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RESEARCH ARTICLE

Micro-Computed Tomographic Evaluation of Sealing Ability of Different Bioceramic Endodontic Sealers: An *in vitro* Study

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ABSTRACT

The aim of the present study was to compare sealing ability of three bioceramic sealers, including Endosequence BC and BioRoot RCS, with bioceramic-coated gutta-percha cone (BCGP) or GP in comparison to a clinical reference standard AH Plus sealer using high-resolution micro-computed tomography (micro-CT) analysis. Fifty freshly extracted single canal premolars were included in the study. All the samples were prepared using 2 Shape rotary system TS1 (25/0.04), TS2 (25/0.06), and F35 (35/0.06) file. After completion of the instrumentation, the samples were randomly divided based on the sealer material used and GP into four experimental groups and one control group with Endosequence, BioRoot, and AH Plus sealers with BCGP and conventional GP. All groups were obturated with (35/0.06) single cone obturation and high resolution of micro-CT was used to determine percentage of voids within the canals. BCGP showed better result than GP at all groups. In BCGP group, there were no significant differences in the percentage of the voids except internal voids. However, in all conventional GP groups, there were significant differences. Within the limitations of this study, it could be concluded that none of the groups were free of voids, BCGP with bioceramic sealer significantly better than GP.

Keywords: AH Plus; BioRoot RCS; Endosequence; Micro-computed tomography; Sealing ability

INTRODUCTION

The main objective of a root filling is to obturate the entire root canal system and produce an impervious apical seal, to leave no voids for bacteria to populate and proliferate (Hammad et al., 2009). The long-term success of endodontic treatments relies on complete filling after root canal obturation (Gomes-Filho et al., 2012). Root canal treatment without obturation or with incorrect obturation is named as incomplete root canal treatment. Ingle et al. radiographically studied endodontic success and failure; they indicated that 58% of treatment failures were due to incomplete obturation (Ingle et al., 2007).

Obturation of a root canal is carried out by two foremost common materials that are core and sealer. Core can be cold or thermo-plasticized, warm condensation multi-phase (gutta-percha [GP] sealer) technique is contemplated as "golden" standard for endodontic treatment that results in a friction fit, "cork-in-the-bottle" type sealing (Nunes et al., 2008).

The main functions of root canal sealers are (i) sealing off of voids, patent accessory canals, and multiple foramina, (ii) forming a bond between the core of the filling material and the root canal wall, and (iii) acting as a lubricant while facilitating the placement of the filling core and entombing any remaining bacteria (Kaur et al., 2015).

There are various kinds of endodontic sealers which are available, including sealers based on glass ionomer, zinc oxide-eugenol, resin based, calcium hydroxide, silicone, MTA based, and bioceramic-based root canal sealers. In particular, bioceramic-based materials that usually contain calcium silicate and/or calcium phosphate have gained significant attention due to their physical and biological properties such as their alkaline pH, chemical stability in the biological environment, and lack of shrinkage. They are also nontoxic and biocompatible (de Miranda Candeiro et al., 2012; Loushine et al., 2011; Zhang et al., 2009).

There are two crucial significances related to the utilization of bioceramic materials as intracanal sealers. First, their biocompatibility prevents rejection by the surrounding tissues (Koch and Brave, 2009). Second, these materials have ability to form hydroxyapatite and ultimately form a bond between dentin and the material (Reyes-Carmona

et al., 2009). However, one main negative point of these materials is in the complexity in removing them from the root canal once they are set for future reendodontic treatment or post-space preparation (Cherng et al., 2001).

Small gaps and voids can produce microleakage along the obturated root canal, making passage for bacterial penetration and reaccumulation of microorganisms (Adib et al., 2004). Thus, goal of root canal obturation is to offer bacterial-tight seal, achieved by good obturation quality with minimal voids and gaps formation in the obturated root canal (Yanpiset et al., 2018).

With the wide use of micro-computed tomography (CT), it has been possible to provide 2- and 3-dimensional (2D and 3D, respectively) views of the filled root canals and adaptation as well as the presence of gap and void volume in different root canal fillings and techniques (Celikten et al., 2015; Huang et al., 2018).

To the best of our knowledge, there are limited studies about the sealing ability with using micro-CT analysis of different bioceramic sealers with bioceramic-coated GP cone or traditional GP to determine which obturation material has the best sealing ability and therefore the main objective of root canal treatment may be achieved. The purpose of the present study was to compare sealing ability of three bioceramic sealers, including Endosequence BC sealer (Brasseler USA) and Biodentine (Septodont, Saint-Maur-des-Fossés Cedex, France), with bioceramic-impregnated GP cone (BCC) or GP in comparison to a clinical reference standard AH Plus sealer using high-resolution micro-CT analysis.

MATERIALS AND METHODS

Fifty freshly extracted single canal premolars between the age of 15 and 35 years were included in the study after the agreement from ethical committee. The age was restricted because it has an influence on the nature of the dentin and dentinal tubules (Kaya et al., 2011).

The samples were radiographed in buccolingual and mesiodistal projections to confirm the existence of a single and straight canal. Exclusion criteria were being teeth with root caries, restorations or immature apices, internal\external resorption, fractured or cracked teeth, calcified\curved canals more than 30 degrees according to Schneider's method, and less than 4 mm of the radius of curvature which were be confirmed by radiographs. All samples were decontaminated in 5.25% sodium hypochlorite for 2 h (Celikten et al., 2015; Huang et al., 2018) and dried. Later, they were cleaned with ultrasonic scaler to remove surface soft tissue and calculus then rinsed and stored in normal saline till the working day.

The coronal portion of all samples was removed under the cement-enamel junction to standardize the root length of 12 mm from the anatomic apex. All samples were being viewed under operating microscope cross-sectionally for rounded canal shape confirmation. An ISO size #10 K-File (Maillefer, Ballaigues, Switzerland) was inserted into the root canal until the tip was just visible beyond the apex. Working length was determined by subtracting 0.5 mm from this length. The cementum of each root was coated with tray adhesive. The root apex to be covered with hot, flexible glue that allowed to solidify before the root was inserted into a polyvinyl silicone filled polyvinyl chloride pipe. The roots were mounted into the center of the tube. This system was permit recapitulation of canal patency but prevent fluid extrusion from the apical foramen during canal preparation and irrigation to prepare a closed canal system according to Saber and Hashim (Saber and Hashem, 2011).

All the samples were prepared using a crown down technique with a 2 Shape rotary system TS1 (25/.04), TS2 (25/0.06), and (35/0.06) file with an endodontic rotary device from ENDO-MATE DT instruction at 400 rpm and a torque of 2.6 Ncm. First size 15 hand file was used to produce glide path, then TS1 (25/0.04), TS2 (25/0.06), and last F35 (35/0.06) file were used up to working length. Irrigation and recapitulation with a size 15 hand file were performed after each file. Canals were irrigated using a disposable double-sided vented needle with 31 G between each file with 4 mL of freshly prepared 5.25% sodium hypochlorite. The needle was placed 1 mm from the working length (Gao et al., 2009). A flush of 4 ml 17% ethylenediaminetetraacetic acid (EDTA) was applied for 1 min to eliminate the smear layer. Finally, the canals were washed with 3 ml of 5.25% sodium hypochlorite and 3 mL normal saline. Then, all the canals were dried with sterile paper points (Huang et al., 2017).

Root Canal Filling

After completion of the instrumentation, the samples were randomly divided based on the sealer material used and GP into four experimental groups and one control group (n = 10 of each).

- Group A1: Endosequence BC (Brasseler USA) sealer was used with single cone GP
- Group A2: Endosequence BC (Brasseler USA) sealer was used with bioceramic-coated GP cone (Endosequence BC Point, Brasseler USA)
- Group B1: BioRoot RCS (Septodont, Saint-Maur-des-Fossés Cedex, France) BC sealer was used with single cone GP

- Group B2: BioRoot RCS (Septodont, Saint-Maur-des-Fossés Cedex, France) BC sealer was used with bioceramic-coated GP cone (Endosequence BC Point, Brasseler USA)
- Group C1 (Control Group): AH Plus sealer (Dentsply, Germany) was be used with single cone GP.

In all of the groups, root canal obturation was carried out using the single-cone obturation technique. A standardized GP cone of the same size as the master apical file was placed into the root canal up to the working length and the tug back was confirmed.

The sealer first was placed on dental mixing pad and it was delivered into the prepared canal with size #30 lentulo spiral (Dentsply Maillefer). The two-thirds of the lentulo was coated with the sealer and placed into the canal 1 mm short of the working length. The handpiece was operated at 500 rpm according to manufacture instructions when the lentulo was in the canal. For standardization, lentulo spiral was used for 15 s only in all the canals for 3 times. The lentulo was slowly removed from the canal as the handpiece was being rotated continuously. In group (A1, B1, and C1) (35/06), single cone GP was used. In group (A2 and B2) size (35/06), bioceramic-coated GP (Endosequence BC Point, Brasseler USA) was used. The obturation was completed with master GP which was placed to working length and pumped twice in the canal to ensure maximum sealer distribution inside the canal. Excess coronal GP was cut and removed by heat carrier. Afterward, the roots were stored at 37°C and 100% humidity for 7 days to allow the sealer to set entirely (Sakr et al., 2017; Wang et al., 2018).

MICRO-CT EVALUATION

A high-resolution, desktop micro-CT system (Bruker Skyscan 1275, Kontich, Belgium) was used to scan the specimens. The scanning conditions were 100 kVp, 100 mA beam current, 0.5 mm Al/Cu filter, 9.93 μm pixel size, and rotation at 0.5 step. To minimize ring artifacts, air calibration of the detector was carried out before each scanning. Each sample was rotated 360° within an integration time of 5 min. The mean time of scanning was around 2 h. Other settings included beam-hardening correction, as described, and input of optimal contrast limits according to manufacturer's instructions, based on prior scanning and reconstruction of the teeth.

Micro-CT Image Analysis

The NRecon software (ver. 1.6.7.2, SkyScan, Kontich, Belgium) and CTAn (ver. 1.17.7.2, SkyScan) were used for the visualization and quantitative measurements of the samples, which used the modified algorithm described by Feldkamp et al., 1989, to obtain axial, two- dimensional, 1000 × 1000 pixel images. For the reconstruction parameters, ring artifact correction and smoothing were fixed at zero and the beam artifact correction was set at 40%. Contrast limits were applied following SkyScan's instructions. Using the NRecon software (Skyscan, Kontich, Belgium), the images obtained by the scanner were reconstructed to show 2-dimensional slices of the roots. In total, 1023 cross-sectional images were reconstructed from whole volume. Moreover, the CTAn (Skyscan, Aartselaar, Belgium) software was used for the 3-dimensional volumetric visualization, analysis, and volume of the root canal measurement.

The presence of voids was assessed in 2D slices as mentioned by Orhan et al., 2018, study in each section on a 21.3 inch flat-panel color active matrix TFT medical display (NEC MultiSync MD215MG, Munich, Germany) with a resolution of 2048–2560 at 75 Hz and 0.17 mm dot pitch operated at 11.9 bits. New cross-section images were prepared perpendicular to the long axis of the root, starting at the most apical part of the root. The sections had an interval of 0.5 mm which resulted 254 average number of cross-section images. The micro-CT images of the sections were then converted to tiff files and coded. Each section was assessed by two observers independently, using a binary registration scale: Internal, external, and combined voids. The observers were allowed to adjust the magnification of sections and were blinded with regard to the root filling technique. In the case of disagreement between the observers, the sections were reexamined and consensus was reached.

For calculation of the voids in 3D volumes, the original grayscale images were processed with a Gaussian low-pass filter for noise reduction and an automatic segmentation threshold was used to subtract dentin from GP, sealer, and voids using CTAn software. A thresholding (binarization) process was used, which entails processing the range of gray levels to obtain an imposed image of black/white pixels only. Then, separately for each slice, a region of interest was chosen to contain a single object entirely to allow calculation of void volumes. Each tooth was divided into three regions for the evaluation of voids, from the apical end of the root at a level of 0–4 (apical), 4–8 (middle), and 8–12 mm (coronal). Three-dimensional visualization and qualitative evaluation of the root canal obturation were performed with CTVox software (version 3.3.0, Bruker micro-CT) [Figure 1].

The mean percentages of the root filling volume (sum of the volume of the GP and the endodontic sealer), the volume of internal voids distributed inside the root canal filling material, the external voids along the canal walls,

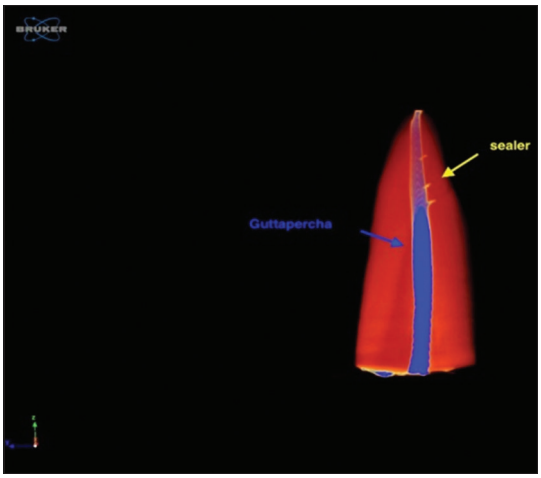

Figure 1: Reconstructed 3-D image for a sample showing the distribution of gutta-percha and sealer

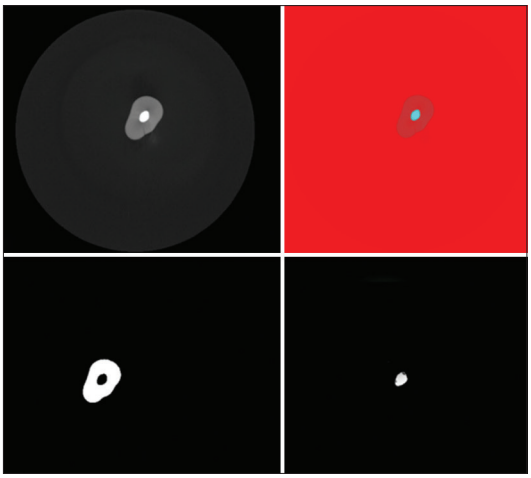

Figure 2: Calculation of mean percentages of the root filling volume and voids with micro-computed tomography analysis

and the combined voids in materials communicating with the canal walls were calculated with the micro-CT analysis [Figure 2].

Statistical Analysis

Correlation between groups was tested using the Mann–Whitney *U*-test. The Kruskal–Wallis *H*-test was used to assess differences between groups. SPSS software was used for all analyses. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Performances of the groups for both materials bioceramic-coated GP (BCGP) and GP used for obturation of the teeth regarding to root dimension are shown in Figure 3. It is worth commenting that BCGP material left result better than GP at all groups. At Endosequence group and using BCGP material, the root position was filled by 98.64% in average, whereas this amount reduced by about 3% and it recorded 95.47% in GP. The same differences were found for BioRoot group with BCGP 98.73% and GP with 96.71%. Moreover, the BCGP material at BioRoot RCS group recorded higher percentage of root filling than Endosequence and AH Plus groups.

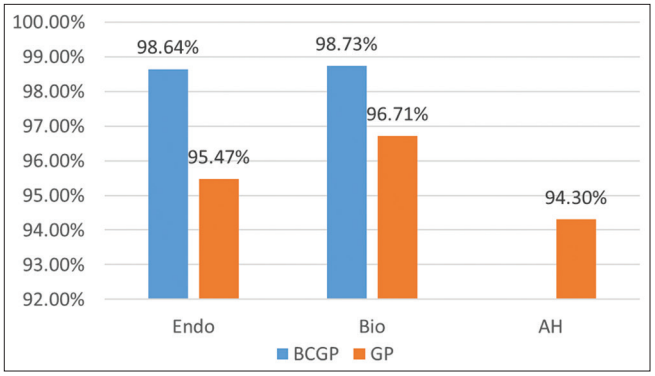

Figure 3: Root filling % according to the groups and materials

Tables 1 and 2 show mean percentage and SD of root canal filling voids in 3D volumes, along with Kruskal and Mann–Whitney *post hoc* test for BCGP and GP. Non-significant differences were found with respect to root filling percentage between both sealers in BCGP. There were non-significant differences in relation to filling material voids except for internal voids there was significantly difference between Endosequence and BioRoot bioceramic sealers in BCGP that Endosequence shows less internal voids than BioRoot in BCGP. However, in GP groups, there was a significant difference in all groups bioceramic sealers show better result than AH Plus sealer.

Tables 3 and 4 show the mean percentages (SD) of root canal filling voids of the sealers in 3D volumes in the apical, middle, and coronal thirds, along with Mann–Whitney *post hoc* test for each area with GP and BCGP. Endosequence in root filling percentage exhibits better results in both groups GP and BCGP in the apical third, while in the middle third, there were no relative differences between the sealers in both groups GP and BCGP. At the same time in the coronal third, the BioRoot and Endosequence with BCGP, there was no significant difference in root filling percentage, however, with normal GP BioRoot showed best result with AH Plus and Endosequence bioceramic.

DISCUSSION

3D obturation and decontamination are crucial following root canal treatments (Pawar et al., 2014). For effective treatment, surviving microorganisms must be coated with an appropriate root filling materials, which should block

Table 1: An overview of the percentage root filling and the void percentage (internal, external, and combined) of bioceramic-coated gutta-percha groups in the whole root canal filling materials in micro-computed tomography images

BCGP	Endosequence	BioRoot RCS
Root filling (%)		
n	30	30
Mean	0.986	0.987
SD	0.013	0.007
Mann–Whitney U (*P*)	0.095 (non-significant differences)	
Internal voids (%)		
n	30	30
Mean	0.000	0.001
SD	0.000	0.001
Mann–Whitney U (*P*)	0.000 (significant difference)	
External voids (%)		
n	30	30
Mean	0.010	0.009
SD	0.008	0.004
Kruskal test (*P*)	0.506 (non-significant differences)	
Combined voids (%)		
n	30	30
Mean	0.003	0.003
SD	0.005	0.003
Kruskal test (*P*)	0.287 (non-significant differences)	

BCGP: Bioceramic-coated gutta-percha, SD: Standard deviation

Table 2: An overview of the percentage root filling and the void percentage (internal, external, and combined) of gutta-percha groups in the whole root canal filling materials in micro-computed tomography images

GP	Endo	Bio	AH
Root filling (%)			
n	30	30	30
Mean	0.955	0.967	0.943
SD	0.033	0.027	0.024
Kruskal test (*P*)	0.003		
Pairwise comparison (Mann–Whitney U)	Bio versus AH		
Internal voids (%)			
n	30	30	30
Mean	0.001	0.002	0.002
SD	0.001	0.002	0.007
Kruskal test (*P*)	0.038		
Pairwise comparison (Mann–Whitney U)	Endo versus AH		
External voids (%)			
n	30	30	30
Mean	0.029	0.021	0.035
SD	0.02	0.016	0.016
Kruskal test (*P*)	0.006		
Pairwise comparison (Mann–Whitney U)	Bio versus AH		
Combined voids (%)			
n	30	30	30
Mean	0.016	0.011	0.02
SD	0.013	0.011	0.01
Kruskal test (*P*)	0.006		
Pairwise comparison (Mann–Whitney U)	Bio versus AH		

GP: Gutta-percha, SD: Standard deviation

microorganisms and toxins from reentering the root canal system (Saunders and Saunders, 1994). Because of

Table 3: The root filling and void percentage of the root canal filling materials of bioceramic-coated gutta-percha groups in micro-computed tomography images at different root regions (apical, middle, and coronal thirds)

BCGP groups with regions	Endo	Bio	Mann–Whitney U (*P*)	Result
Apical				
Root filling (%)				
Mean	0.993	0.987	0.005	Significant difference between Endo and Bio
SD	0.002	0.008		
Internal voids (%)				
Mean	0	0.001	0.001	Significant difference between Endo and Bio
SD	0	0.001		
External voids (%)				
Mean	0.006	0.01	0.010	Significant difference between Endo and Bio
SD	0.001	0.005		
Combined voids (%)				
Mean	0.001	0.003	0.010	Significant difference between Endo and Bio
SD	0.001	0.003		
Middle				
Root filling (%)				
Mean	0.994	0.991	0.019	Significant difference between Endo and Bio
SD	0.002	0.002		
Internal voids (%)				
Mean	0	0.001	0.496	No difference
SD	0	0.002		
External voids (%)				
Mean	0.006	0.007	0.29	No difference
SD	0.001	0.001		
Combined voids (%)				
Mean	0.001	0.001	0.29	No difference
SD	0.001	0.001		
Coronal				
Root filling (%)				
Mean	0.972	0.984	0.05	No significant difference
SD	0.013	0.007		
Internal voids (%)				
Mean	0	0.001	0.023	Significant difference between Endo and Bio
SD	0	0.001		
External voids (%)				
Mean	0.019	0.01	0.016	Significant difference between Endo and Bio
SD	0.008	0.005		
Combined voids (%)				
Mean	0.009	0.004	0.05	No significant difference
SD	0.005	0.003		

SD: Standard deviation

this, root canal filling materials are continually improving, and bioceramic sealers with coated bioceramic GP are becoming increasingly popular. This new concept helps to overwhelmed the disadvantages of conventional GP points and sealer obturation, like its incapability of the conventional GP to stick to the sealer and inability of the conventional sealers to adhere to the dentin, the solubility of the sealer, and consequently the microleakage of the root canal filling which makes the prognosis a questionable one (Nunes et al., 2008).

Table 4: The root filling and void percentage of the root canal filling materials of gutta-percha groups in micro-computed tomography images at different root regions (apical, middle, and coronal thirds)

GP groups with regions	Endo	Bio	AH	Kruskal–Wallis H (*P*)	Mann-Whitney U pairwise comparison
Apical					
Root filling (%)					
Mean	0.977	0.964	0.953	0.013	Endo versus AH
SD	0.019	0.019	0.015		
Internal voids (%)					
Mean	0.002	0.003	0.001	0.035	Bio versus AH
SD	0.001	0.003	0.001		
External voids (%)					
Mean	0.015	0.022	0.03	0.015	Endo versus AH
SD	0.012	0.012	0.009		
Combined voids (%)					
Mean	0.007	0.011	0.017	0.015	Endo versus AH
SD	0.008	0.008	0.006		
Middle					
Root filling (%)					
Mean	0.947	0.97	0.951	0.086	No difference
SD	0.026	0.024	0.025		
Internal voids (%)					
Mean	0.001	0.001	0.001	0.411	No difference
SD	0.001	0.002	0		
External voids (%)					
Mean	0.033	0.02	0.03	0.146	No difference
SD	0.016	0.015	0.017		
Combined voids (%)					
Mean	0.019	0.01	0.017	0.146	No difference
SD	0.011	0.01	0.011		
Coronal					
Root filling (%)					
Mean	0.94	0.968	0.925	0.02	Bio versus AH
SD	0.041	0.037	0.021		
Internal voids (%)					
Mean	0.001	0	0.006	0.042	Endo versus AH
SD	0.001	0	0.012		
External voids (%)					
Mean	0.038	0.021	0.044	0.024	Bio versus AH
SD	0.025	0.022	0.016		
Combined voids (%)					
Mean	0.022	0.011	0.026	0.024	Bio versus AH
SD	0.016	0.015	0.011		

SD: Standard deviation

In this study, two sealers were selected; Endosequence BC sealer is one of the ideal bioceramic sealers that contain very small nanoparticles facilitating the diffusion into dentinal tubules (Al-Haddad et al., 2015) and BioRoot RCS is a bioactive sealer that is composed of tricalcium silicate and zirconium oxide. It is estimated that the amount of calcium that leaches from BioRoot RCS is double the calcium that leaches from a similar type of bioceramic sealer, Endosequence BC sealer (Veríssimo and do Vale, 2006). These calcium ions on contact with the physiological fluids form a phase of calcium phosphate forming a zone of mineral infiltration between the sealer and dentine of the root canal. This zone aids in the biomineralization process of the sealer (Prüllage et al., 2016). Therefore, this sealer exhibits its higher sealing property.

The previous studies reported that AH Plus can be considered the gold standard for root canal sealants (Barrieshi et al., 1997); therefore, we compared this sealant to the Endosequence BC sealer and BioRoot RCS sealer with the respect of two different GP (coated with bioceramic and conventional GP).

In the present study, single-rooted teeth with single patent canals were used to minimize the variations in the canal anatomy that could possibly affect the results. The canal diameter was enlarged to a standard size 35/06 and the root length was standardized to 12 mm to standardize the samples.

Removing smear layer before obturation enhances the sealing of the root canal (Kahn et al., 1997). Hence, 17% EDTA was applied for 1 min to effectively open the dentin tubules. The low surface tension of EDTA also facilitates its access into the dentin tubules to remove the smear layer (Yilmaz et al., 2011).

By the concept, bioceramic sealers have been developed for using with bioceramic-coated GP cone to achieve single unit of root canal obturation. Sealing effectiveness of bioceramic impregnated cone sealer system was superior to that of conventional GP sealer combinations in this study that there was less void present in bioceramic-coated GP groups than conventional GP groups. This result may because of interestingly, chemical adhesion between bioceramic-coated GP cone and bioceramic sealer (Wang, 2015) could be helpful in avoiding the long-term leakage, which would be superior to the conventional obturation materials, the minimal gaps could still be detected in the obturated root canals from the micro-CT image. It seems that irregularities in the prepared root canals could not be completely filled with the sealer, and this could be a pathway of bacterial leakage.

In the present study, micro-CT was performed to reveal void and void-free regions in the obturated root canals. None of the obturated canals were void or gap free. This is consistent with other previous findings, which showed that minor voids were detected in all obturated root canals using different obturation techniques (Epley et al., 2006; Hammad et al., 2009; James et al., 2007). As demonstrated, all tested sealers had less void percentage in the apical part and more void percentage in the cervical part.

Bioceramic materials contain alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates (Koch and Brave, 2012). The alkaline nature of bioceramic by-products has been documented to denature collagen fibers, which assists the sealer penetration into the dentin tubules (Balguerie et al., 2011). However, AH Plus is certainly acidic, thus its bonding may limit to dentin. Moreover, AH Plus contains a polymer that contracts on polymerization, which may result in sealant cracking and deterioration. Thus, it is expected for these reasons that generally bioceramic sealer showed less void than AH Plus, but has yet to be confirmed by further *in vivo* follow-up studies.

CONCLUSION

Within the limitations of this study, it could be concluded that none of the groups were free of voids, bioceramic-coated GP with bioceramic sealer significantly better than conventional GP. BioRoot and Endosequence bioceramic sealers with bioceramic-coated GP have no significant difference and best result among all groups.

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