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Evaluation of Root Canal Sealer Filling Quality Using a Single-Cone Technique in Oval Shaped Canals: An *In Vitro* Micro-CT Study

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Summary: The aim of this study was to evaluate and compare the presence of voids in oval root canals filled with different root canal sealers (EndoSequence BC Sealer, Smartpaste bio, ActiV GP) and to compare those with root canals filled with AH Plus sealer using micro-CT. In total, 40 freshly extracted human single-root maxillary premolars were used. Specimens instrumented with the EndoSequence NiTi rotary instrument were assigned randomly into four groups. In each group, root canals were filled with single-cone gutta-percha and one of the tested sealers. Each specimen was then scanned using micro-CT at a voxel resolution of 13.47 μm. Proportions of sections with voids in cross-sectional images and void volumes for each sealer were calculated in the apical, middle, and coronal thirds. Differences according to root canal sealers were evaluated statistically using the Kruskal–Wallis test and the Mann–Whitney U-test at a significance level of 5%. The analysis showed a decrease in void formation in the apical third, with a significant difference between the apical and coronal thirds among bioceramic sealers, ActiV GP, and AH Plus ($p < 0.05$) but no significant difference between the apical and middle thirds or between the middle and coronal thirds was found for the sealers tested ($p > 0.05$). All root canal sealers tested resulted in voids. The bioceramic sealers (EndoSequence BC Sealer, Smartpaste bio) produced similar voids which had the fewest in the apical third of root canals among the sealers tested which can be related due to root canal anatomy variations. SCANNING 38:133–140, 2016. © 2015 Wiley Periodicals, Inc.

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Introduction

Success in endodontic treatment depends on the 3-dimensional filling of the root canal to prevent residual bacteria and their toxins from affecting the periapical tissues (Michaud *et al*., 2008; Ozok *et al*., 2008). Many endodontic root canal filling materials, techniques, and sealers have been developed for this purpose. Gutta-percha is commonly used with sealers to provide a fluid-tight seal. Root canal sealers fill the voids between gutta-percha points and between gutta-percha and root canal walls; for this reason, sealers are essential to optimize the outcome of the root canal treatments and prevent reinfection (Sönmez *et al*., 2012).

Microleakage is a major cause of endodontic failure, which may occur between the gutta-percha and sealer, between the sealer and dentin, or through voids within the sealer (Hovland and Dumsha, 1985). Thus, the quality of a root canal filling and the success rate of endodontic treatment depend greatly on the sealing ability of a root canal sealer (Wu *et al*., '94).

Although gutta-percha and traditional sealers have been the most commonly used materials for obturation of endodontically treated teeth, this standard approach fails to prevent leakage effectively within the root canal system and has been referred to as the "weak link" in endodontic treatment (Magura *et al*., '91). Thus, new materials are being developed continuously to improve the seal of endodontic obturation and to help minimize the likelihood of microleakage.

Calcium phosphate silicate ceramic-based sealers, which can also serve as repair cements, have been introduced in endodontics recently (Damas *et al*., 2011; Zoufan *et al*., 2011; Nagas *et al*., 2012). EndoSequence BC Sealer (Brasseler USA, Savannah, GA; also previously known as iRoot SP Injectable Root Canal Sealer, Innovative BioCeramix, Inc., Vancouver, BC, Canada) and Smartpaste bio (DRFP Ltd., Stamford, UK) are examples of calcium phosphate silicate ceramic-based sealers. EndoSequence BC Sealer and Smartpaste bio were developed to improve the sealing of root canal fillings. According to their manufacturers, they consist of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agents. These materials are premixed, ready-to-use, injectable bioceramic cement pastes with calibrated intracanal tips (Hess *et al*., 2011). ActiV GP (Brasseler USA) is a root canal filling system that consists of glass ionomer-coated gutta-percha (ActiV GP cone) cones that are bondable to intra-radicular dentin through the use of a glass ionomer sealer (ActiV GP sealer). It has been described as a tertiary monoblock system in which there are three interfaces between the bonding substrate and the bulk material core (Tay and Pashley, 2007; Nikhil *et al*., 2012).

The knowledge of morphological characteristics and variations of root canal plays an important role in the success of treatment (Vertucci, ’84). Oval or ribbon-shaped canals occur in approximately 25% of teeth which the preparation and filling of these canals are challenging (Wu *et al*., 2000). Rotary nickel titanium (Ni–Ti) instruments are tending to prepare a central circular bulge. However, using Ni–Ti instruments can lead to non-instrumented areas in the buccal and lingual sides of the root canal which occur irregular shape after root canal preparation (Rödig *et al*., 2002). Moreover, for irregular shaped canals together with round profiles and smooth taper are lead to difficulties of the root canals fillings because of the post-preparations of the canals.

Currently, various filling techniques are being used for 3-dimensional homogenous filling, of root canal system. Using the single-cone technique with matched-taper gutta-percha cones is popular after root canal preparation with rotary instruments. Because it allows better adaptation in 3-dimensional preparation (Cavenago *et al*., 2012), and it reduces the time spent on the lateral compaction technique (Tasdemir *et al*., 2009). However, the single cone technique may result in voids in irregular-shaped canal (Weis *et al*., 2004; Bergmans *et al*., 2005). Furthermore, Gordon *et al*. (2005) reported that despite of effectiveness of a single cone technique in filling of canals prepared by rotary Ni–Ti instruments, its ability to fill an oval or irregular canal space was clearly diminished by its shape.

Because of the bioceramic sealer does not shrink upon setting which approximately expands 0.002%, has an excellent flowability and dimensional stability. The use of a single-cone filling technique is recommended by studies. Moreover, it was indicated that bioceramic root canal filling materials can be used for filling root canals with or without gutta-percha points (Zhang *et al*., 2010; Taşdemir *et al*., 2014) and requires the presence of water to set and harden (Zhang *et al*., 2009).

However, the studies on sealing capability of bioceramic root canal filling materials in terms of void for various root canal anatomy especially in oval canals using single cone techniques are limited. Hence, the aim of this study was to evaluate and compare the presence of voids in oval root canals filled with different root canal sealers (EndoSequence BC Sealer, Smartpaste bio, ActiV GP) and to compare those with root canals filled with AH Plus sealer using micro-CT. The null hypothesis was that, the bioceramic root canal filling materials can fill effectively the root canal with similar voids occurrence in comparison to resin and glass ionomer based root canal sealers using single cone techniques.

Materials and Methods

A power analysis (Power and Precision software, Biostat, Englewood, NJ) was conducted that indicated the detection of differences between root canal root canal root canal filling materials could be obtained with at least 35 teeth at a power of 0.8 ($\alpha = 0.05$). Thus, this study was conducted using 40 extracted teeth.

Sample Preparation

In our study, 40 extracted human single-root maxillary premolars without caries, root resorption, or fractures were used. Root surfaces were scaled with a Gracey curette to remove soft tissue, calculus, and bone. Each tooth was placed in 5.25% sodium hypochlorite (NaOCl) for 2 h for surface disinfection, and then stored in distilled water until testing was performed.

Before starting the experiment, a pre-operative radiograph was taken to evaluate the anatomy of the extracted teeth before their selection. After inspection with an optical microscope (OPMI pico; Zeiss Co., Jena, Germany), only specimens with oval canals were selected, to standardize the root canal 3D configuration.

All teeth were decoronated at the cemento–enamel junction and adjusted so that each root was approximately 12 mm in length. Subsequently, a size #10 K-File (Maillefer, Ballaiges, Switzerland) was inserted into the root canal until the tip was just visible beyond the apex. Working length was determined by subtracting 1 mm from this length. All samples treated and measured had the same taper, of 0.06. Accordingly, 40 experimental teeth were instrumented to a size 40/06 using a crown-down technique with an EndoSequence 0.06 taper NiTi rotary instruments (Brasseler, USA). Irrigation was performed with 2 mL 2.5% NaOCl between each instrument. A final rinse with 2 mL 2.5% NaOCl, 2 mL 17% EDTA (Patterson Dental Supply, Dallas, TX) for 1 min, and 10 mL distilled water was performed. Then, the canals were dried with paper points (Dentsply Tulsa Dental, Johnson City, TN).

Root Canal Filling

Teeth were assigned randomly into four experimental groups (10 roots each). For the random selection, lots were drawn by an investigator who was blinded to the treatments. Root canal sealers were prepared in accordance with the manufacturers' recommendations and then the experimental groups of teeth were filled with a single-cone technique. Group 1 was filled with AH Plus root canal sealer (Dentsply DeTrey, Konstanz, Germany) and 40.06 gutta-percha (Maillefer). Group 2 was filled with EndoSequence BC Sealer and 40.06 gutta-percha (Maillefer). Group 3 was filled with Smartpaste bio and 40.06 gutta-percha (Maillefer), and Group 4 was filled with ActiV GP and a 40.06 ActiV GP cone. After the filling process, roots were stored at 37°C at 100% humidity for 5 days to ensure the sealer was set. The application and the approximate amount of sealers used in each root canal followed Gandolfi *et al.* (2013).

Micro-CT Evaluation

A high-resolution, desktop micro-CT system (Bruker Skyscan 1172, Kontich, Belgium) was used to scan the specimens. The scanning conditions were: 100 kVp, 100-mA beam current, 0.5-mm Al/Cu filter, 13.47 μm pixel size, rotation at 0.5 step. To minimize ring artifacts, air calibration of the detector was carried out prior to 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. 1.12.9, SkyScan) were used for the visualization and quantitative measurements of the samples, which used the modified algorithm described by Feldkamp *et al.* ('89) to obtain axial, 2-dimensional, $1000 \times 1{,}000$-pixel images. For the reconstruction parameters, ring artifact correction and smoothing were fixed at 0 and the beam artifact correction was set at 40%. Contrast limits were applied following SkyScan's instructions. By 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 1,023 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 were assessed in 2D slices following Moeller et al.'s (2013) 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 2,048–2,560 at 75 Hz and 0.17-mm dot pitch operated at 11.9 bits. New cross-sections 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-sections images. The micro-CT images of the sections were then converted to tiff files and coded. Each section was assessed by two observers (BC, KO) independently, using a binary registration scale: internal, external, and combined voids (Fig. 1). 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 re-examined 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 gutta-percha, sealer, and voids using CtAn (ver. 1.12.9, SkyScan). 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).

The mean percentages of the root filling volume (sum of the volume of the gutta-percha and the endodontic sealer), the volume of internal voids distributed inside the root canal root canal filling material, the external voids along the canal walls, and the combined voids in materials communicating with the canal walls, were calculated with the micro-CT analysis (Fig. 2).

Statistical Analysis

Kruskal Wallis test was performed first, and then, in cases where statistically significant p-values were

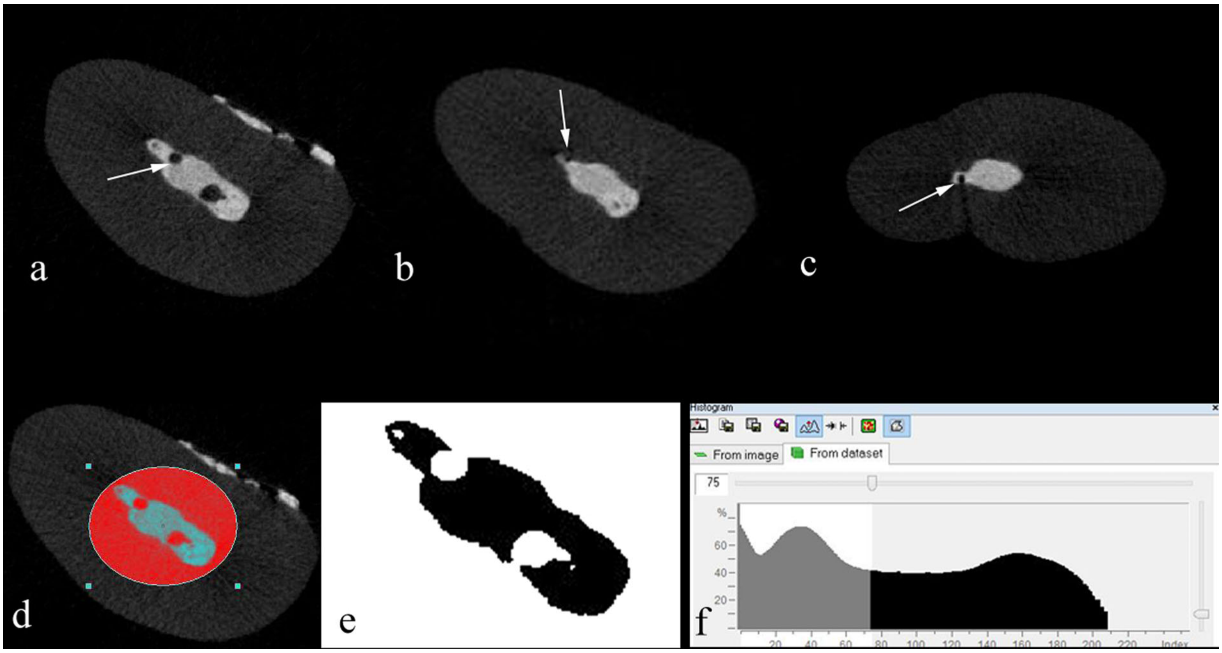

Fig 1. Micro CT images showing (a) internal void, (b) external void, and (c) combined void, (d) ROI selection on images, (e) void detection inside ROI, (f) binarization of the image by the gray-level histogram.

found, Mann–Whitney's U post-hoc tests carried out for pairwise comparisons. These analyses were performed with the SPSS software (ver. 20; Chicago, IL) at significance level of $\alpha = 0.05$.

Results

Table I summarizes the mean percentage values ($\pm$ SD) of root canal filling materials and voids. A high

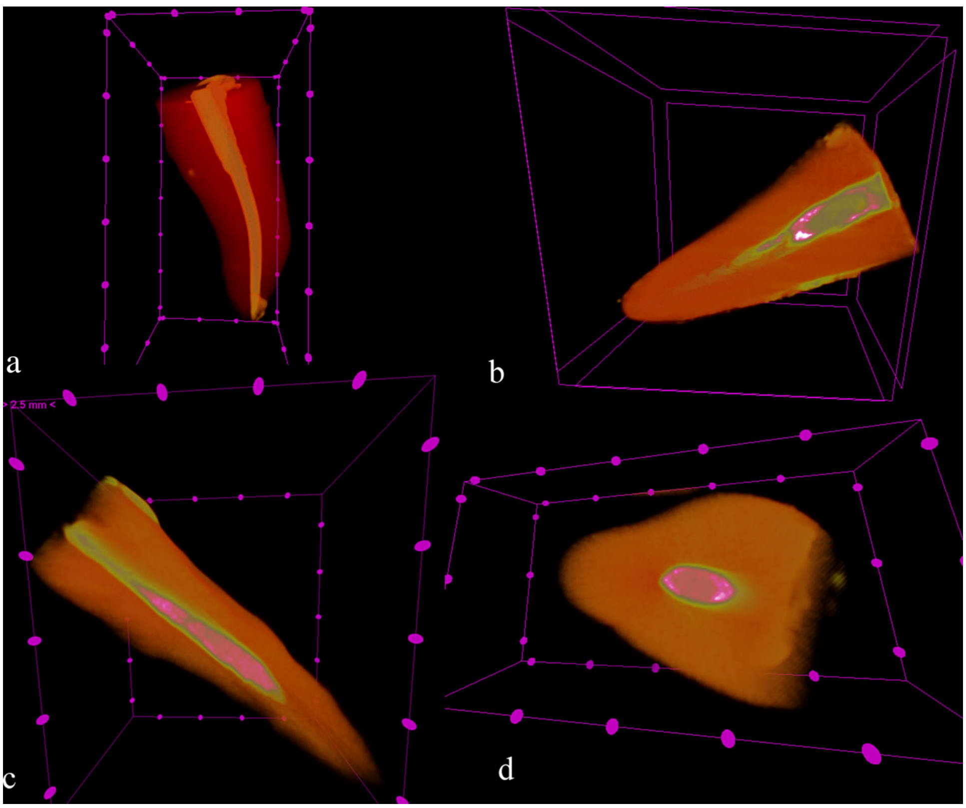

Fig 2. (a) 3D representation of the root, (b, c, d) images showing gutta-percha in pink, sealer in green, and voids in white.

TABLE I Table showing the mean percentage (standard deviation) both the propotions of section with voids in 2D slices and the root canal filling materials volume voids in 3D images

	Root canal filling materials	n	Mean	SD	Statistical analysis: p	Statistical analysis: Pairwise comparisions
Propotions of section with voids (%)	EndoSequence BC sealer	10	65.5 (24.4–100)	22.8	0.456	p > 0.05
	Smartpaste bio	10	61.4 (22.4–100)	21.5		
	ActiV GP	10	64.3 (25.4–100)	18.8		
	AH plus	10	72.7 (38.2–100)	20.7		
	Total	40	66.0 (22.4–100)	20.6		
Root filling (%)	EndoSequence BC sealer	10	98.424	1.245	0.738	p > 0.05
	Smartpaste bio	10	97.276	1.202		
	ActiV GP	10	98.212	1.327		
	AH Plus	10	97.890	1.236		
	Total	40	97.950	1.252		
Internal voids (%)	EndoSequence BC sealer	10	0.389	0.214	0.826	p > 0.05
	Smartpaste bio	10	0.414	0.315		
	ActiV GP	10	0.487	0.362		
	AH plus	10	0.478	0.331		
	Total	40	0.442	0.305		
External voids (%)	EndoSequence BC sealer	10	0.859	0.645	0.792	p > 0.05
	Smartpaste bio	10	0.738	0.558		
	ActiV GP	10	0.652	0.489		
	AH plus	10	0.70	0.523		
	Total	40	0.884	0.625		
Combined voids (%)	EndoSequence BC sealer	10	0.624	0.524	0.786	p > 0.05
	Smartpaste bio	10	0.576	0.402		
	ActiV GP	10	0.565	0.569		
	AH plus	10	0.589	0.442		
	Total	40	0.663	0.484		

frequency of voids was found with all root canal filling materials. Overall, in relation to the proportion of micro-CT sections with voids, the root filling techniques did not differ significantly ($p = 0.456$; Table I). Moreover, no significant difference was found in the percentage of root canal filling material volume and voids. All root canal filling materials used showed similar filling abilities.

Table II shows the percentage of volume of voids in detail, in the apical, middle, and coronal thirds according to root canal filling materials. The analysis showed considerable reduction of voids in terms of combined voids in the apical third, with significant differences between apical and coronal third of the teeth ($p < 0.05$). However, no significant difference was found between the

TABLE II Mean percentage (standard deviation) percentage of the root canal filling materials voids in 3D volumes according regions (apical, middle, coronal thirds)

Regions	Root canal filling materials	n	Mean	SD	p-value	Pairwise comparisions
Apical third	EndoSequence BC sealer	10	0.214	0.196	p < 0.05*	p > 0.05
	Smartpaste bio	10	0.278	0.262		
	ActiV GP	10	0.358	0.218		
	AH plus	10	0.299	0.287		
Middle third	EndoSequence BC sealer	10	0.388	0.328	p > 0.05	p > 0.05
	Smartpaste bio	10	0.487	0.37		
	ActiV GP	10	0.525	0.36		
	AH Plus	10	0.504	0.34		
Coronal third	EndoSequence BC sealer	10	0.818	0.805	p < 0.05*	p > 0.05
	Smartpaste bio	10	0.976	0.834		
	ActiV GP	10	1.545	0.907		
	AH plus	10	1.564	0.905		
Overall total		40	0.663	0.484		

*Statistically significant differences of apical and coronal third ($p < 0.05$).

apical and middle or middle and coronal thirds ($p > 0.05$) for any root canal filling material tested in this study.

Discussion

Conventional methods include leakage studies using dyes or alternative tracers (Wu and Wesselink, '93), such as fluid filtration, dye penetration, radioisotopes, bacterial penetration, and saliva leakage. Previous studies have indicated that conventional methods have disadvantages, such as being time-consuming, and cannot be standardized (Siqueira *et al.*, 2000). Moreover, the pressure used in the fluid filtration method cannot be appropriately standardized (Pommel and Camps, 2001), dye penetration studies do not simulate the true clinical situation, and dye studies demonstrate that air entrapped in voids along the root canal filling may hinder fluid movement (Verśsimo and do Vale, 2006). Bacterial micro-leakage studies involve long periods of observation and do not allow quantification of the number of penetrating bacteria (Siqueira *et al.*, 2000). Recently, micro-CT analysis began to be used because it is a non-destructive analytical method that provides objective data. Specimens can be examined both quantitatively and qualitatively: volumes can be calculated with dedicated software, while it is also possible to localize specific details with visual image analysis. This technology is capable of distinguishing root canal filling materials, voids, and tooth structures with high accuracy and spatial resolution (Jung *et al.*, 2005).

Several studies have been reported using micro-CT data for the evaluation of voids and the filling quality of sealers (Metzger *et al.*, 2010; Flores *et al.*, 2011; Somma *et al.*, 2011; Zogheib *et al.*, 2012; Naseri *et al.*, 2013; Keleş *et al.*, 2014; Li *et al.*, 2014; Wolf *et al.*, 2014). Naseri *et al.* (2013) studied voids with different filling techniques with micro-CT using AH-26 and determined that all samples had some voids with all the different filling techniques. Somma *et al*. (2011) used three different filling techniques with AH Plus and indicated no significant difference in detecting voids. The mean values of root filling for the methods were between 98.167% and 99.023%, while the internal voids were between 0.059% and 0.322%. Similarly, the external voids were between 0.485% and 0.94%, and the combined voids were 0.273–0.828%.

Gandolfi *et al.* (2013) investigated the voids with AH Plus and MTA Flow sealers using Thermofill filling. They separated the teeth into three parts apical, middle, and coronal thirds in assessing voids. They indicated that MTA Flow had fewer voids in the apical third than AH Plus, whereas similar voids were seen in the middle and coronal thirds after 7 days of storage. When examined separately, the apical part of the teeth had the fewest gaps. Moeller *et al.* (2013) used AH Plus with two different techniques in micro-CT examinations. They found that a high frequency of voids was present with both techniques, increasing from the apical towards the cervical part. They also indicated that the proportion of micro-CT examinations with voids was between 15.8% and 100%, with a mean of 65.9% for lateral compaction and 66.9% for a hybrid technique. In a similar study, Wolf *et al.* (2014) used three canal sealers that were investigated using micro-CT. In the coronal, middle, and apical thirds, root canals filled with 2 Seal showed the highest percentage of volume of voids, whereas canals filled with RoekoSeal showed the lowest percentage ($p < 0.05$).

In this study, the region-dependent increase in voids and gap formation from the coronal to the apical was more pronounced in the 2 Seal-treated groups than the others. Keleş *et al.* (2014) investigated voids with AH Plus with two different techniques in the apical, coronal, and middle thirds of the teeth. The mean volumes of root fillings were between 95.74% and 99.43%. For all sections, the mean voids were between 0.57% and 4.26%. In this study, none of the filling techniques was able to completely fill the root canal spaces. Li *et al.* (2014) performed a similar study to ours also using micro-CT. They divided the teeth into three regions as (0–4, 4–8, and 8–12 mm) and investigated three different filling techniques using ThermaSeal Plus sealer. They stated that canals filled with Guttacore carriers had the lowest incidence of interfacial gaps and voids, although the results were not significantly different from canals filled by warm vertical compaction.

In our study, we measured the mean percentage volume of root fillings and voids in detail in the apical, middle, and coronal thirds using various root canal sealers. The mean volumes of root filling were between 97.276% and 98.424%, similar to previous studies (Somma *et al.*, 2011; Naseri *et al.*, 2013). It seems that even when filling techniques differ, similar results can be obtained for root canal fillings, as noted by Somma *et al.* (2011). Based on our results, all materials resulted in some voids in all three thirds of the teeth. All root canal root canal filling materials had similar performances in root canal filling. However, significant differences were found in terms of combined voids in the apical third versus the coronal third of the teeth. Moreover, in the Moeller *et al.* (2013) study, the mean proportion of micro-CT examinations with voids was between 65.9% and 66.9%, which is similar to our results, 64.3–72.7%, with no significant difference among the root canal sealers tested.

Based on our results, similar gaps and voids were found for all the root canal sealers tested. In all preparation techniques and also sealers produced voids. In our opinion, these voids are closely related to the root canal anatomy rather than the root canal filling material or technique. Only very limited studies were conducted on root canal anatomy esp. using Micro-CT level (Filpo-Perez *et al.*, 2015). Generally, the morphology of the root canal varies greatly in shape and transversal cross-sections in different groups of teeth (Wu *et al.*, 2000; Versiani *et al.*, 2013). In a recent study, the roundness of the root canal were investigated from apical third at every 1-mm interval from the apical foramen to the 5-mm level. It was figured out that the morphology of the canals were changing dramatically. The median values of roundness and aspect ratio indicated a prevalence of oval-shaped canals in the last 2 mm and long oval-shaped canals at the 3-, 4-, and 5-mm levels. In our opinion, the root canal anatomy is a dynamic anatomy that can be changing from the level of the root. Thus, this anatomical variation leads to voids in coronal more than middle and apical thirds without significant difference of the materials.

In terms of root canal sealer comparison, although EndoSequence BC sealer and Smartpaste bio and the other root canal filling materials provided similar filling quality ($p > 0.05$), bioceramic root canal filling materials can be recommend because of small particle size (less than 2 μm), and excellent level of viscosity which are capable of flow into dentinal tubules based on the results of this study (Ersahan and Aydin, 2010; Koch and Brave, 2012; Nagas *et al.*, 2012). Additionally, the calcium silicate content of the root canal fillings helps to exhibit minimal or no shrinkage during the setting phase (Nagas *et al.*, 2012). When comparing AH plus, the chosen AH plus, nowadays strongly recommends for its excellent physicochemical and biological properties (Guinesi *et al.*, 2014). The reason for the similar results can be interpreted by the expansion during their setting reaction (Storm et al., 2008; Gandolfi *et al.*, 2013). On the other hand, no differences were observed between roots filled with ActiV GP system and the others. This could be also related to the tertiary monoblock system in which there are three interfaces between the bonding substrate and the bulk material core. Thus, combined using of ActiV GP and its glass-ionomer sealer produce a true monoblock. Tay and Pashley (2007) have reported the superior bonding of ActiV GP to root canal dentin.

The results of this can be interpreted in terms of clinical relevance as; Bioceramics and ActiV GP sealers can be used effectively as and AH Plus in oval root canals using single cone technique. However, clinical long-term studies are necessary to support the confident use of these materials.

In this study, an *in vitro* model was used therefore the expansion of sealing capacity could not be tested in due time. In a recent review by Prati and Gandolfi (2015) stated that bioceramic root canal filling materials can expand by 0.2–6% of the initial volume. Water sorption induces some expansion and makes a strong contribution to the sealing capacity. It was also pointed out by Gandolfi *et al.* (2009), proteins (for example from soaking medium or from *in vivo* body fluids) reduce the expansion of hydraulic calcium silicate cements and increase the setting time. As it was stated, there may be expansion of the bioceramic materials *in vivo* conditions. In our opinion, further researchers should be performed according to figure out this expansion property of the materials.

A limitation of this study is that it was based on the single-cone technique; the bioceramic root canal sealers used in this study should also be tested with other filling techniques. Thus, further studies should be conducted for full comparisons using both different filling techniques and root canal sealers in combination.

Conclusions

All root canal sealers tested resulted in voids. The null hypothesis was accepted as the bioceramic root canal filling materials can fill effectively the root canal with similar voids. The bioceramic sealers (EndoSequence BC Sealer, Smartpaste bio) produced similar voids which had the fewest in the apical third of root canals among the sealers tested which can be related due to root canal anatomy variations.

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