	31.35 (11.02)	30.85 (22.89 - 37.40)	AH Plus BC vs BioRoot	0.068 / 0.407
BioRoot	14	38.82 (17.92)	33.53 (24.66 - 56.12)	AH Plus BC vs CeraSeal	**<0.001 / <0.001**
				AH Plus BC vs TotalFill	**<0.001 / <0.001**
CeraSeal	14	16.40 (5.22)	17.17 (13.16 - 19.88)	BioRoot vs CeraSeal	**<0.001 / <0.001**
TotalFill	15	13.13 (2.28)	12.96 (11.72 - 14.29)	BioRoot vs TotalFill	**<0.001 / <0.001**
				CeraSeal vs TotalFill	0.419 / **0.061**

* Two-sample t test

* Wilcoxon rank sum test

- In the **Coronal third,** mean and median penetration depths of AH Plus BC and BioRoot did not differ significantly (p>0.05). Likewise, the mean and median penetration depths of CeraSeal and TotalFill also did not differ significantly (p>0.05).

- In the **Middle third** the mean penetration depths of AH Plus BC and BioRoot differed significantly (p=0.008). The median values, however, did not differ significantly (p>0.05). The mean and median penetration depths of CeraSeal and TotalFill also did not differ significantly (p>0.05).

- In the **Apical third,** the mean and median penetration depths of AH Plus BC and BioRoot did not differ significantly (p>0.05). Likewise, the mean and median penetration depths of CeraSeal and TotalFill also did not differ significantly (p>0.05).

- For the **Overall average of coronal, middle and apical root thirds,** the mean and median penetration depths of AH Plus BC and BioRoot did not differ significantly (p>0.05). Likewise, the mean and median penetration depths of CeraSeal and TotalFill also did not differ significantly (p>0.05).

- At all three levels, as well as on the **Overall average**, the maximum mean penetration depth was achieved with BioRoot.

The mean and median averages about overall sealer penetration depth can be seen in Figure 10 below.

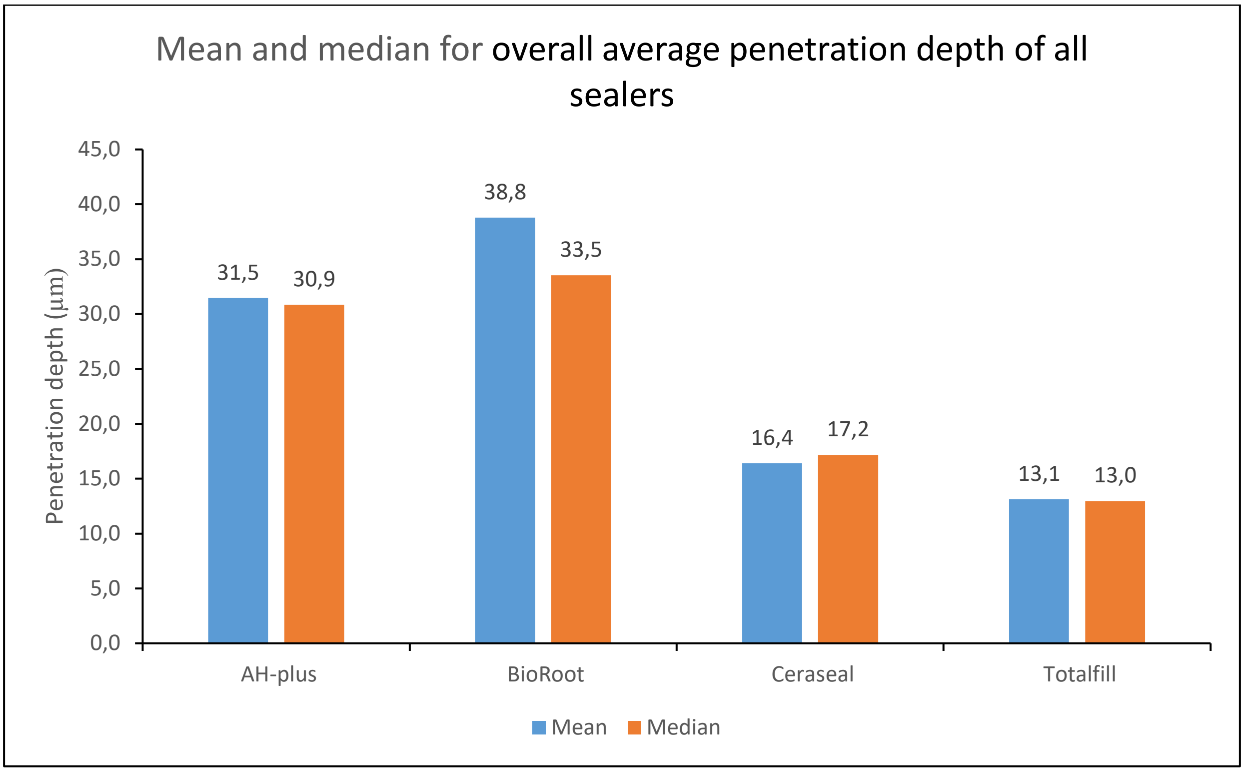


Figure 10. The mean and median overall averages for penetration depths of all the sealers

CHAPTER 5: DISCUSSION

The aims of this *in vitro* study were to assess the depth of dentinal tubule penetration of four different sealers (AH Plus Bioceramic, TotalFill, CeraSeal, and BioRoot RCS) using scanning electron microscope (SEM), and to measure the total surface area of voids present in each sealer material using micro-computed tomography (micro-CT). Significant differences were found between the materials in terms of both dentinal tubule penetration, as well as total surface areas of void present. The null hypotheses were therefore rejected within the limitations of the study sample destruction technique.

The term “single-cone technique” was associated with poor quality obturations for many years due to the need for large amounts of sealer, particularly in oval shaped canals, which resulted in void formation and subsequent sealer shrinkage, fluid leakage and sealer degradation.[23] In the last decade, with the introduction of bioceramic sealers demonstrating low contraction rates, and the introduction of single-file systems combined with matching gutta-percha points, the single-cone technique has become the ideal choice for root canal obturation.[23, 85] For this reason, the present study used the WaveOne Gold file system, combined with matching gutta-percha points.

5.1. Void formation

Micro-CT allows for the quantification of sealer void formation and the evaluation of three-dimensional (3D) changes immediately after obturation.[86] The present study, therefore, used micro-CT analysis, owing to its optimal results in several previous studies that quantified voids present in root canal fillings.[27, 86]

Voids and marginal gaps are frequently found in root canal fillings, and such defects reduce the overall sealing ability, creating the conditions for bacterial and fluid microleakage from crown to apex.[68, 80] In this study, void formation was observed in all the tested calcium silicate-based sealers (BioRoot, CeraSeal, TotalFill and AH Plus BC).

Teeth were decoronated in this study to achieve uniform lengths of samples (10.0 ± 1.5 mm), in accordance with previous studies.[68, 87] While the use of single-rooted premolars with straight roots and root canals minimised the anatomical variables, the wide buccolingual configuration of the root canal system created a challenge to achieve void-free root fillings using the single-cone obturation technique.[34] Celikten *et al*.[34] found that, at all levels, void volumes were the highest for the single cone technique with the tested bioceramic sealer.[34] The distribution of the sealer and, therefore, the formation of voids within the root canal filling has previously been reported to be unpredictable, irrespective of the obturation method used.[67, 68]

A smaller number of void areas inside the sealer and less gap regions at the root canal wall have been reported when iRoot SP was compared to the resin-based sealer AH Plus.[42] This finding has been supported by several other investigations.[43, 69, 88] The surface area of closed pores showed the largest values in the coronal sections, followed by the middle and the apical sections for both EndoSequence BC sealer and AH Plus BC ($p<0.05$).[24, 69] The small particle size and improved viscosity of bioceramic sealers favours their capacity to fill root canal systems, thereby decreasing the presence of voids in these materials.[2, 34, 89] In the present study, the overall average surface area of voids at all three positions showed no significant differences in mean values. The median surface area of voids for AH Plus BC and BioRoot, however, differed significantly (p=0.049), implying these two materials showed larger void sizes compared to CeraSeal and TotalFill. This may be due to flow and film thickness of BioRoot.[86] Micro-CT evaluation has previously found the coronal region of the root canal space to have a larger amount of sealer than middle or apical regions. The increased amount of sealer may be the cause of greater void formation found in this region.[86]

It has been reported that the porosity reduction of EndoSequence BC sealer may be attributed to its premixed delivery system, because hand-mixed root canal sealers, like BioRoot, appear to be prone to subjective operator-induced factors, thus producing more structural defects (voids).[86] This finding was not observed in the present study.

It has been suggested that standardisation of voids may be beneficial in order to distinguish a baseline threshold for evaluating other reconstructions.[90] This may be accomplished by using an internal void of known dimensions for each material, thereby distinguishing different thresholds for each reconstruction.[86, 90] However, it has been reported that such approaches are not commonly used in these types of study.[86] The present study similarly did not implement any baseline threshold measurements. Different methods for determining threshold values have been used in previous studies,[80, 86, 90-92] however, these studies did not include internal voids of known dimensions. Future research may benefit from the inclusion of void standardisation and the determination of threshold values prior to the evaluation of this parameter.

Previous studies focused mainly on quantitative aspects of void formation (i.e. percentage of voids or volume of voids) when evaluating different sealers.[43, 68, 88, 93] However, in these studies no data was reported related to the surface area of voids at a particular region of interest. The findings of the present study should, therefore, be considered novel, adding to the existing body of evidence regarding void formation in these materials.

In a study by Waltimo *et al.,*[91] voids were divided into open and closed categories.[94] The rationale for dividing the voids into the two categories is due to the fact that unfilled spaces (i.e. open voids/pores) may lead to regrowth of microorganisms and/or allow their ingression by microleakage.[94] Closed voids/pores, on the other hand, may be considered isolated unfilled spaces with much less potential for bacterial growth and migration.[94] Other studies found that the preparation and mixing methods of endodontic sealers were found to affect the porosity and voids in their mixtures.[48, 95-97] The present study did not classify the voids present into open or closed categories, but, instead, calculated the surface area of voids present at the region of interest.

A limitation related to the use of micro-CT as a study methodology is that the different radiopacity and rheological properties of the root canal sealers may affect the detection of voids.[88, 89] Moreover, the resolution of the micro-CT images obtained is important regarding the detection of small voids.[88, 89] The present study used 10 µm as the voxel size, since it has been shown that 11.2 µm is a reliable cut-off value for the evaluation of root canal filling voids when using micro-CT imaging.[88, 89]

With regards to the findings of the present study concerning overall mean void formation, the null hypothesis was accepted, as no significant differences were detected in the surface area of voids analysed for each region of interest combined.

5.2. Penetration depth

The tubule penetration depth of endodontic sealers depends mainly on their physicochemical properties, smear layer removal and dentinal permeability.[51] Another factor which may influence dentin tubule penetration depth is the anatomical root canal zone (i.e. coronal, middle, or apical third).[51] Akcay *et al*.[98] showed that laser activation of either sodium hypochlorite (NaOCl) or ethylenediaminetetraacetic acid (EDTA) was better than NaOCl irrigation alone for sealer penetration into dentinal tubules, and as effective as an EDTA final flush.[98] For this reason, in the present study each canal was irrigated using 5 ml 3.5% NaOCl during instrumentation and was then thoroughly rinsed after completion of instrumentation using 3 ml EDTA (17%) for 1 minute to remove the smear layer.[66]

Different microscopic techniques, such as light microscopy, confocal laser scanning microscopy (CLSM) and SEM have been used to demonstrate sealer penetration depth of root canal sealers within dentinal tubules.[18] Sealer penetration depth is commonly investigated using CLSM. In this technique, sealers are mixed with organic dyes, such as rhodamine B, and indirectly detected by fluorescence of the organic dye. It has, however, been demonstrated that penetration depth of rhodamine B dye does not necessarily correlate with the detection of the sealer in the dentinal tubules by SEM.[83] For this reason, rhodamine B dye was not used in the present study.

One major disadvantage is that SEM only allows sealer detection at the surface of the specimen, and, therefore, specimen preparation using a low-speed precision cutter could influence the results, as the sealer could be washed out or mechanically removed from the tubules.[83] This finding was supported in the present study, as some specimens were lost to evaluation for this reason.

The single-cone obturation technique was recommended to be used with calcium silicate-based (CSB) sealers in order to prevent any changes in the sealer properties when using the warm vertical obturation technique.[99] For this reason warm obturation techniques were not used in the present study. The present study compared and evaluated the dentinal tubule penetration of BioRoot, AH Plus BC, CeraSeal and TotalFill in the three defined thirds of the root canal system (coronal, middle and apical) after a final irrigation protocol that included EDTA 17% for smear layer removal. Dentinal tubule penetration as deep as 2 mm has previously been reported for iRoot SP.[30, 42] In the present study, sealer penetration of all samples was detected in close proximity to the root canal interface with the sealer. Other studies comparing the sealer penetration depths of AH Plus and MTA Fillapex have reported penetration depths varying from 23.4 to 84.3µm.[100, 101] These shorter penetration depths into the dentinal tubules were attributed to the limitation of SEM, which only allows the specimen surfaces to be viewed.[18]

In the present study, BioRoot showed significantly higher penetration depth values than the other three sealers, followed by AH Plus BC and then CeraSeal and TotalFill at all root levels investigated. This finding is supported by those of Viapiana *et. al.*[43] as well as Turker *et. al.*[102] Milanovic *et al.*[86] and El-Hachem *et al.*[18] who found that the sealer depth of penetration at 5 mm from the apex was significantly lower for AH Plus, followed by the novel tricalcium silicate (NTS), and it was higher for BC Sealer.[18, 86]

Bioceramic sealers have been found to demonstrated better dentin tubule penetration when compared to traditional resin-based sealers. When comparing conventional resin-based AH Plus and TotalFill bioceramic sealer, Turkel *et al.*[103] found that the bioceramic sealer had better tubule penetration than AH Plus, regardless of adjunctive irrigation activation techniques.[103, 104] This may be due to the small particle size or the viscosity of the CSB materials that facilitate the flow of the sealant into the dentinal tubules.[24, 51] When comparing these sealers in conjunction with a single cone technique, Akcay *et al.*[98] showed that the bioceramic sealer iRoot SP had significantly greater penetration area than the other tested sealers, including AH Plus.[98, 104]

The studies by Reynolds *et al.*[104] and Huang *et al.*[24] showed that the depth and percentage of sealer penetration was greater in the coronal- compared to the apical section, and the texture of the sealers was more homogenous.[24, 104] These results are consistent with the findings of previous studies.[30, 105-107] This may be due to the fact that the number and diameter of dentinal tubules decrease apically in the root canal system. Another explanation may be the better removal of the smear layer in the coronal region.[104] This finding was supported in the present study and by several previous studies including Wang *et al.*,[2] McMichael *et al.*,[30] and Eymiril *et al.*,[108] who all reported less sealer tubule penetration in the apical third compared to the middle- and coronal root canal thirds.[51]

Eltair *et al.*[66] found bioceramic sealer used in conjunction with conventional gutta percha to be associated with a lower percentage of interfacial gaps between gutta-percha and sealer ($p < 0.001$) and between sealer and dentin ($p = 0.04$), whereas the AH Plus sealer showed more gaps between sealer and dentin (p= 0.04).[66] This finding motivated the use of conventional gutta-percha for the present study.

Turker *et al.*[102] found that the retention of CSB sealers in the dentin tubules was higher than epoxy resin-based sealer when the smear layer was preserved.[102] Furthermore, BioRoot also demonstrated higher retention compared to MTA Plus and AH Plus when the smear layer was removed.[102] The present study demonstrated BioRoot to have the highest tubule penetration values of the tested bioceramic sealers, which support the findings of Turker *et al.*[102]

With regards to the findings of the present study concerning penetration depth of CSB sealers into dentin tubules, the null hypothesis was rejected, as there were significant differences detected in the measurements between all four sealers.

5.3. Retreatability of bioceramic sealers

A desirable property of an ideal root filling material or sealer, as outlined by Grossman,[26] was the ability to be easily removed from the root canal if necessary.[26] Retreatment consists of the removal of existing filling material to allow disinfection of the root canal system to promote periapical healing.[70, 72, 109]

Several parameters have been used to assess the retreatability of endodontically treated teeth, including the ability to regain the working length (WL) and patency, the time to reach the WL, and the amount of remaining root canal filling material or debris.[110-112]

CSB sealers possess high hydraulic conductance, which tends to tightly seal dentinal tubules, and re-establishing patency and WL in retreatment cases may be more challenging compared to conventional sealers.[109] Furthermore, CSB sealers have been shown to create hydroxyapatite crystals in the interface between dentin and sealer, and their retrieval from the dentin tubules may be more difficult.[113] The findings of the present study, demonstrating good dentinal tubule penetration of bioceramic materials, may support this assertion.

Evidence to the contrary however exists. Kim *et.al*.[113] found that there was no significant difference in retrievability of patency and working length between the AH Plus and the bioceramic sealer groups,[113] which was supported by the findings of Yang *et al*.[109] Conventional retreatment techniques however failed to fully remove either CSB or epoxy resin-based sealers from the dentin walls, indicating that endodontic retreatment may be challenging regardless of the type of sealer used.[109]

5.4. Limitations of the study

- It is important to note that the results of the present *in vitro* study may not necessarily translate into clinical practice.
- The age of the teeth may have influenced the degree of sealer penetration, as factors, including dentinal changes, were evident in some of the SEM slices.
- Due to the method of slicing the teeth for scanning with SEM, some gaps in the interface between the sealers and tooth structure were created, evident as detachments in some of the scans.
- Some samples were lost to evaluation and could not be measured due to the sectioning method employed.
- Analysis of the samples in this study was performed by one operator, and, therefore, reproducibility cannot be guaranteed.

- A limitation of the micro-CT system and the software used are that two voids are at times counted as a single large void if those two voids are in contact with each other.
- A limitation of SEM is that only the surface of the samples provided information that could be analysed, which led to the loss of some samples.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

- None of the four tested bioceramic sealers demonstrated void-free fillings and voids were found in all samples to varying degrees.
- The overall mean surface area of the voids did not differ significantly between the four sealers evaluated ($P > 0.05$).
- BioRoot showed significantly higher penetration depth values compared to the other three bioceramic sealers (mean and median = $p<0.001$), with a mean penetration depth of 38.82 µm.
- The lowest sealer penetration depth was found for TotalFill at 13.32 µm, which was not significantly different from Ceraseal, with a mean penetration depth of 16.40 µm.
- The depth of penetration of the tested sealers into the dentin tubules may be influenced by the method of application, i.e., premixed versus hand mixed forms.
- Microcomputed tomography (micro-CT) is an appropriate methodology to assess the presence of voids in bioceramic sealers.
- Scanning electron microscopy (SEM) can be used for the evaluation of dentin penetration depth of bioceramic sealers.

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APPENDIX A: ETHICAL CLEARANCE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through June 30, 2025 and Expires 07/28/2026.

Faculty of Health Sciences **Research Ethics Committee**

10 October 2023

Approval Certificate
Annual Renewal

Dear Dr LP Shabalala,

Ethics Reference No.: 627/2021 – Line 3
Title: An in vitro, SEM and micro-CT comparison of dentinal tubule penetration and void formation by four bio-ceramic sealers in premolars

The **Annual Renewal** as supported by documents received between 2023-09-18 and 2023-10-09 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-10-09 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-10-10.
- Please remember to use your protocol number (627/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo