Original papers

Evaluation of new bioceramic endodontic sealers: An in vitro study

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Abstract

Background. The biophysical properties of root canal sealers (RCSs) positively affect the success of endodontic treatment. It is important to ensure an impermeable apical seal after the thorough eradication of the infection. Since bioceramic sealers release bioactive and concomitantly biocompatible products after setting, chemical bonding to dentin and favorable healing is achieved.

Objectives. This study evaluated the chemical composition and elemental distribution of 4 RCSs (1 resin-based and 3 bioceramic-based) by using energy dispersive X-ray spectroscopy (EDX), field emission scanning electron microscopy (FE-SEM) and elemental mapping after root canal obturation, both coronally and apically.

Material and methods. Forty extracted single-rooted teeth were shaped, cleaned and randomly divided into 4 groups according to the type of sealer used for obturation. After the sealer set, the teeth were sectioned horizontally to obtain coronal and apical standardized sections. The sections were qualitatively and quantitatively assessed in terms of chemical composition of the tested sealers, using SEM images and elemental mapping as well as the EDX analysis.

Results. All of the calcium silicate sealers showed significantly higher peaks of calcium at the periphery of the root canals, contacting dentinal moisture, and high peaks of zirconium, while tungsten was significantly high in AH Plus™. TotalFill® BC™ and BioRoot™ RCS showed higher calcium, oxygen and silicon content coronally than apically, while phosphorus was only detected more apically, which was different for EndoSeal® MTA. All sealers revealed small amounts of different heavy metals, not described by their manufacturers, and a uniform particle distribution with almost regular surfaces.

Conclusions. All of the tested sealers except AH Plus revealed high calcium/phosphorus ratio peaks, suggesting regenerative potential in vivo, with acceptable purity and surface texture, and supporting their biocompatibility, with chemical bonding to root dentin.

Keywords: bioceramic, root canal sealers, energy dispersive X-ray spectroscopy, scanning electron microscopy, heavy metals

Introduction

An adequate impermeable three-dimensional (3D) seal of the thoroughly disinfected root canal system is traditionally achieved using a gutta-percha core material and accessories cemented with a root canal sealer (RCS). Different types of sealers, including zinc oxide–eugenol (ZnO/E), calcium hydroxide ($CaOH_2$), glass ionomer (GI), silicone, epoxy resin or methacrylate resin, and bioceramic-based sealers, have been introduced into the market. These sealers differ in terms of their main chemical composition.[1]

Cytotoxicity and the absence of bonding to dentin, as well as the absence of hard tissue deposition, have been detected with ZnO/E sealers.[2] To overcome these shortcomings, resin-based sealers were introduced.[3] AH Plus™, the most widely used epoxy resin-based sealer, revealed good sealing ability, lower solubility in tissue fluids and minimal cytotoxic reactions, but no bioactivity with the surrounding tissues.[3]

The single-cone obturation method has been reintroduced to decrease the time, effort and extra forces required when using the lateral compaction technique. However, then, lower bond strength and adaptation to root canal dentin were observed. Hence, there is great demand to achieve the 'monoblock concept' through bonding to the core material and the dentinal walls, thus improving bond strength and adaptation.[4]

Since the 1970s, the application of ceramics in biomedicine has greatly expanded[5,6]; they can be either bioactive or bioinert in nature, depending on their interaction with vital tissues. Unlike bioinert ceramics, bioactive materials (bioactive glass, calcium silicates (Ca_2SiO_4) and calcium phosphates ($Ca_3(PO_4)_2$)) interact with the surrounding tissues to encourage the growth and regeneration of more durable mineralized tissues.[1,5,7]

Hydraulic calcium silicates, or a combination of Ca_2SiO_4 and $Ca_3(PO_4)_2$ have been used as bioceramic-based sealers. They show unique bioactivity, as they set and harden in the presence of moisture, finally forming hydroxyapatite at the interface and creating a bond to dentin. These can be mineral trioxide aggregate (MTA)-based, non-MTA-based or calcium phosphate-based types.[1,6] In the presence of moisture, phosphate ($(PO_4)_2^{3-}$) partially reacts with Ca_2SiO_4 hydrogel and $CaOH_2$ to form hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) along the mineral infiltration zone.[1] The excellent biocompatibility of bioceramic sealers is most likely due to their similarity to biological $Ca_{10}(PO_4)_6(OH)_2$, as well as the presence of $Ca_3(PO_4)_2$, the main inorganic component of hard tissues. Bioceramic-based sealers have been observed to promote bone regeneration when unintentionally extruded periapically during root canal filling.[8]

The chemical composition of sealers determines their biophysical properties, which influence the formation of a hermetic seal, thus promoting the healing and regeneration of periapical tissues.[9]

Therefore, the aim of this study was to use energy dispersive X-ray spectroscopy (EDX) and field emission scanning electron microscopy (FE-SEM) to identify and characterize chemical elements and their distribution in AH Plus, EndoSeal® MTA, TotalFill® BC™, and BioRoot™ RCS sealers, along with comparing their bioactive and heavy metal content.

Material and methods

The chemical composition of the 4 different endodontic sealers is presented in Table 1. Forty sound, freshly extracted, single-rooted, single-canaled teeth with complete apices were used in this study (Fig. 1A).

Prior to sample preparation, the teeth were disinfected through immersion in 2.5% sodium hypochlorite solution (NaOCl) for 2 h, rinsed, and finally stored in saline solution.

The crowns were cut perpendicular to the long axis of the teeth with a diamond disk mounted on a slow-speed handpiece with a coolant, obtaining a root length

Table 1. Description of the materials used in the present study

Material	Type	Composition	Batch/ Manufacturer
AH Plus	epoxy resin-based sealer	Paste A (epoxide paste): diepoxide; calcium tungstate; zirconium oxide; iron oxide; and pigments Paste B (amine paste): 1-adamantanamine; N,N′-dibenzyl-5-oxanonandiamine-1,9; TCD-diamine; calcium tungstate; zirconium oxide; aerosil; and silicon oil	1705000906 Dentsply De Trey, Konstanz, Germany
EndoSeal MTA	bioceramic (mineral trioxide aggregate) sealer	Premixed syringe contains: calcium silicate; calcium aluminates; calcium sulfate; radiopacifier (zirconium oxide); bismuth oxide; thickening agent (hydroxypropyl methylcellulose); and N-methyl-2-pyrrolidone solvent	CI 171027A Maruchi, Wonju, South Korea
TotalFill BC	bioceramic sealer	Premixed syringe contains: zirconium oxide; calcium silicate; calcium phosphate; monobasic calcium hydroxide; fillers; and thickening agents	160045P FKG Dentaire, La Chaux-de-Fonds, Switzerland
BioRoot RCS	bioceramic sealer	Powder: tricalcium silicate; opacifier (zirconium oxide); and povidone (hydrophilic biocompatible polymer) Liquid: water; calcium chloride; and polycarboxylate (water-reducing agent)	B20645 Septodont, Saint-Maur-des-Fossés, Cedex – France

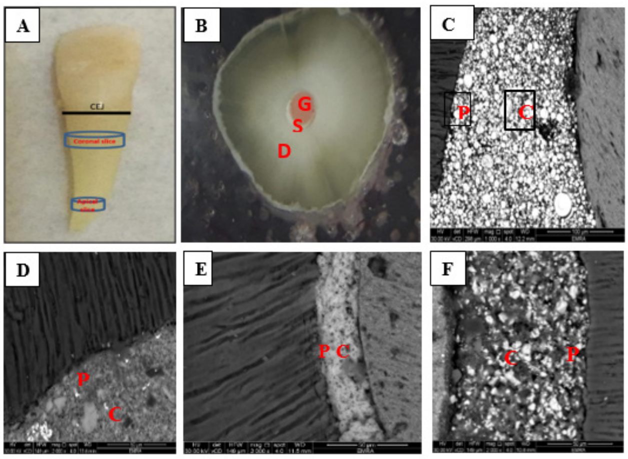

Fig. 1. A – photograph showing the sites of the planned apical and coronal slices (each of a thickness of 2 mm) below the cemento-enamel junction (CEJ), used for the energy dispersive X-ray spectroscopy (EDX) and field emission scanning electron microscopy (FE-SEM) assessments; B – photograph of the sliced root portion, showing the master cone (G), the sealer (S) and dentin (D); C–F – SEM images with the planned areas of the EDX analysis at the core (C) and periphery (P) of different sealers – AH Plus, EndoSeal MTA, TotalFill BC, and BioRoot RCS, respectively

of 15 ±1 mm. A K-file size 10 (Mani Inc., Takanezawa, Japan) was introduced to ensure the patency of the root canal and the working length of the canal was determined with a K-file size 15 (Mani Inc.). Each canal was irrigated with 5 mL of 2.5% NaOCl and instrumented up to size 20. An R40 reciprocal file (tip size 40 and a taper of 0.06 mm, VDW.SILVER® RECIPROC® endomotor; VDW, Munich, Germany) was used according to the manufacturer's instructions with a reciprocal motion to ensure a uniform internal width of the canal lumen.

A total of 5 mL of 2.5% NaOCl was used between the instrumentation steps, followed by 17% ethylenediaminetetraacetic acid (EDTA) (Prevest DenPro, Bari Brahmana, India) to remove the smear layer, and finally the canals were irrigated with 3 mL of 9% saline solution. The canals were gently dried using a dental ENDO Aspirator® root canal tip (Cerkamed, Stalowa Wola, Poland).

The teeth were randomly and equally divided into 4 groups according to the sealer used (n = 10 per group): AH Plus (group 1); EndoSeal MTA (group 2); TotalFill BC (group 3); and BioRoot RCS (group 4).

Each sealer was mixed and introduced into the canals according to the manufacturers' instructions; for AH Plus, additional dryness was achieved using matched paper points size 40. For obturation, a gutta-percha cone (tip size 40 and a taper of 0.06 mm) was dipped into the corresponding sealer, and then inserted and pressed to the full working length by means of a carrier. Excess material was removed with a heat carrier by applying a vertical compaction motion. After initial setting, the cavity was finally sealed with a GI filling material (Medicem; Promedica Dental Material, Neumünster, Germany). The teeth were placed on Petri dishes and stored in an incubator (37°C at 100% relative humidity) for 7 days.

Next, the teeth were placed into auto-polymerizing methyl methacrylate acrylic resin blocks. The roots were sectioned horizontally at the coronal (10 mm from the apex) and apical (4 mm from the apex) levels to obtain 2-millimeter-thick coronal and apical slices (Fig. 1A,1B).

The surfaces of both coronal and apical slices were examined at the core and periphery of the set sealers by using FE-SEM (model Quanta 250 FEG, Field Emission Gun; FEI Europe, Eindhoven, the Netherlands) with an accelerating voltage of 30 kV, magnification of ×14 up to ×2,000, a resolution of 132 eV, and an amplification time of 12.8 μs under vacuum. The chemical composition and elemental distribution of the sealers were determined using an EDX unit with the Noran System SIX (NSS) spectral analysis software, v. 2.3 (Thermo Fisher Scientific, Suwanee, USA) in the non-standard analysis mode (for automatic elemental identification) and with the phi-rho-Z (PROZA) correction. A dimensionally standardized rectangular area of analysis was set for all samples at the central and peripheral regions of the apical and coronal slices (Fig. 1C–1F).

Elemental maps were built according to the Net Counts Method microanalysis[10] at high resolution, showing surface regularity, elemental distribution, and the particle size and shape. The results were evaluated qualitatively, based on FE-SEM images and elemental mapping, and quantitatively, taking into account the element weight (wt%) and atomic (at%) percentages recorded during the EDX analysis. The particle size was measured using the ImageJ software (ImageJ 1.52d, Wayne Rasband, National Institutes of Health, USA). The scale measurements were calibrated for every image, marking the area of the particle size measurement, with differentiation from the surrounding area. The color threshold was adjusted, and the area and diameter of the distinguished particles were measured.

Statistical analysis

The data was statistically analyzed and tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The one-way and two-way analyses of variance (ANOVA) were performed to evaluate the effect of the sealer type and its position within the root canal on the activity of the different elements available. This was followed by multiple comparisons using Duncan's post hoc test (α = 0.05). The IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA), was used for statistical analysis.

Results

The EDX spectral microanalysis and elemental mapping for the tested sealers revealed different elemental composition and distribution in terms of bioactive and heavy metal content expressed as wt% and at% (Table 2, Fig. 2–5).

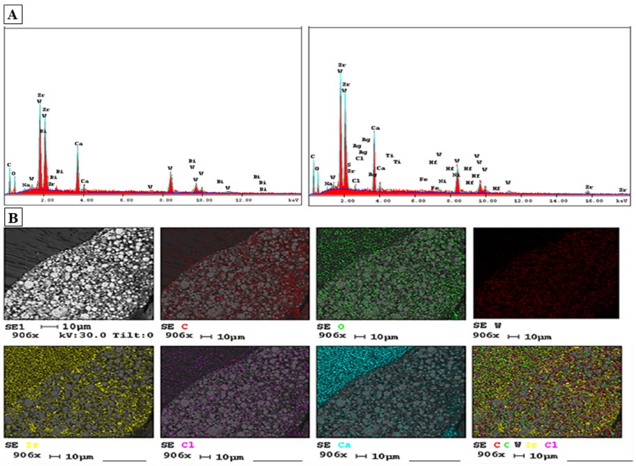

Fig. 2. A – EDX spectral microanalysis of AH Plus at magnification ×2,000 revealed primarily the highest peaks of W and Zr, with comparably lower peaks of C, O and Ca; furthermore, the material revealed minute peaks of Hf, Cl, Ti, Ni, and Fe; B – SEM image and elemental mapping of AH Plus at magnification ×906 showed C (reddish), O (green), W (brown-red), Zr (yellow), Cl (violet), and Ca (blue) uniformly distributed on the surface

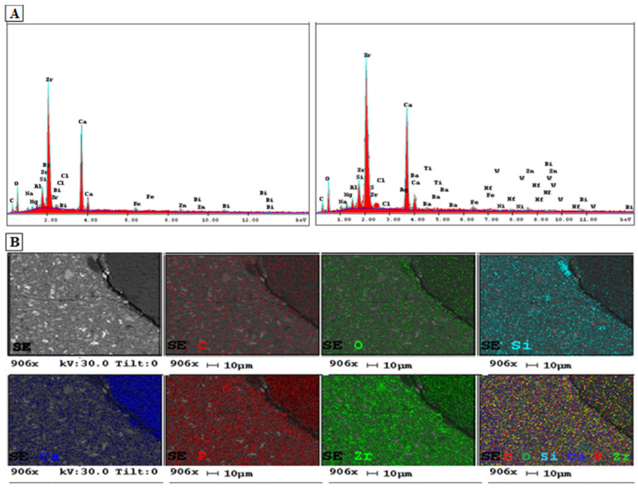

Fig. 3. A – EDX spectral microanalysis of EndoSeal MTA at magnification ×2,000 revealed higher peaks of Zr, Ca, and O, as well as lower peaks of C, Bi and Si; minute amounts of Mg, Na, Al, Ti, Hf, Ni, Zn, W, and Fe were also detected; B – SEM image and elemental mapping of EndoSeal MTA at magnification ×906 showed a uniform distribution of C (faint red), O (green), Si (blue), Ca (navy blue), P (red), and Zr (fluorescent green)

At the apical level, the EDX analysis showed that the highest calcium (Ca) wt% values were recorded for BioRoot RCS (19.22 ±0.5) and Endoseal MTA (19.32±0.6), followed by TotalFill BC (12.77 ±0.5). The highest carbon (C) wt% values were recorded for AH Plus (25.95 ±0.2), followed by BioRoot RCS (18.94 ±0.3), TotalFill BC (18.09 ±0.5) and EndoSeal MTA (11.81 ±0.5) (Table 2, Fig. 2A,3A,4A,5A).

Table 2. Concentration of elements expressed as weight (wt%) and atomic (at%) percentages in the tested root canal sealers (RCSs) at the apical level

Element	AH Plus		EndoSeal MTA		TotalFill BC		BioRoot RCS		*p*-value
	wt%	at%	wt%	at%	wt%	at%	wt%	at%	
Ca†	10.06 ± 0.4	6.70 ±0.4	19.32 ±0.6	12.31 ±0.5	12.77 ±0.5	8.49 ±0.3	19.22 ±0.5	11.24 ±0.8	≤0.001*
C†	25.95 ±0.2	58.44 ±0.5	11.81 ±0.5	25.71 ±0.4	18.09 ±0.5	39.55 ±0.3	18.94 ±0.3	38.27 ±0.2	≤0.001*
O†	15.04 ±0.7	25.01 ±0.7	26.02 ±1.0	43.74 ±0.6	22.70 ±0.8	36.06 ±0.4	24.20 ±1.2	38.07 ±1.1	≤0.001*
Si†	1.21 ±0.3	1.09 ±0.1	3.57 ±0.2	3.42 ±0.4	2.94 ±0.3	2.64 ±0.3	2.58 ±0.4	2.66 ±0.3	≤0.001*
P†	0	0	1.25 ±0.4	1.15 ±0.0	2.88 ±0.1	2.56 ±0.1	1.97 ±0.7	1.76 ±0.0	≤0.001*
Ca/P	0	0	15.46 ±2.9	10.31 ±3.3	4.43 ±0.5	3.32 ±0.3	9.75 ±2.6	6.38 ±2.8	≤0.001*
Zr#	12.88 ±0.6	3.77 ±0.4	26.37 ±1.0	7.72 ±0.2	38.64 ±0.7	10.91 ±0.4	27.10 ±0.6	7.37 ±0.4	≤0.001*
Bi#	0.34 ±0.0	0.05 ±0.0	6.59 ±0.4	0.88 ±0.1	1.53 ±0.2	0.19 ±0.0	1.71 ±0.1	0.19 ±0.0	≤0.001*
Cl	0.41 ±0.3	0.12 ±0.2	0.04 ±0.1	0.07 ±0.3	0.01 ±0.0	0.02 ±0.0	0.01 ±0.0	0.01 ±0.0	≤0.001*
Ti#	0.31 ±0.1	0.18 ±0.0	0.24 ±0.1	0.15 ±0.0	0.11 ±0.0	0.05 ±0.0	0.00 ±0.0	0.02 ±0.0	≤0.001*
W#	33.76 ±0.5	4.99 ±0.1	0.81 ±0.2	0.12 ±0.0	0.93 ±0.1	0.14 ±0.0	0.91 ±0.1	0.12 ±0.0	≤0.001*
Hf#	1.66 ±0.2	0.29 ±0.2	1.46 ±0.3	0.28 ±0.2	2.63 ±0.1	0.38 ±0.0	2.08 ±0.0	0.32 ±0.1	≤0.001*
S	0.02 ±0.0	0.01 ±0.0	0.18 ±0.0	0.29 ±0.2	0.07 ±0.1	0.06 ±0.1	0.17 ±0.3	0.19 ±0.3	≤0.001*
Ag#	0.01 ±0.0	0.01 ±0.0	0.01 ±0.0	0	0	0	0.03 ±0.0	0.02 ±0.0	≥0.05
Ba#	0	0	0.38 ±0.3	0.09 ±0.0	0	0	0.35 ±0.3	0.06 ±0.0	≤0.001*
Fe#	0.45 ±0.1	0.25 ±0.0	1.33 ±0.1	0.67 ±0.1	0.43 ±0.1	0.25 ±0.1	0.26 ±0.0	0.12 ±0.0	≤0.001*
Ni#	0.71 ±0.1	0.32 ±0.1	0.24 ±0.1	0.11 ±0.0	0.54 ±0.2	0.25 ±0.1	0.35 ±0.2	0.13 ±0.0	≤0.001*
Zn#	0	0	1.45 ±0.6	0.62 ±0.1	1.92 ±0.5	0.76 ±0.1	5.81 ±1.2	2.13 ±0.1	≤0.001*
Mg	0	0	1.23 ±0.1	1.31 ±0.0	0.37 ±0.1	0.39 ±0.0	0.63 ±0.3	0.62 ±0.0	≤0.001*
Al#	0	0	1.59 ±0.1	1.63 ±0.1	0.17 ±0.1	0.16 ±0.0	0	0	≤0.001*
Na	0.78 ±0.0	0.85 ±0.0	1.67 ±0.1	1.90 ±0.0	0.32 ±0.1	0.35 ±0.0	0.95 ±0.3	1.06 ±0.0	≤0.001*

Data presented as mean ± standard deviation (*M* ±*SD*). Ca – calcium; C – carbon; O – oxygen; Si – silicon; P – phosphorus; Ca/P – calcium/phosphorus ratio; Zr – zirconium; Bi – bismuth; Cl – chlorine; Ti – titanium; W – tungsten; Hf – hafnium; S – sulfur; Ag – silver; Ba – barium; Fe – iron; Ni – nickel; Zn – zinc; Mg – magnesium; Al – aluminum; Na – sodium; † bioactive element; # heavy metal; * statistically significant.

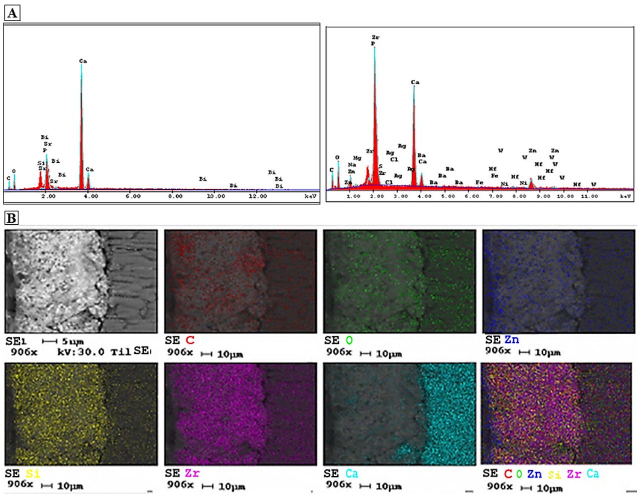

Fig. 4. A – EDX spectral microanalysis of TotalFill BC at magnification ×2,000 revealed higher peaks of Ca, Zr and P, with lower peaks of C, O, Zn, and Si; minute peaks of Na, W, Bi, and Cl were also detected; B – SEM image and elemental mapping of TotalFill BC at magnification ×906 showed a uniform distribution of C (greyish-red), O (green), Zn (navy blue), Si (yellow), Zr (violet), and Ca (blue)

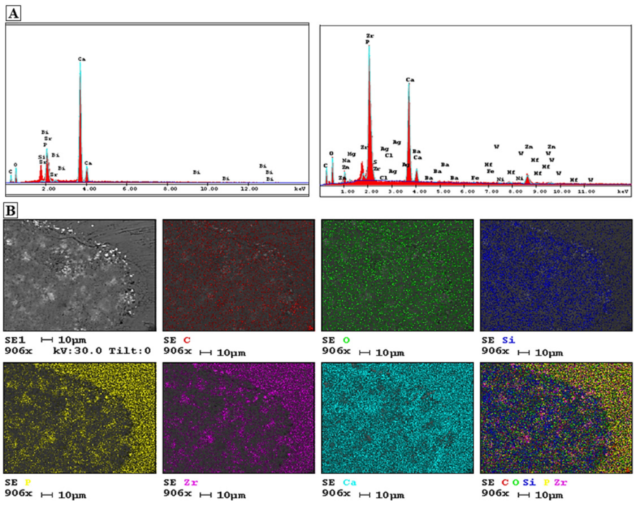

Fig. 5. A – EDX spectral microanalysis of BioRoot RCS at magnification ×2,000 revealed higher peaks for Ca and Zr, with lower peaks of O, C, Si, Zn, Bi, and P; minute peaks of Hf, Ni, Ba, Fe, and Cl were also detected; B – SEM image and elemental mapping of BioRoot RCS at magnification ×906 showed a uniform distribution of prominent Ca (blue), in addition to C (red), O (green), Si (navy blue), P (greenish-yellow), and Zr (violet)

Higher silicon (Si) wt% values were found in EndoSeal MTA (3.57 ±0.2), followed by TotalFill BC (2.94 ±0.3) and BioRoot RCS (2.58 ±0.4). EndoSeal MTA presented the highest oxygen (O) and calcium/phosphorus ratio (Ca/P) wt% values (26.02 ±1.0 and 15.46 ±2.9, respectively), followed by BioRoot RCS (24.20 ±1.2 and 9.75 ±2.6, respectively) and TotalFill BC (22.70 ±0.8 and 4.43 ±0.5, respectively). A more significant wt% value of phosphorus (P) was detected in TotalFill BC (2.88 ±0.1) than in BioRoot RCS (1.97 ±0.7) and EndoSeal MTA (1.25 ±0.4) (Table 2, Fig. 2A,3A,4A,5A).

All the tested sealers revealed the content of heavy metals (zirconium (Zr), bismuth (Bi), tungsten (W), hafnium (Hf), iron (Fe), and nickel (Ni)) at significantly different proportions, with Zr being the most prominent. TotalFill BC showed the highest Zr wt% value, followed by BioRoot RCS, EndoSeal MTA, and AH Plus. Tungsten was the most prominent heavy metal in terms of wt% in the AH Plus sealer, while the wt% value of Bi was significantly higher in EndoSeal MTA than in the other tested sealers. Iron and Ni were found in small amounts in all the tested sealers (Table 2).

Traces of other heavy metals (titanium (Ti), silver (Ag), barium (Ba), zinc (Zn), and aluminum (Al)) were present in some of the tested sealers, while others showed their absence. Titanium was absent in BioRoot RCS, Ag was absent in TotalFill BC, Ba was absent in AH Plus and TotalFill BC, and Al was absent in AH Plus and BioRoot RCS. Zinc was absent in AH Plus, but significantly prominent in BioRoot RCS. Traces of chlorine (Cl), sulfur (S), magnesium (Mg), and sodium (Na) were also detected in all sealers except AH Plus, where Mg was absent (Table 2).

The comparison of the wt% values of bioactive elements in the different sealers tested at different tooth levels (the coronal core, the coronal periphery, the apical core, and the apical periphery) showed statistically significant differences in the concentration of nearly all bioactive elements at various root canal levels, except for AH Plus, which showed the complete absence of P, and thus no Ca/P, the lowest concentration of Ca, O and Si, but the highest concentration of C at all root canal levels (Table 3).

For all calcium silicate sealers, significantly higher peaks of Ca were detected at the periphery of the root canals as compared to their core, but Si revealed a higher concentration at the core than at the periphery. For TotalFill BC and BioRoot RCS, the Ca, O and Si content was higher at the coronal portion, while the P content was higher more apically. These results were reversed for EndoSeal MTA (Table 3).

The highest Ca/P ratio for EndoSeal MTA (15.46 ±2.9) was recorded at the apical peripheral root level, while the highest values for TotalFill BC (8.69 ±3.1) and BioRoot RCS (34.25 ±4.8) were recorded at the coronal peripheral root level. (Table 3).

The SEM images of the AH Plus specimens revealed regular surfaces with globular-like particles (mean area of 6.1 ±11 μm^2 and diameter of 2.1 ±1.8 μm) (Fig. 1C,2B). The EndoSeal MTA specimens showed slightly irregular crystalline surfaces, including particles of different shapes (needle-like, globular-like and matrix-like) and sizes (mean area of 2.6 ±7.9 μm^2 and diameter of 1.2 ±1.4 μm) (Fig. 1D,3B). TotalFill BC revealed regular surfaces with homogenous, smaller and matrix-like particles (mean area of 0.6 ±1.4 μm^2 and diameter of 0.6 ±0.6 μm) (Fig. 1E,4B). The SEM analysis of the BioRoot RCS surface revealed regular globular-like particles embedded in a similarly shaped matrix (mean area of 5.5 ±12.2 μm^2 and diameter of 1.9 ±1.8 μm) (Fig. 1F,5B).

Table 3. Concentration of bioactive elements expressed as weight percentage (wt%) at the core and periphery of both coronal and apical levels for the tested root canal sealers (RCSs)

Element	Slice area	AH Plus	*p*-value	EndoSeal MTA	*p*-value	TotalFill BC	*p*-value	BioRoot RCS	*p*-value
Ca	Cc	9.57^{a} ±0.3	≥0.05	15.01^{c} ±0.5	≤0.05*	11.43^{b} ±4.2	≤0.05*	33.65^{b} ±7.1	≤0.05*
	Cp	10.63^{a} ±0.5		17.13^{b} ±0.7		16.69^{a} ±4.8		39.04^{a} ±1.2	
	Ac	10.49^{a} ±0.6		17.11^{b} ±0.6		11.03^{b} ±0.7		16.50^{c} ±0.7	
	Ap	10.06^{a} ±0.4		19.32^{a} ±0.6		12.77^{b} ±0.5		19.22^{c} ±0.5	
C	Cc	27.70^{a} ±3.7	≤0.05*	11.40^{b} ±0.9	≤0.05*	26.12^{a} ±1.9	≤0.05*	12.21^{c} ±0.5	≤0.05*
	Cp	26.95^{a} ±3.4		12.41^{a} ±2.7		17.07^{c} ±3.3		12.90^{b} ±0.5	
	Ac	22.54^{c} ±0.5		12.12^{a} ±0.4		19.47^{b} ±0.6		19.04^{a} ±0.4	
	Ap	25.95^{b} ±0.2		11.81^{b} ±0.5		18.09^{c} ±0.5		18.94^{a} ±0.3	
O	Cc	12.46^{b} ±1.3	≤0.05*	25.74^{b} ±1.0	≤0.05*	24.88^{b} ±3.2	≤0.05*	32.92^{a} ±3.5	≤0.05*
	Cp	12.85^{b} ±1.2		26.09^{b} ±1.5		30.72^{a} ±1.7		32.01^{a} ±1.4	
	Ac	13.32^{b} ±0.3		28.05^{a} ±0.6		24.35^{c} ±0.7		25.39^{b} ±0.1	
	Ap	15.04^{a} ±0.7		26.02^{b} ±1.0		22.70^{c} ±0.8		24.20^{b} ±1.2	
Si	Cc	1.40^{a} ± 0.0	≤0.05*	4.12^{a} ±0.2	≤0.05*	5.99^{a} ±1.4	≤0.05*	5.12^{a} ±1.8	≤0.05*
	Cp	1.41^{a} ±0.2		3.45^{b} ±0.7		4.53^{b} ±0.5		2.89^{c} ±0.5	
	Ac	1.33^{b} ±0.3		4.52^{a} ±0.3		3.99^{b} ±0.3		4.41^{b} ±0.4	
	Ap	1.21^{c} ±0.3		3.57^{b} ±0.2		2.94^{c} ±0.3		2.58^{c} ±0.3	
P	Cc	0^{a}	≥0.05	1.45^{a} ±0.2	≤0.05*	1.48^{c} ±0.2	≤0.05*	1.76^{a} ±0.3	≤0.05*
	Cp	0^{a}		1.46^{a} ±0.3		1.92^{b} ±0.1		1.14^{c} ±0.1	
	Ac	0^{a}		1.23^{b} ±0.1		1.67^{c} ±0.1		1.44^{b} ±0.0	
	Ap	0^{a}		1.25^{b} ±0.0		2.88^{a} ±0.1		1.97^{a} ±0.0	
Ca/P	Cc	0^{a}	≥0.05	10.35^{b} ±3.0	≤0.05*	7.72^{b} ±2.1	≤0.05*	19.11^{b} ±4.2	≤0.05*
	Cp	0^{a}		11.73^{b} ±2.3		8.69^{a} ±3.1		34.25^{a} ±4.8	
	Ac	0^{a}		13.91^{a} ±3.3		6.60^{bc} ±1.4		11.45^{b} ±3.5	
	Ap	0^{a}		15.46^{a} ±2.9		4.43^{c} ±0.5		9.75^{c} ±2.6	

Data presented as *M* ±*SD*. Cc – coronal core; Cp – coronal periphery; Ac – apical core; Ap – apical periphery; * statistically significant. Different uppercase letters denote statistically significant differences between the w% of particular elements in various sealers at various root canal levels ($p < 0.05$).

Discussion

To control the quality of endodontic materials, their tissue tolerance and interaction with the tooth structure should be tested. Many standardized technological tests according to the American National Standards Institute/American Dental Association (ANSI/ADA) in the USA and the International Organization for Standardization (ISO) internationally have been applied for this purpose. Such tests include the combined EDX microanalysis and SEM elemental mapping analysis, which describe all trace elements and the surfaces of chemical elements quantitatively and qualitatively.[7,9,10]

X-ray spectrometry is not capable of distinguishing between ionic and non-ionic types of elements, and it also has a detection limit of ~0.1%, which makes the detection of light elements sometimes inaccurate, depending on the element itself.[9] Field emission scanning electron microscopy was used in this study, as it neglects the surface treatment or manipulation step prior to analysis. Accordingly, low-energy X-rays, which are characteristic of light elements, were not absorbed in the gold surface layer, as was observed in previous studies.[9,11] Also, X-ray detection is not affected by the chemical state of elements, but it is influenced by inter-element interference, known in X-ray spectrometry as the peak overlap. This may cause serious problems in the analysis of elements with similar energy peaks, such as W and Si in AH Plus.[9] As a trial to overcome this problem, this study used net counts, which are representative of the chemical element of each energy peak, and also provide an accurate analysis of the chemical composition of the material. Accordingly, some authors suggest some additional analysis to SEM-EDX, such as atomic absorption spectrometry (AAS), X-ray fluorescence (XRF), X-ray diffraction analysis (XRD), and inductively coupled plasma optical emission spectrometry (ICP-OES), to increase the accuracy of elemental characterization.[12]

AH Plus, an epoxy resin-based sealer, was used in this study as a reference, as it is considered to be the gold standard in terms of sealing ability and low solubility, according to the literature.

Al-Haddad et al. stated that the chemical composition of the set bioceramic sealers varied according to the surrounding environment during the setting reaction.[13] Thus, it was important to simulate natural oral conditions by conducting the study on freshly extracted teeth[13] instead of using cylindrical molds or polyethylene tubes, which were commonly employed in different studies.[9,11,14,15] Thereby, the effect of microstructure and dentinal moisture at different tooth levels on the bioactivity of the tested sealers and their clinical significance in terms of bonding to dentin and sealing ability could be evaluated.[11,14]

Unlike in the case of the AH Plus sealer, the concentration (wt%) of bioactive elements in the tested bioceramic sealers varied significantly at different root canal levels. The TotalFill BC and BioRoot RCS sealers revealed a higher concentration of bioactive elements (Ca, O and Si) coronally. This may be due to the larger diameter of dentinal tubules coronally (4.32 μm), allowing more sealer penetration and greater moisture contact than in the middle (3.74 μm) and apical (1.73 μm) zones.[16,17]

Due to the modified setting reaction,[18] the resultant change in the composition of the bioactive products occurred with different levels of hydration.[13,19]

The difficult removal of the smear layer at the apical third might also act as a physical barrier, interfering with the sealer adaptation to root canal dentin moisture.[20]

EndoSeal MTA showed different bioactive element distribution at variable root levels as compared to the other sealers, which may be due to differences in the flow and the setting time, making it less sensitive to changes in dentin moisture.[20]

The higher Ca/P ratios detected at the peripheral root portions confirm the effect of dentin moisture on the bioactivity of bioceramic sealers through the complex hydration reaction.

The Ca/P atomic ratios detected in this study for BioRoot RCS and TotalFill BC (6.38 ±2.8 at% and 3.32 ±0.3 at%, respectively) are more or less in agreement with those found in BioRoot RCS (3.20–5.21 at%) by Siboni et al.[21] However, the EndoSeal MTA Ca/P atomic ratio (10.70 ±3.3 at%) is not in accordance with those obtained by Yoo et al. (1.45–1.89 at%).[22]

Unfortunately, the Ca/P ratios for all the bioceramic sealers tested in the present study contradicted the manufacturers' claims, being different from those of the tooth structure (1.5–1.67 wt%).[23] Such differences might be the result of not using phosphate buffered saline (PBS) or Hank's Balanced Salt Solution (HBSS) in any treatment or storage, as these are quite important for the formation of a mineralized apatite structure.[5,13,21,22]

In the present study, a lower presentation of P wt% was observed for the EndoSeal MTA, TotalFill BC and BioRoot RCS samples, which explains an increase in the Ca/P ratio.[5]

Due to the complete absence of P and Ca/P, and the lowest Ca concentration, AH Plus revealed a low capacity of inducing periapical repair as compared to the rest of the endodontic sealers that were investigated.[6,24] This is consistent with the findings of Sampaio et al.[9] and Reszka et al.[11] However, Siboni et al. detected a thin Ca/P deposit on AH Plus immersed in HBSS.[21]

All the tested sealers showed regular surfaces with uniformly distributed particles that were similar in shape, but different in size, except for EndoSeal MTA, which showed different particle shapes and slightly irregular surfaces.

According to Balto and Al-Nazhan[25] and Sampaio et al.,[9] better cell adhesion and biocompatibility are expected with the AH Plus, TotalFill BC and BioRoot RCS sealers. However, EndoSeal MTA showed more cell adhesion and viability than AH Plus in other cytological and histological studies,[26] which emphasizes the impact of other factors, such as bioactivity and heavy metal toxicity, on biological behavior.

Based on the literature, AH Plus has cytotoxic effects due to its epoxy resin content and the release of toxic monomers, such as bisphenol A diglycidyl ether, which delays periapical healing when extruded apically.[6,24]

Due to the clinical importance of the direct and indirect contact of the sealer with periapical tissues, elemental composition and concentration in the tested sealers were compared at the apical level. Consistent with the literature, dissimilarity was detected in the tested sealers at the apical level,[5,11,13,21] although Ca_2SiO_4 is the main component in the 3 bioceramic sealers tested.[19] This dissimilarity was due to the different sources of Ca and additives provided by their manufacturers, and their different presentation forms.

According to the literature, heavy elements (Zr, Bi, Ti, W, and Hf) have been added to sealers to increase their radiopacity. High peaks of Zr and low peaks of Hf were detected in all the tested sealers, as well as Bi in EndoSeal MTA and W in AH Plus (from calcium tungstate ($CaWO_4$)).[27] This explains the high radiopacity of AH Plus reported in the literature,[26,28] higher than in the case of BioRoot RCS,[21] followed by the EndoSeal MTA and EndoSequence® BC™ sealers.[28] Although zirconium dioxide (ZrO_2) provides a lower contrast than other radiopacifiers (W and bismuth oxide (Bi_2O_3)), it seems to be more inert,[7,19] allowing a longer release of calcium ions, thus making the tricalcium silicate (Ca_3SiO_5) cements more biocompatible.[13,29]

Several heavy metals (Bi, W, Hf, Ag, Ba, Ni, Zn, and Al) were detected in the tested sealers, which could possibly endanger periapical cells, and also affect tissue healing.

Some of the sealers showed certain elements that had not been mentioned by the manufacturers (Bi, Hf, Fe, Ni, Zn, Mg, and Al) in small wt%, either due to contamination during manufacturing or as industrial secrets.[29]

Differences in heavy metal composition between the cited literature[11,13,30] and the present study may be due to variations in the experimental conditions.

Further studies should be conducted to evaluate the effect of elemental composition on the biological and physicochemical features of calcium silicate-based sealers, aiming to minimize the harmful effects while enhancing endodontic repair.

Conclusions

When in contact with root dentin moisture, the tested bioceramic sealers released different percentages of bioactive elements at the periphery, which could possibly enhance chemical bonding to root dentin. All the tested sealers except AH Plus revealed high peaks of the Ca/P ratio, suggesting regenerative potential in vivo, which supports their biocompatibility. The Ca/P ratio and heavy metal content were not in complete agreement with those suggested by the manufacturers.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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