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RESEARCH ARTICLE

DENTINAL TUBULE PENETRATION OF AH PLUS, ENDOSEAL MTA AND BIO-C ROOT CANAL SEALERS WITH TWO OBTURATION TECHNIQUES – A CONFOCAL MICROSCOPIC EVALUATION

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ABSTRACT

Background: Sealer penetration increases the contact area between the dentinal walls and the sealer, thus improving the sealing and antimicrobial efficacy of the sealers. **Objectives:** Evaluation the maximum depth and percentage of AH Plus, Endoseal MTA and Bio-C sealer penetration into the dentinal tubules using single cone and warm vertical compaction (WVC) obturation techniques. **Materials and methods:** Ninety single rooted mandibular premolars decoronated at 13mm from apex. After instrumentation, samples were randomly divided into 6 groups (n=15) according to the sealers and obturation techniques used. The obturated samples were then kept in 100% humidity at 37°C for 7 days and sectioned at 3mm, 5mm and 7mm from the terminus of filling. The sectioned samples were observed under confocal laser scanning microscope to determine maximum penetration depth and percentage of sealer penetration. Statistical analyses was performed using one way ANOVA with Tukey Post-HOC test for multiple comparison and independent t-test. **Results:** At all the levels, the maximum depth and percentage of penetration of Bio-C and Endoseal MTA were significantly better ($p \leq 0.05$) as compared to AH Plus when the samples were obturated with single cone and warm vertical compaction obturation techniques. However, Endoseal MTA and Bio-C sealers showed no statistically significant difference ($p > 0.05$). Irrespective of the sealer used, warm vertical compaction obturation technique showed a statistically significant ($p \leq 0.05$) difference in depth and percentage of sealer penetration at all the levels. **Conclusion:** Bio-ceramic sealer (Endoseal MTA and Bio-C) had greater depth and percentage of sealer penetration as compared to AH Plus with both obturation techniques at all the evaluated levels. Furthermore, warm vertical compaction obturation technique achieved greater depth and percentage of sealer penetration as compared to single cone technique.

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INTRODUCTION

A three-dimensional hermetically sealed root canal obturation with an inert, biocompatible material effectively impedes the communication between the periodontal space, periapical tissue and the root canal thereby isolating the remaining bacteria within the root canal system. Gutta percha has been widely used as a solid core material for root canal obturation[1]. However, solely using gutta percha does not fill the irregularities between the canal wall and filling material, necessitating the use of sealer. Root canal sealers creates a union between a core material and root canal wall andcanpenetrate the accessory canals, lateral canal, and dentinal tubules. Penetration of the root canal sealers into the dentinal tubules is an important characteristic as it enhances the sealing potential and antimicrobial efficacy of sealers and entomb any residual bacteria within the tubules thereby preventing reinfection.Furthermore, it increases the surface area of contact between filling material and dentinal walls thereby mechanically interlocking the sealers to the dentinal walls[1]. Epoxy resin-based sealers offers longer sealing duration, great dimensional stability and can form regular, homogenous and streamline chemical adhesion with root canal wall[2]. AH Plus is considered as a gold standard among sealers to which the other investigated sealers are compared.

Recently developed calcium silicate-based bioceramic sealers are favored as they possess the ability torelease calcium. Endoseal MTA, a premixed bio-ceramic pozzolanbased sealeris known for its intratubular biomineralization, thereby leading to good sealing ability and dentine bonding. Endoseal MTA has a smaller particle size, low solubility, expansion during setting, good flow and becomesthixotropic when the material is released from the needle tip[3].Bio-C sealeris ready to use bio-ceramic sealer which shows high biocompatibility, bioactivity, anti-bacterial action, and high adhesion with dentine. It has 65% bio-ceramic composition, which stimulates the formation of mineralized tissue content. Bio-C sealer has a small particle size, short setting time, alkalization ability, setting expansion propertyand adequate flow[4]. In addition to different types of sealers, obturation techniques have a profound impact on the root canal sealing quality. Single cone (SC)is simple and robust technique that uses a single matched gutta percha cone coated with sealer and does not require either accessory points or the lateral condensation. Therefore, it does not exert any longitudinal or lateral pressure on the root canal walls[5].Warm vertical compaction (WVC) technique (Schilder's technique[6]**)** uses heat source and pluggers to produce a three-dimensional homogenous obturation that allows filling of canal irregularities and accessorycanals. Literature reports thatwarm vertical compactionproduced a greater volume of gutta percha and lower percentage of voids in root canal filling[7]. In this study, the confocal laser scanning microscope (CLSM) method was used to assess sealer penetration into dentinal tubules as it does not require any special sample preparation technique and the integrity of the sealer is retained. CLSM also provides the means to visualize the full extent of sealer penetration as well as the sealer penetration depth[8]**.** Although several valuable research studies can be found on the investigation of AH Plus sealer penetration,information on the comparison of AH Plus, Endoseal MTA and Bio-C sealer penetration into the dentinal tubules using different obturation techniques in the available scientific literature is scarce. Hence, a comparative study was undertaken to compare of the dentinal tubule penetration of AH Plus, Endoseal MTA and Bio-C sealers using single cone and warm vertical compaction obturation techniques.

MATERIALS AND METHODOS

Approval for the study protocol was obtained from the ethical committee (approval no. 3040). Ninety recently extracted single-rooted human mandibular premolars with single straight root canal were collected.The selected teeth were decoronated at 13mm from the apex to standardize the root length and canal patency was determined with 10 K-file.Preparation of root canal was carried out using ProTaper universal rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to F3 (30/0.09).The root canals were irrigated with 3 ml of 5% sodium hypochlorite between each file change and finally using 5ml of 17% EDTA for 3min to eliminate the smear layer. Then canals were flushed with 5ml of distilled waterand dried with paper points. The root samples were randomly allocated into six groups (n=15 each), according to the type of sealer and obturation technique.

GROUP 1: AH Plus (Dentsply De Trey, Germany) with single cone.
GROUP 2: Endoseal MTA (Maruchi, Wonju, Korea) with single cone.
GROUP 3: Bio-C sealer (Angelus, Londrina, Brazil) with single cone.
GROUP 4: AH Plus with warm vertical compaction.
GROUP 5: Endoseal MTA with warm vertical compaction.
GROUP 6:Bio-C sealer with warm vertical compaction.

Sealers were prepared according to the manufacturers guidelines and labeled with 0.1% Rhodamine B dye for visualization under confocal laser scanning microscope. The dye-sealer mixture (approximately 0.05ml) was applied along the entire length of each canal using lentulospiral for 10 seconds with a slow speed handpiece to minimize the deviations associated with the sealer application. In SC technique, a corresponding ProTaper universal F3 gutta-percha cone was coated with the sealer and placed in the root canal up to the working length. During WVC obturation, a heating woodpecker obturating pen device and pluggers (Guilin Woodpecker Medical Instruments, China) were used to down pack the placed gutta-percha cone and fill the apical 5mm of canal space**.** A room temperature plugger was used to compact the heated gutta-percha. The remaining coronal space was then filled by warming and packing 3-4mm segments of sealer coated gutta percha. The heat was applied to each segment and the mass was compacted with the plugger. Obturated specimens were placed in an incubator at 37°C in 100% humidity for 7 days to allow sealers to set. Each root was sectioned transversely at 3mm, 5mm and 7mm from the root apex. The disadvantage of longitudinal sectioning is that it does not allow for the complete observation of the entire dentine surrounding the canal and there is a potential to miss areas of deep penetration. Each cross-section, 2mm thick, was examined under confocal laser scanning microscope (Nikon, Tokyo, Japan) and Images at 4x magnification were recorded using the fluorescent mode with laser emission wavelengths at 546nm-674nm following the excitation at 543nm.The images obtained from CLSM for both the obturation techniques with each sealer are shown in Figure 1 and Figure 2.

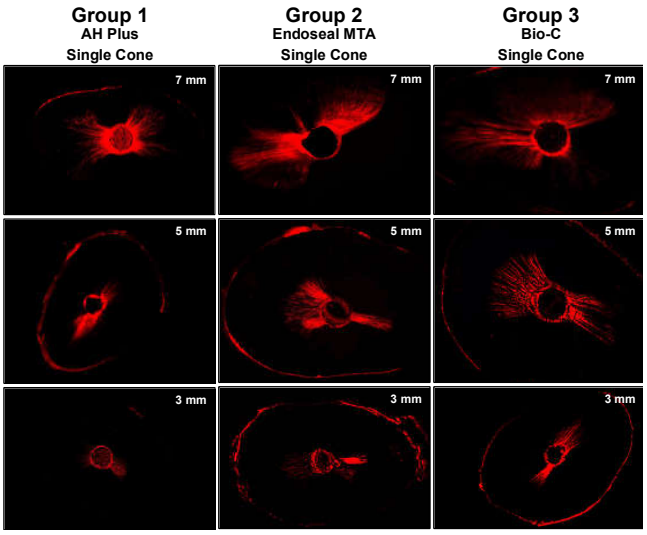

Figure 1. CLSM images of root samples obturated with single cone technique

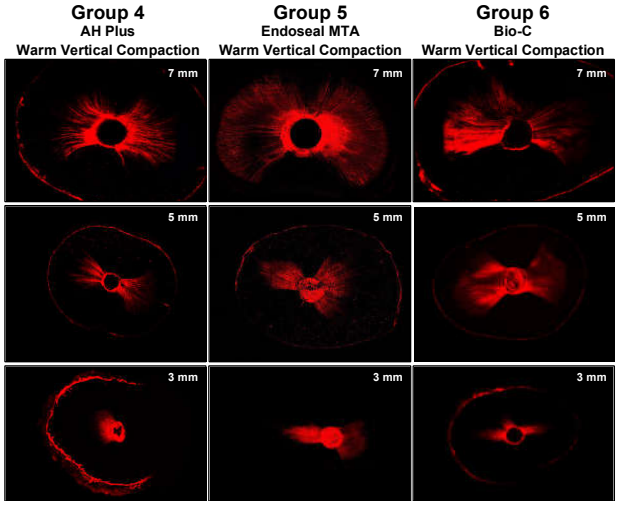

Figure 2. CLSM images of root samples obturated with warm vertical compaction technique

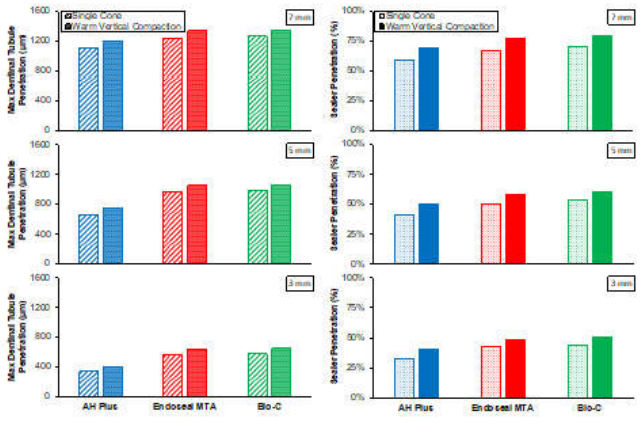

Figure 3. Mean maximum dentinal tubule depth and percentage of penetration for tested dealers and obturation techniques

Digital images were then imported into the ImageJ software program (ImageJ, NIH) to determine the maximum tubule penetration depth. The deepest penetration was calculated from the canal wall to the point of maximum sealer penetration using the measuring tool. To determine the percentage of sealer penetration, the circumference of the root canal wall was measured. Next, areas along the canal walls into which the sealer penetrated the dentinal tubules with any distance were outlined and measured. The outlined distances were divided by the canal circumference to calculate the percentage of sealer penetration. All the analysis results were recorded and evaluated by single operator to rule out any errors. Each measurement was performed twice to ensure consistency and repeatability. Statistical analysis was performed using one way ANOVA test of variance with Tukey Post-HOC and independent t-test in statistical package for social science software, Chicago, IL, USA.

RESULTS

The overall resultsof the dentinal tubule penetration depth and percentage computation are summarized in Table 1 and Table 2 respectively. The mean maximum dentinal tubule penetration, as observed from Table 1, decreased with statistically significant differences ($p \leq 0.05$) when moving towards the apical terminus in all groups. Among the tested sealers, Bio-C (bio-ceramic) exhibited maximum penetration depth followed by Endoseal MTA (bio-ceramic) and lastly AH Plus (resin-based) using both obturation techniques. For instance, at 7mm level, using SC obturation, Bio-C penetrated to a depth of 1270µm, Endoseal MTA 1241.5 µm and AH Plus 1111.6µm. Similar trends were observed at all levels and WVC technique as well. The Percentage of dentinal tubule penetration demonstrated the same behavior (both in mean values and statistics) as seen in penetration depth i.e., dentinal tubule penetration percentage decreased when moving towards apical terminus, and was maximum for Bio-C, followed by Endoseal MTA and least for AH Plus. Additionally, for all the tested sealers, WVC showed a higher maximum depth and percentage of dentinal tubule penetration with statistically significant differences ($p \leq 0.05$) at all levels as compared to SC obturation technique. Statistical analysis further revealed statistically insignificant differences ($p > 0.05$) in both depth and percentage of dentinal tubule penetration between the two bio-ceramic sealers, whereas a comparison between resin-based and bio-ceramic sealers showed statistically significant differences ($p \leq 0.05$).

DISCUSSION

Root canal sealers bond core filling material with the dentinal walls, seal off voids, patent accessory canals and multiple foramina, and enshrining the remaining microorganisms, thus promoting peri-apical healing[9].Sealer penetration into the dentinal tubules (maximum depth and percentage of sealer penetration) aids in the retention of root canal fillings by developing mechanical interlocking, thus increasing the contact area between the dentinal walls and the sealer, and improving the sealing efficacy of the root canal system[10]. Sealer penetration into the dentinal tubule is influenced by several factors such as physical and chemical properties of sealers (including flow, surface tension, particle size and viscosity), filling technique, dentine permeability (number and diameter of tubules), smear layer removal, root canal dimensions and the presence of water[11].In this in-vitro comparative study, the dentinal tubule penetration of AH Plus, Endoseal MTA and Bio-C sealers using single cone and warm vertical compaction obturation techniques was evaluated.

Table 1. Mean maximum dentinal tubule penetration depth (all values in µm)

Level		7mm		5mm		3mm	
Obturation		SC	WVC	SC	WVC	SC	WVC
AH Plus	Mean	1111.6	1205.1	664.48	750.08	342.03	408.63
	±St. Dev.	125.9	89.76	99.91	105	70.18	92.34
	p-value	0.027		0.03		0.034	
Endoseal MTA	Mean	1241.5	1337.2	976	1052	573.31	641.95
	±St. Dev.	126.39	131.42	104.84	98.52	81.28	84.52
	p-value	0.045		0.045		0.031	
Bio-C	Mean	1270	1351.1	987.7	1064.6	578.44	653.3
	±St. Dev.	116.74	86.21	112.35	87.49	98.29	93.95
	p-value	0.039		0.046		0.042	

Table 2. Mean percentage of dentinal tubule penetration (all values in %)

Level		7mm		5mm		3mm	
Obturation		SC	WVC	SC	WVC	SC	WVC
AH Plus	Mean	59.04	69.54	41.37	49.95	32.37	40.95
	±St. Dev.	7.03	7.82	6.62	6.54	6.31	8.34
	p-value	0.001		0.001		0.004	
Endoseal MTA	Mean	67.16	77.82	50.38	58.17	42.7	48.89
	±St. Dev.	7.48	12.1	7.47	8.6	6.6	8.7
	p-value	0.007		0.013		0.037	
Bio-C	Mean	70.52	79.4	53.92	60.67	44.23	50.76
	±St. Dev.	7.69	9.7	6.73	8.63	6.54	7.87
	p-value	0.01		0.024		0.02	

The single-rooted mandibular premolars were chosen as variations in different root canals may introduce additional uncertainties in the results.Selected teeth were prepared using pro-taper universal rotary file system as it has been reported to result in uniform and better centered canal preparation. Additionally, it has good flexibility, high cutting efficiency and ability to create regular canal patterns[12]. The root canals were irrigated with a combination of 5% NaOCl solutionand 17% EDTA solution. Published literature has demonstrated an effective smear layer removal using NaOCI and EDTA solutions as irrigants during root canal instrumentation[13].Moon YM et al. (2010)[14] reported that the use of EDTA as final irrigant resulted in significantly better sealer penetration at both coronal and apical levels.

The sealers were labeled with Rhodamine B fluorescent dye to allow visualization under confocal laser scanning microscope without any potential interference with the physical properties, handling characteristics and setting times of the sealer[15]. CLSM allows clear visualization of the sealer within the dentinal tubules with the control of the depth of field, exclusion of artifacts, reduction in background noise away from the focal plane and ability to assemble many optical sections at various depths, even from thick specimens,making the technique highly suitable for this study[16]. From the results presented in Table 1 and Table 2, it is evident that bio-ceramic sealers (Bio-C sealer and Endoseal MTA) showed higher maximum depth and percentage of dentinal tubule penetrationas compared to the AH Plus in both single cone and warm vertical compaction obturation techniques. For easier and clearer observation, these results have been depicted graphically in Figure 3. Higher depth and percentage of penetration of bio-ceramic sealers is attributed to their good flow. Flow of sealer reflects its ability to penetrate into the dentinal tubules, small irregularities and ramifications of the root canal system, and enter un-instrumented accessory root canal anatomy[17].Lim ES et al. (2015)[18]andFilho MT et al. (2020)[19]reported the greater flow and filling ability of Endoseal MTA and Bio-C sealers as compared to AH Plus, respectively. Particle size of Bio-C sealer is less than 2 µmand that of Endoseal MTA is 1.5 µm, which further promotes their penetration into the dentinal tubules (which typically have the diameter between 2 to 3 µm)[3,20]. Due to small particle size, the sealers become thixotropic when released via the needle tip and infiltrates the dentinal tubules to form apatite precursors and sealer tags[3]. In contrast, the epoxy resin-based sealer, AH Plus has relatively low flow compared with that of Bio-C sealer and Endoseal MTA, is available in paste/paste formulation, undergoes slight polymerization shrinkage and is hydrophobic in nature (i.e. moisture can negatively impact its ability to penetrate and adapt to the dentinal tubules)[20]. AH Plus has mixed particle size of 8-26 µm, further explaining the observed less penetration depth and percentage of sealer penetration in this study[21]. CLSM evaluation showed that there was no statistical significant difference ($p>0.05$) between Endoseal MTA and Bio-C root canal sealers when depth and percentage of dentinal tubule penetration was compared. However, a comparison between resin-basedsealer (AH Plus) and bio-ceramic sealers (Endoseal MTA and Bio-C) showed statistically significant differences ($p\leq0.05$). Ha JH et al. (2018)[22] reported that bio-ceramic sealer (Endoseal MTA) showed superior wetting and adhesion properties. Moreover, both Bio-C sealer and Endoseal MTA show 0.2% expansion as they utilize moisture from the dentinal tubules to complete the setting reaction favoring better sealing ability. Alkaline nature of by-products of bio-ceramic sealers has been reported to denature the dentinal collagen fibers, thereby enhancing the penetration of root canal sealers into the dentinal tubules[23]. Moreover, both Endoseal MTA and Bio-C are available as premixed injectable sealers. A study by Muedra P et al. (2021)[24] showed that pre-mixed calcium silicate-based bio-ceramic sealer exhibited better dentinal tubule penetration depth and percentage of sealer penetration than their powder/liquid counterparts. These results are in accordance with the published studies[3,20,25,26]comparing calcium silicate-based and resin-based sealers to the other sealers used in the study.

Bio-ceramic sealers are recommended to be used with single cone obturation technique because heat might deteriorate the physical properties of sealers such as flow, setting time and bond strength. However, Heran J et al. (2019)[27] showed that calcium silicate-based sealers were minimally influenced by heat and therefore, could potentially be used with techniques like warm vertical compaction. The results of present study indicated that AH Plus, Endoseal MTA and Bio-C sealers had higher depth and percentage of penetration when used with warm vertical compaction compared with the single cone technique. This could be attributed to the greater compressive forces applied coronally during warm vertical compaction obturation. These results are in agreement with the published studies by Eid D et al. (2021)[28], Macedo LMD et al (2017)[29] and Kim Y et al. (2019)[8]. Additionally, statistically significant ($p \leq 0.05$) differences were observed among the three root sections. The maximum dentinal tubule penetration and percentage of sealer penetration of all the tested sealers was higher at the 7 mm, followed by 5 mm and least at 3 mmfrom the apical terminus of filling in both single cone and warm vertical compaction obturation technique. This may be a possible consequence of the reduction in dentin permeability because of lower tubule density at apical region of the root canal with the tubules being smaller in diameter and some areas likely being completely devoid of tubules. The density of dentinal tubules increases in the apical-coronal direction towards the root surface and in external-to-internal direction from the root surface[11]. The sclerosis, sporadic mineralization and ineffective delivery of irrigants to the apical region leads to a reduction in the efficacy of smear layer removal closer to the apex which contributes to the lesser penetration. Another possible explanation for deeper penetration of all the sealers in coronal third is application of greater compressive (vertical) forces during obturation[1]. Similar results have been observed in the published studies[26,28,30,31]. The tendency of tubule penetration increased apico-coronally regardless of the nature of sealer and obturation technique.

CONCLUSION

This in-vitro study presented a comparison of sealer penetration depth and percentage among AH Plus, Endoseal MTA and Bio-C root canal sealers using two different obturation techniques, single cone and warm vertical compaction. Within the limitations of this study, both bio-ceramic sealers (Endoseal MTA and Bio-C) achieved greater penetration depth and percentage compared with epoxy resin-based sealer (AH Plus) using both obturation techniques. Nevertheless, no significant differences were evident between the two bio-ceramic sealers. Warm vertical compaction consistently resulted in higher penetration depth and percentage, regardless of the sealer used as compared to the single cone technique. Additionally, irrespective of the obturation technique used, the three tested sealers showed maximum penetration at 7 mm, followed by 5 mm and least at 3 mm from the apical terminus of filling. Thus, according to the presented results, a combination of Bio-C sealer and Endoseal MTA with warm vertical compaction results in higher depth and percentage of sealer penetration among the tested groups and maybe recommended for root canal obturation. However, before drawing any definitive conclusions, clinical in-vivo evaluation with sufficiently large sample size and extensive research should be performed to verify the obtained results.

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