Evaluation of filling ability of Guttaflow Bioseal sealer to the simulated lateral canal by scanning electron microscope: An in vitro study

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Background and objectives: Lateral canals establish a connection between the main root canal and periodontal ligament. Any necrotic tissue and bacteria residing in the lateral canals influence the efficiency of the filling of the root canal and the outcome of root canal therapy. The aim of this study was to determine which type of root canal sealer among GuttaFlow Bioseal, AH-Plus and Endosequence BC sealer provides deeper penetration into artificially prepared lateral canals.
Methods: Thirty mandibular first premolars with single canals and mature roots were instrumented using Protaper universal rotary system after decoronation and standardisation of length (12mm). Three lateral canals were prepared in the coronal, middle and apical thirds in each root (at 3, 6, and 9 mm) using #15 engine-driven reamer. The specimens were divided into three groups (n = 10), according to the filling material, (I): Guttaflow Bioseal seal, (II): AH Plus sealer, (III): Endosequence Bioceramic sealer. In all groups, specimens were obturated using the corresponding Protaper universal gutta-percha and accessory cones. Specimens in each group were cross sectioned. Depth of sealer penetration was measured using a scanning electron microscope.
Results: The data were subjected to ANOVA and Tukey's HSD tests, with significance level of 5%. There was no difference among the tested sealers for filling the lateral canals. Concerning the positions of lateral canals, no differences were also found among sealer types, except for middle portion (at 7 mm from the apex); statistically there is a significant difference between three types of sealer (P value= 0.05), where GuttaFlow Bioseal showed a significantly greater penetration depth than AH Plus sealer.

Keywords: Lateral canal, scanning, electron microscope, Guttaflow, Bioceramic, AH Plus, Root canal treatment.

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Introduction

Root canal treatment (RCT) consists of the eradication of bacterial load in the root canal and obturation of the root canal system three-dimensionally. The root canal space generally becomes infected as a result of caries, defective restorations, dentine cracks, traumatic injuries, tooth wear, or periodontal disease.[1] The root canal system has complex anatomy with irregularities, isthmuses, and lateral canals that may contain bacteria and necrotic tissue.[2] Even with great advances in endodontic technology, it is not possible to clean and shape every irregularity present in the root canal system.[3]

Root canal ramifications can establish a connection between the main root canal and periodontal ligament, as well as the apical foramen. Accessory canals are minute canals that extend in a horizontal, vertical, or lateral direction from the pulp to the periodontium. Lateral canals may be single or multiple or large or small. They may occur anywhere along the root but are most common in the apical third.[4] Accessory canals are formed by the entrapment of periodontal vessels in Hertwig's epithelial root sheath during calcification.[5]

Incomplete obturation of the root canal accounts for about 58% of endodontic failures.[6] The incomplete obturation may be because of inadequate instrumentation or improper obturation technique.[7] Laboratory studies have shown that gutta-percha seals significantly better when used in combination with a sealer.[8,9] The root canal sealer is crucial not only to assist in filling irregular spaces but also to enhance the seal and to penetrate into small, normally inaccessible areas[10]; it also acts as a lubricant while facilitating the placement of the filling core and entombing any remaining bacteria. The depth of sealer penetration is affected by the physical properties of the root canal sealer, how it mixes, root canal anatomy, techniques of application and the presence of the smear layer.[11]

Several microscopic techniques are currently used to evaluate the sealer penetration capacity, including stereomicroscope, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM).[12] A scanning electron microscope is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.[13,14]

New obturation materials have been introduced into the endodontic market over the last decade. Some of these are modifications of materials developed for restorative dentistry, for example Polydimethylsiloxane (PDMS) which is a polymer that has elastic behaviour, excellent biocompatibility, is capable of sealing materials of a various nature, and is resistant to high temperatures, to light degradation, to electricity, to weathering and chemical attack.[15,16]

GuttaFlow Bioseal (GFB) sealer (Coltene/Whaledent, Altstätten, Switzerland) is a polydimethylsiloxane sealer which is composed of Gutta-percha powder particles, polydimethylsiloxane, a platinum catalyst, zirconium dioxide, calcium salicylate, nanosilver particles, colouring, and bioactive glass-ceramic. It has the same formulation as the GuttaFlow sealer but also includes calcium silicate, which, upon contact with biological tissues, releases natural repair constituents and aids in the regeneration of periapical tissues.[17] Silicon is one of the main components of GuttaFlow Bioseal and GuttaFlow.[2] GuttaFlow Bioseal differs from other GuttaFlow sealers as it also contains bioactive glass, which consists of silica, calcium oxide, sodium oxide and phosphorus oxide.[18]

Endosequence BC sealer (Brasseler, Savannah, GA, USA), is a premixed, ready-to-use, and injectable bio-ceramic sealer. It is composed of calcium silicates, calcium phosphate monobasic, calcium hydroxide, zirconium oxide, filler, and thickening agents. It has favourable flowability, small particle size, no setting shrinkage, and shows some extent of volume expansion which directly affects the root canal filling quality.[19]

AH Plus sealer consists of a paste-to-paste system (Paste A: epoxy resins, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: amines, calcium tungstate, zirconium oxide, silica, silicone oil). It is characterised by very low shrinkage, high dimensional stability, good adhesion to dentin and by very good sealing ability, so it is considered as a “Gold Standard” sealer. AH Plus was harmless and safe when tested for possible interactions with living tissue.[20]

Materials and methods

Sample selection. For this in-vitro study, 30 extracted single-rooted human teeth (mandibular first premolar) with fully developed apices from patients requiring extractions for orthodontic reasons were selected. Soft tissue and calculus were removed mechanically from the root surfaces. The teeth were stored in 0.5% sodium chloride (RICCA, USA) for 48 hours for disinfection, and then the teeth were stored in 0.9% sterile normal saline at room temperature, and the water changed every 2 days until used. Digital periapical radiographs (Fona, China) were taken from the buccolingual direction to confirm the presence of a single straight canal and to rule out open apex, root caries, restoration, calcified root canal, dilaceration, crack, root resorption, and fracture.All samples were examined under blue light transillumination to determine that the enamel was free from cracks.[21] All teeth with such variations were excluded from this study.

Sample preparation. All the teeth were decoronated leaving 12 mm root length by using a slow speed conventional straight hand-piece with a diamond disc (D+Z, Germany), with water coolant[22] which was executed in such a way that the disc was perpendicular to the long axis of the teeth. Then all roots were measured using digital calliper (Christ, Germany). Pulp tissue was removed with a barbed broach. The patency of the canal was checked by passing #10 Stainless steel K-file (Dentsply, Maillefer) 1mm through the apical foramen. Artificial sockets were made using a disposable syringe (10 ml) which was cut (height=3cm) using a diamond disk with parallel milling device (AMANN, Germany) and filled with vinyl polysiloxane impression material (Muller-Omicron, Germany), which was mixed (base and catalyst) according to manufacturer instructions. The roots were placed in the centre of putty material. The material was left to set, in this manner, forming a small block to facilitate handling of the roots during instrumentation and obturation.[23]

Instrumentation and irrigation of samples. For standardisation, during instrumentation, the moulds were fixed by a bench vice. The teeth were then prepared with a ProTaper universal rotary system (Dentsply, Ballaiques, Switzerland) using the crown-down technique with an electric motor (NSK, End mate, Japan). The rotational direction of the ProTaper was forward (clockwise) continuous rotation. The rotational speed was 300 rpm, and the torque limit value was 3.0 ncm.[24]

Reproducible glide path (RGP) was ensured by no. 15/0.2 ISO K-file which was smoothly inserted and removed from the canal before starting instrumentation. The instrumentation started with an S1 shaping file up to two-thirds of the canal in a gentle in and out pecking motion according to the manufacturer instructions, and then the irrigation needles were inserted into two-thirds of the root and irrigated with 2 ml sodium hypochlorite solution. After irrigation, RGP was checked with # 15 K-file.

The SX instruments were used to increase the taper of the coronal third, and the S1, S2, F1 and F2 instruments were used sequentially to the full working length, reaching the apical diameter of size 25, .06 taper. After the use of each rotary instrument, the canals were irrigated and recapitulated; irrigation was carried out with 2ml 5.25% sodium hypochlorite (NaOCl). The smear layer was removed by applying 3 ml of 17% aqueous ethylene diamine tetraacetic acid (Spident, Korea) for 1 minute. The flutes of the instruments were cleaned with ethyl alcohol and dried after each use.[25]

Generation of artificial lateral canals. To facilitate the creation of simulated lateral canals, plaster of Paris was used to make a plate. Plaster was poured into a basic plastic mould, and root blocks were placed horizontally in the centre of the impression. Then, the plaster plate was fixed on the magnetic table of a parallel milling device through a movable screw on the device which was of modular design. Next, three simulated lateral canals were drilled on the mesial surface of the root, perpendicular to the main canal at 3 mm, 6 mm, and 9 mm from the apex, using a #15 engine reamer (Thomas, France), which created the perforations until reached the main canal. Patency of simulated lateral canals was confirmed by inserting a size 08 K-file (Dentsply, Maillefer) into each simulated lateral canal up to the main root canal, and a periapical radiograph was taken. If the file did not penetrate the main root canal, the tooth was discarded (3). Final irrigation was done with 5mL 5.25% NaOCl and generous rinsing with 5mL of physiological (saline) solution (3). The main root canals were dried with sterile F2 ProTaper paper points (Dentsply, Ballaiques, Switzerland).

Sample grouping. After root canal preparation, the specimens were divided randomly into 3 groups (each group containing 10 samples) according to the type of root canal sealer used:

Group I: Gutta-percha cone and Guttaflow bio-seal sealer.

Group II: Gutta-percha cone and AH Plus sealer.

Group III: 10 samples were obturated with gutta-percha cone and Endosequence bioceramic sealer.

Samples obturation

For standardisation, during obturation, the moulds were fixed by a bench vice for all 3 groups.

Group I: Guttaflow bioseal sealer. All the canals were obturated by Protaper gutta-percha size # F2. Once the master gutta-percha cone was fitted at the working length with slight resistance (tug back) effect, the protective cap of the Guttaflow bio-seal applicator was removed and replaced with a flexible mixing tip. After slight pressure on the plunger the sealer flows homogeneously and spread onto a mixing glass slab and inserted into the canal with stainless steel k-file (#15), however, any deficiency would be substituted immediately by sealer coat on the gutta-percha cone, and master gutta-percha cone also was covered with the sealer and placed in the canal. For oval-shaped canals, the spreader was placed in the canal a long side the cone 2mm shorter than final working length. A lateral pressure was applied to push the cone away laterally as far as possible and spread from side to side compressing the gutta-percha and sealer against the walls after that the spreader was removed and accessory gutta-percha introduced to the canal. Again the spreader was placed in the canal and the same process repeated until there is no more room in the canal for additional gutta-percha. The gutta-percha then was cut by a hot instrument. However, condensation with instruments is not necessary.

Group II: AH Plus sealer. The two pastes of AH plus sealer were mixed according to the manufacturer instructions on a cold dry clean glass slap using cement mixing spatula to get a homogenous consistency. Then, the mixed sealer was inserted into the canal with stainless steel k- file (#15); however, any deficiency would be substituted immediately by a sealer coat on the gutta-percha cone. Master gutta-percha cone #F2 also was covered with the sealer and placed in the canal to the working length.

Group III: EndoSequence Bioceramic sealer. No mixing is required so that it can be applied immediately and introduced directly into the root canal. EndoSequence BC sealer was placed inside the root canal using disposable tips. The tip of the syringe was inserted into the canal no deeper than the coronal one third. A small amount (1-2 calibration markings) of sealer was dispensed into the root canal by compressing the plunger of the syringe. Then the master gutta-percha cone #F2 was coated with a thin layer of sealer and very slowly inserted into the canal to its final working length.

After completing the filling procedure, all roots were maintained for 7 days at high humidity and were stored in pieces of wrapped cotton at 37°C in an incubator to allow the sealers to set completely. The silicone blocks were removed, and digital periapical radiographs were taken from the buccolingual direction for all samples.

Sectioning and image analysis. After one week, the teeth were returned to the mould, and plaster was poured into basic plastic moulds and root blocks vertically placed in the centre of impression. Then, a plaster plate was obtained and fixed onto the magnetic table of the parallel milling device using a movable screw of the device which was of modular design. The samples were cross-sectioned using a diamond disc and continuous water cooling to prevent frictional heat, placed perpendicular to the main canal directly above the point of making the lateral canal at 4 mm, 7 mm, and 10 mm from the apex. Thus, 90 specimens were obtained. Then, the surfaces were polished using fine and medium sandpaper disks (WILLIAM TDV, Malaysia) under running water to eliminate debris during the cutting procedure. The samples submitted to scanning electron microscopy had 2 mm thickness.

After cross-sectioning, each specimen was labelled by a blue marker on the side containing the lateral canal to identify the area to be magnified. Each group was placed in dry gauze and kept inside a plastic container, and the containers were labelled.[26] The sections were then coated with a thin gold coating before observation by scanning electron microscope (SEM). Samples were viewed using the 20× lens to measure the depth of sealer penetration into a simulated lateral canal.

Statistical analysis. The collected data were analysed using SPSS (Statistical Package for Social Science) software version 21.0 for Windows (SPSS, Chicago, Illinois, USA). Descriptive statistics were used which Include mean, sampe numbers, standard deviation, minimum and maximum values and graphical presentations by column charts. One Way Analysis of Variance (ANOVA) was used to test any statistically significant difference between the groups. Tukey's HSD (Honest Significant Difference) test also was used for comparison among the groups after using ANOVA test, the level of significance was set at 0.05 and a confidence interval of 95%.

Results

Sealer penetration into the simulated lateral canal.

The mean and standard deviations of sealer penetration depths are present in Table 1, The GFB sealer showed the highest penetration depth into the simulated lateral canal, while AH Plus showed the lowest penetration depth. The mean value for cervical, middle and coronal portion are present in Table 2.

Table 1: Mean and standard deviation for the depth of penetration of three sealers

Material	N	Mean	Std. Deviation	Minimum	Maximum
GFB	30	1.2003	0.41180	.42	1.89
AH+	30	1.0090	0.31766	.20	1.74
Endo. BC.	30	1.0187	0.43905	.32	2.02
Total	90	1.0760	0.39847	.20	2.02

(N= number of samples., mm= millimetre, AH+ = AH Plus)

Table 2: Descriptive statistic of each section of different group

Material	Site	Sample No	Mean ± St. Deviation (mm)	Minimum (mm)	Maximum (mm)
GFB	Cervical	10	1.33 ± 0.44	0.58	1.84
	Middle	10	1.24 ± 0.37	0.69	1.89
	Apical	10	1.03 ± 0.40	0.42	1.59
AH+	Cervical	10	1.10 ± 0.43	0.20	1.74
	Middle	10	1.04 ± 0.21	0.81	1.43
	Apical	10	0.88 ± 0.26	0.50	1.33
Endo.BC.	Cervical	10	1.43 ± 0.41	0.64	2.02
	Middle	10	0.89 ± 0.32	0.32	1.22
	Apical	10	0.74 ± 0.25	0.35	0.97

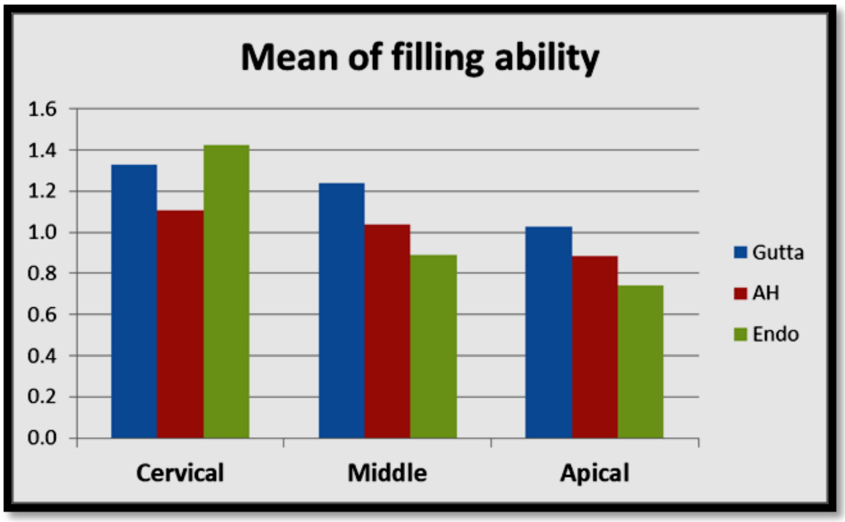

Figure 1: Column chart represents the mean value of the depth of penetration of three sealer types in each section of root.

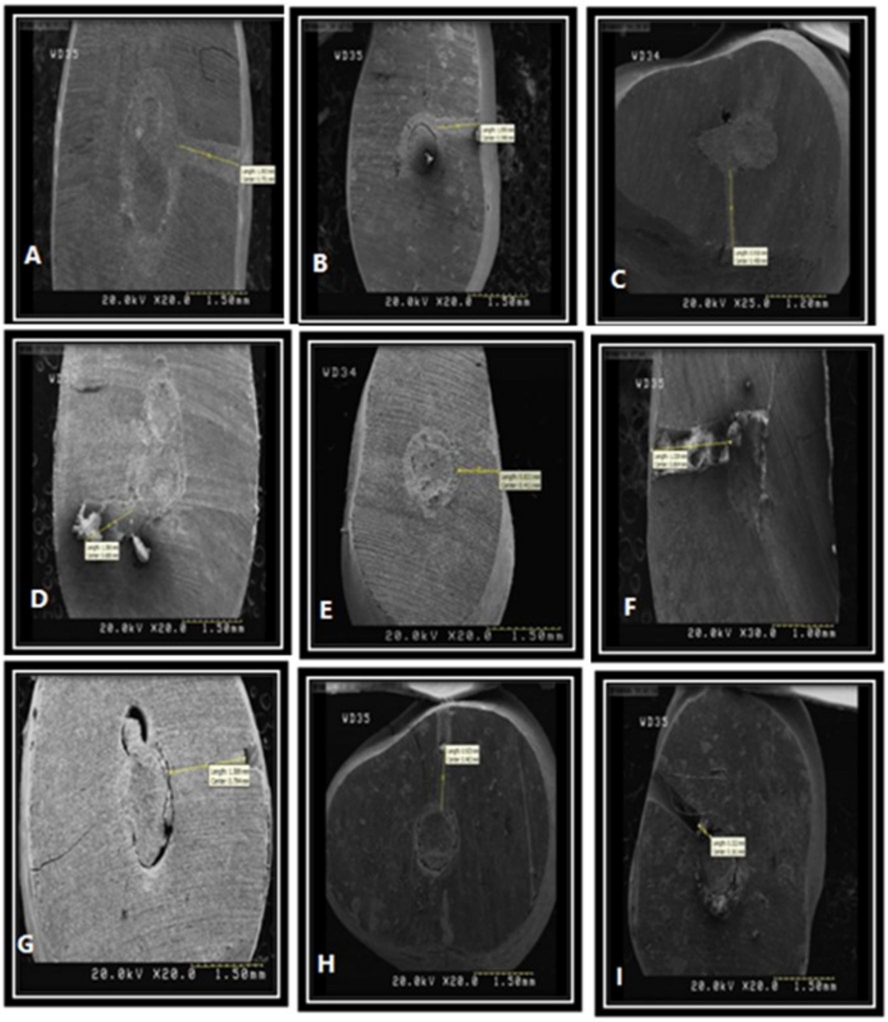

Figure 2: SEM images of three sealers which were obtained in the coronal, middle and apical portions for the depth of penetration of root canal sealer into the lateral canal. A: GFB sealer – coronal section, B: GFB sealer- middle portion, C: GFB sealer- apical portion. D, E, F: AH+ sealer (Coronal, middle, and apical portion). G, H, I: Endosequence BC sealer (Coronal, middle, and apical portion).

Comparison of the penetration depth of three sealers. ANOVA along with Tukey HSD[a] was used to compare the depth of sealer penetration of three groups of sealer into the simulated lateral canals, comparing the outcome of each group to determine which sealer type showed deepest penetration and which showed shallowest penetration [into simulated lateral canal]. The statistical results showed no significant difference between the three sealer groups (P-value > 0.05) (Table 3).

Comparison of the penetration depth capacity of sealers in three sections of root. GuttaFlow Bioseal group, when comparing within groups, gave no statistically significant difference in the length of penetration between the coronal, apical and middle parts of the root (P-value = 0.25). For AH+ group, the statistical result was also not significant (P-value = 0.29). While Endosequence BC. Group, when comparing within groups, the coronal, middle and apical third showed statistically highly significant difference (P-value < 0.05). The Endosequence BC group showed poor penetration in the apical third (0.74 ± 0.25) in comparison with middle (0.89 ± 0.32), and cervical third (1.43 ± 0.41) of the root canal (Table 4).

Table 3: Tukey HSD[a] test for comparison between three groups of sealer

(I) Material	(J) Material	Mean Diff. (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GFB	AH+	0.19133	0.10146	0.149	-.00506	0.4333
	Endo.BC	0.18167	0.10146	0.179	-0.0603	0.4236
AH+	GFB	-0.19133	0.10146	0.149	-0.4333	0.0506
	Endo.BC	-0.00967	0.10146	0.995	-0.2516	0.2323
Endo. BC	GFB	-0.18167	0.10146	0.179	-0.4236	0.0603
	AH+	0.00967	0.10146	0.995	-0.2323	0.2516

(GFB= GuttaFlow Bioseal, Endosequence bioceramic, Sig., Level of significant)

Table 4: ANOVA-Tukey HSD tests for comparison between depths of penetration in three sections of the root of three sealer types

Material	Mean ± SD			P-value
	Cervical	Middle	Apical	
GFB	1.33 ± 0.44	1.24 ± 0.37	1.03 ± 0.40	0.25
AH+	1.1 ± 0.43	1.04 ± 0.21	0.88 ± 0.26	0.29
Endo.BC	1.43 ± 0.41	0.89 ± 0.32	0.74 ± 0.25	< 0.001

Table 5: ANOVA-Tukey HSD tests for comparison between the depth of penetration of three sealer types in three sections of the root.

Site	Mean ± SD			P-value
	GFB	AH	Endo. BC.	
Cervical	1.33 ± 0.44	1.1 ± 0.43	1.43 ± 0.41	0.30
Middle	1.24 ± 0.37	1.04 ± 0.21	0.89 ± 0.32	0.05
Apical	1.03 ± 0.40	0.88 ± 0.26	0.74 ± 0.25	0.13

When three sections of root were filled with different sealer types compare to each other; statistically there was no significant difference between three sealers in cervical portion (P-value = 0.30). For apical portion also there is no statistically significant difference between three sealers (P-value = 0.13), while for middle portion statistically there is significant difference between three types of sealer (P value= 0.05); the deepest penetration was found in GFB group, shallowest penetration was for Endosequence BC group (Table 5).

Discussion

Success or failure of endodontic treatment is critically affected by the root canal obturation process.[27] A non-filled lateral canal makes a two-way passage for bacteria and tissue products between the root canal space and the periodontal tissues, so the lateral canals are considered a constant challenge to endodontists. Depth of sealer penetration into irregularities of the root canal system was affected by different physical and chemical properties of the sealer[28] and different irrigants.[29, 30] Therefore, the aim of this in vitro study was to assess the penetration depth of three different sealers (Guttaflow Bioseal, AH Plus, EndoSequence Bioceramic) into the simulated lateral canal.

Extracted teeth were used in this study instead of epoxy resin blocks because, despite the ease of making narrow ramification, their surface texture and condition could influence the flow properties of gutta-percha and sealer[31]; extracted teeth offer a more accurate simulation of the clinical situation. In order to produce experimental conditions as close as possible to the clinical reality, in this study lateral canals were created by using a size 15 engine reamer adapted to a low -speed handpiece. The lateral canals were made after the shaping of the main canal to avoid the smear layer formation, which could obliterate them, following the methodology proposed by Goldberg et al.[32] and modified by Pécora et al.[33]

Scanning electron microscopy has been used in this study because this form of microscopy can be used to measure sealer penetration depth at high magnification. The scanning electron microscope (SEM) is an essential instrument for examining solid specimens and furnishing qualitative and quantitative information. SEM is a linear accelerator of electrons in which the surface of a specimen is scanned by a beam of electrons that are reflected to form an image. Topography, morphology, chemistry and crystallography can be studied by SEM.[34] The SEM uses electromagnets rather than lenses, thereby, amplifying magnification and facilitating clear images.[35,36]

The sealers were selected based upon their potential properties for the filling of minimally instrumented root canals. AH Plus has a considerably low flowability, low film thickness, and slight shrinkage upon setting in comparison to other sealers.[13] Polydimethylsiloxane has been used in dentistry for a long time owing to its properties. It has an extremely low surface tension, which provides a high flow rate, limited dimensional change on setting (0.6-0.2%) and low water sorption.[37] EndoSequence BC Sealer has desirable properties such as osteoconductivity, being hydrophilic, having adhesion and ability to form a chemical bond with the dentine walls of the root canal.[38]

The comparison among the three tested sealers did not exhibit any statistical difference regarding the filling of the lateral canal, in agreement with the study of Almeida et al.[35] who compared five different sealers: AH Plus®, Epiphany®, Endométhasone®, Pulp Canal Sealer® and Sealapex®. According to a scanning electron microscopic study by Leski and Pawlicka, RoekoSeal and GuttaFlow have shown good adaptation to the root canal walls and better penetration into the dentinal tubules.[37]

In the analysis of the influence of the lateral canal location only EndoSequence BC. Group, when compared within group for the coronal, middle and apical third (4, 7, 10mm), showed statistically highly significant difference in the depth of penetration of root canal sealers. The EndoSequence BC group showed poor penetration in the apical third (0.74 ± 0.25) in comparison with middle (0.89 ± 0.32), and cervical third (1.43 ± 0.41) of the root canal. When three sections of root that filled with different sealer types compare to each other statistically there was no significant difference between three sealers in cervical portion. For apical portion also there is no statistically significant difference between three sealers, while for middle portion statistically there is significant difference between three types of sealer; the deepest penetration was found in GFB group, shallowest penetration was for EndoSequence BC group.

In the studies of Dulac et al.[37] and Venturi et al.[3] a greater root canal filling rate in the canal closest to the cervical third was observed than in the canals at medium and apical thirds, but the result of the studies of Almeida et al.[35] did not verify statistically significant differences. Akcay et al. assessed dentinal tubule penetration by different root canal sealers, including GuttaFlow Bioseal, using laser scanning confocal microscopy. They showed that GuttaFlow Bioseal has similar dentinal tubule penetration to that of MTA Fillapex and AH-Plus[17] Souza et al.[38] compared the depth of penetration of seven sealers into artificial lateral canals (Epiphany, AH Plus, EndoRez, EndoFill, Endomethasone, Sealapex and Sealer 26) and they found that AH Plus exhibited the greatest depth. The dentinal tubule penetration of the AH Plus and MTA Fillapex root canal sealers has been evaluated with Amorosa-Silva et al. reporting that their penetration into the dentinal tubules was statistically similar.[37]

Akcay et al. assessed dentinal tubule penetration by different root canal sealers, including GuttaFlow Bioseal, using laser scanning confocal microscopy. They showed that GuttaFlow Bioseal has similar dentinal tubule penetration to that of MTA Fillapex and AH Plus.[17] These results were in accordance with the view that the depth of dentinal tubule penetration of a sealer appears to be influenced by the chemical and physical characteristics of the materials that make up the sealer.[39] Souza et al[38] compared the depth of penetration of seven sealers into artificial lateral canals (Epiphany, AH Plus, EndoRez, EndoFill, Endomethasone, Sealapex and Sealer 26) and they found that AH Plus exhibited the greatest depth. The dentinal tubule penetration of the AH Plus and MTA Fillapex root canal sealers has been evaluated with Amorosa-Silva et al[40] reporting that their penetration into the dentinal tubules was statistically similar.

Conclusion

According to the results found, it can be concluded that there were no differences among the endodontic sealers tested regarding the filling of artificial lateral canals. Concerning the positions of lateral canals, no differences were found among sealer types, except for the middle portion, where GFB showed a significantly greater filling than AH Plus and EndoSequence Bioceramic sealer. For EndoSequence BC group when three sections also compared with each other, there is statistically highly significant difference between them.

Conflicts of interest

The author reported no conflict of interests.

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