THE QUALITY OF ROOT CANAL FILLING IN MANDIBULAR MOLARS UTILIZING WARM VERTICAL AND SINGLE CONE TECHNIQUE: A THREE-DIMENSIONAL MICRO-COMPUTED TOMOGRAPHIC STUDY

by

Wendy Wing Man Lai

B.DSc., The University of British Columbia, 2007
D.M.D., The University of Pennsylvania, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Craniofacial Science)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

July 2016

Abstract

The goal of the root canal filling procedure is the total 3-dimensional filling of root canals and accessory canals. With the development of innovative sealers and gutta percha in matching taper and diameter as rotary instruments, the single cone technique is gaining popularity.

Objective: To compare the percentage of voids and gaps in the coronal, middle and apical third of mandibular molar root canals obturated with different sealers and techniques using micro-computed tomography.

Hypothesis: No differences in the percentage of voids and gaps are found between: 1) the obturation groups; 2) the mesial or distal canals of the mandibular molars; and 3) the root canal thirds.

Methods: Thirty extracted two-rooted human mandibular molars were divided into three experimental groups: 1) single cone technique using ThermaSeal Plus sealer; 2) warm vertical technique using ThermaSeal Plus sealer; and 3) single cone technique using BC sealer. All canals were instrumented with Vortex Blue 0.04 files to an apical size of #35 (mesial) and #40 (distal). The teeth were mounted on a custom attachment for post-instrumentation and post-obturation micro-CT scan. The scans were examined for the relative proportions of voids and gaps in the coronal, middle and apical third of mandibular molar root canals. Data were analyzed with mixed effects models and Wald chi-square test.

Results: A statistically higher percentage of gaps was found in the apical third compared to the coronal third and the middle third of the canal ($p < 0.05$). No significant differences in voids were found in the root canal thirds. No significant differences in voids and gaps were found between the three obturation groups or between the mesial and distal canals ($p > 0.05$). None of the

methods were able to produce a void-free root filling and voids occurred in both mesial and distal canals with no predilection for any part of the canals.

Conclusion: Within the limitations of this study, it appears that the single cone technique utilizing gutta percha in matching taper and size as rotary instruments is a suitable alternative for obturation of mandibular molars as compared to the warm vertical technique.

Preface

The research question and study design were identified by Dr. Wendy Wing Man Lai and were subsequently revised with contributions from Dr. Ya Shen and Dr. Markus Haapasalo. Collection and preparation of the samples and performance of the research were carried out by Dr. Wendy Wing Man Lai. Micro-CT scans were done by Mr. John Schipilow at the UBC Faculty of Dentistry Centre for High Throughput Phenogenomics. Micro-CT data collection and analysis were performed by Dr. Wendy Wing Man Lai with assistance from Dr. Yan Yang and Mr. John Schipilow. Statistical analysis was performed by Ms. Ting Ting Zhao (UBC Statistical Consulting and Research Laboratory) and Dr. Wendy Wing ManLai. Ethics approval was acquired and granted from the University of British Columbia Clinical Research Ethics Board (certificate number H15-02793).

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AHP--AH Plus sealer

AHWV--AH Plus sealer warm vertical group

BCS--BC sealer

BCSC---BC sealer single cone group

BCWV--BC sealer warm vertical group

CLSM---Confocal laser scanning microscopy analysis

CLC-- Cold lateral compaction

ISO---International Organization for Standardization

MCT-- Micro-computed tomography

μA ---microampere

μm --micrometer

mm ---millimeter

ms--millisecond

kVP ---Peak kilovoltage

SC---Single cone

NaOCl ---Sodium hypochlorite

RPM---Rotations per minute

N.cm---Newton centimeters

SEM---Scanning electron microscopy

S.D.---Standard deviation

TSP--- ThermaSeal Plus sealer

TSWV---ThermaSeal sealer warm vertical group

TSSC--ThermaSeal sealer single cone group

GP--Gutta percha

WV---Warm vertical

WL---Working length

ZO--Zinc oxide

List of Symbols

^0C--Degree Celcius

Acknowledgements

I feel very honored to have had the opportunity to work with the most dedicated and exceptional research committee members. My sincerest thank you to my supervisor Dr. Ya Shen for her patience, guidance and mentorship throughout this challenging project. She was there for me every step of the way. I am also very grateful to my co-supervisor Dr. Markus Haapasalo for enlightening me with his views on this research project and for sharing his words of wisdom. His passion for research and quality education is an inspiration. I am very thankful for my committee member Dr. Rick Carvalho for his insightful perspectives. I greatly appreciate his suggestions which further improved my research. As well, I would like to thank Dr. Jeff Coil, who was very supportive and guided me in my professional development throughout this program.

I am thankful to the UBC Faculty of Dentistry, my clinical supervisors, staff members of the Grad Endo program and all my co-residents for the love, encouragement and assistance during the past three years, particularly this last year. I am very grateful to have your support during the most challenging times of my life. A huge thank you to Dr. Carly Dool for keeping me company during endless nights of studying and thesis writing. I could not have done this without her by my side and I am fortunate to have gone through the graduate program with her.

I would also like to specially thank Mr. John Schipilow for his assistance with MicroCT scans and Dr. Yan Yang for guiding me through the micro-CT analysis process. As well, I would also like to thank my co-resident Dr. Ahmed Hieawy for his emotional support and assistance throughout my program. Also, I would like to thank Dr. Rick White and Ms. Ting Ting Zhao for their assistance with statistical analysis. Lastly, I would like to thank the Canadian Academy of Endodontics for the research grant and Sirona Dentsply and Brasseler for the products donation.

Dedication

I wholeheartedly dedicate my education and accomplishments to my family, especially my late father, whose unconditional love and support have allowed me to realize my dreams. I would like to thank them for believing in me and for being there for me during the hardest times. I have finally reached the end of my educational marathon and I could not have done this without their patience and encouragement.

I would also like to dedicate my research to my friends and especially Alan for keeping me sane and keeping my spirits up during the stressful times.

Last but not least, I would like to dedicate my dental hygiene, dental and endodontic achievements to the late Ms. Dianne Gallagher. Her passion for education and strong work ethic have inspired me to be persistent and strong in the face of adversity. I would like to thank her for believing in me and for following my career. This thesis is the fruit of her words of wisdom and encouragement.

1. Introduction

Bacteria and their byproducts are the main cause of pulpal inflammation and apical periodontitis (1-3). The goal of endodontic treatment is to eliminate and prevent microbial infection in the periradicular region via proper instrumentation and irrigation, disinfect the root canal system, and hermetically seal the canal space with a root canal filling (4, 5). However, as complete eradication of bacteria in the canal system is impossible at present, the root canal filling should prevent ingress of bacteria, entomb surviving microbes, and maintain an environment that would inhibit bacterial growth (4, 6, 7). The association of apical pathosis with the presence of inadequate root canal filling in retreatment cases has been noted in outcome studies (8, 9). Hence, there is great interest to evaluate the efficacy of obturation material via *in vitro* studies such as dye leakage studies, fluid filtration, bacterial penetration setup, scanning electron microscopy, and recently microcomputed tomography (MCT) (10-12).

Dye leakage studies have been criticized to have small sample size and application of low power of statistical tests which could conclude significant differences as insignificant (13). As well, many of the old leakage studies focused on the apical region and are based on the assumption that the apical disease was due to the fluid at the apical portion of the root filling and not necessarily due to the passage of toxins or microbes (12). The fluid filtration model and the bacterial penetration setup enable the volume of water or bacteria passing through the filling to be determined as a function of time and experimental variables (12, 14, 15). However, these techniques are unable to quantify the presence of voids (12). In addition, SEM studies require the samples to be sectioned, dehydrated and coated with gold which inherently damages the sample (16, 17).

Thus, MCT is gaining popularity for studying the obturation quality in root canal filled teeth as it can quantify the presence of voids in a nondestructive manner (17). However, the resolution of micro-CT may be a limiting factor and small size voids are likely to remain undetected also with this technique.

1.1. Root Filling

Gutta percha (GP) is the most commonly used obturation material for root canal treatment in the past century (6). GP is derived from the dried juices of the Taban tree and consists of 20% GP and approximately 80% of Zinc oxide in addition to dyes and radiopaque material (12, 18, 19). Crystalline GP may exist in the α or the β form in which GP for endodontic use usually exist (12, 20). GP transforms from the β form to the α form upon heating to 42-49 ºC and transforms from α form to amorphous state upon heating to 53-59 ºC (20). The phase transformation properties of GP is important in thermoplasticized obturation techniques (21). GP does not exhibit any systemic cytotoxic effects and can be easily removed during retreatment as it is dissolvable in chloroform (22, 23). Although GP fulfills many of Grossman's requirement for the ideal root filling material (Table 1.1) (24), it is unable to seal the root canal system completely and relies on the sealer to fill the space between the GP and the dentin wall (6).

Table 1. Grossman's requirements for the ideal root filling material (1936)

Easily introduced into the root canal system	Radiopaque
Seal the canal laterally as well as apically	Not stain tooth structure
Not shrink after being inserted	Not irritate periodontal tissue
Impervious to moisture	Easily removed from the canal, if needed
Bactericidal or at least bacteriostatic	Sterile or easily sterilized immediately before insertion

1.2. Sealers

Many different sealer types are used in endodontics including those based on zinc oxide eugenol, resin, glass ionomer, silicone, calcium hydroxide, and bioceramic (e.g. calcium silicate) materials (12, 25). As the sealer is the component that is in contact with periapical tissues and pulp stump, it is critical for the sealer to be biocompatible and ideally exhibit qualities proposed by Grossman in (Table 1.2) (24).

Table 2. Grossman's requirement for the ideal sealer (1936)

Exhibit **tackiness** when mixed to **provide good adhesion** between it and the canal wall when set	**Bacteriostatic,** not encourage bacterial growth
Hermetic seal	**Insoluble in tissue fluids**
Radiopaque	**Slow setting time**
Contains fine powder particles so they can **mix easily with liquid**	**Tissue tolerant**, not irritating to periradicular tissue
No shrinkage upon setting	**Soluble in common solvent** if it is necessary to remove the root canal filling
Non staining to tooth structure	

1.2.1. ThermaSeal Plus Sealer

ThermaSeal Plus sealer (TSP) (Dentsply Tulsa Dental, Tulsa, OK) is an epoxy-amine-based sealer and is the same product as AH Plus sealer (AHP) (Dentsply International Inc, York, PA) (26). The difference in names is only due to marketing purpose and the sealer is considered to be the most successful one amongst resin-based sealers (12). AHP has been shown to demonstrate good apical sealing ability, working time, setting time, flow rate, solubility and dimensional stability, biocompatibility, and antimicrobial activity (27-29). It also has an initial alkaline pH which greatly reduce to neutral at 24 hours (27, 30). The manufacturer recommends TSP to be used with the master point technique (single cone technique), lateral condensation or warm compaction techniques (ThermaSeal leaflet, Dentsply Tulsa Dental). With heat, the setting time of AHP is reduced and the film thickness is increased (31). In addition, the flow rate of AHP increases upon heating and this is in accordance to ISO standards (31).

1.2.2. BC Sealer

The Endosequence BC Sealer (BCS) (Brasseler USA Dental LLC, Savannah, GA); also previously known as iRootSP root canal sealer (Innovative BioCermaix, Inc., Vancouver, BC, Canada) is a bioceramic sealer which is gaining popularity due to its biocompatibility, alkalinity, non-toxicity, lack of shrinkage upon setting, and chemical stability (11, 30). BCS contains zirconium oxide, tricalcium silicate, dicalcium silicate, colloidal silica, calcium silicates, calcium phosphate monobasic, and calcium hydroxide (11). BCS has also been shown to demonstrate a high pH which contributes to its osteogenic potential, biocompatibility and antibacterial properties (6, 30). Despite the fact that BCS has the higher solubility compared to AHP, its solubility has no impact on its sealing ability or dimensional stability (30). In the presence of moisture, BCS expands slightly upon setting which contributes to its sealing ability (6, 30). Upon coming in contact with tissue fluids, BCS has been shown to be release calcium hydroxide which interacts with phosphates to form hydroxyapatite, a component of bone (6, 11). BCS is hydrophilic and has a working time of over 30 minutes (6). The setting time is 4 hours in normal conditions but the setting reaction is also dependent on the available moisture (6).

With these good mechanical, biological and handling properties, BCS has been proposed to be used as the main component of the root filling with the GP as the delivery device to facilitate the hydraulic movement of the sealer into the canal irregularities(6). Although studies have been performed on the effect of heat on tricalcium silicate based-sealers, varied results were noted on the different tricaclium silicate-based sealers (31). Despite their similar chemical compositions, these varied results indicate that the tricalcium silicate-based sealers should be tested individually to determine the effect of heat on their physical and chemical properties. No study has been performed to determine the effect of heat on BCS specifically and the manufacturer recommended BCS to be used with the single cone and lateral condensation technique.

1.3. Obturation Techniques

Various gutta percha obturation techniques have been used in endodontics including the cold lateral condensation technique, warm vertical compaction technique, and single cone technique (32, 33).

1.3.1. Cold Lateral Condensation Technique (CLC)

CLC is the most commonly taught and practiced filling technique world-wide (33). It requires a canal preparation that is continuously tapered from the orifice to the apical region (33). A master cone which coincides with the master apical file size preparation will be selected (32). Upon coating the master cone with a sealer, a spreader is placed lateral to the cone to create space for accessory GP cones (Figure 1) (33). The placement of accessory cones continues until spreader cannot reach more than 2-3 mm into the canal (33). At that time, a heat source will sear off the cones and allow for consolidation of the filling (33). This technique allows for a positive apical seal and produces a dimensionally stable and dense filling in the coronal and middle third of the canal (32) .

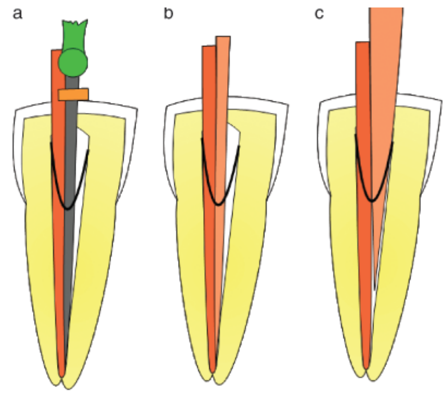

Figure 1. Cold lateral condensation technique (33)

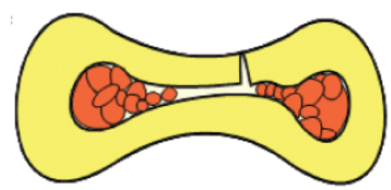

Figure 2. Cross sectional view of canal filled with CLC (33)

1.3.2. Warm Vertical Technique (WV)

The warm vertical condensation technique was popularized by Herbert Schilder in 1960's (32). Schilder stated that the technique can produce a consistently dense three-dimensional filling especially in the apical portion as it also fills the accessory and lateral canals (32). This technique requires the canal shaping to be: 1) a continuously tapered funnel shape, 2) maintain original anatomy, 3) maintain position of the apical foramen and 4) keep the foramen diameter as small as practicable (33, 34). Canal preparation is considered adequate when a taper-matched cone or a fine medium or medium cone can fit to working length (33, 34). The master cone selected must have a taper that is more gradual than the taper of the root canal to prevent the cone from binding with the body of the canal and not near the apex (32). The cone should then be fitted to the radiographic terminus and trimmed to be short (0.5-1 mm) of this length while ensuring that it possesses good tugback (resistance to pulling out) (26, 35). Pluggers should also be pre-fitted to ensure the instrument can compact GP in the coronal, middle and apical third of the canal (26, 32). Only a small amount of zinc oxide sealer is applied in the canal as the condensation pressure applied to the warm GP can spread the sealer evenly over the canal wall(26, 32).

The coronal part of the cone is seared off with a heated instrument which allows GP to be deformed from compaction with a plugger (26). At this temperature, the GP retains its crystalline beta form with minimal shrinkage as it cools back to body temperature (26). The instrument can heat up to 2-3 mm of GP apical to the instrument tip (21). Through successions of heat waves and compaction cycles, the warm filling material can flow into lateral canals and apical ramifications (26). This "downpack" phase continues until the apical 4-5 mm of the canal space is "corked" with the obturation material (26, 33). This was performed based on the recommendation that the removal of GP to a level less than 6 mm from the apex can minimize the amount of sealer within the GP mass (36, 37). The canal is then "backfilled" with injection molded GP in 3-4 mm increments and vertically compacted with a plugger (33). This obturation technique is also known as the multiple wave vertical condensation technique (33).

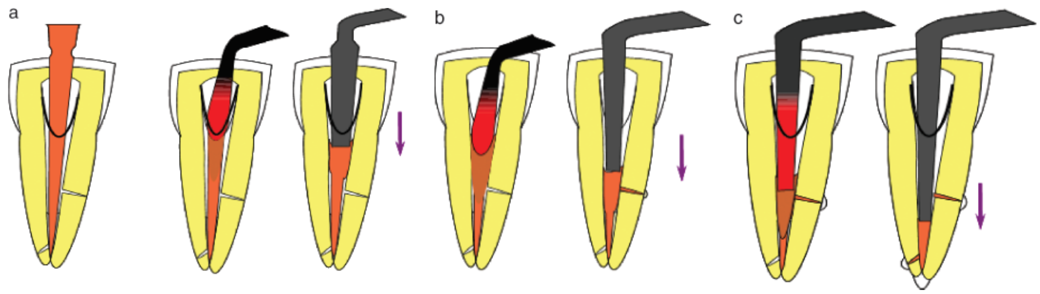

Figure 3. Warm vertical compaction technique (Image from Whitworth, 2005 (33)

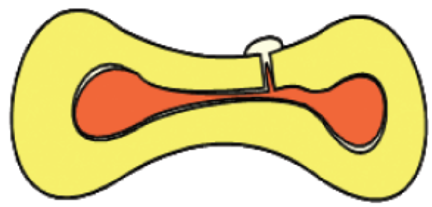

Figure 4. Cross-section of canal filled with warm vertical compaction technique (33)

Another variation of this technique is known as the "continuous wave" obturation technique which was developed by Buchanan (38). System B, a heat source developed by Buchanan, consists of tips that can be heated rapidly to deliver a precise amount of heat for an indefinite amount of time and can be cooled down rapidly (26, 38). Therefore the heating tip could be also be used as a "cold" plugger to compact the GP (26, 38). In this technique, the GP will be removed in one continuous wave of heat (26, 33). Therefore, it would be critical to pre-select a heating tip that binds 4-5 mm from the working length (38). The backfilling of the canal will be the same as described above.

One of the critiques for WV is that when the heated GP cools down, it shrinks more than the sealer does on setting (6, 39). As well, the shrinkage of GP and sealer as opposed to just the sealer results in a bigger gap between the GP and the sealer (6). Other studies have also pointed out that in order to create space for the plugger to reach 4-5 mm from the WL, a larger taper canal preparation in the coronal third is needed, which could produce microfractures (6, 40, 41).

1.3.3. Single Cone Technique (SC)

The SC technique was developed in 1960s when ISO sized instruments and GP cones were developed (33, 42). This technique was recommended to be used in canals that are reasonably parallel so that the master cone fits tightly in the apical third of the root canal (42). After reaming a circular apical stop 2 mm short of the canal, a single GP cone with good tug back was cemented with sealer to fill the canal space(33, 43). The SC technique has been criticized to be a "sealer heavy" technique and was found to have more apical leakage than the CLC technique due to the dissolution of sealer (33, 44, 45). However, the thickness of the sealer is dependent on the fit of the cone to the root canal walls after cleaning and shaping (46). Thus, the use rotary instrument size and corresponding GP cones would ensure a high volume of GP in the canal and decrease the amount of sealer used (47, 48). It is also possible that the new epoxy resin and bioceramic sealers are not susceptible to dissolution, which may change the situation regarding leakage.

The combined use of a matching GP cone and a sealer which is dimensionally stable and insoluble in fluids, such as BCS, has been advocated to be used with the single cone technique (6). The SC technique would eliminate the need for the space required for the plugger to be placed 4 mm from the WL (needed for WV) or the space for a spreader (needed for CLC). SC technique would thus allow for a more conservative canal preparation and thereby more remaining root dentin (6).

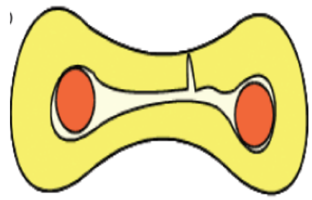

Figure 5. Cross-section of canal obturated with single cone technique(33)

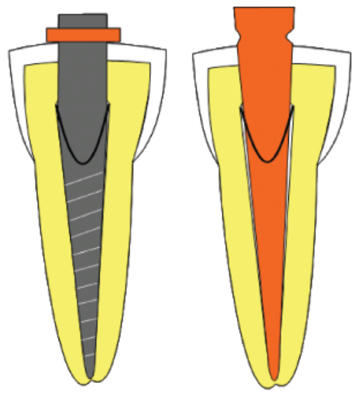

Figure 6. Single cone technique using matched files and gutta percha

1.4. Monoblock

Recently, GP cones impregnated with glass ionomer (Activ GP) or bioceramic particles have been developed (6, 35). These cones are designed for use with the single cone technique as they might provide a bond between the canal wall and the master cone forming a monoblock (35, 49). The term monoblock, meaning "a single unit", may be used to determine the number of interfaces between the material and the root canal dentin which can also relate to the material's sealing quality and tooth strengthening ability(50). This is of significance as endodontically treated teeth may be susceptible to fracture due to the reduction of remaining tooth structure from extensive restorative procedure in addition to endodontic instrumentation (50).

Replacement monoblocks are classified as primary, secondary and tertiary (Figure 7). A primary monoblock has only one interface between the material and the root canal wall while a secondary monoblock has two circumferential interfaces between the cement and the core material as well as the cement and the root canal dentin (50). A tertiary monoblock has a third circumferential interface in between the bonding substrate and the abutment material (50). In order for the material to be classified as a primary monoblock, it needs to bond strongly and mutually to one another as well as to the substrate it is intended to reinforce (50). As well, the material should have a similar modulus of elasticity as the substrate as that influences its ability to strengthen the remaining tooth structure(50, 51). Historically, root canal fillings are classified as secondary monoblocks as the sealer neither bonds tightly to dentin or GP nor do they function as mechanically homogenous units with the radicular dentin(50). However, with the use of BCS and GP cones impregnated with a nano particle layer of bioceramic, the interface between the core and the sealer has been suggested to be eliminated and the root filling material would thus be considered a primary monoblock (6, 50). Such fillings have been shown to improve its sealing ability (6, 52, 53).

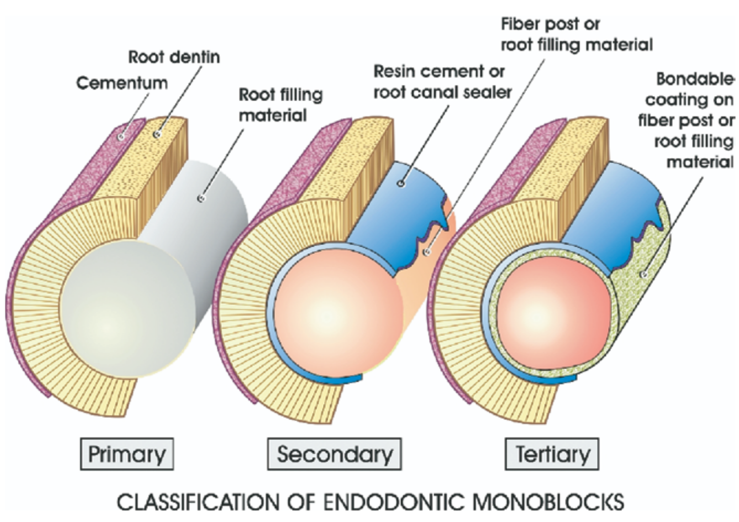

Figure 7. Classification of endodontic monoblocks(50)

1.5. Micro-Computed Tomography (MCT)

Micro-computed tomography is a modern non-destructive, three-dimensional imaging technology that is increasing in popularity to study dental hard tissues (17, 54). It was first suggested for use to study human teeth by Tachibana and Matsumoto (55). It was subsequently used to measure enamel thickness (56), geometry of root canal and root canal volume post instrumentation (17, 57-62), obturation quality (10, 43, 54, 60, 62-64) and retreatment efficacy (65). Medical computed tomography units provide pixel space of approximately 1 mm, which is insufficient to provide the accuracy of details needed in endodontics (59). MCT voxel size is determined by slice spacing and pixel size, which enable enhanced resolution (59). Compared to the conventional imaging techniques (e.g. scanning electronic microscopy, confocal microscopy, and stereomicroscopy), MCT enables the sample to be analyzed without sectioning and allows for repeated scanning and three dimensional reconstruction of images using software such as Amira, NRecon, CTAn and CTVol for further data analysis (43, 59, 62, 64). In addition, a study by Jung *et al.* (2005) has shown that there was a good qualitative correlations ($p < 0.001$) between the images obtained from MCT sections and histological sections and that images from the MCT sections were able to discern between GP, sealer and voids (64). Thus MCT is the method of choice for the evaluation of quality of various obturation material as it allows the specimen to be examined quantitatively and qualitatively without destruction (66).

1.6. Quality of Obturation

The quality of obturation can be evaluated by the percentage of voids and gaps (54). Voids could be classified as internal, external and combined as shown in figure 9. Internal voids are found inside the filling material, whereas external and combined voids (collectively known as gaps) are found between the filling material and the root canal wall dentin (54). Voids are of less clinical significance because bacteria, if present, will be entrapped within the filling (54). In contrast, the presence of gaps may negatively impact the treatment outcome as they are in direct contact with potentially infected dentinal walls and may promote failure of the sealer and lead to leakage (54). In addition, the shrinkage of the root canal sealer of as little as 1% has been reported to be large enough for bacteria and noxious byproduct penetration (19, 67). Thus, the differentiation and the identification of the location of voids and gaps are of clinical relevance and the quantity of voids and gaps is an important part of evaluation of obturation techniques and materials (68).

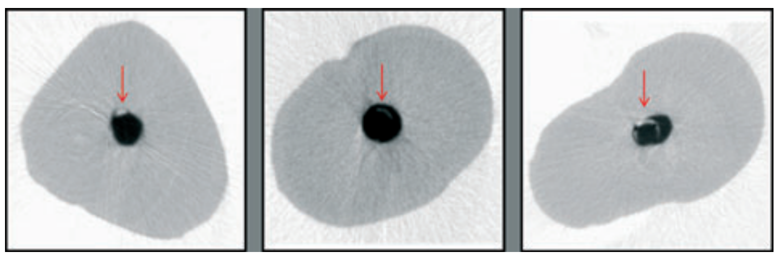

Figure 8. Classification of voids after canal filling: external (left), internal (middle) and combined (right) void, indicated by arrow (Somma *et al.,* 2011) (54)

1.7. Rationale

WV technique has been shown to approximately double the number of lateral canals filled as compared to CLC (69). Historically, when SC technique was performed with conventional sealers, it has been reported to be less effective in sealing root canals than WV technique (44, 45). With the development of innovative sealers and GP cones with matching taper and diameter of rotary NiTi instruments, SC technique is regaining popularity as studies showed no difference in obturation quality (percentage of voids) between SC and CLC and WV techniques (70-72).

In addition, SC takes less time and may provide an obturation similar in sealing ability, bond strength, radiographic quality and percentage of GP and sealer-filled areas and void obtained with CLC or WV technique (47). The dimensional stability of BCS and the primary monoblock formed with matching GP cones impregnated and coated with bioceramic nanoparticles would theoretically eliminate interfacial gaps and produce a "perfect coronal and apical seal" (6, 68).

AHP is considered the most successful one amongst resin-based sealers and is used by many studies to compare the different obturation techniques. TSP, which is the same product as AHP, will be used in this study to draw comparisons to BCS (12). With MCT, the samples can undergo micro-CT scanning post-instrumentation and post-obturation to evaluate the obturation quality without any destruction to the samples (17, 73).

Many existing studies have used single-rooted teeth to evaluate various obturation techniques as they aim to standardize their samples for comparative purposes and to improve data analysis (74). However, the presence of anatomical variations in teeth, especially molars, often presents significant instrumentation and obturation challenges for clinicians (74). The distal root in mandibular molars often has one canal whereas the mesial root often has two canals with various canal configurations and isthmuses in the middle and apical third (75). As it has been suggested that the difference in obturation quality in the different thirds of the root could be due to differences in anatomical variation, it would be of interest to compare the quality of filling in the different canal thirds of the mesial and distal root in mandibular molars (66).

Previous MCT studies have been done to compare the quality of obturation performed with the CLC and WV technique with AHP sealer (73), SC and WV technique with AHP (43, 54), as well as CLC technique with different sealers (10). As the effect of heat and the optimal heating temperature for BCS has yet to be established, the BCS product leaflet recommended BCS to be used with the SC technique. No MCT study has yet compared the quality of obturation performed with SC technique with BCS as compared to SC or WV technique using TSP. As well, no published MCT study has been done to compare the obturation quality between the different canal thirds of the mesial and distal canals of mandibular molars. Therefore, it is of interest to compare the obturation quality in the different thirds of the mesial and distal canals of mandibular molars obturated with single cone technique using ThermaSeal Plus sealer (TSSC), warm vertical technique using ThermaSeal Plus sealer (TSWV) and single cone technique using BC sealer (BCSC) in mandibular molars.

1.8. Objectives

The objectives of the study are:

i) to compare the percentage volume of voids and gaps of mandibular molars obturated with a) single cone technique using ThermaSeal Plus sealer (TSSC), b) warm vertical technique using ThermaSeal Plus sealer (TSWV) and c) single cone technique using BC sealer (BCSC) in mandibular molars

ii) to compare the percentage volume of voids and gaps in the mesial and distal canals of mandibular molars obturated with TSSC, TSWV and BCSC.

iii) to compare the percentage volume of voids and gaps in the coronal, middle, apical third of root canals of mandibular molars obturated with TSSC, TSWV, and BCSC.

1.9. Null Hypothesis

The null hypothesis (H_0) is:

i) there is no overall difference in the percentage volume of voids and gaps in mandibular molars obturated with TSSC, TSWV or BCSC.

ii) there is no difference in percentage volume of voids and gaps between the mesial and distal canals of mandibular molars obturated with TSSC, TSWV or BCSC.

iii) there is no difference in the percentage volume of voids and gaps among the coronal, middle and apical third of root canals of mandibular molars obturated with TSSC, TSWV or BCSC.

2. Materials and Methods

2.1. Sample Size Calculation

The sample size was determined by calculating the effect size from a similar MCT study on the obturation quality of premolars by Keles *et al.* (73). The appropriate effect size was determined from the mean and the standard deviations obtained from the percentage of voids in the WV group (3.09+_2.17) and from the CLC group (0.59± 0.74) in their study (73). The appropriate effect size was determined to be 1.54, the alpha-type error was specified to be 0.05 and the power beta was specified to be be 0.95. The minimum sample size per group was 10.

2.2. Sample Selection

Thirty-three extracted human permanent mandibular molars were used in this study. The teeth were collected from the various dental offices in Vancouver, British Columbia, Canada (certificate number H15-02793). The teeth were extracted for reasons unrelated to the present study and donated anonymously. Upon extraction, the teeth were stored in 0.05% NaOCl at room temperature. The inclusion criteria for the samples were: permanent mandibular molar with two separate roots with intact pulp chambers. The exclusion criteria for the samples were: teeth with visible cracks, resorptive defects, horizontal or vertical root fracture, previous endodontic treatment, or open apices. Samples were examined clinically under the operating microscope (Global Surgical Corporations, St. Louis, MO). In addition, the samples were examined radiographically with one bucco-lingual digital radiograph and one mesio-distal digital radiograph) with intraoral photostimulable phosphor storage plates, ScanX Classic Digital Imaging system (Air Techniques, Melville, NY) and digital radiography imaging software (Planmeca Romexis, Helsinki, Finland) to ensure the selection and exclusion criteria were met. Selected teeth were assigned a unique sample number and the samples were allocated to the three groups of 11 via stratified sampling based on the number of canals and the Vertucci (1984) canal classification (76).

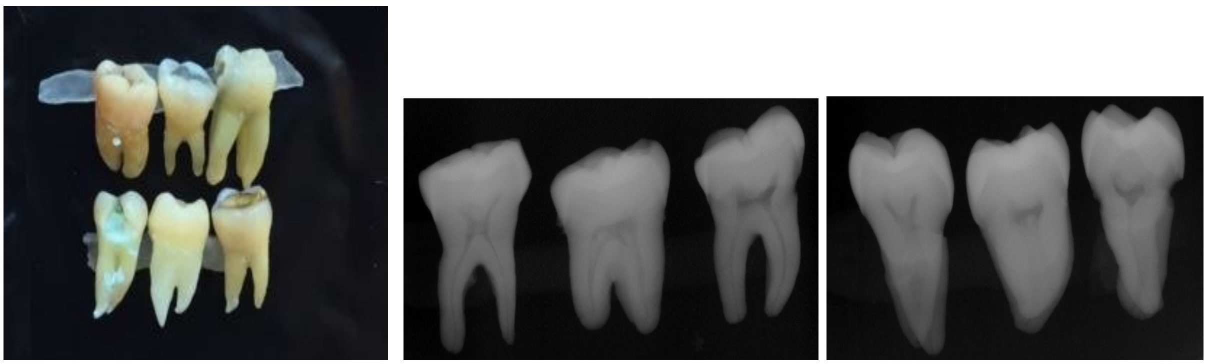

Figure 9. Sample selection: Periapical radiographs (bucco-lingual view and mesio-distal view)

2.3. Sample Preparation and Root Canal Instrumentation

All existing restorations on the samples were removed to prevent any interference from the materials on micro-CT scans. The root canals were exposed with a 169 carbide bur and the access were further refined with a LA Axxess (Kerr Dental, Orange, CA, USA). In cases with calcifications in the pulp chamber (pulp stones), a ProUltra Piezo Ultrasonic unit and ProUltra Endo Tips (Dentsply International, York, PA) were used to remove the calcifications (Figure 11). Upon establishing patency with a size 10-hand K file, the working length was determined by subtracting 1 mm from the length at which the file emerged from the apical foramen. Each canal was then instrumented up to size 15 using a hand K file (Lexicon, Dentsply Tulsa Dental Specialties).

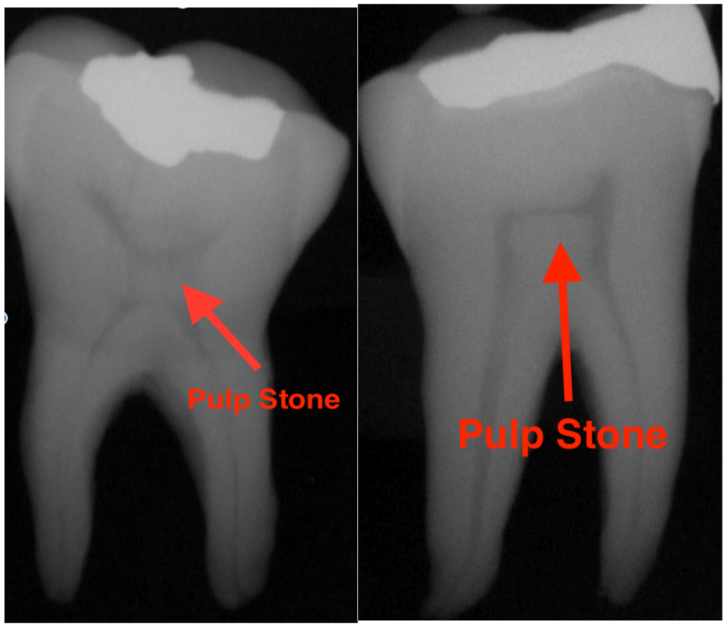

Figure 10. Pulp calcifications in two sample teeth

Due to the various curvatures exhibited by various samples, coronal flaring was performed to reduce risk of file separation (77). A Vortex nickel titanium rotary endodontic orifice opener file (size 25/0.08) was used (Dentsply Tulsa Dental Specialties) with an Aspetico DTC Torque Control Motor (Dentsply Tulsa Dental Specialties) with a W&H 8:1 gear reduction electric contra angle endodontic handpiece (Dentsply Tulsa Dental Specialties) at 500 RPM.

Root canal instrumentation was then carried out with a crown down approach, beginning with a Vortex Blue Rotary file size 35/0.04 for mesial canals and a size 40/0.04 for distal canal proceeding to the next smaller file size until the file reaches the WL. Then the canals were instrumented to WL with files in increasing file sizes until the final apical size of 35/0.04 was reached for the mesial canals and 40/0.04 for the distal canals. Copious irrigation with 6% NaOCl was used in between files to flush out debris. After final canal preparation, water and 1mL of Qmix (Dentsply Tulsa Dental Specialties, Tulsa, OK) were used with needle irrigation for each canal before drying with paper points of corresponding sizes. The samples were then wrapped in moist gauze to prevent desiccation and kept at +37^{o}C before the root filling.

2.4. Micro-CT Post Instrumentation Scan

Up to four teeth were mounted on each level of the custom attachment (Figure 10). All samples were scanned using a MicroCT 100 (SCANCO Medical AG, Brüttisellen, Switzerland) with the following settings: isotropic voxel size of 30 μm, energy of 90 kVp, tube current of 200 μA, integration time of 500 ms, a 0.1 mm copper filter and a x2 frame averaging. The scan resolution was determined by a previous endodontic micro-CT study and pilot scans utilizing different voxel sizes (65). The scan time was approximately 41minutes per one layer of teeth in the specimen holder. The samples were wrapped in moist cotton gauzes after the scanning was completed.

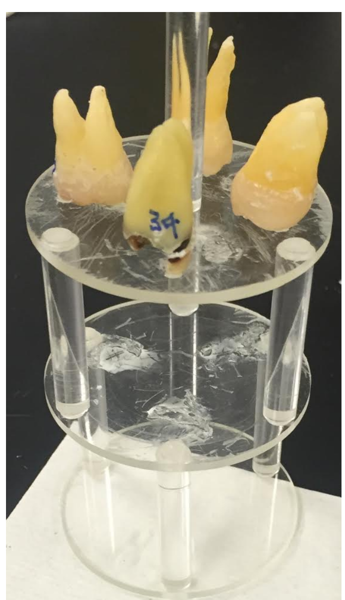

Figure 11. Samples mounted on a custom attachment for the micro-CT scan

2.5. Root Canal Filling

Thirty-three samples were equally divided into 3 obturation groups: Group A: ThermaSeal Plus sealer with single cone technique (TSSC), Group B: ThermaSeal Plus sealer with warm vertical technique (TSWV), and Group C: BC Sealer with single cone technique (BCSC).

In all the groups, a matching 35/.04 GP and a matching 40/.04 GP were fit in the mesial and distal canals of the molar respectively to achieve tugback at the WL (Brasseler USA Dental LLC, Savannah, GA). The sealer (TSP for Group 1 and 2 and BCS for Group 3) was applied by injection into the canal and a size 15-hand K file was spun counter-clockwise to evenly coat the canal with the sealer. The apical 5 mm of the selected GP was then lightly coated with a thin layer of sealer and the GP was reinserted gently until the working length was reached.

<u>Group A: TSSC Group</u>

The GP cone was seared off with a SuperEndo Alpha A2 Heat Source (B&L Biotech USA, Bala Cynwyd, PA) at 200 ^{o}C at approximately 1 mm above the canal orifice. The excess GP was then compacted with the Schilder pluggers (sizes 9 and 9.5) (Dentsply Maillefer, Ballaigues, Switzerland) until the GP was flush with the orifice opening. Excess GP was removed with the Alpha A2 heat source. This methodology to vertically compact the excess GP was based on the study by Horsted-Bindslev et al. (2007).

<u>Group B: TSWV</u>

Using the multiple wave warm vertical compaction technique as described previously, the coronal GP was removed with a SuperEndo Alpha A2 Heat Source (B&L Biotech USA, Bala Cynwyd, PA) at 200 ^{o}C and downpacked with Schilder pluggers (size 8.5, 9, 9.5) (Dentsply Maillefer, Ballaigues, Switzerland) in segments until the apical 5 mm of GP remained. Digital periapical radiographs (one from Bucco-lingual direction and one from mesio-distal direction) were taken prior to backfilling. The backfill was performed with the Calamus Flow Obturation Delivery System with a 25-guage injection tip cartridges (Dentsply Tulsa Dental Specialties) and Schilder plugger sizes 9, 9.5 and 10. The obturation was considered complete when the coronal portion of the root filling was flush with the canal orifice opening.

Group C: BCSC Group

The same procedure as Group A was performed for Group C except BCS was used.

Procedures after root filling

After the obturation was completed, all the samples were stored at 100% humidity and 37 ^{o}C for at least 24 hours to allow setting of the sealer prior to the post-obturation micro-CT scan (78).

2.6. Micro-CT Post Obturation Scan

All the samples were scanned with the MicroCT 100 (SCANCO Medical AG) using the same settings as described previously for the post-instrumentation scan.

2.7. Micro-CT Image Analysis

Three micro-CT softwares were used for the visualization, reconstruction and quantitative measurements of the MCT images. Amira v. 6.0 (FEI Visualization Sciences Group, SAS, Burlington, MA) was used to reconstruct and crop the MCT images, as well as to create images and animations. MeVisLab v 6.4 (MeVis Medical Solutions AG) was used to visualize and register (match) the post-instrumentation and the post-obturation MCT images along the axial, sagittal and coronal plane (Figure 12). The number of image slices for each root was then determined visually by going through the axial slices with MeVis Lab v6.4 (MeVis Medical Solutions AG). The slice in which the filling was first visualized in the coronal third was determined to be the first slice and the slice in which the filling could be last visualized in the apical third was determined to be the final slice. The total number of root slices was divided by three for the segmentation of the coronal, middle and apical third of the root (Figure 13) (67). The average total number of root slices are approximately 350-450 slices per root.

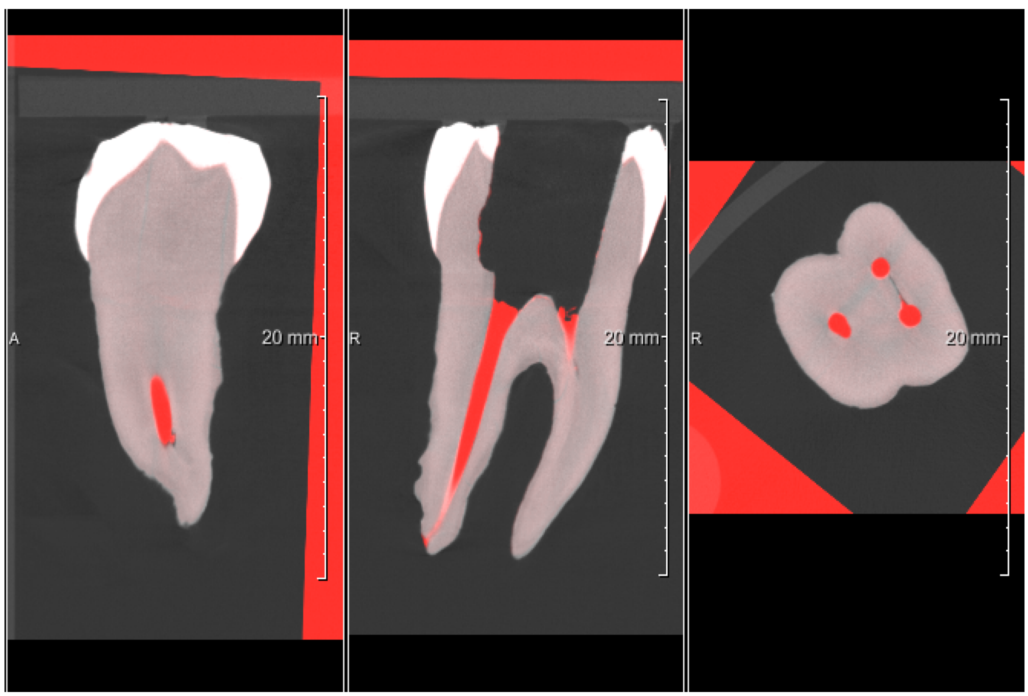

Figure 12. Registering (matching) the post-obturation scan to the post-instrumentation scan in the axial, sagittal and coronal planes.

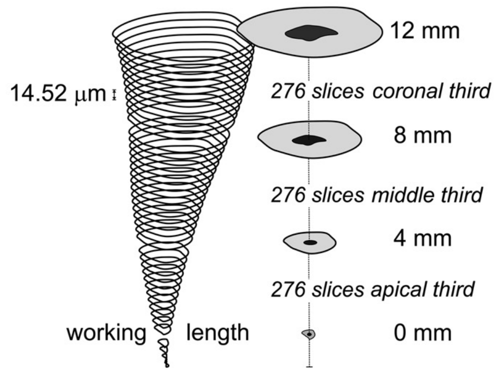

Figure 13. Schematic illustration of using the axial view to equally divide the MCT slices to the coronal, middle and apical third from the study by Li *et al.,* 2014)(67)

Utilizing the threshold function, the volume of the root canal space and the root canal filling were separately identified and quantified (Figures 14 & 15). The empty root canal space often included the isthmus area.

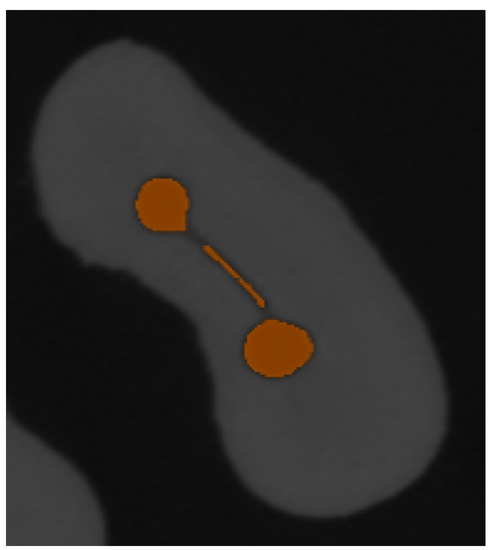

Figure 14. Utilizing the threshold parameter in MeVisLab v 6.4 (MeVis Medical Solutions AG) to determine the volume of the root canal space

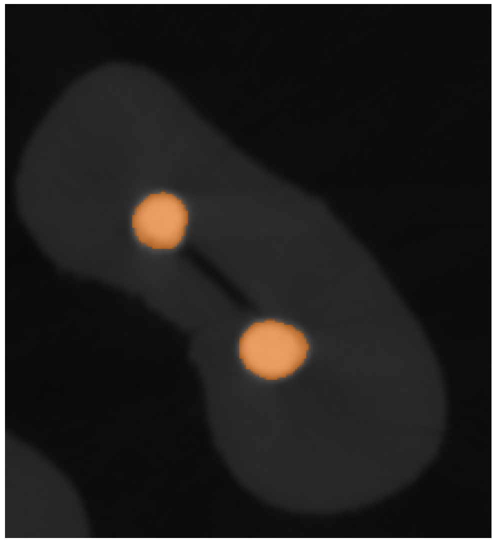

Figure 15. Utilizing the threshold parameter in MeVisLab v 6.4 (MeVis Medical Solutions AG) to determine the volume of the filling in the root canal

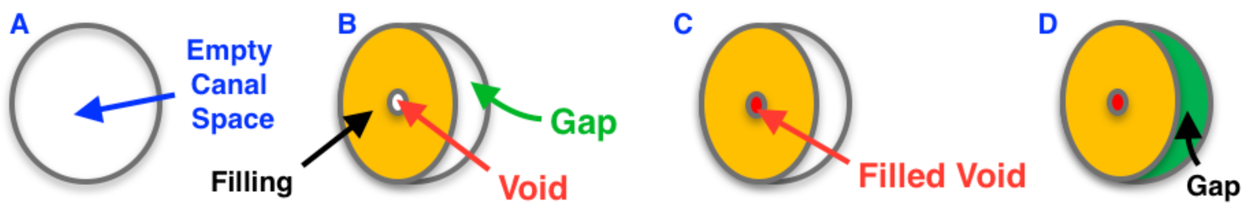

Figure 16. Schematic drawing of: a) empty canal space b) root canal filing, void and gap in canal space c) filled void within root canal filling d) determination of volume of gap after subtraction of volume of filled void within root canal filling (c) from the volume of empty canal space (a)

The segmented data was then analyzed with ImageJ v 1.49 (National Institutes of Health, public domain) for binary registration to detect the voids and "fill" them within the root canal filling (Figure 16). The "fill" data was then re-loaded to MeVisLab v 6.4 (MeVis Medical Solutions AG) to determine the new volume of the root canal filling which included the "filled" void (Figure 17). The volume of the interfacial gaps was determined by subtracting the volume of the root canal filling and the filled void (Figure 17c) from the total volume of the root canal space (Figure 17a). Subsequently the volume of the voids could be determined by subtraction of volume of filling (Figure 17 b) from volume of filled void (figure 17c). The volume of voids and gaps were expressed as a percentage of the root canal space after instrumentation.

Figure 17. Void detection (left) and void filled (right) with Image J v 1.49 (National Institutes of Health, public domain)

2.8. Data Analysis

Due to the repeated measurements of correlated variables (e.g. multiple measurements were made for the different canal thirds and the different roots on the same tooth), a mixed effects model was used. In a mixed effects model, the fixed and random effects were both simultaneously accounted. In this study, the obturation techniques, the roots and the level of the canal thirds were considered as fixed effects while the general variability among the teeth were considered as random effects. Using SPSS v.20 (SPSS INC. Chicago, IL), the Kolmogrov-Smirnov test and non-parametric Levene's test were used to test the assumption of normality and variance. Since the percentage volume of voids and the percentage volume of gaps were not normally distributed and showed skewness, a logit transformation was performed for the percentage volume of voids and the percentage volume of gaps. After the logit transformation, the percentage volume of voids and the percentage volume of gaps were treated as response variables. A Wald chi-square test was then used to test the significance of the coefficients in regression at a significance level of 0.05. For further information on statistical analysis, please refer to Appendix A.

3.Results

3.1. Comparison of the Mean Percentage of Voids and Gaps Between the Obturation Groups

The mean percentage volume of voids and gaps (± S.D.) for each obturation group, each root, and each canal thirds are tabulated in Table 3.1. No significant difference on the effects on the percentage volume of voids were contributed from different obturation groups, canals and root canal thirds ($p > 0.05$) as shown in Table 3.2. No significant difference on the effects on the percentage volume of gaps were contributed from the obturation groups ($p > 0.05$) and canals ($p > 0.05$) as shown in Table 3.3. A significant effect on the percentage volume of gaps were contributed from the root canal thirds ($p < 0.05$). In particular, the apical third contribute significantly more to the percentage volume of gaps than the coronal third ($p < 0.05$) and the middle third ($p < 0.05$) as shown in Table 3.4. Although it was not the objective to compare the percentage of gaps to the percentage of voids in each sample, a higher percentage of gaps than voids were noted in all three obturation groups as shown in table 3.1.

Table 3. Mean and S.D. of percentage volume of voids and gaps of the different obturation groups

		TSSC		TSWV		BCSC	
		Mesial	**Distal**	**Mesial**	**Distal**	**Mesial**	**Distal**
Coronal	**Voids**	0.46±0.81	0.48±1.4	0.17±0.17	1.06±2.40	0.18±0.27	0.68±1.66
	Gaps	4.24±2.25	3.38±2.74	3.92±3.15	4.48±3.13	3.56±2.46	4.90±7.25
Middle	**Voids**	0.13±0.17	0.37±0.91	0.22±0.40	0.43±0.54	0.13±0.23	0.84±1.81
	Gaps	3.74±4.13	5.45±4.62	3.13±2.28	3.24±3.52	4.51±2.81	4.80±4.27
Apical	**Voids**	0.93±2.13	0.10±0.17	0.55±0.94	0.52±1.05	1.25±2.49	0.99±2.30
	Gaps	3.5±2.43	6.88±3.61	5.59±3.50	5.87±3.50	6.13±3.79	5.83±3.77

Table 4. Wald chi-square test results for the percentage volume of voids.
P > 0.05 indicates no statistical significance.

Covariates	Chi-Square	Degree of Freedom	P-value
Obturation techniques	0.93	2	0.63
Canal	0.00	1	0.97
Root Canal thirds	0.10	2	0.95

Table 5. Wald Chi Square Test results for the percentage volume of gaps. P <0.05 indicates the covariate contribute significantly to the percentage volume of gaps.

Covariates	Chi-Square	Degree of Freedom	P-value
Obturation techniques	0.28	2	0.8705
Canal	1.18	1	0.2775
Root Canal thirds	9.94	2	**0.0069**

Table 6. Multiple comparisons of the effect from the roots canal thirds on the percentage volume of gaps after a logit transformation. P < 0.05 indicates the effects observed between the different parts of the roots contribute significantly to the percentage volume of gaps.

Root canal third comparisons	P value
Apical - Coronal	**0.0388**
Apical - Middle	**0.0107**
Coronal - Middle	0.8875

3.2. Comparison of the Percentage of Voids and Gaps Between the Mesial and Distal Canals

No statistical difference in the percentage volume of voids and the percentage volume of gaps was noted between the mesial and distal canals ($p > 0.05$). Again, a higher percentage volume of gaps compared to the percentage volume of voids were noted for the mesial and distal canals.

3.3. Comparison of the Percentage of Voids and Gaps Between the Level of the Canal Thirds

A significant difference in the percentage volume of gaps ($p < 0.05$) and no significant difference in the percentage volume of voids ($p > 0.05$) were noted between the levels of the canal thirds. In all canals, regardless of the obturation techniques, a higher percentage volume of gaps were noted in the apical third compared to the coronal third ($p < 0.05$) and the middle third ($p < 0.05$).

3.4. Images

Reconstructed MCT images post-obturation, periapical radiographs taken during screening and post-obturation, as well as horizontal cross-section images showing the root filling, voids and gaps of one representative sample from each obturation group are shown in Figures 12-20.

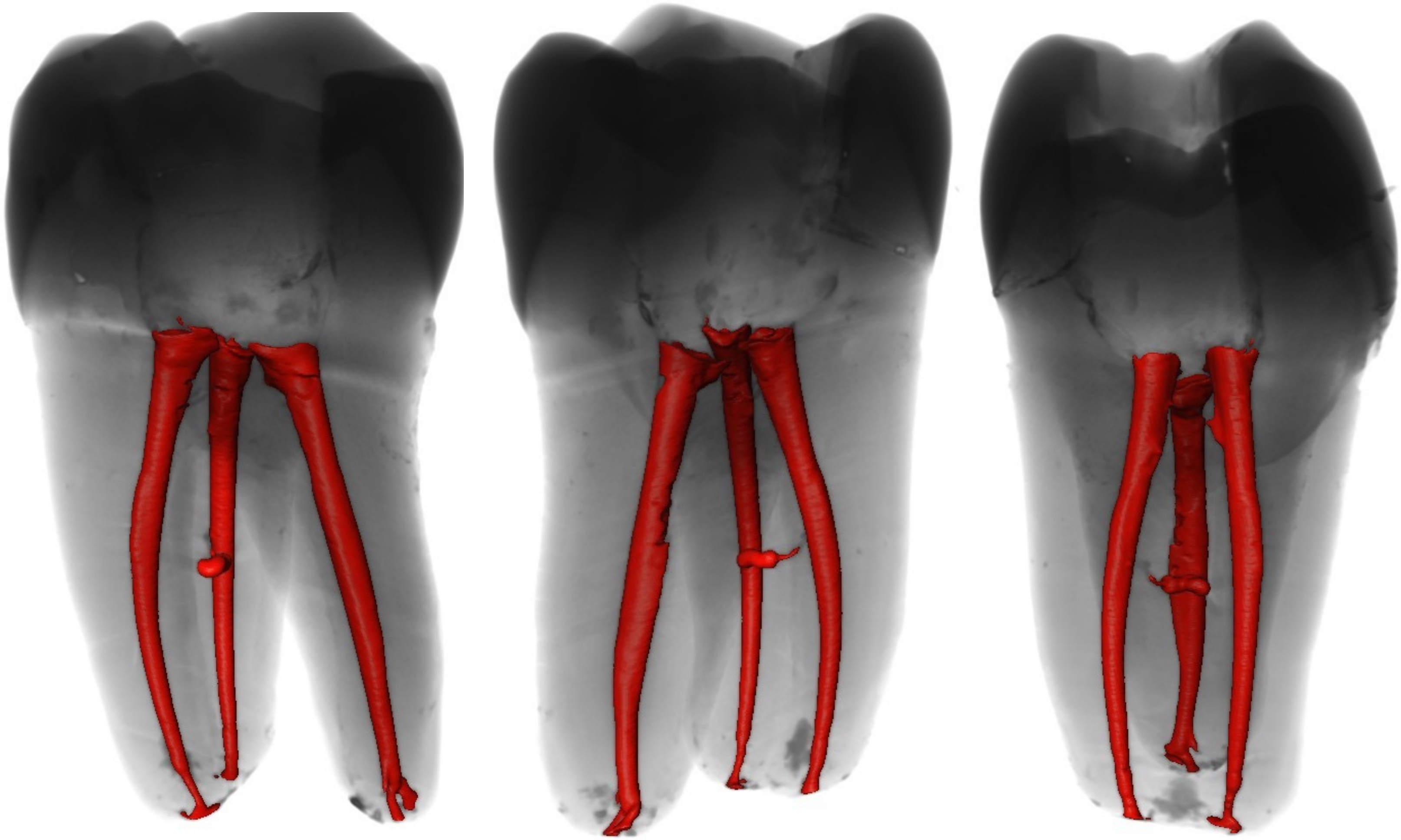

Figure 18. 3D volumetric reconstruction of a mandibular molar obturated with ThermaSeal Plus sealer with single cone technique (TSSC)

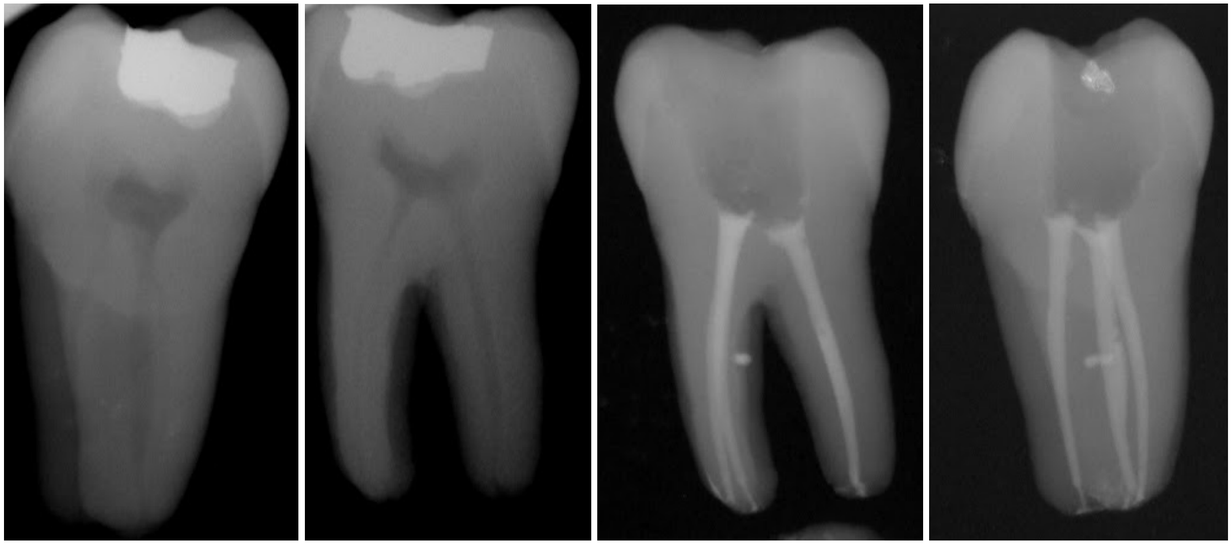

Figure 19. Periapical radiographs taken mesio-distally and bucco-lingually of a mandibular molar during screening and post-obturation with TSSC

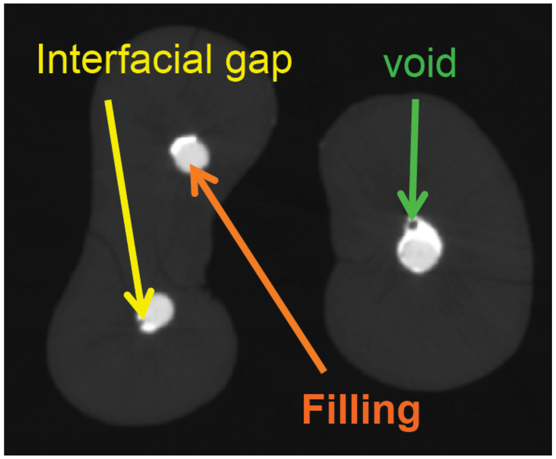

Figure 20. Horizontal cross-section of mandibular molar obturated with TSSC showing the filling, interfacial gap and void

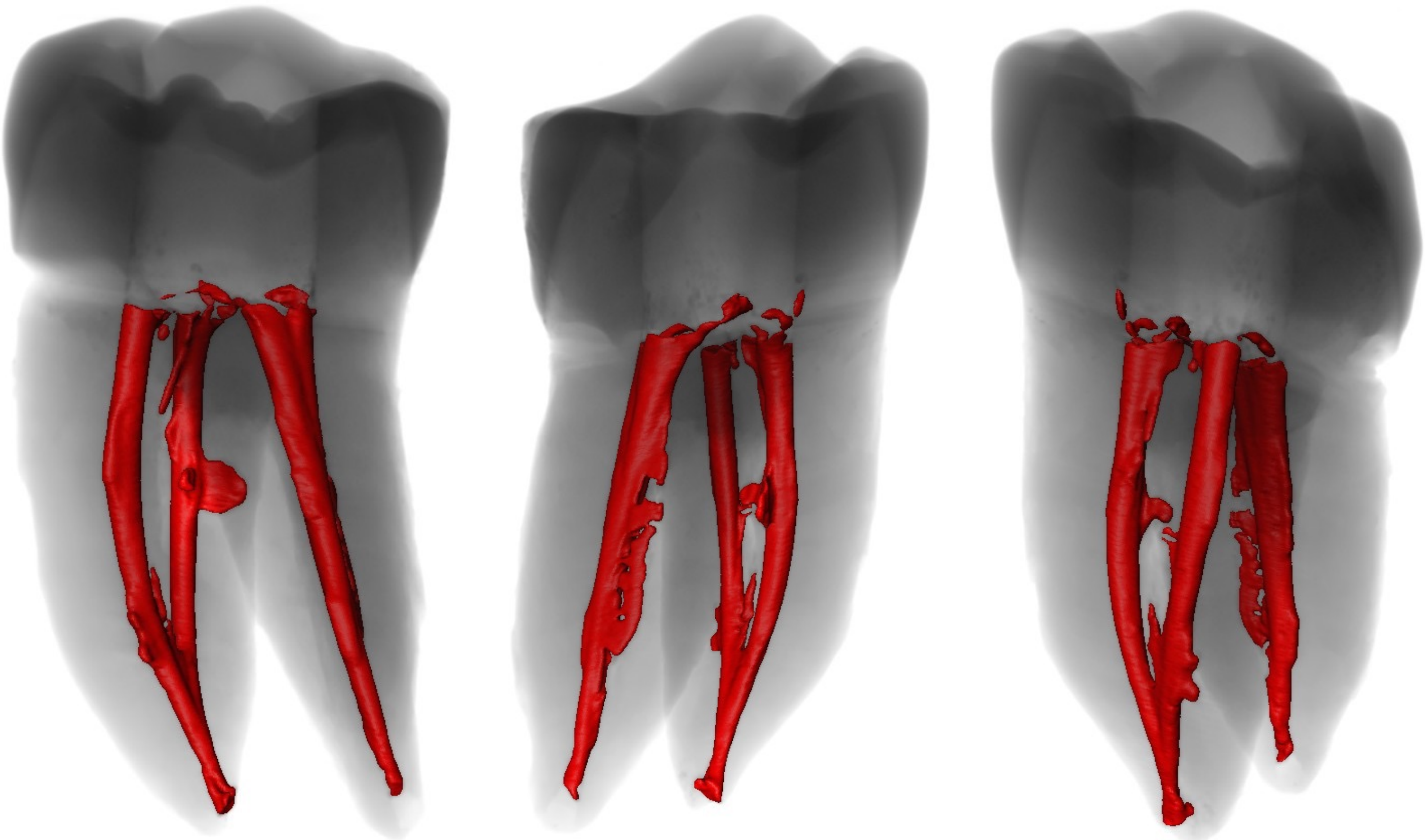

Figure 21. 3D volumetric reconstruction of a mandibular molar obturated with ThermaSeal Plus sealer with warm vertical technique (TSWV)

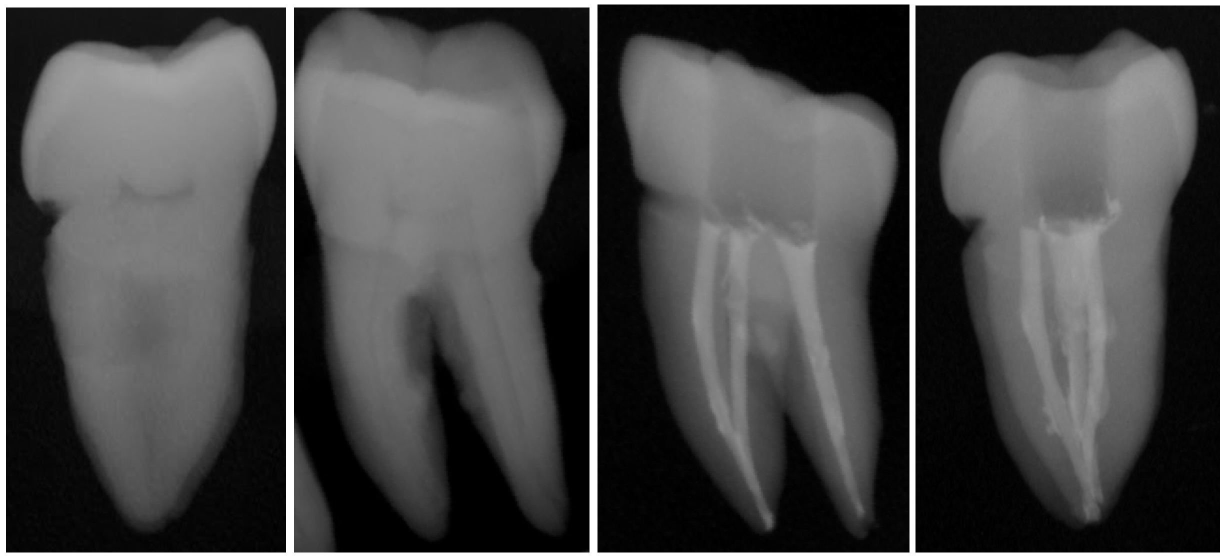

Figure 22. Periapical radiographs taken mesio-distally and bucco-lingually of a mandibular molar during screening and post-obturation with TSWV

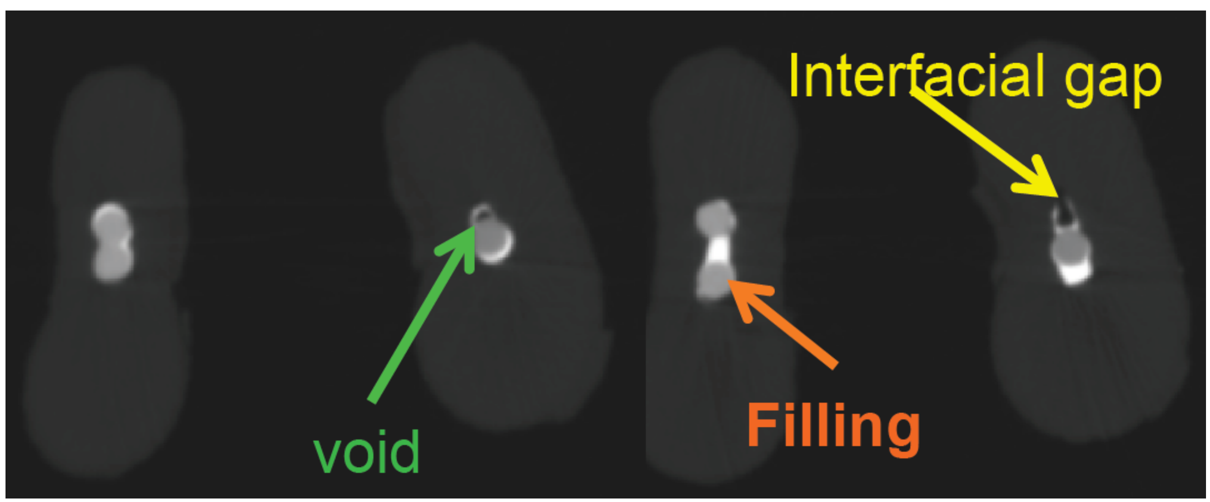

Figure 23. Horizontal cross-section of a mandibular molar obturated with TSWV showing the filling, interfacial gap and void

Figure 24. 3D volumetric reconstruction of a mandibular molar obturated with BC sealer and single cone technique (BCSC)

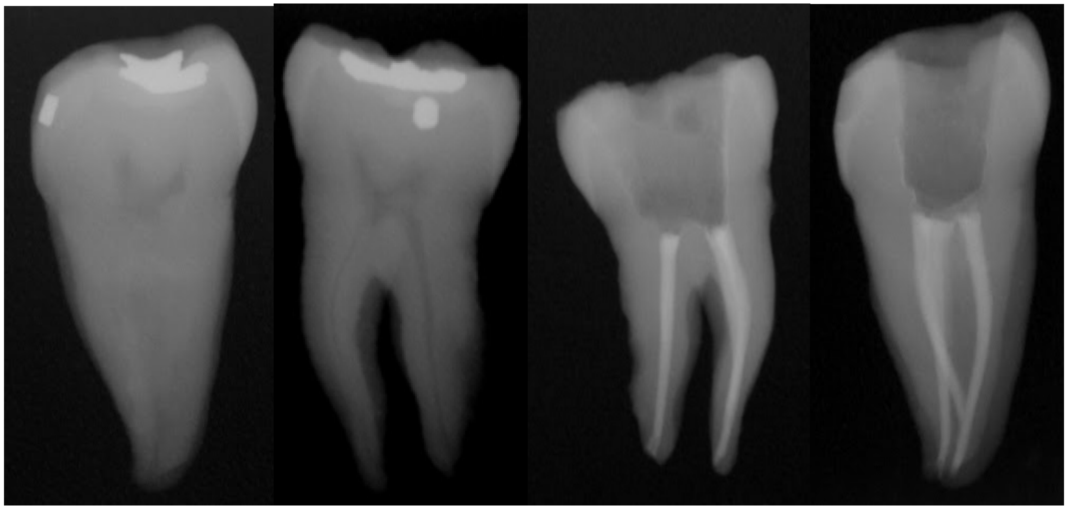

Figure 25. Periapical radiographs taken mesio-distally and bucco-lingually of a mandibular molar during screening and post-obturation with BCSC

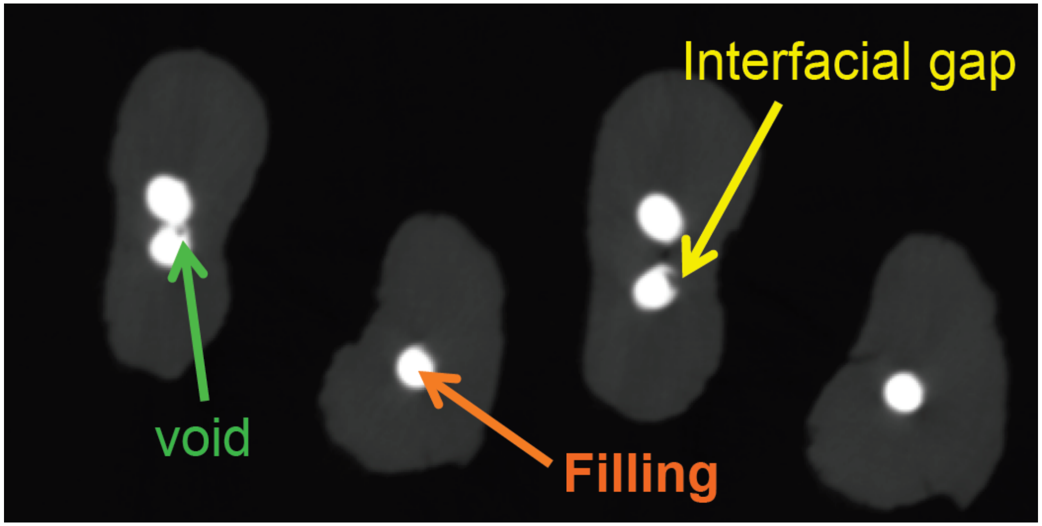

Figure 26. Horizontal cross-section of a mandibular obturated with BCSC showing the filling, interfacial gap and void

4.Discussion

As BCS is a new sealer, most of the existing studies have been done to determine its biological (4, 79) and physical properties (4, 80) as well as its apical sealing abilities (78). To date, there is only one published MCT study which investigated the root canal filling quality of teeth obturated with BCS (66). A MCT study by Celikten *et al*. (2015), compared the obturation quality in single-rooted maxillary premolars using the single cone technique together with various root sealers (BCS, Smartpaste bio, ActiV GP and AHP) (66). However, in their study, the WV technique was not examined (66). There are currently no published MCT studies that have compared the obturation quality between the mesial and distal canals of mandibular molars. Thus, this study represents the first attempt to use MCT technology to compare the obturation quality (percentage volume of voids and gaps) in the mesial and the distal canals of mandibular molars obturated with the TSSC, TSWV and BCSC technique.

The incidence of voids within the root fillings can be affected by the root canal anatomical variations, canal preparation, sealer distribution and volume, and operator experience (73, 81). Unfilled canal spaces were often collectively reported as voids in previous studies (43, 73). In this study, the internal voids were reported as voids and the external and combined voids were collectively reported as gaps. However, many studies reported any unfilled spaces as voids only (43, 63, 73). Hence, with differences in methodologies, statistical analysis and parameters measured, direct comparisons between the existing studies and the current study are challenging.

4.1. Differences Between the Obturation Groups

No significant differences in the percentage volume of voids and the percentage volume of gaps were found between the TSSC, TSWV and BCSC groups ($p > 0.05$). This corroborated the study by Celikten *et al*. (66) who also found no difference in the percentage of voids between their BCSC and AHSC group. In addition, this finding is also supported by studies which compared the percentage of voids in teeth obturated with AHP sealer using the WV and the SC technique (43), the WV, CLC, SC technique (48) and the Thermafil, SC, and WV technique (54). In the current study, the root canal third of each root was analyzed separately as the presence of voids and gaps in each third are of clinical significance and could provide insight on the behavior of the different obturation techniques and different sealers in different anatomical locations.

The lack of difference between the obturation groups could also be due to the fact that the heat source only reached the area 5 mm from the WL during downpacking in the TSWV group. Although the heat carrier was recommended to be inserted 3-5 mm short of WL for the WV technique (82), it has been reported that only 1-2 mm of the GP apical to the heat carrier are prone to plastic deformation (83). Hence, the apical 2-4 mm of the canal was essentially filled with a SC technique for all the obturation groups. This corroborated the MCT study by Li *et al.* (67) which compared the quality of obturation of teeth obturated with TSP with GuttaCore, WV and CLC techniques also stated that the apical third of their WV group was essentially filled with a SC technique. The root length of the samples was not standardized and may vary from 8-12mm. For samples with shorter roots in the TSWV group, the apical third and a portion of the middle third could essentially be obturated with a SC technique as the effect of the heat does not deform GP

1-2m beyond the heat source. Hence, the various root lengths could be a confounding variable for this study.

In the TSSC and the BCSC group, the GP in the coronal third was seared off approximately 1 mm above the orifice with the heat carrier and vertically compacted to seal off the orifice. This methodology for the SC technique was adopted in a previous study by Horsted-Bindslev *et al*. (84). Hence, in all the samples, the apical 5 mm was essentially obturated with the SC technique and part of the coronal third of the canals was vertically compacted. The difference between the samples in the different obturation groups should therefore be found mainly in the middle third of the root. An interesting finding is that although the most apical GP is not affected in the WV technique, the sealer is often better pressed into canal irregularities and even beyond the foramen than in the SC technique (48).

Furthermore, none of the obturation groups can produce a root canal filling without voids or gaps. This finding corroborated with previous studies using MCT (10, 43, 73) or digital image analysis of root cross- sections (47, 48, 85). Our findings also indicate that voids and gaps are randomly distributed along the canals when these combinations of obturation techniques and sealers are used and this finding is also in agreement with other studies (10, 54, 62, 67, 73).

4.2. Differences Between the Mesial and Distal Canals

In the mesial root of the mandibular molars, the incidence of isthmuses was 54-59% and they were often found 3-6 mm from the apex (74, 86, 87). In a study by Von Arx (2005), 83% of the mesial roots of the mandibular molars had two canals with the presence of an isthmus, 11% had two separate canals with no isthmus and only 6% had a single canal (88). In contrast, less anatomical variations were often present in the distal root of mandibular molars and a single oval shape canal was often found (75, 89). Hence, due to these anatomical differences, it is of interest to determine the influence of anatomy on the quality of the obturation.

In this study, the incidence of isthmuses was approximately 40- 50 % in the mesial canals and the isthmus location varies along the length of the root. No significant difference in the percentage volume of voids and gaps were found between the mesial and distal canals of the obturated mandibular molars across all obturation groups and for the canal thirds ($p > 0.05$). This finding is in agreement with a CLSM study by Marciano *et al*. (74), which found that the presence of isthmuses did not increase the presence of voids in the mesial canals of mandibular molars obturated with AHP sealer using the WV or the SC technique. Their study also found no relation between the presence of isthmus and sealer distribution with the WV, SC, CLC and thermoplastized GP techniques (74). However, as it was a CLSM study and sections were only taken at the 2mm, 4mm and 6mm level, additional isthmuses may remain undetected and the effects of isthmuses on void formation may not be fully investigated (74). As there are no published MCT studies that compared the obturation quality of the mesial canal to the distal canal of mandibular molars, additional research is required to confirm the results of the present study. The lack of difference between the mesial and distal canals could be because the isthmuses were not specifically evaluated in this study.

In the distal root of mandibular molars, less anatomical variations and often just a single oval shape canal has been found (75, 89). In the past, it was believed that the use of single-cone obturation should be limited to round canals as the oval shape canals could create a filling with a higher percentage of voids and gaps (47, 54, 74, 90). This is because resin based sealers could undergo shrinkage, and the use of large volume of such sealers could further increase the presence of voids and gaps (73). However, this concept is now re-examined with the introduction of bioceramic sealers such as BCS that expands slightly upon setting (6). Despite the differences in the behavior of the resin based sealers and BCS, a study which compared the apical sealing ability of teeth obturated with BCSC, AHWV and BCWV technique found no difference in the apical sealing ability of the BCSC and the AHWV groups (78). These findings are in agreement with the present results that no statistically significant difference in the percentage volume of voids and gaps was noted between the distal and mesial canals regardless of the obturation techniques.

Although the isthmuses or lateral canals were not specifically evaluated in this study, extensions of filling material or dentinal debris into the isthmuses or lateral canals were noted in some of the 3D reconstruction images of all the obturation groups. This finding was in agreement with the CLSM study by Somma *et al*. (54) which found obturation material in the isthmus of the mesial canals of mandibular molars obturated with TSP with Thermafil, WV, CLC, and SC technique. This finding was also noted in several MCT studies which studied the obturation quality of the mesial canals of mandibular molars (43, 74).

Whether having obturation material in the isthmus or lateral canal may be considered an advantage remains questionable. It could be perceived as advantageous as the antibacterial activity of sealers could reduce the residual bacteria in these ramifications (43). However, despite the radiographic appearance of some of the filling extending to the isthmus or the lateral canal, it has been shown that the space actually remained unfilled and the filling material could damage the remaining tissue and cause inflammation surrounding the material (91). This is due to the fact that the tissues within the ramifications remain unaffected by chemomechanical preparation and the filling material was unable to disinfect or seal the space adequately (91). Thus, efforts should be focused on finding ways to adequately disinfect these lateral canals and ramifications to optimize treatment outcome (91).

4.3. Differences Between the Coronal, Middle and Apical Root Canal Thirds

Only a few MCT studies have examined the quality of obturation by different techniques across the root canal thirds (66, 73). In this study, no significant difference in the percentage volume of voids was noted in the canal thirds in all the canals in all the obturation groups ($p > 0.05$). However, a significantly higher percentage volume of gaps was noted in the apical third compared to the middle third ($p < 0.05$) and the coronal third ($p < 0.05$) in all canals and all the obturation groups. No significant differences in the total percentage volume of gaps were noted in the middle third compared to the coronal third ($p > 0.05$) of all canals and all obturation groups.

The significant difference in the percentage volume of gaps but not the voids in the canal thirds could be due to the fact that a higher percentage volume of gaps than voids was found in all obturation groups, canals, and canal thirds. Hence, most of the unfilled space was found between the interface of the sealer and the canal wall. This finding corroborated the MCT study by Somma *et al*. who compared the percentage of voids (internal, external and combined) in straight, single-rooted teeth obturated with Thermafil, SC and WV technique using AHP sealer

(54). A higher percentage of gaps (external voids and combined voids) were noted compared to the percentage of voids in all the obturation groups (54). The MCT study by Celikten *et al*. (2015) also found a higher percentage of external and combined voids (gaps) compared to the internal voids in the BCSC and AHSC group (66). This is likely to be of clinical significance as the gaps are in contact with potentially infected canal walls, which may promote failure of the sealer and lead to leakage (54). Due to the potential of gaps to negatively impact treatment outcome, an emphasis should be placed on finding solutions to optimally disinfect the root canal system (54).

A significantly higher percentage volume of gaps in the apical third with no significant between the coronal third and the middle third of the obturated root canals were also noted in the study by Keles *et al.* (73). In that study, the obturation quality of oval canals in premolars obturated with AHP sealer with CLC and WV techniques were compared (73). No differentiation was made between the voids and the gaps and any unfilled root canal space was collectively reported as voids in their study (73). Despite the differences in reporting their data, a significantly higher percentage of unfilled space was noted in the apical third with no significant difference in the percentage volume of unfilled space was noted between the coronal third and middle third of the samples in the AHWV groups (73).

In contrast, the MCT study from Celikten *et al.* (2015) found a significantly lower percentage of combined voids (gaps) in the apical third as compared to the coronal third in premolars obturated with the BCSC and AHSC technique (66). The authors stated that the presence of more anatomical variations in the coronal third could explain the lower percentage of combined voids in the apical third (66). However, the majority of lateral canals and apical ramifications has been known to be found in the apical 3 mm of the root (92). Hence, in agreement with their reasons that voids are closely related to the canal anatomy rather than the filling technique or material, the anatomical variation in the apical third of mandibular molars could be the reason that more gaps are noted in the apical third than the coronal third or the middle third of the roots in this study (92).

4.4. Other Observations

Dentin debris was found in the isthmus between the canals in occasion. This is a common observation after rotary instrumentation, irrespective of the conventional irrigation strategies (67, 73). The dentin debris may prevent the compaction of the root canal filling into the isthmus area (54). Following the current positive pressure needle irrigation recommendation, a 30 gauge, side-vented needle was used within 1 mm of the WL (93-95). However, due to the curvature of the root canal, the needle was not able to reach the area 1 mm short of WL in some samples.

A recent study by Freire *et al*. (95) found that the dentin debris occupied approximately 3.4% of the root canal space in the mesial roots of mandibular molars, with greater accumulation in the anatomical retentive areas. In their study, a 55.55% and a 53.65% reduction in dentin debris was noted when passive ultrasonic irrigation or a negative pressure irrigation system EndoVac were used, respectively (96). This finding corroborates another study, which noted cleaner isthmus and more debris removal in mandibular molars using EndoVac as compared to the manual agitation of the irrigant solution with a well-fitted GP cone (97). Recently, the GentleWave System (Sonendo Inc., Laguna Hills, CA), a new device which utilizes multisonic energy to create a strong hydrodynamic cavitation cloud, has demonstrated greater cleaning capacity and reduction in residual debris within the mesiobuccal and mesiolingual canals of mandibular molars (98) and in palatal and distobuccal canals in maxillary molars (99) as compared to those cleaned with conventional methods. As no irrigation activation was used in this study, it would be of interest to determine if the obturation quality would be enhanced with an irrigation activation protocol in future MCT studies. The isthmus was not particularly treated in this study and an irrigation activation protocol may better clean these anatomical retentive areas and decrease the percentage volume of voids and gaps.

In addition, the setting behavior of the BCS in this study may be different than in the clinical situation. A study by Xuereb *et al*. (99) found that the hydration reaction and bioactivity of BCS *in vivo* is not the same as in the *in vitro* situations. Hence, the use of moist gauze to wrap around the samples may not provide adequate moisture needed for BCS to set and consequently, voids and gaps may have formed. Future studies should focus on ways to secure similar moisture in the tooth and root dentin as found *in vivo* to simulate the setting reaction of the calcium silicate sealer in clinical situations and further evaluate the quality of obturation using calcium silicate sealers in such conditions.

5.Limitations of Study

Direct comparisons between the current study and previous studies are challenging due to the different methodologies, sample selection, operator differences, measuring parameters, MCT scan setting, and interpretation of MCT scans (73, 81). In particular, many studies do not differentiate between voids and gaps. However, as gaps are of more clinical significance than voids due to leakage, future studies should differentiate between the presence of voids and gaps in their findings. As this is one of the first MCT studies that compared the obturation quality between the mesial and distal canals of the mandibular molars, additional MCT studies should be performed to confirm the findings of this study.

Due to the complexity of the anatomical variations in mandibular molar mesial canals, various samples in this study exhibited various canal configurations as described by Vertucci (76). The variations in canal anatomy were taken into account and each obturation group contained an equal number of samples which exhibited one Vertucci type I canal configuration, nine type II canal configuration and one type V configuration (76). With these anatomical complexities, dentin debris and tissues have been known to be trapped in the isthmus and ramifications and could influence the obturation quality (96). Although no conventional irrigation technique can completely remove all the debris accumulated during instrumentation, irrigant activation has been reported to be more effective than conventional needle irrigation (97, 100). In future studies, irrigant activation and agitation would be recommended to determine if it would improve the quality of the root canal obturation.

As well, an orifice opener was used for the coronal third of all the roots. Hence, even though GP cones which corresponded to the final file size were used in the SC groups, the fit of the GP cone no longer matched the instrument as more space in the coronal third was created. The root lengths and root curvatures were also not standardized and not equally allocated into the obturation groups in this study. The root lengths of the samples vary from 8-12mm. The use of a heat carrier to the level of 5 mm coronal to the WL may not adequately cause plastic deformation of the GP (83) and the most apical portion of the TSWV root filling could essentially be filled as if with the SC technique. In samples with shorter root length in the TSWV group, the apical third or even a portion of the middle third could essentially be filled as if with the SC technique. In addition, the setting of BCS is hard to replicate in an in vitro setting (101). Hence, this could also affect the results for the BCSC group and the results from this study may have limited implications in the clinical setting.

With severe curvatures, the irrigation needle was not able to reach the level 1 mm coronal to the WL. As well, downpacking the GP using the WV technique in these teeth was challenging. Hence, the dentin debris and tissues could still be trapped in the isthmus and ramifications and could influence the obturation quality (96). A more stringent sample selection criteria and sample distribution criteria should be adopted in future studies.

6. Conclusion

Within the limitation of this study, none of the filling techniques were able to completely fill the root canal space and produce a void-free root canal filing. There was no difference in the percentage volume of voids and the percentage volume of gaps in mandibular molars obturated with ThermaSeal plus sealer using the single cone technique or the warm vertical technique or using BC sealer with the single cone technique. In addition, there was no difference in the percentage volume of voids and percentage volume of gaps between the mesial and distal canals of the mandibular molars. A significantly higher percentage volume of voids was noted in the apical third compared to the coronal and middle third of the mandibular molars in both roots and in all obturation groups. The incidence of voids within the root fillings can be affected by the root canal anatomical variations, canal preparation, sealer distribution and volume and operator experience (73).

Although it has been shown that root canal fillings with no voids are associated with an improved treatment outcome, it is impossible to determine the specific threshold of voids below which a favorable treatment outcome is expected (73, 102). Therefore efforts should be focused on finding ways to effectively disinfect the root canal space, lateral canals and ramifications in order to optimize treatment outcome (91).

Future research could compare the retreatability of canals obturated with TSWV and TSSC and BCSC technique in a MCT study. A study by Hess *et al*. compared the retreatability of mandibular molars obturated with AHWV and BCSC technique in a SEM study (103). However, with a

MCT study, the samples could be preserved and the presence of other potentially interesting aspects such as dentin fractures after retreatment can also be evaluated.

The effect of heat for the quality of the root filling with BCS is not fully known. In future studies, upon determining the effect of heat on the physical and biological properties of BCS, an optimal heating temperature could be established. It would be of interest to compare the obturation quality of teeth obturated with BC sealer or ThermaSeal Plus sealer using the single cone technique or the warm vertical technique and determine the influence of the presence of isthmuses on the measured parameters in a MCT study.

This study is one of the first MCT studies that compared the obturation quality in the mesial and the distal canals of mandibular molars. Despite the inherent anatomical differences in the samples and the difficulties in standardizing the samples for comparisons, the findings may be of clinical interest and relevance for clinicians who face these anatomical challenges in clinical practice (74). Within the limitations of this study, it appears that the single cone technique utilizing gutta percha in matching taper and size is a suitable alternative for obturation of mandibular molars as compared to the warm vertical technique.

Bibliography

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol. 1965; 20:340-349.

2. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy. 1974; 25(4):347-358.

3. Sundqvist G. Bacteriological studies of necrotic dental pulps. Umae Univ Odontological Dissertations. 1976.

4. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009; 35(7):1051-1055.

5. Trope M, Bergenholtz G. Microbiological basis for endodontic treatment: can a maximal outcome be achieved in one visit? Endod Topics. 2002; 1:40-53.

6. Trope M, Bunes A, Debelian G. Root filling materials and techniques: bioceramics a new hope? Endod Topics. 2015; (32):86-96.

7. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85(1):86-93.

8. Sjogren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. J Endod. 1990; 16(10):498-504.

9. Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto study. Phases I and II: Orthograde retreatment. J Endod. 2004; 30(9):627-633.

10. Hammad M, Qualtrough A, Silikas N. Evaluation of root canal obturation: a three-dimensional in vitro study. J Endod. 2009; 35(4):541-544.

11. Wang Z. Bioceramic materials in endodontics. Endod Topics. 2015; 32:3-30.

12. Orstavik D. Materials used for root canal obturation: technical, biological and clinical testing. Endod Topics. 2005; 12:25-38.

13. Schuurs AH, Wu MK, Wesselink PR, Duivenvoorden HJ. Endodontic leakage studies reconsidered. Part II. Statistical aspects. Int Endod J. 1993; 26(1):44-52.

14. Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. J Endod. 1990; 16(12):566-569.

15. Derkson GD, Pashley DH, Derkson ME. Microleakage measurement of selected restorative materials: a new in vitro method. J Prosthet Dent. 1986; 56(4):435-440.

16. Souza Sde F, Francci C, Bombana AC, Kenshima S, Barroso LP, D'Agostino LZ, et al. Qualitative SEM/EDS analysis of microleakage and apical gap formation of adhesive root-filling materials. J Appl Oral Sci. 2012; 20(3):329-334.

17. Dowker SE, Davis GR, Elliott JC. X-ray microtomography: nondestructive three-dimensional imaging for in vitro endodontic studies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997; 83(4):510-516.

18. Carrotte P. Endodontics: Part 5. Basic instruments and materials for root canal treatment. Br Dent J. 2004; 197(8):455-464.

19. Orstavik D, Nordahl I, Tibballs JE. Dimensional change following setting of root canal sealer materials. Dent Mater. 2001; 17(6):512-519.

20. Schilder H, Goodman A, Aldrich W. The thermomechanical properties of gutta-percha. 3. Determination of phase transition temperatures for gutta-percha. Oral Surg Oral Med Oral Pathol. 1974; 38(1):109-114.

21. Goodman A, Schilder H, Aldrich W. The thermomechanical properties of gutta-percha. Part IV. A thermal profile of the warm gutta-percha packing procedure. Oral Surg Oral Med Oral Pathol. 1981; 51(5):544-551.

22. Duncan H, Chong B. Removal of root filling materials. Endod Topics. 2011; 19(33-57).

23. Dahl J. Toxicity of endodontic filling materials. Endod Topics. 2005; 12:39-43.

24. Grossman L. Endodontic Practice. Philadelphia: Lea & Febiger; 1981.

25. Orstavik D. Endodontic filling materials. Endod Topics. 2014; (31):53-67.

26. Ingle JN, West J, Gutmann J, Glickman G, Korzon B, Martin H. Obturation of the radicular space. In: Ingle J, Bakland, editor. Endodontics. Hamilton: BC Decker; 2002. p. 571-668.

27. Siqueira JF, Jr., Rocas IN, Valois CR. Apical sealing ability of five endodontic sealers. Aist Endod J. 2001; 27(1):33-35.

28. Siqueira JF, Jr., Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod. 2000; 26(5):274-277.

29. Leonardo MR, Almeida WA, Utrilla LS. Tissue response to an epoxy resin-based root canal sealer. Endod Dent Traumatol. 2007; 12:28-32.

30. Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. J Endod. 2013; 39(10):1281-1286.

31. Camilleri J. Sealers and warm gutta-percha obturation techniques. J Endod. 2015; 41(1):72-78.

32. Schilder H. Filling root canals in three dimensions. Dent Clin North Am. 1967; 723-744.

33. Whitworth JM. Methods of filling root canals: principles and practices. Endod Topics. 2005; 12:2-24.

34. Ruddle J. Chapter 8: Cleaning and Shaping the Root Canal System. In: Burns CA, editor. Pathways of the Pulp. St. Louis; 2002. p. 231-291.

35. Hargreaves KC, Berman, L. Pathways of the Pulp. 10th ed. St. Louis: Mosby; 2011.

36. Eguchi DS, Peters DD, Hollinger JO, Lorton L. A comparison of the area of the canal space occupied by gutta-percha following four gutta-percha obturation techniques using Procosol sealer. J Endod. 1985; 11(4):166-175.

37. Silver GK, Love RM, Purton DG. Comparison of two vertical condensation obturation techniques: Touch 'n Heat modified and System B. Int Endod J. 1999; 32(4):287-295.

38. Buchanan LS. Continuous wave of condensation technique. Endod Prac. 1998; 1(4):7-18.

39. Tsukada G, Tanaka T, Torii M, Inoue K. Shear modulus and thermal properties of gutta percha for root canal filling. J Oral Rehabil. 2004; 31(11):1139-1144.

40. Wilcox LR, Roskelley C, Sutton T. The relationship of root canal enlargement to finger-spreader induced vertical root fracture. J Endod. 1997; 23(8):533-534.

41. Zandbiglari T, Davids H, Schafer E. Influence of instrument taper on the resistance to fracture of endodontically treated roots. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(1):126-131.

42. Cohen SB, RC. Pathways of the pulp. 2nd ed. St. Louis: Mosby; 1980.

43. Alshehri M, Alamri HM, Alshwaimi E, Kujan O. Micro-computed tomographic assessment of quality of obturation in the apical third with continuous wave vertical compaction and single match taper sized cone obturation techniques. Scanning. 2015; 9999: 1-5.

44. Beatty RG, Vertucci FJ, Zakariasen KL. Apical sealing efficacy of endodontic obturation techniques. Int Endod J. 1986; 19(5):237-241.

45. Beatty RG. The effect of standard or serial preparation on single cone obturation. Int Endod J. 1987; 20(6):276-281.

46. Wu MK, van der Sluis LW, Ardila CN, Wesselink PR. Fluid movement along the coronal two-thirds of root fillings placed by three different gutta-percha techniques. Int Endod J. 2003; 36(8):533-540.

47. Schafer E, Koster M, Burklein S. Percentage of gutta-percha-filled areas in canals instrumented with nickel-titanium systems and obturated with matching single cones. J Endod. 2013; 39(7):924-928.

48. Schafer E, Nelius B, Burklein S. A comparative evaluation of gutta-percha filled areas in curved root canals obturated with different techniques. Clin Oral Investig. 2012; 16(1):225-230.

49. Monticelli F, Sadek FT, Schuster GS, Volkmann KR, Looney SW, Ferrari M, et al. Efficacy of two contemporary single-cone filling techniques in preventing bacterial leakage. J Endod. 2007; 33(3):310-313.

50. Tay FR, Pashley DH. Monoblocks in root canals: a hypothetical or a tangible goal. J Endod. 2007; 33(4):391-398.

51. Li LL, Niu L, Selem LC, Eid AA, Bergeron BE,Chen JH, et al. Quality of obturation achieved by an endodontic core-carrier system with crosslinked gutta-percha carrier in single-rooted canals. J Dent. 2014; 42:1124-1134.

52. Ghoneim AG, Lutfy RA, Sabet NE, Fayyad DM. Resistance to fracture of roots obturated with novel canal-filling systems. J Endod. 2011; 37(11):1590-1592.

53. Gandolfi MG, Iacono F, Agee K, Siboni F, Tay F, Pashley DH, et al. Setting time and expansion in different soaking media of experimental accelerated calcium-silicate cements and ProRoot MTA. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 108(6):e39-45.

54. Somma F, Cretella G, Carotenuto M, Pecci R, Bedini R, De Biasi M, et al. Quality of thermoplasticized and single point root fillings assessed by micro-computed tomography. Int Endod J. 2011; 44(4):362-369.

55. Tachibana H, Matsumoto K. Applicability of X-ray computerized tomography in endodontics. Endod Dent Traumatol. 1990; 6(1):16-20.

56. Spoor CF, Zonneveld FW, Macho GA. Linear measurements of cortical bone and dental enamel by computed tomography: applications and problems. Am J Phys Anthropol. 1993; 91(4):469-484.

57. Bjorndal L, Carlsen O, Thuesen G, Darvann T, Kreiborg S. External and internal macromorphology in 3D-reconstructed maxillary molars using computerized X-ray microtomography. Int Endod J. 1999; 32(1):3-9.

58. Peters OA, Laib A, Ruegsegger P, Barbakow F. Three-dimensional analysis of root canal geometry by high-resolution computed tomography. J Dent Res. 2000; 79(6):1405-1409.

59. Nielsen RB, Alyassin AM, Peters DD, Carnes DL, Lancaster J. Microcomputed tomography: an advanced system for detailed endodontic research. J Endod. 1995; 21(11):561-568.

60. Endal U, Shen Y, Knut A, Gao Y, Haapasalo M. A high-resolution computed tomographic study of changes in root canal isthmus area by instrumentation and root filling. J Endod. 2011; 37(2):223-227.

61. Rhodes JS, Ford TR, Lynch JA, Liepins PJ, Curtis RV. A comparison of two nickel-titanium instrumentation techniques in teeth using microcomputed tomography. Int Endod J. 2000; 33(3):279-285.

62. Mirfendereski M, Roth K, Fan B, Dubrowski A, Carnahan H, Azarpazhooh A, et al. Technique acquisition in the use of two thermoplasticized root filling methods by inexperienced dental students: a microcomputed tomography analysis. J Endod. 2009; 35(11):1512-1517.

63. Zogheib C, Naaman A, Medioni E, Arbab-Chirani R. Influence of apical taper on the quality of thermoplasticized root fillings assessed by micro-computed tomography. Clin Oral Investig. 2012; 16(5):1493-1498.

64. Jung M, Lommel D, Klimek J. The imaging of root canal obturation using micro-CT. Int Endod J. 2005; 38(9):617-626.

65. Ma J, Al-Ashaw AJ, Shen Y, Gao Y, Yang Y, Zhang C, et al. Efficacy of ProTaper Universal Rotary Retreatment system for gutta-percha removal from oval root canals: a micro-computed tomography study. J Endod. 2012; 38(11):1516-1520.

66. Celikten B, Uzuntas CF, Orhan AI, Orhan K, Tufenkci P, Kursun S, et al. Evaluation of root canal sealer filling quality using a single-cone technique in oval shaped canals: An In vitro Micro-CT study. Scanning. 2016; 38(2):133-140.

67. Li GH, Niu LN, Selem LC, Eid AA, Bergeron BE, Chen JH, et al. Quality of obturation achieved by an endodontic core-carrier system with crosslinked gutta-percha carrier in single-rooted canals. J Dent. 2014; 42(9):1124-1134.

68. Li GH, Niu LN, Zhang W, Olsen M, De-Deus G, Eid AA, et al. Ability of new obturation materials to improve the seal of the root canal system: a review. Acta Biomater. 2014; 10(3):1050-1063.

69. Brothman P. A comparative study of the vertical and the lateral condensation of gutta-percha. J Endod. 1981; 7(1):27-30.

70. Gordon MP, Love RM, Chandler NP. An evaluation of .06 tapered gutta-percha cones for filling of .06 taper prepared curved root canals. Int Endod J. 2005; 38(2):87-96.

71. Romania C, Beltes P, Boutsioukis C, Dandakis C. Ex-vivo area-metric analysis of root canal obturation using gutta-percha cones of different taper. Int Endod J. 2009; 42(6):491-498.

72. Zmener O, Pameijer CH, Macri E. Evaluation of the apical seal in root canals prepared with a new rotary system and obturated with a methacrylate based endodontic sealer: an in vitro study. J Endod. 2005; 31(5):392-395.

73. Keles A, Alcin H, Kamalak A, Versiani MA. Micro-CT evaluation of root filling quality in oval-shaped canals. Int Endod J. 2014; 47(12):1177-1184.

74. Marciano MA, Ordinola-Zapata R, Cunha TV, Duarte MA, Cavenago BC, Garcia RB, et al. Analysis of four gutta-percha techniques used to fill mesial root canals of mandibular molars. Int Endod J. 2011; 44(4):321-329.

75. Kerekes K, Tronstad L. Morphometric observations on the root canals of human molars. J Endod. 1977; 3(3):114-118.

76. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol. 1984; 58(5):589-599.

77. Roland DD, Andelin WE, Browning DF, Hsu GH, Torabinejad M. The effect of preflaring on the rates of separation for 0.04 taper nickel titanium rotary instruments. J Endod. 2002; 28(7):543-545.

78. Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 107(6):e79-82.

79. Zhou HM, Du TF, Shen Y, Wang ZJ, Zheng YF, Haapasalo M. In vitro cytotoxicity of calcium silicate-containing endodontic sealers. J Endod. 2015; 41(1):56-61.

80. Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. J Endod. 2011; 37(5):673-677.

81. Zaslansky P, Fratzl P, Rack A, Wu MK, Wesselink PR, Shemesh H. Identification of root filling interfaces by microscopy and tomography methods. Int Endod J. 2011; 44(5):395-401.

82. Buchanan LS. The continuous wave of condensation technique: a convergence of conceptual and procedural advances in obturation. Dentistry today 1994; 13(10):80-85.

83. Briseno Marroquin B, Wolf TG, Schurger D, Willershausen B. Thermoplastic properties of endodontic gutta-percha: a thermographic in vitro study. J Endod. 2015; 41(1):79-82.

84. Horsted-Bindslev P, Andersen MA, Jensen MF, Nilsson JH, Wenzel A. Quality of molar root canal fillings performed with the lateral compaction and the single-cone technique. J Endod. 2007; 33(4):468-471.

85. De-Deus G, Reis C, Beznos D, de Abranches AM, Coutinho-Filho T, Paciornik S. Limited ability of three commonly used thermoplasticized gutta-percha techniques in filling oval-shaped canals. J Endod. 2008; 34(11):1401-1405.

86. Gu L, Wei X, Ling J, Huang X. A microcomputed tomographic study of canal isthmuses in the mesial root of mandibular first molars in a Chinese population. J Endod. 2009; 35(3):353-356.

87. Jung IY, Seo MA, Fouad AF, Spangberg LS, Lee SJ, Kim HJ, et al. Apical anatomy in mesial and mesiobuccal roots of permanent first molars. J Endod. 2005; 31(5):364-368.

88. Von Arx, T. Frequency and type of canal isthmuses in first molars detected by endoscopic inspection during periradicular surgery. Int Endod J. 2005; 38 (3): 160-8.

89. Filpo-Perez C, Bramante CM, Villas-Boas MH, Hungaro Duarte MA, Versiani MA, Ordinola-Zapata R. Micro-computed tomographic analysis of the root canal morphology of the distal root of mandibular first molar. J Endod. 2015; 41(2):231-236.

90. Robberecht L, Colard T, Claisse-Crinquette A. Qualitative evaluation of two endodontic obturation techniques: tapered single-cone method versus warm vertical condensation and injection system: an in vitro study. Journal of oral science 2012; 54(1):99-104.

91. Ricucci D, Siqueira JF, Jr. Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. J Endod. 2010; 36(1):1-15.

92. Kim S, Pecora, G, Rubinstein, R. Color atlas of microsurgery in endodontics. Philadelphia: Saunders; 2001.

93. Shen Y, Gao Y, Qian W, Ruse ND, Zhou X, Wu H, et al. Three-dimensional numeric simulation of root canal irrigant flow with different irrigation needles. J Endod. 2010; 36(5):884-889.

94. Park E, Shen Y, Haapasalo M. Irrigation fo the apical root canal. Endod Topics. 2012; 27:54-73.

95. Freire LG, Iglecias EF, Cunha RS, Dos Santos M, Gavini G. Micro-Computed Tomographic Evaluation of Hard Tissue Debris Removal after Different Irrigation Methods and Its Influence on the Filling of Curved Canals. J Endod. 2015; 41(10):1660-1666.

96. Susin L, Liu Y, Yoon JC, Parente JM, Loushine RJ, Ricucci D, et al. Canal and isthmus debridement efficacies of two irrigant agitation techniques in a closed system. Int Endod J. 2010; 43(12):1077-1090.

97. Molina B, Glickman G, Vandrangi P, Khakpour M. Evaluation of Root Canal Debridement of Human Molars Using the GentleWave System. J Endod. 2015; 41(10):1701-1705.

98. Haapasalo M, Shen Y, Wang Z, Park E, Curtis A, Patel P, et al. Apical pressure created during irrigation with the GentleWave system compared to conventional syringe irrigation. Clin Oral Investig. 2015; 1-10.

99. Xuereb M, Vella P, Damidot D, Sammut CV, Camilleri J. In situ assessment of the setting of tricalcium silicate-based sealers using a dentin pressure model. J Endod. 2015; 41(1):111-124.

100. Teplitsky PE, Chenail BL, Mack B, Machnee CH. Endodontic irrigation--a comparison of endosonic and syringe delivery systems. Int Endod J. 1987; 20(5):233-241.

101. Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature -- Part 2. Influence of clinical factors. Int Endod J. 2008; 41(1):6-31.

102. Hess D, Solomon E, Spears R, He J. Retreatability of a bioceramic root canal sealing material. J Endod. 2011; 37(11):1547-1549.

Appendix

Appendix A: Statistical Consulting Report

Statistical Consulting Report

Tingting Zhao

May 16, 2016

1 Question of interest

- To compare the filling quality (the pecentage of gaps (%gaps) and the percentage of voids (%voids)) in molars filled with TSWV, TSSC and BCSC
- To compare the filling quality (%gaps and voids) in the mesial and distal canal of molars filled with TSWV, TSSC and BCSC.
- To compare the filling quality (%gaps and voids) of the coronal (top), middle and apical (bottom) part of the root of molars filled with TSWV, TSSC and BCSC.
- If any differences were found between the techniques, the root or the root thirds, I would like to know the interaction between the factors.

2 Data Manipulation

Before introducing the models, since both measures we use to evaluate the filling quality such as %gaps and %voids are not Normally distributed and show skewness, we consider a logit transformation. For example, if the original percentage is p, after a logit transformation, it becomes $\log\left(\frac{p}{100-p}\right)$. However, we notice that there are zero values in %voids, which bring issues for a logit transformation. To solve the issue, we first add 0.01% to those zero %voids values and then perform the transformation.

3 Mixed effects models

3.1 Motivation

Linear mixed effects models are adequate to solve all the questions in Section 1. The motivation to use linear mixed effects models is that we have repeated measures for each tooth involved in the study and they are not independent observations. For example, measures in the medial and distal root of molars from the same tooth are biologically connected. This is also true when we have observations of the filling quality on the top, middle, and bottom part of the same tooth. Mixed effects models refer to the simultaneous use of both fixed and random effects. Subject effects are almost always treated as random effects and treatment effects are almost always treated as fixed effects. For example, in this study, different filling techniques, different root canals, and different parts of the root can be considered as fixed effects. The fixed effects parameter for "technique" tells how population means differ between different filling techniques and the random effect parameters incorporate the general variability among different teeth.

3.2 Model specification

Since we evaluate the filling quality via %voids and %gaps, we will fit models with %voids and %gaps after a logit transformation as the response variable respectively. When we evaluate the filling quality, we consider two candidate models.

The simpler model incorporates "techniques", "canals" and "partofroot" as fixed effects and "toothid" as the random effects, which is denoted as **Model 1**:

$$y_i = \beta_0 + \sum_{k=1}^{2} \beta_{1k} 1(\text{technique}_i = k) + \beta_2 \text{canals}_i + \sum_{j=1}^{2} \beta_{3j} 1(\text{partofroot}_i = j) + b_i + \epsilon_i$$

where

- y_i is the ith observation of "%gaps" or "%voids" after adjustment for zeros and the logit transformation, which is described in Section 2.
- $\boldsymbol{\beta}_1 = (\beta_{11}, \beta_{12}), \beta_2, \boldsymbol{\beta}_3 = (\beta_{31}, \beta_{32})$ are fixed effect coefficients, where $\boldsymbol{\beta}_1$ and $\boldsymbol{\beta}_3$ are vectors of two elements since both "techniques" and "partofroot" has three levels and one of the levels is treated as the baseline level. For example, if we select "Apical" as baseline, β_{31} and β_{32} represents the the change in the response variable when we consider either the "Coronal" or "Middle" part of the root compared with the baseline "Apical" part while keeping other variables fixed.
- b_i is the random effect coefficient for the ith tooth. We need to note that if two observations y_i and y_j come from the same tooth, they share the same random effect coefficient. Different teeth have distinctive random effect coefficients
- ϵ_i is the random error for the ith tooth, which follows a Normal distribution.
- "techniques", "canals" and "partofroot" are all categorical variables.
- $1(\cdot)$ is an indicator function. For example, if we consider $1(\text{technique}_i = k)$, if $\text{technique}_i = k$ is true, the function takes value of one, otherwise it takes value of zero.

The more complex model, besides the fixed effects considered in Model 1, which also incorporates all pairs of interactions between any two of the fixed effects and is represented by **Model 2**:

$$\begin{aligned} y_i = {} & \beta_0 + \sum_{k=1}^{2} \beta_{1k} 1(\text{technique}_i = k) + \beta_2 \text{canals}_i + \sum_{j=1}^{2} \beta_{3j} 1(\text{partofroot}_i = j) \\ & + \sum_{j=1}^{2}\sum_{k=1}^{2} \beta_{4kj} 1(\text{technique}_i = k) \times 1(\text{partofroot}_i = j) \\ & + \sum_{k=1}^{2} \beta_{5k} 1(\text{technique}_i = k) \times \text{canals}_i \\ & + \sum_{j=1}^{2} \beta_{6j} 1(\text{partofroot}_i = j) \times \text{canals}_i \\ & + b_i + \epsilon_i. \end{aligned}$$

4 Results

Throughout this report, we choose the significance level $\alpha = 0.05$. The response variables are %gaps and %voids after adjustment to zero values and a logit transformation. We summarize our results in this section.

Covariates	Chi-square	Df	P-value
techniques	0.28	2	0.8705
canals	1.18	1	0.2775
partofroot	9.94	2	0.0069

Table 1: Analysis of Deviance Table for Model 1 (Wald chi-square test) for %gaps.

partofroot	Mean	SE	df	lower.CL	upper.CL
Apical	0.0439	0.1288	126.03	0.0344	0.0560
Coronal	0.0292	0.1288	126.03	0.0228	0.0373
Middle	0.0270	0.1288	126.03	0.0210	0.0346

Results are averaged over the levels of: techniques, canals

Confidence level used: 0.95

Table 2: Marginal means and 95% confidence intervals of coefficients for the main effects are provided after adjusting the effects of different techniques and canals.

4.1 Results of Model 1 using %gaps after a logit transformation as the response variable

We fit Model 1 to our data with %gaps after a logit transformation as the response variable. To test the significance of the coefficients for the main effects in Model 1, we perform Wald chi-square test on the fitted model and the result is summarized in Table 1. We can see that the P-value in Table 1 for "partofroot" is smaller than the significance level 0.05. It reflects that different parts of the root contribute significantly different to %gaps. To summarize the effects of different part of roots on the %gaps after adjusting the effects of different techniques and canals, we obtain the marginal estimates of the coefficients for the main effects in Model 1 via "lsmeans" package in R. The interpretation for the mean value of "Apical" as 0.0439 can be "Apical" can contribute 0.0439% to %gaps after averaging different levels of techniques and canals. Finally, to find out which pairs among all three parts of the root have significant different effects on the %gaps, we conduct multiple comparisons still via the "lsmeans" package. The result in Table 3 shows that the P-values for "Apical - Coronal" and "Apical - Middle" are smaller than 0.05, which indicates the effect on the %gaps after a logit transformation between "Apical" and "Coronal" or "Apical" and "Middle" are significantly different. The estimates for both of them are positive, which reflects that "Apical" part of the root contributes significantly more than "Coronal" and "Middle" to the %gaps after a logit transformation. That means the filling quality evaluated by %gaps in the "Apical" (bottom) part is worse than the "Coronal" (top) and "Middle" part.

We also perform model diagnostics for Model 1 via the residual plot and the QQ plot of the residuals. Figure 1 shows no pattern, which indicates the appropriateness of the model. We also show the QQ plot of the residuals in Figure 2, which reflects that a Normal assumption for the %gaps after a logit transformation is appropriate.

4.2 Results of Model 2 using %gaps after a logit transformation as response variable

We fit Model 2 to the data with %gaps after a logit transformation as the response variable. Besides the main effects in Model 1, Model 2 incorporates all pairs of interactions between the main effects into the model. To test the significance of the coefficients in Model 2, we also perform Wald chi-square test on the fitted model and summarize the result in Table 4. We can see that only the coefficient for different parts of the root is significant.

However, a good model is considered to fit the data well and is as simple as possible. Since Model 1 and Model 2 are nested, we conduct model comparison between the two models using

contrast	Estimate	SE	df	t.ratio	P-value
Apical - Coronal	0.42430	0.172	157	2.467	0.0388
Apical - Middle	0.50437	0.172	157	2.933	0.0107
Coronal - Middle	0.08007	0.172	157	0.466	0.8875

Table 3: Multiple comparisons of the effects from different parts of roots on %gaps after a logit transformation. A P-value smaller than 0.05 indicates the effects of two different parts of the root in comparison on %gaps after a logit transformation are significantly different.

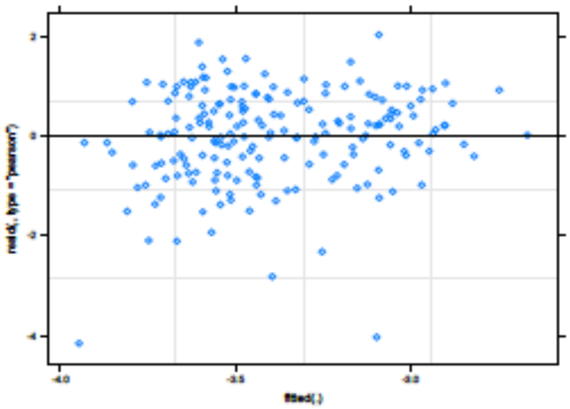

Figure 1: Residual plot against fitted values for %gaps after a logit transformation. It is a scatter plot of residuals on the y axis and fitted values (estimated responses) on the x axis.

ANOVA. The result is summarized in Table 5. The P-value is $0.55 > 0.05$, which indicates there is no significant difference between the two models. Thus, we should choose Model 1 since it is simpler and easier to interpret.

4.3 Results of Model 1 when using %voids after a logit transformation as the response variable

When we use %voids to evaluate the filling quality, we treat %voids after a logit transformation as the response variable. One thing we need to note is that since %voids has zero values in the observations, we adjust these zeros first and then perform the logit transformation. The details of how to adjust zeros and perform the logit transformation is described in Section 2. We perform Wald chi-square test to test the significance of the coefficients and summarize the result in Table 6. We can see that none of the coefficient for techniques, canals or partofroot is significant. It shows that there is no significant difference between the effects on %voids from different techniques, canals and parts of root.

4.4 Results of Model 2 when using %voids after a logit transformation as the response variable

We also fit Model 2 to the data when %voids after zero adjustment and a logit transformation serves as the response variable but non-significance is found for any main effects or the interaction terms so that we do not report the result here. Instead, we show the model comparison result in Table 7. It reflects that there is no significant difference between the two models so that we should choose the simpler model, which is Model 1.

We also perform model diagnostics via the residual plot and the QQ plot of residuals in

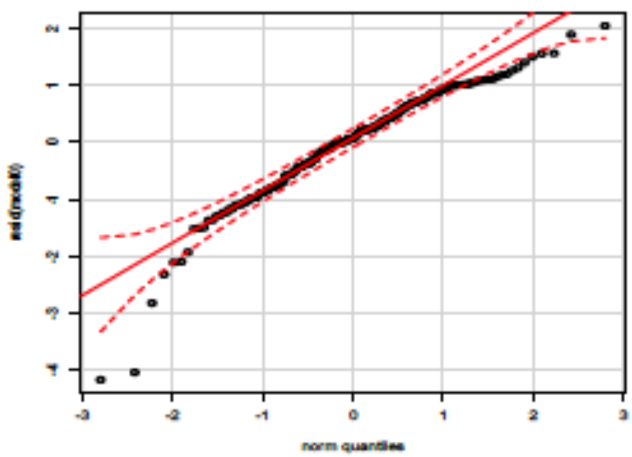

Figure 2: QQ plot of residuals for Model 1 when %gaps after a logit transformation serves as the response variable.

Covariates	Chisq	Df	P-value
techniques	0.28	2	0.8705
canals	1.17	1	0.2797
partofroot	9.85	2	0.0073
techniques:canals	2.20	2	0.3326
techniques:partofroot	3.34	4	0.5023
canals:partofroot	1.00	2	0.6061

Table 4: Analysis of Deviance Table for Model 2 (Wald chi-square test) for %gaps.

Figure 3 and Figure 4. In general, most of the points in the residual plot show no pattern except there are some points on the bottom-left corner show a downward trend. This pattern is due to the manual adjustment of zeros in %voids. This should not be a big issue and the QQ plot shows the appropriateness of a Normal assumption.

5 Conclusions

In this report, to solve all the questions of interest in Section 1, we fit two candidate models to the data when %gaps and %voids after zero adjustment and a logit transformation serve as the response variables, respectively. For both cases, a simpler model incorporating all the main effects is supported via model selection procedure. There is significant difference in %gaps among different parts of the root. Moreover, "Apical" (bottom) part of the root contributes significantly more to %gaps than the other two parts. It indicates the filling quality evaluated by %gaps in "Apical" part is worse than the other two parts. When we use %voids to evaluate the filling quality, there is no significant difference between the effects on %voids from different techniques, canals and parts of the root.

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	P-value
Model 1	8	554	580	-269	538			
Model 2	16	563	615	-266	531	6.88	8	0.55

Table 5: Model Comparison between Model 1 and Model 2 when %gaps after a logit transformation is the response variable.

Covariates	Chisq	Df	P-value
techniques	0.93	2	0.63
canals	0.00	1	0.97
partofroot	0.10	2	0.95

Table 6: Analysis of Deviance Table for Model 1 (Wald chi-square test) for %voids.

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	P-value
Model 1	8	830	856	-407	814			
Model 2	16	834	886	-401	802	12	8	0.15

Table 7: Model Comparison between Model 1 and Model 2 when %voids after zero adjustment and a logit transformation is the response variable.

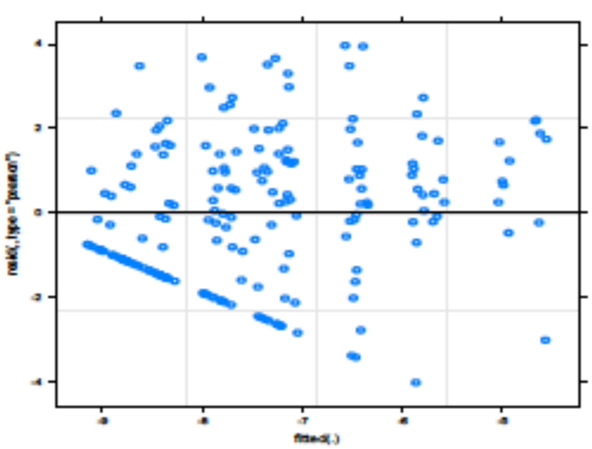

Figure 3: Residual plot against fitted values from Model 1 for %voids after a logit transformation.

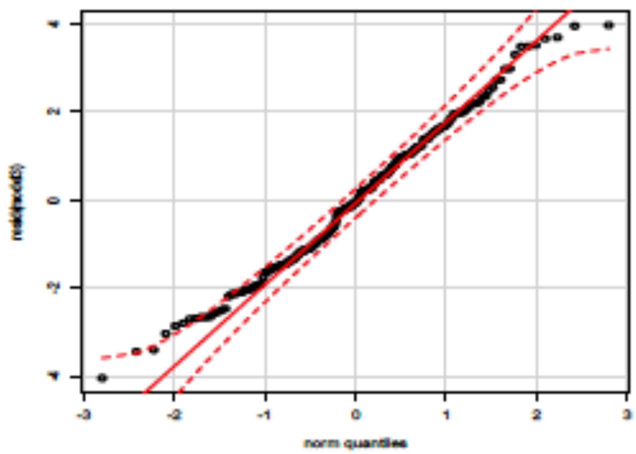

Figure 4: QQ plot of residuals when %void after zero adjustment and a logit transformation is the response variable.