Chemical Elements Characterization of Root Canal Sealers using Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis

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Abstract

Introduction: The aim of this study was to evaluate the chemical elements composition of root canal filling materials using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX).

Methods: Eighteen standard polyethylene tubes were filled with the following materials: Sealapex®, Sealer 26®, MTA Fillapex®, Pulp Canal Sealer®, Endofill® and AH Plus®. After 48 hours at 37°C and 95% relative humidity, the samples were surface-sputtered with gold and analyzed using SEM at 5000X magnification. Their chemical composition and element distributionwere determined using EDX. Results were evaluated qualitatively (SEM images and elemental mapping) and quantitatively (weight percentage).

Results: Calcium oxide- and hydroxide-based sealers (Sealapex® and Sealer 26®) had calcium peaks of 53.58 wt% and 65.00 wt%. MTA Fillapex® had 30.58 wt% of calcium and high amounts of silicon (31.02 wt%) and bismuth (27.38 wt%). Zinc oxide- and eugenol-based sealers, Pulp Canal Sealer® and Endofill®, had 67.74 wt% and 63.16 wt% of zinc each. AH Plus® had a higher amount of zirconium (64.24 wt%). All materials had elements other than those described by the manufacturers. Surface analysis using EDX revealed that regularity varied, element distribution was uniform, and particles had similar sizes and variable shapes.

Conclusions: Most chemical elements were those described by the manufacturers, but percentages were different. The surface of root canal sealers had different regularity findings, uniform distribution and particles of similar sizes but variable shapes.

Key Words: Scanning Electron Microscopy, Energy-dispersive X-ray Analysis, Chemical Properties, Root Canl Filling Materials

Introduction

Root canal sealers should fill the spaces between gutta-percha points and dentin walls, and due of their properties, root canal sealers should not interfere the healing of periapical tissues [1]. Therefore, materials should be carefully selected after evaluating the chemical composition of sealers. Bond strength to dentin walls should also be considered [2]. New root canal sealers should provide adequate alternatives defined by their specificities and the conditions under which they will be used [1].

The chemical characteristics of the compounds found in root canal sealers may define important correlations with tissue tolerance, dentin bond strength and antimicrobial properties [3]. Resende et al. [4] evaluated the physicochemical properties and the surface morphology of several sealers: AH Plus®, Epiphany® and Epiphany SE®. AH Plus® met ANSI/ADA specifications for all properties, differently from the others. According to Haragushiku et al. [5], AH Plus® had the best adhesion to dentin walls when compared with Apexit® and Epiphany.

The results of bond strength tests with Sealapex® and Apexit® were poorer than those found for Sealer 26® and CRCS®. EDTA increased the adhesion of root canal sealers to dentin walls, except in the case of Sealapex® [6]. Confocal microscopy revealed that Sealapex® penetrated dentin tubules [7]. Calcium hydroxide had an excellent potential to induce the formation of a mineralized barrier. Sealapex®, a root canal sealer that contains calcium oxide, also stimulated the deposition of mineralized tissue after root canal fillings in a study with dogs and monkeys [8]. Tagger et al. [9] evaluated the release of calcium and hydroxyl ions by Sealapex®, CRCS® and Hermetic®. Sealapex® released the highest number of ions, and its solubility might explain its higher rate of ion release than that of the other sealers tested. MTA Fillapex® is a root canal filling material that is well tolerated by tissues [10].

Sound knowledge about the chemical composition of root canal filling materials, whose elements are distributed on the surface of their structure, may facilitate the understanding of their properties and their interaction with the tissues with which they are in contact. One of their characteristics, biocompatibility, may be directly affected by their chemical composition because they may explain the presence of irritant compounds near biological tissues [11]. Therefore, the study of the chemical composition of the surface of root canal sealers may bring new perspectives to the analysis of the interaction between biological, physical and chemical properties.

This study used Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX) to describe the chemical elements found on the surface of root canal sealers.

Materials and Methods

Specimen preparation

Six commercially available root canal sealers were used for the experiments: Sealapex® (Sybron Endo, Orange, CA), Sealer 26® (Dentsply, De Tray, Konstanz, Germany), MTA Fillapex® (Angelus Soluções Odontológicas, Londrina, Brazil), Pulp Canal Sealer® (Sybron Endo, Orange, CA), Endofill® (Dentsply, Petropolis, Brazil), AH Plus® (Dentsply, De Tray, Konstanz, Germany). *Table 1* shows the composition of the materials under evaluation, as described by their manufacturers.

Eighteen standard polyethylene tubes (number 12 Levine Probe, Embramed, Jurubatuba, Brazil), measuring 3 mm in internal diameter and 3 mm long, were prepared using a digital caliper reading to 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan) and a #11 scalpel blade (Swann Morton, Sheffield, United Kingdom). Three tubes

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Table 1. *Chemical composition of sealers under evaluation.*

Material tested	Components	
Sealapex®	Catalyst	Base
	Isobutyl salicylate resin	N-ethyltoluenesulfonamideresin
	Fumed silica (silicon dioxide)	Fumed silica (silicon dioxide)
	Bismuth trioxide	Zinc oxide
	Titanium dioxide pigment	Calcium oxide
Pulp Canal Sealer®	Powder	Liquid
	Zinc oxide	Oil of cloves
	Precipitated molecular silver	Canada Balsam
	Oleoresins (white resin)	
	Thymoliodide	
Sealer 26®	Powder	Resin
	Calcium hydroxide	Bisphenol A ether
	Bismuth oxide	
	Hexamethylenetetramine	
AH Plus®	Paste A	Paste B
	Bisphenol-A epoxy resin	Dibenzyldiamine
	Bisphenol-F epoxy resin	Aminoadamantane
	Calcium tungstate	Tricyclodecane-diamine
	Zirconium oxide	Calcium tungstate
	Silica	Zirconium oxide
	Iron oxide pigments	Silica
		Silicone oil
MTA Fillapex®	Components	Components
	Natural resin	Nanoparticulate silica
	Salicylate resin	MTA
	Diluting resin	Pigments
	Bismuth oxide	
EndoFill®	Powder	Liquid
	Zinc oxide	Eugenol
	Hydrogenated resin	Sweet almond oil
	Bismuth subcarbonate	
	Barium sulfate	
	Sodium borate	

for each group were placed on a polished glass slab (75 x 25 x 1 mm) and filled with the test materials using a #24 spatula (SS White Duflex, Rio de Janeiro, Brazil). All sealers were mixed according to their manufacturer's instructions. After that, the specimens were placed in a chamber at 95% relative humidity and 37°C for 48 hours.

Microscopy and elemental analysis

The specimens were surface-sputtered with gold and examined using a Leo Stereoscan 420i Scanning Electron Microscope (SEM) (Leica Electron Optics, Cambridge instruments, Cambridge, UK) at 8-10 kV and 2-nm resolution, without any preparation or manipulation. Images at 5000X magnification were captured to determine surface regularity.

Chemical elements were analyzed using the Energy Dispersive X-ray unit (EDX) NSS Spectral Analysis System 2.3 (Thermo Fisher Scientific Inc., Suwanee, GA). EDX measurements to define elemental composition and distribution were made from the central region of each specimen using electron beam spot sizes lower than 50 nm, accelerating voltage of 25 kV and 110 mA beam current determined according to the composition described by the manufacturers. Spectra were obtained for 100 seconds live time.

Measurements were made by two experienced professionals trained in a pilot study with 10% of the samples. They used the NSS Spectral Analysis System 2.3 in non-standard analysis mode and phi-rho-Z (PROZA) correction. Elemental maps were built according to the Net Counts method, at high resolution and using the same software. SEM surface analysis and EDX microanalysis were qualitative and descriptive to define surface regularity (regular and irregular), distribution of elements (uniform or non-uniform), and particle shape (globular-like, needle-like, matrix) and size (similarities between particles without measurements).

Results

Quantitative results of elements according to EDX microanalysis are described in *Table 2*. Calcium oxide- or hydroxide-based sealers (Sealapex® and Sealer 26®) had higher calcium peaks (53.58 wt% and 65.00 wt%) than the MTA-based material (MTA Fillapex, 30.58 wt%), which also had high amounts of silicon (31.02 wt%) and bismuth (27.38 wt%). Zinc oxide- and eugenol-based sealers (Pulp Canal Sealer® and Endofill®) had zinc peaks (67.74 wt% and 63.16 wt%), whereas the resin-based material (AH Plus®) had a greater amount of zirconium (64.24 wt%).

In *Figures 1-6*, SEM images show the distribution maps of the 2 main elements detected by EDX microanalysis and the surface structure (element distribution, size and shape of particles) of the sealers under evaluation.

The Sealapex® specimens had an irregular surface (*Figure 1A*) and a uniform distribution of elements, mostly globular-like particles of calcium and needle-like particles of bismuth of different sizes (*Figure 1B*).

Table 2. *Elements found in the root canal sealers using energy dispersive X-ray analysis (EDX).*

Element	Sealapex®		Sealer 26®		MTA Fillapex®		Pulp Canal Sealer®		Endofill®		AH Plus®	
	at%	wt%	at%	wt%	at%	wt%	at%	wt%	at%	wt%	at%	wt%
Ag	-----	-----	-----	-----	-----	-----	21.69	31.58	-----	-----	-----	-----
Al	0.96	0.51	1.07	0.53	0.97	0.61	1.04	0.38	1.62	0.56	4.16	1.21
Ba	-----	-----	-----	-----	-----	-----	-----	-----	7.91	13.92	-----	-----
Bi	6.15	25.37	8.84	33.80	5.63	27.38	-----	-----	7.10	19.03	-----	-----
Ca	67.74	53.58	88.58	65.00	32.81	30.58	-----	-----	-----	-----	14.56	6.31
Cl	-----	-----	-----	-----	-----	-----	0.36	0.17	-----	-----	1.96	0.75
Fe	0.13	0.14	-----	-----	-----	-----	-----	-----	-----	-----	0.37	0.23
Hf	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.63	1.22
Mg	0.33	0.16	1.51	0.67	-----	-----	-----	-----	-----	-----	-----	-----
Ni	0.18	0.21	-----	-----	-----	-----	0.16	0.12	0.13	0.10	-----	-----
S	5.90	3.73	-----	-----	11.31	8.43	-----	-----	7.87	3.23	-----	-----
Si	7.97	4.42	-----	-----	47.50	31.02	-----	-----	-----	-----	-----	-----
Ti	5.40	5.11	-----	-----	1.78	1.98	-----	-----	-----	-----	-----	-----
W	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	13.11	26.04
Zn	5.24	6.76	-----	-----	-----	-----	76.75	67.74	75.37	63.16	-----	-----
Zr	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	65.20	64.24

Light elements were excluded from EDX microanalysis because fluorescence production decrease as atomic number is lower, and quantitative analysis of these elements may be imprecise (Vaughan, 1999).

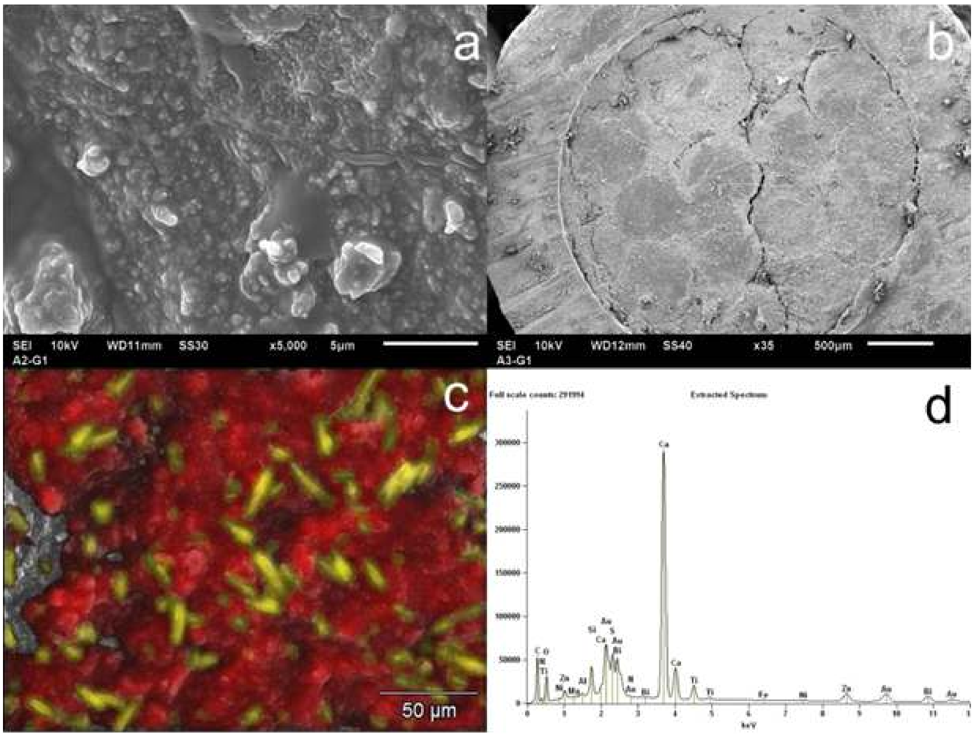

Figure 1. *Sealapex® SEM images with 5,000X magnification (A), showing irregular surface. Elements distribution maps of calcium (red) and bismuth (yellow) at the surface of Sealapex® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of variable sizes and shapes.*

The Sealer 26® specimens had a regular surface (*Figure 2A*) primarily composed of uniformly distributed particles of calcium and bismuth of different sizes and shapes.

The MTA Fillapex® specimens had an irregular surface (*Figure 3A*) and a uniform distribution of elements, primarily a silicon matrix with globular-like particles of calcium and bismuth particles of different sizes and shapes (*Figure 3B*).

The Pulp Canal Sealer® specimens had a regular surface (*Figure 4A*) and a uniform distribution of elements, mostly globular-like particles of zinc and silver (*Figure 4B*).

The Endofill® specimens had an irregular surface (*Figure 5A*) and a uniform distribution of elements, mostly globular-like particles of zinc and bismuth (*Figure 5B*).

The AH Plus® specimen had a regular surface (*Figure 6A*) and uniformly distributed globular-like particles, primarily zirconium (*Figure 6B*).

Discussion

The chemical composition of root canal sealers that are used in close contact with periapical tissues is a predictive factor to understand their physical, chemical and biological properties [11,12]. The knowledge of their chemical composition informs the selection of the best material to be used in clinical conditions. In this study, the surface of the specimens showed different regularities for each cement, but a uniform distribution of elements and particles of similar sizes and shapes for most sealers.

Surface regularity is important for cell adhesion to the material and, therefore, essential to evaluate biocompatibility [13]. Our

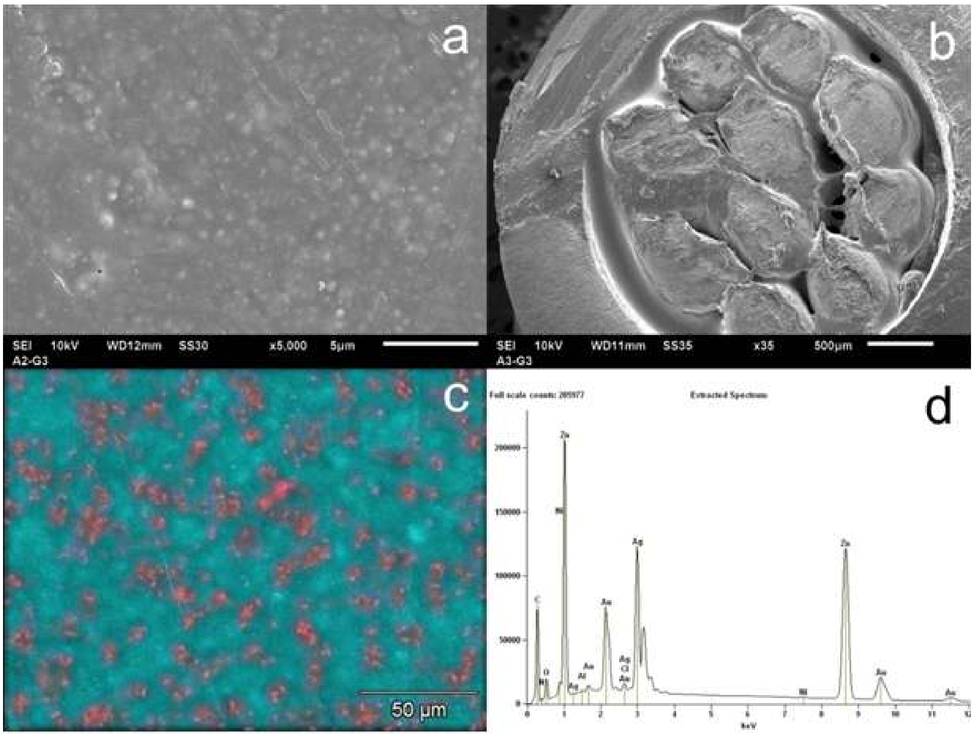

Figure 2. *Sealer 26® SEM images with 5,000X magnification (A), showing regular surface. Elements distribution maps of calcium (red) and bismuth (yellow) at the surface of Sealer 26® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of variable sizes and shapes.*

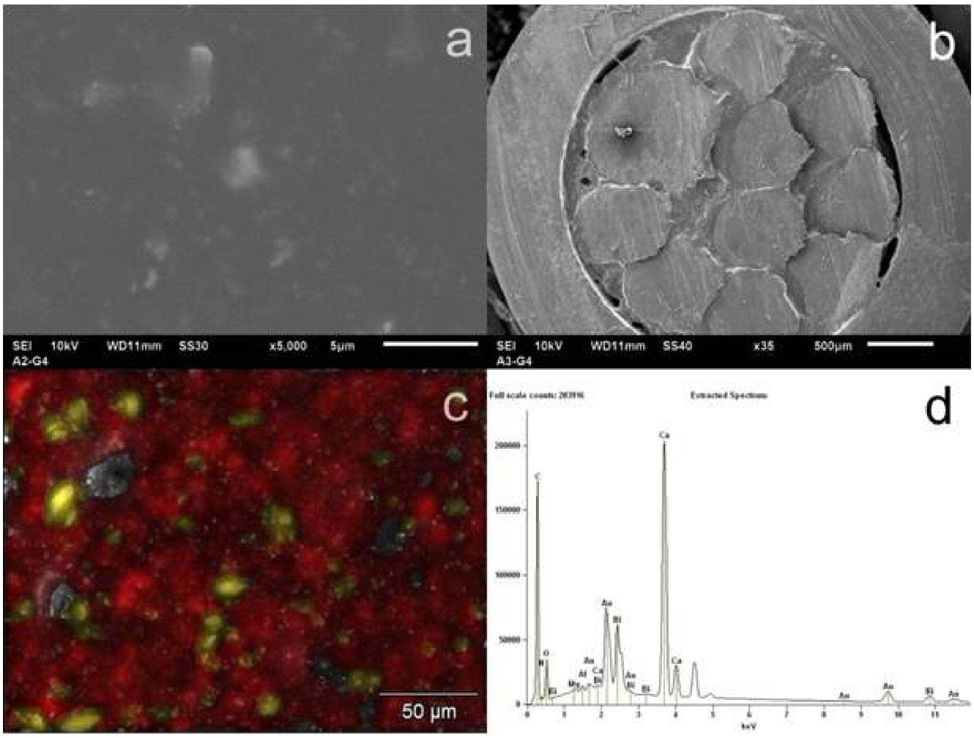

Figure 3. *MTA Fillapex® SEM images with 5,000X magnification (A), showing irregular surface. Elements distribution maps of silicon (blue) and calcium (red) at the surface of MTA Fillapex® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and variable shapes.*

results showed that Sealer 26®, Pulp Canal Sealer® and AH Plus® produced regular surfaces. Therefore, better cell adhesion results should be expected when using these root canal sealers. However, other factors, such as chemical composition, may also affect cell adhesion and biocompatibility, and surface regularity should not be analyzed in isolation.

EDX microanalysis of the root canal sealers revealed similarities between the elements found in our study and the main compounds described by their manufacturers. Sealapex® (53.58 wt%) and Sealer 26® (65.00 wt%) had high amounts of calcium, which confirms that the main components were calcium oxide in Sealapex® and calcium hydroxide in Sealer 26®. MTA Fillapex® had high amounts of silicon (31.02 wt%) from silicon dioxide and MTA silicates. Pulp Canal Sealer® and Endofill® had high values of zinc (67.74 wt% and 63.16 wt%), found in zinc oxide, the main component of their formulas. AH Plus®, an epoxy resin-based sealer, had higher amounts of zirconium (64.24 wt%), from zirconium dioxide, and also tungsten (26.04 wt%), from calcium tungstate.

All the materials under analysis had elements not described by their manufacturers: Sealapex® had traces of Al, Fe, Mg and Ni; Sealer 26®, of Al and Mg; MTA Fillapex®, of Ti; Pulp Canal Sealer®, of Cl and Ni; Endofill®, of Al and Ni; and AH Plus®, of Al, Hf and Mg. These results might be attributed to contamination during manufacture or to industrial secrets.

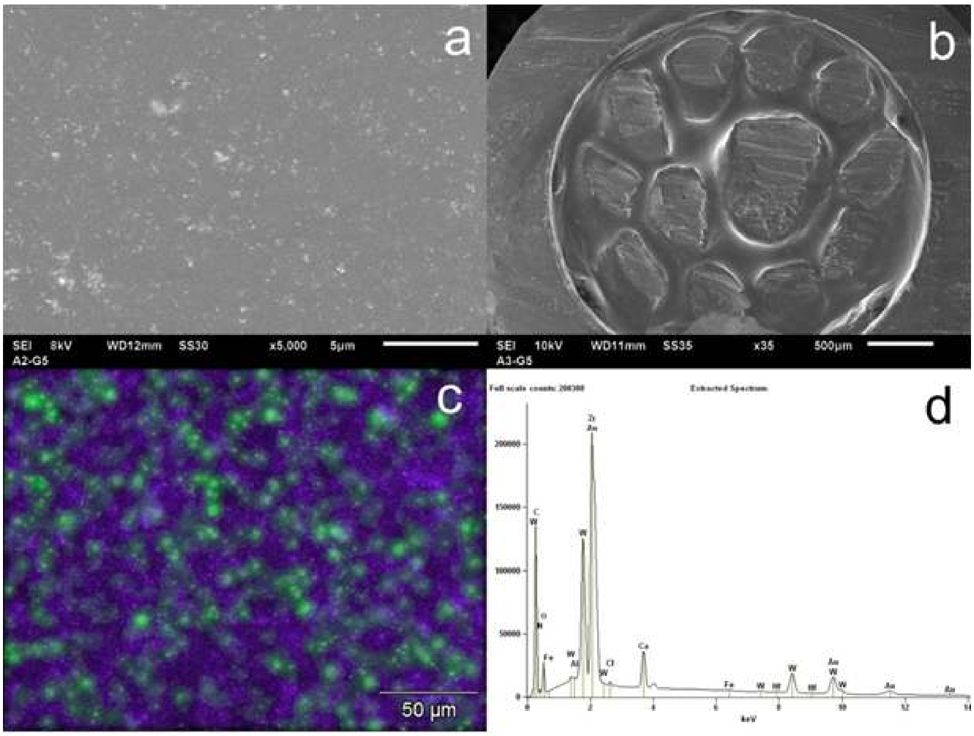

Figure 4. *Pulp Canal Sealer® SEM images with 5,000X magnification (A), showing regular surface. Elements distribution maps of zinc (light blue) and silver (pink) at the surface of Pulp Canal Sealer® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.*

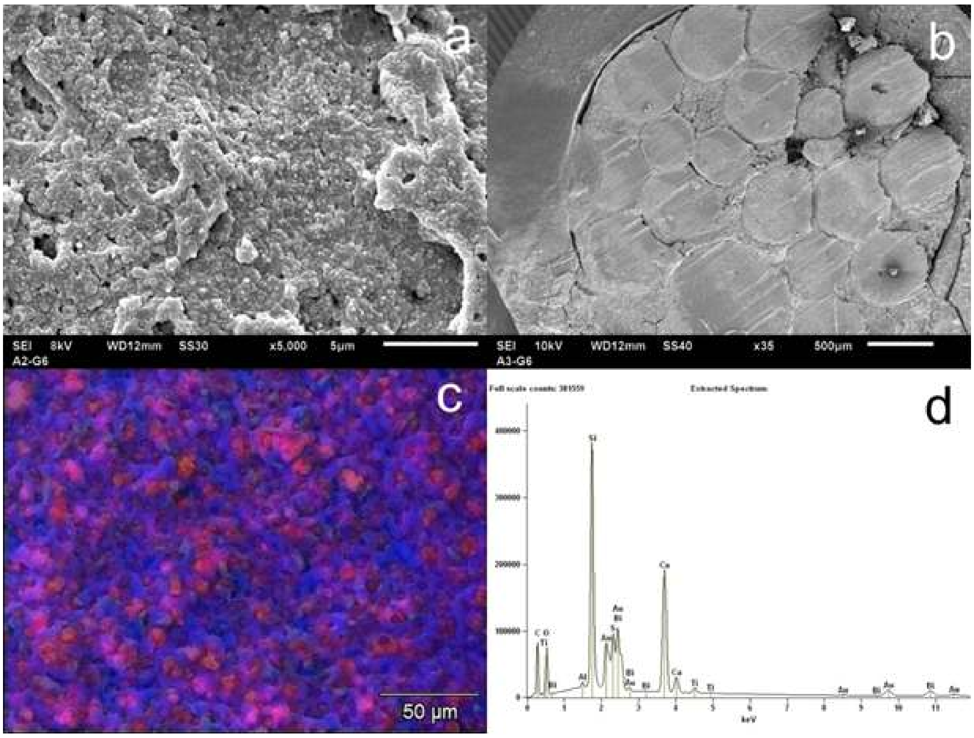

Figure 5. *Endofill® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of zinc (light blue) and bismuth (yellow) at the surface of Endofill® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.*

Some elements described by the manufacturers were not found by means of EDX microanalysis. Pulp Canal Sealer® had no iodine, which should be derived from thymol iodine, whereas Endofill® did not have boron, sodium or chlorine, which should be present in sodium borate and hydrogenated resins.

AH Plus® was the most radiopaque root canal sealer of all the materials under evaluation [14,15]. Evidence clearly shows that heavy elements have greater radiopacity. Elements such as silicon, which has a low atomic number, should result in radiolucent materials, whereas elements with a high atomic number, such as barium, are found in radiopaque materials [16]. Therefore, the presence of zirconium and tungsten in AH Plus® may justify its high radiopacity.

Sealapex® has low radiopacity, but recent studies found greater radiopacity, at a level similar to that of Sealer 26® [17]. This enhanced radiopacity must be due to changes in Sealapex® composition, which now seems to have an increased percentage of bismuth oxide. The results of our study showed that the levels of bismuth in Sealapex® were similar to those found in Sealer 26® and MTA Fillapex®.

Sealer adhesion to dentin is essential for good canal sealing [18]. AH Plus® had the best bond strength [5,19,20], a property associated with the formation of covalent bonds between epoxide rings and amine groups exposed in the collagen net [21]. The presence of chlorine in AH Plus® is explained by the presence of bisphenol-A and bisphenol-F epoxy resins in its composition.

Sealer 26® also had good bond strength values, whereas Sealapex®

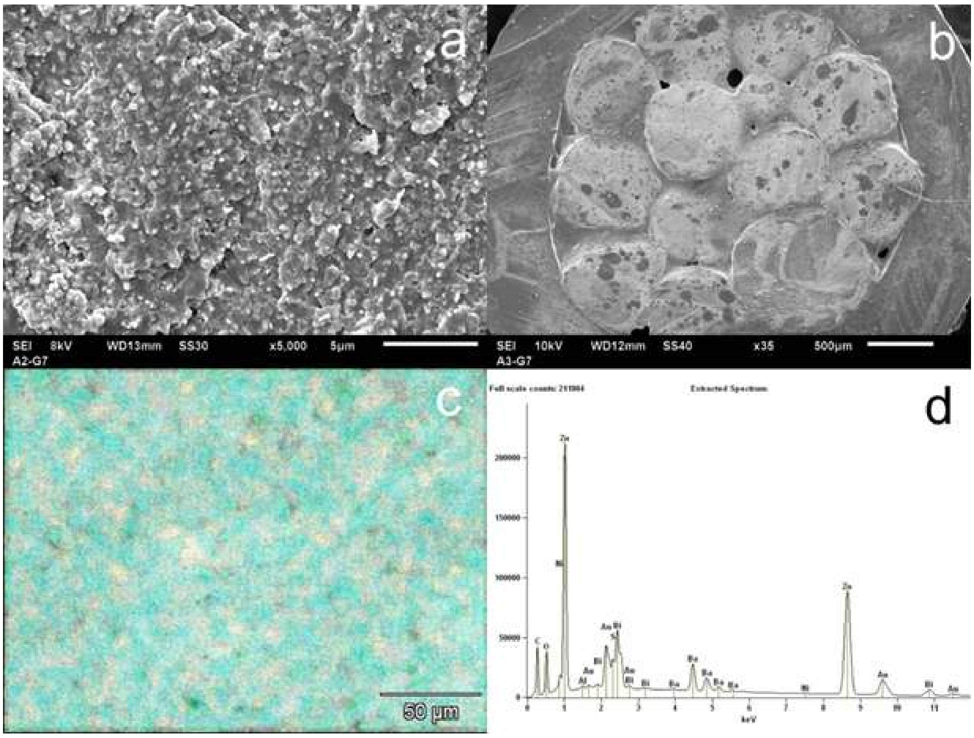

Figure 6. *AH Plus® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of zirconium (purple) and tungsten (green) at the surface of AH Plus® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.*

had poor adhesion to dentin. Calcium oxide or hydroxide may affect ealer bond strength to dentin, which may vary substantially [22].

Several elements, such as Ag, Al, Ba, Bi, Hf, Ni, S, Zn, Zr and W, may harm human cells at certain concentrations. These elements were found in the filling materials under evaluation, which may explain why their results were suggestive of cytotoxicity or genotoxicity [23-27].

Sealapex® had a high amount of bismuth, in addition to Al, Ni, S and Zn. These elements have been associated with cytotoxicity. Moreover, the instability of this sealer in an aqueous environment increases the release of these elements [28]. Studies showed that Sealer 26® might have cytotoxic effects [24,25], may be due to the high amount of Bi and the presence of Al. MTA Fillapex®, developed in an attempt to combine the biological, physical and chemical properties of MTA, has also been associated with aggressive effects on cells [26]. These effects may be explained by the high amounts of bismuth, as well as the presence of Al and S, found in these compounds.

Pulp Canal Sealer® and Endofill® are root canal sealers with eugenol in their composition, a compound known for its cytotoxic effects to human cells [23]. This study found high amounts of silver and zinc in Pulp Canal Sealer®, along with Al and Ni. High amounts of zinc and the presence of Al, Ba, Bi, Ni and S were found in Endofill®, a sealer with cytotoxic effects. AH Plus® may have cytotoxic effects, which may be explained by the fact that its main component is epoxy resin, and that it releases amines, or formaldehyde [29]. The high amounts of zirconium and tungsten, along with Al and Hf, may explain part of this cytotoxic mechanism.

As the chemicals in root canal sealers may harm cells or have genotoxic effects, root canal filling should be kept inside the canal and not reach the periapical region.

Antimicrobial properties are directly associated with the chemical composition of the sealers. Elements such as Ag, Bi, Cl, S and Ti are known for their bactericide activity. Calcium oxide turns into calcium hydroxide in the presence of water, which explains the antimicrobial mechanism of Sealapex®, Sealer 26® and MTA Fillapex®. The release of hydroxyl ions elevates Ph [30,31]. MTA Fillapex® had a considerably lower amount of calcium than Sealer 26® and Sealapex®.

Pulp Canal Sealer® and Endofill® seem to have antimicrobial properties due to the presence of eugenol and zinc oxide [32]. However, the irritant effect of eugenol on biological tissues precludes its use as an antimicrobial agent of a root canal sealer. The antimicrobial properties of Pulp Canal Sealer® may be associated with the presence of nanoparticulate silver. The antimicrobial action of AH Plus® is due to the presence of bisphenol-A epoxy resin and amines in its composition [33].

Several studies reported that calcium is actively involved in periapical repair [30,34,35]. Estrela et al. [30] described the mechanism by which calcium ions form calcite crystals and directly participate in the construction of a mineralized barrier. Seux et al. [35] described the affinity of fibronectin for calcite crystals, the promotion of cell adhesion and differentiation and, consequently, the deposition of hard tissue. Therefore, materials with high levels of calcium, such as Sealapex® and Sealer 26®, may result in better filling. MTA Fillapex® had considerably lower levels of calcium than the other two materials, and a lower capacity of inducing periapical repair should be expected from this sealer. However, the higher amount of calcium and hydroxyl ions from calcium hydroxide, as well as their mechanical action as a matrix, may protect against overfilling and justify the use of this medication before root canal filling, as a means to induce periapical repair and the formation of a mineralized barrier in the root apex [12].

EDX microanalysis has been used to describe the chemical composition of root canal sealers because of its efficiency inaccurately detecting chemicals in solid materials, mainly heavy elements [13]. However, this method has some limitations in, for example, detecting light elements. Also, the fluorescent yield (the proportion of ionization events that result in the emission of an X-ray) decreases with decreasing atomic number of the element. Moreover, the low energy X-rays, a characteristic of light elements, are absorbed in the Au contact layer, which was part of the method used in this study.

For this reason, the quantitative description of organic compounds containing carbon, oxygen, hydrogen and nitrogen was not accurate.

The use of Net Counts ruled out questions about the representative chemical element of each energy peak and provided an accurate analysis of the chemical composition of the materials. However, the interpretation of results may be difficult when the sample has elements with similar energy peaks. The analysis of the AH Plus® samples revealed the presence of W, but its energy peak was very similar to that of another element described by the manufacturer, Si, and no conclusion could be reached about whether AH Plus® had silicon or not.

The knowledge and evidence of the characteristics of the chemical elements of root canal sealers provided by the manufacturers are important due to influence other cytotoxicity and physicochemical properties [36-38].

Further studies should assess the effects of the main chemical elements and chemical compounds on the periapical tissue, as well as their antimicrobial properties.

Conclusions

The root canal sealers under analysis had different surface structures. Particles were distributed uniformly and had similar sizes but variable shapes. Some of the chemical elements found in the root canal sealers are not described by their manufacturers.

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