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GuttaFlow Bioseal as Monocone Obturation Technique in Curved Root Canals: A Scanning Electron Microscopy Study

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ABSTRACT

The obturation quality of GuttaFlow Bioseal in curved root canals is not commonly investigated although there has been a current approach toward utilizing this material in extracted molars in recent years. This study assessed the obturated surface area, extrusion of root filling material beyond the apical foramen and duration of obturation procedure in curved root canals using monocone obturation technique. Access cavity was prepared in 20 human mandibular molars. Root canals with curvature of more than 10° as determined according to Schneider's method were included. Samples were prepared using Hyflex CM rotary files and divided into two groups (n=10): Group 1 [gutta-percha cone and GuttaFlow Bioseal] and Group 2 [gutta-percha cone and RoekoSeal Automix root canal sealer]. The duration of obturation procedure was recorded and obturation radiographs were taken. Samples were bisected and the mesial roots were sectioned horizontally to obtain 3 root segments; apical, middle and coronal. All resected roots were mounted on brass stubs, sputter-coated with thin platinum coating and observed under scanning electron microscope (SEM) at 70x magnification. The SEM images were transferred to the SketchAndCalc Area Calculator software. No statistically significant differences in the obturated surface area and extrusion of root filling material were observed between Group 1 and 2, irrespective of the status of root canal curvature. Duration for obturation in severe root canal curvatures between Group 1 and 2 were statistically significant. Obturated surface area and extrusion of root filling material

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were not affected by the root canal curvature, however duration for obturation using GuttaFlow Bioseal in severe root canal curvatures was slightly longer.

Keywords: *Curved Root Canal, mandibular Molars, GuttaFlow Bioseal, obturation, scanning electron microscopy*

INTRODUCTION

After the completion of root canal preparation, the root canal is obturated to diminish the chance for microorganisms to grow [1,2]. Conventional root filling materials used to seal the root canal system include gutta-percha (GP) and root canal sealer. There are various methods used for obturation such as cold and warm lateral compaction, continuous or interrupted waves of thermoplastic obturation, thermomechanical compaction, carrier-based and single cone techniques [1,3] each with its own advantages and disadvantages.

A thermoplastic GP obturation technique advocated by Schilder [2] seals the main root canal and its eccentricities, however, it is a technique-sensitive procedure which requires careful handling of the heat source and good clinical skills, making it time-consuming. Studies have shown that this obturation technique provides a good adaptation to the root canal wall [3-6] but leakage associated with this technique has also been highlighted [7]. Despite contradicting findings, majority of the studies reported good adaptation of GP when using thermoplastic obturation techniques compared to cold lateral compaction techniques.

The matched-taper monocone obturation technique uses GP cones that are of similar taper and size with the endodontic file that is used to prepare the root canal. When fitting a matched-taper GP cone into the root canal, the hydraulic pressure facilitates the flow of root canal sealer to conform to the root canal irregularities. This obturation technique has been well-studied due to it being simpler to execute, less time-consuming and appropriate for the comparison studies purposes.

GuttaFlow Bioseal consists of bioactive substances incorporated into a mixture of GP, polydimethylsiloxane, platinum catalyzer and zirconium dioxide [8]. The bioactive substances enable the formation of hydroxyapatite crystals upon contact with moisture. Having a setting time of approximately 12-16 minutes, better flow properties and being a less time-consuming procedure, GuttaFlow Bioseal could be a promising root filling material to seal the root canal system. Previous studies have investigated the properties of GuttaFlow Bioseal, including penetration into the dentinal tubules following the agitation procedure [9], cytotoxicity [10-14], physicochemical properties [15-17], sealing ability [18-22], osteogenic activity [23], retreatability [8,24] and fracture strength of the root canal treated teeth [25] but scientific evidence of the other

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aspects of obturation using this material is still lacking. Hence, continuous investigation is required to warrant its clinical use.

The evaluation of obturation quality in molars has been the subject of interest that has gained popularity in the recent years [19,26-28]. In the past, the obturation quality has been evaluated in the incisors [29-32] and premolars [9,33-38], however, changing approaches reflect the need to evaluate the effectiveness of obturation procedures in more complex root canal systems such as multirooted teeth with various root canal configurations and curvatures.

Scanning electron microscopy (SEM) is a common method for microscopic evaluation of the obturation quality [4,21,39-47]. Apart from SEM, confocal laser scanning microscopy (CLSM) [9,48-50] and micro computed tomography (micro-CT) [20,22,26,38,51-56] have also been utilized. The micro-CT approach is limited by the difficulty to differentiate the presence of voids and marginal gaps in the root canal [35]. However, this hindrance can be overcome by using higher resolution nano-CT, as demonstrated by Huang et al., 2017 [56]. CLSM differs from SEM with regards to the source of light, specimen processing and the use of fluorescence [48]. Dye penetration [28,57-59] and fluid filtration methods [21,60] have also been performed, although in current practice, these approaches are less common compared to other techniques. The aims of this study were to evaluate obturated surface area, extrusion of root filling material and duration of obturation procedure using GuttaFlow Bioseal as monocone obturation technique in curved root canals.

EXPERIMENTAL

Ethical Approval

This study was approved by the International Islamic University Malaysia Research Ethics Committee (IREC 2019-021).

Preparation of samples

A total of 4 samples for the training session and 20 samples for the experiment were selected based on the inclusion and exclusion criteria as follows. Inclusion criteria included mandibular molars with moderate to severely curved mesial roots, intact coronal tooth structure, no caries, no restoration, intact root and fully formed apices. Exclusion criteria included severe pathological conditions, calcified canal and presence of crack lines.

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Training session

A training session was carried out involving 2 undergraduate dental students under close supervision of an endodontist to standardize each step of the procedure. This includes the demonstration of access cavity preparation, root canal preparation, obturation, tooth sectioning and observation under scanning electron microscope (SEM). Inter- and intra-examiner reliability was difficult to conduct due to the difference in samples, therefore, the procedures were assessed clinically and radiographically.

Endodontic procedure

The endodontic procedures were conducted by 2 undergraduate dental students as shown in Figure 1. 20 samples were mounted on silicone impression materials and preoperative radiographs were taken in 2 directions; mesio-distal and bucco-lingual to assess the status of the root canal.

Standard access cavity preparation was performed with a cavity access set. Then, 25/.08 Hyflex CM rotary file (Coltène/Whaledent) was used to preflare the coronal aspect of the canal. Size 15 K-file was placed in the mesiobuccal root canal and periapical radiographs were taken. The degree of root canal curvature was determined according to Schneider's method [61]. The value of 10° to 20° was classified as moderate root canal curvature whereas more than 20° was classified as severe root canal curvature. After the determination of root canal curvature, size 15 K-file was placed in the root canals until the tip could be seen at the apical foramen. The apical terminus was set to 0.5 mm short of the apical foramen and confirmed with periapical radiograph. Then, the mesiobuccal and mesiolingual root canals were prepared with 20/.06 and 25/0.6 Hyflex CM rotary files (Coltène/Whaledent) at 500 rpm rotational speed and 2.5 Ncm torque level in accordance with manufacturer's recommendations. The preparation of distal root canals was continued until 30/.06 Hyflex CM rotary file.

Sodium hypochlorite of 5.25% concentration was used as root canal irrigant, and the final flush was performed using 17% Ethylenediaminetetraacetic acid and 5.25% sodium hypochlorite. Then, all samples were randomly divided into 2 groups (n=10 each group); Group 1 [matched GP cone and GuttaFlow Bioseal] and Group 2 [matched GP cone and RoekoSeal Automix root canal sealer]. Group 1 consisted of 6 moderate and 4 severe root canal curvature samples; in Group 2, 3 moderate and 7 severe root canal curvature samples were present, respectively. In both groups, the GuttaFlow Bioseal and RoekoSeal Automix root canal sealer were delivered into the prepared root canals (mesiobuccal, mesiolingual and distal) using a delivery tip and a matching GP cone was fitted into the root canals. A heated endodontic plugger was used to cut gutta-percha at the canal orifices, and the GP was compacted vertically. Excess material in the pulp chamber was removed.

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The duration of obturation procedure was recorded with a digital timer starting from the delivery of root filling material into the root canal until complete removal of the excess material from the access cavity. Radiographs were taken after the obturation procedure and the cavity access was restored with composite resin (NTP remium, Coltene). Then, all samples were stored in a separate vial under room temperature at 100% humidity for 7 days to ensure complete setting of the root filling material.

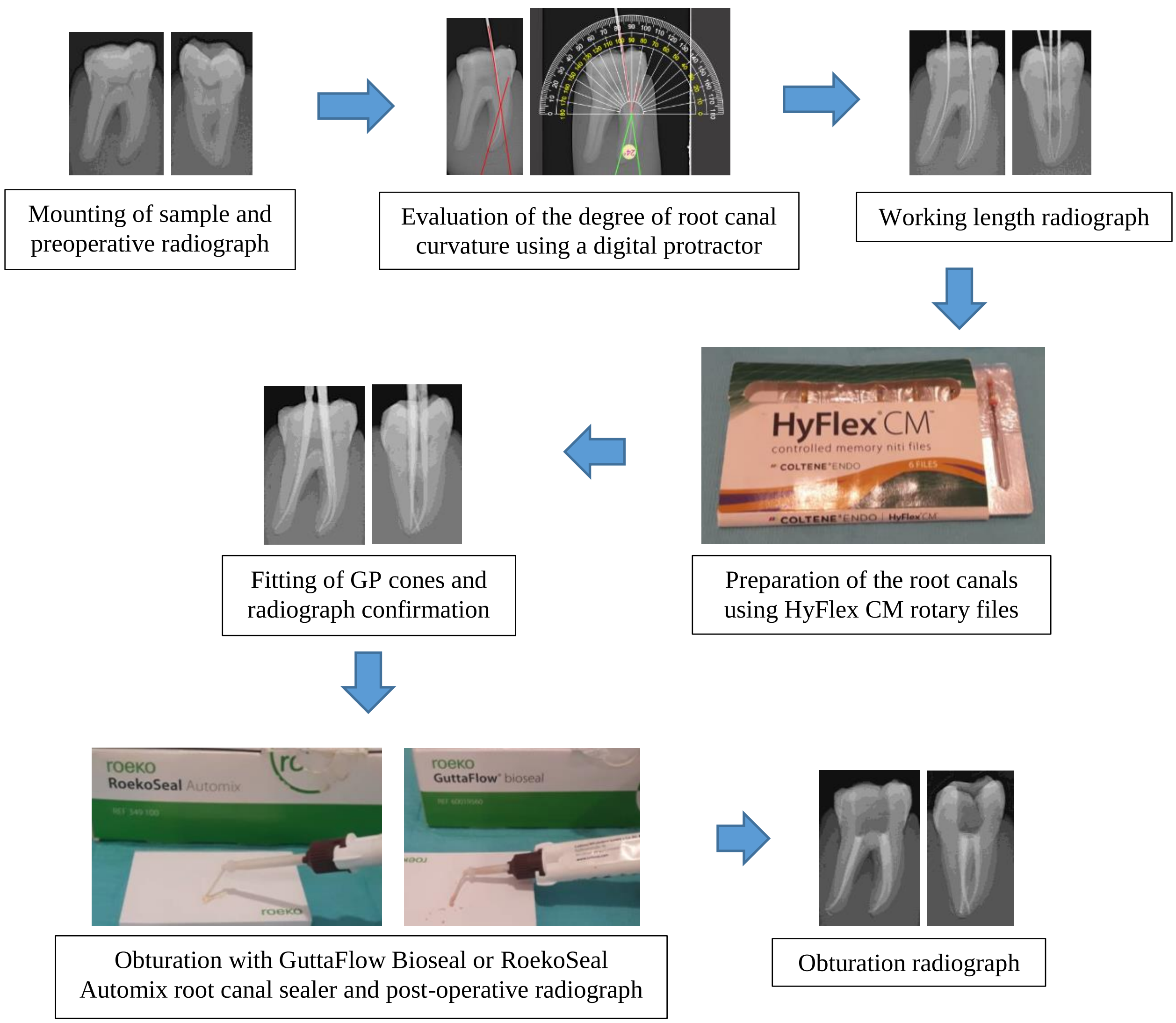

Figure 1: Endodontic procedure involving an access cavity preparation, root canal preparation and obturation

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The samples were sectioned vertically to split the mesial and distal roots. Then, the mesial roots were sectioned horizontally with a diamond saw cutting machine (Isomet 1000, Buehler Ltd., Lake Bluff, IL) with water cooling at 3 regions; the apical, middle and coronal regions. Distal roots were not included in this study. Debris was removed and the resected roots were smoothed with a 600-grit wet silicon carbide sandpaper (Leco, St. Joseph, MI, USA) before the observation under SEM. The resected roots were dehydrated in 25%, 50% and 75% ethanol for 20 minutes at each concentration, in 95% ethanol for 30 minutes and 100% ethanol for 60 minutes, then dried by placement on a filter paper inside a covered glass vial at room temperature for 24 hours.

The resected roots were mounted on brass stubs and sputter-coated with thin platinum coating using Sputter Coater Machine (BAL-TEC SCD005, Scotia, New York) at 70 mA for 70 seconds. Then, the resected roots were placed in the chamber and the surfaces were observed under SEM (FEI ESEM Quanta 450 FEG, Hillsboro, Oregon, USA) at 70x magnification.

SketchAndCalc Area Calculator software

The SEM images were transferred to SketchAndCalc Area Calculator software for the evaluation of obturated surface area, marginal gaps, and voids. The aspects in the obturation images were carefully sketched by following the outline of the root canal, root filling material and empty spaces within the root filling material. The values of obturated surface area, marginal gaps and voids were automatically generated after each sketching. These values were recorded for the calculation of volumetric percentage using Formula 1. The data was analysed with SPSS version 25.0. Preliminary statistical analysis of the data was done using Kolmogorov-Smirnov normality test to assess whether the variables were normally distributed.

Volumetric percentage of the obturated surface area =

$$\frac{\text{Value of the obturated surface area - Value of the void(s)*}}{\text{Value of the root canal space}} \times 100 \qquad (1)$$

*If present

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RESULTS AND DISCUSSION

SEM images of the resected roots at the apical, middle, and coronal regions in Group 1 and 2 at 70x magnification are shown in Figures 2a to f. The SEM images were transferred to SketchAndCalc Area Calculator software for the evaluation of volumetric percentage of the obturated surface area (Figure 3a and b).

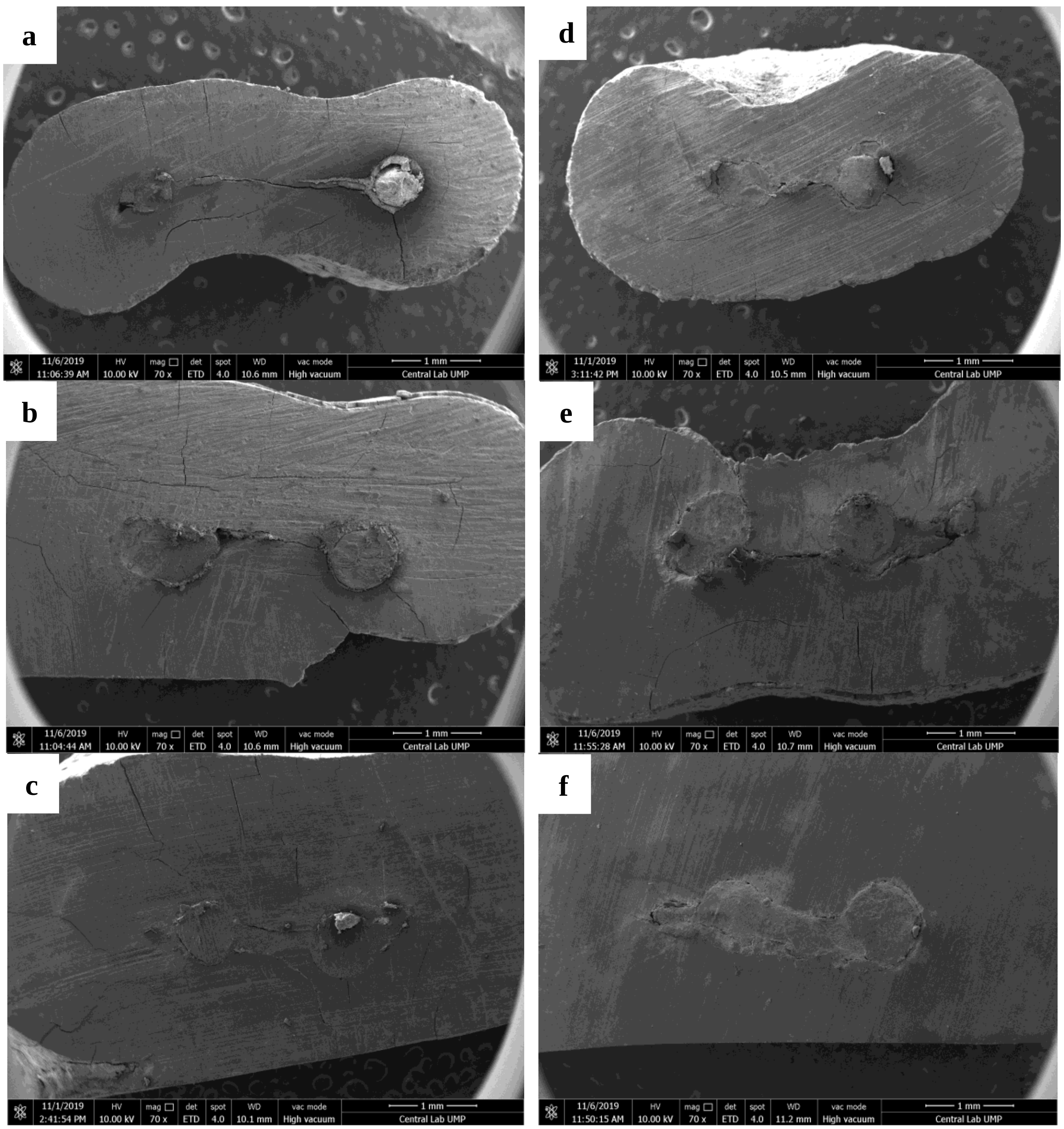

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Figure 2: Obturated surface area in Group 1 a) apical, b) middle, c) coronal, and in Group 2 d) apical, e) middle, f) coronal regions

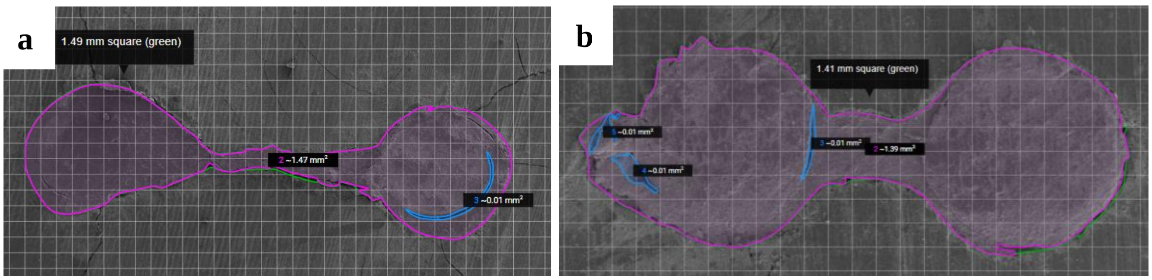

Figure 3: Presence of marginal gaps and voids in the mesial root canals evaluated using SketchAndCalc Area Calculator software. a) Group 1 b) Group 2

Analysis of the volumetric percentage of the obturated surface area was carried out using independent sample t-test with a significance level of $p< 0.05$. The results showed no statistically significant differences between Group 1 and Group 2 at the apical, middle, and coronal regions (Table 1).

Table 1: Percentage obturated surface area

Status of root canal curvature	**Resected roots**	**Group 1 (GuttaFlow Bioseal)**	**Group 2 (RoekoSeal Automix root canal sealer)**	***p* value**
		Mean (SD)	**Mean (SD)**	
Moderate	Apical	89.71 (±5.27)	92.26 (±4.68)	0.502
	Middle	95.10 (±1.94)	97.09 (±1.19)	0.153
	Coronal	89.85 (±12.00)	91.70 (±9.12)	0.823
Severe	Apical	88.71 (±4.47)	94.35 (±2.76)	0.028
	Middle	94.60 (±1.88)	92.35 (±4.38)	0.362
	Coronal	91.47 (±7.12)	94.25 (±2.58)	0.362

Evaluation of the extrusion of root filling material beyond the apical foramen between Group 1 and 2 using Fisher's exact test showed no statistically significant differences (Table 2).

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Table 2: Extrusion of root filling material beyond the apical foramen

Status of root canal curvature	Experimental groups	Extrusion of obturation material n (%)		*p* value
		No	Yes	
Moderate	Group 1 (GuttaFlow Bioseal)	5 (83.3%)	1 (16.7%)	1.00
	Group 2 (RoekoSeal Automix root canal sealer)	2 (66.7%)	1 (33.3%)	
Severe	Group 1 (GuttaFlow Bioseal)	3 (75%)	1 (25%)	1.00
	Group 2 (RoekoSeal Automix root canal sealer)	6 (85.7%)	1 (14.3%)	

Analysis using independent sample t-test showed statistically significant differences in the duration for obturation procedure (in minutes) between Group 1 and 2 in severe root canal curvatures (Table 3).

Table 3: Duration for obturation procedure

Status of root canal curvature	Experimental groups	Duration of obturation procedure Mean (SD)	*p* value
Moderate	Group 1 (GuttaFlow Bioseal)	12.39 (±2.21)	0.324
	Group 2 (RoekoSeal Automix root canal sealer)	10.90 (±1.25)	
Severe	Group 1 (GuttaFlow Bioseal)	13.55 (±1.66)	0.05*
	Group 2 (RoekoSeal Automix root canal sealer)	9.83 (±1.56)	

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The present study evaluates the obturated surface area in curved root canals of mandibular first molars using SEM imaging. Other aspects that have not been investigated previously include extrusion of root filling material beyond the apical foramen and duration for obturation procedure. Therefore, these aspects in obturation are also evaluated. Although the mandibular first molar is not commonly investigated, information obtained from the present study could provide the baseline for a more thorough investigation in the future.

Evaluation of obturation quality in the mesial roots of mandibular molars and the use of GuttaFlow Bioseal was corroborated with the past studies [26,27]. OrthoMTA (BioMTA, Seoul, South Korea) [26] and Endosequence BC (Brasseler USA, Savannah, GA, USA) [27] have both been investigated in the past, but the present study focuses on GuttaFlow Bioseal (Coltène/Whaledent) considering its excellent properties to seal the root canal system.

SEM imaging was selected in the present study because of its ability to produce high resolution images at higher magnifications compared to the conventional microscopy and this was congruent with previous studies [4,39-47]. This method is appropriate for evaluating the irregular surfaces due to the depth of the focal field and the degree of magnification obtainable [62], but the limitation of this approach is that, the high-vacuum nature of SEM complicate distinguishing between genuine and artificial gaps created during vacuum desiccation [63].

SEM is used extensively for the microscopic evaluation of various structures. However, the preparation of samples can result in the potential image distortion, complicating the geometrical measurement of SEM images. Micro-CT, on the other hand, is a non-destructive method for the microscopic evaluation, however the limitation of this method is its inability to effectively differentiate between voids and marginal gaps [35]. This might explain the discrepancy in results observed under a stereomicroscope and micro-CT, which is attributed to the inadequate resolution of micro-CT technique for the detection of small voids [38].

CLSM also does not require samples processing, does not require gold or platinum sputter-coating and is able to preserve the samples in their natural state, resulting in the observation of the samples under close to normal conditions. CLSM does not produce imaging artifacts and is a non-destructive approach [64] similar to micro-CT. This is related to the optical sectioning technique that enables the examination of the samples with enhanced clarity rather than physical sectioning [65]. However, CLSM is not without its limitations whereby confocal imaging quality is dependent on the level of resolution, scanning time and the risk of photo destruction of the sample is present. The use of higher resolution is more time consuming for the scanning and the longer the fluorophore is exposed to the laser. Increasing the level of resolution does not necessarily result in an increase in useful biological information of the sample.

Compared to other microscopic evaluation, the dye penetration method is easier to perform, however, its limitation is that air entrapped in the marginal gaps along the interface may interfere with fluid movement [66] resulting in misleading interpretation of the findings [67]. To date, there

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are various methods for evaluating the obturation quality, each with its own advantages and disadvantages, although some authors have opted for double methods for more comprehensive assessments [19,21,32,33,35,38,56]. Perhaps, the findings in the present study require validation using other methods in the future.

Voids refer to the space surrounded by the same materials, while gaps are the loss of continuity at an interface made of two different materials [35]. In the present study, the obturated surface area between Group 1 and Group 2 at the apical, middle and coronal regions were equivalent irrespective of the status of root canal curvature. This could be attributed to effective delivery of the root filling material into the root canal resulting in equal obturation quality between GuttaFlow Bioseal and the conventional root filling material. However, the marginal gaps and/or voids could be seen in all of the samples and this observation was in agreement with the past studies [30,31,34,68]. This observation could be due to the complexity of the root canal system in the mandibular molars with varying degrees of root canal curvature; thus, perfect adaptation of root filling material to the root canal wall is difficult to obtain.

Extrusion of the root filling material beyond the apical foramen between Group 1 and Group 2 was equivalent regardless of the status of root canal curvature. This could be due to the similar obturation technique used and/or the material viscosity, however the latter could not be confirmed because evaluating material viscosity was beyond the scope of the present study. However, future research needs to be conducted to validate these findings.

The duration for obturation procedure using GuttaFlow Bioseal in severe root canal curvature was 27.5% longer than obturation using conventional root filling material. This observation might not be related to the status of root canal curvature but rather the amount of GP mass due to the combined use of GP cone and GuttaFlow Bioseal. Hence, removal of excess material slightly increased the duration for obturation procedure compared to obturation using GP cone and conventional root canal sealer.

Initially, a similar study on single rooted mandibular premolars was conducted as a preliminary observation of the aforementioned parameters [69]. Although the results were almost identical to the present study, the previous study was evaluated in a less complex root canal system involving extracted teeth that are more commonly investigated compared to molars. However, the limitations of the present study include; (1) The endodontic procedures were conducted by 2 researchers with different skills and experience, even though the study protocols required a training session and were done under close supervision. (2) Anatomical variations of the samples that were difficult to standardize due to the different samples. (3) Limited number of samples in each group. (4) Single method of microscopic evaluation. The aforementioned aspects could possibly influence the results, therefore, future research should focus on other root canal anatomies, different obturation techniques, larger samples sizes and multiple methods of microscopic evaluation to provide insight into the effectiveness of obturation procedures when using GuttaFlow Bioseal.

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CONCLUSION

Within the limitations of the present study, two conclusions could be suggested. Firstly, the obturated surface area and the extrusion of root filling material beyond the apical foramen between Group 1 and Group 2 were comparable irrespective of the status root canal curvature. Secondly, the obturation using GuttaFlow Bioseal in severe root canal curvature was 27.5% longer than the obturation using conventional root filling material.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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