Analysis of Chemical Elements and Heavy Metals in MTA Fillapex and AH Plus

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Abstract

Aim: The aims of this study were to analyze the chemical constitution and the presence of heavy metals in MTA Fillapex and AH Plus sealers.

Methods: The main components of the sealers were analyzed by using a Scanning Electron Microscopy equipped with Energy Dispersive X-ray analysis (SEM/EDX), and the concentration of 11 heavy metals (arsenic, bismuth, cadmium, calcium, copper, chromium, iron, lead, manganese, nickel and zinc) was measured by Atomic Absorption Spectrophotometry (AAS).

Results: SEM/EDX revealed that MTA Fillapex contained silicon (16.79 wt%), calcium (14.87 wt%), bismuth (18.37 wt%), carbon (17.79 wt%) and oxygen (26.29 wt%), and AH Plus, zirconium (31.16 wt%), tungsten (15.72 wt%), carbon (34.98 wt%) and oxygen (14.36 wt%). AAS revealed increased levels of arsenic, copper, chromium, iron, nickel and zinc in AH Plus, and of bismuth, calcium and lead in MTA Fillapex.

Conclusions: The chemical composition of MTA Fillapex and AH Plus was in accordance to MSDS provided by manufacturers. Evidence of the presence of heavy metals was found in both root canal sealers.

Key Words: Atomic absorption spectrophotometry, Chemical elements, Energy dispersive X-ray spectroscopy, Heavy metals, Root canal sealers

Introduction

Root canal sealers (RCS) are used to promote an airtight seal and prevent oral pathogens from colonizing and re-infecting the root and periapical tissues [1]. The sealers currently available are classified according to main chemical components: zinc-oxide-eugenol sealers, sealers containing calcium hydroxide, resin-based, glass-ionomer-based, silicone-based and bioceramic sealers [2-4].

AH Plus is an epoxy-resin-based material used as reference because of its good physicochemical properties, such as long-term dimensional stability, apical sealing ability and adherence to root dentine [5-9]. However, the AH Plus's low solubility has not been fully explained, because its chemical characteristics remain unknown [8]. In addition, a moderate to severe cytotoxicity postoperative effect of AH Plus has been reported, which may be related to release of constitutional compounds [10]. MTA Fillapex® has been recently introduced in the market as a new option for root canal filling. It was designed in an attempt to combine the physicochemical properties of a sealer with the biological properties of Mineral Trioxide Aggregate (MTA) [11,12]. It is composed of MTA, salicylate resin, natural resin, bismuth oxide and silica [3,4,13]. Compared to others RCS, MTA Fillapex has short working and setting times [4,13], adequate radiopacity [12], high flowability [4,12] and alkaline pH [12]. Despite these characteristics, MTA Fillapex presents low compressive strength, which has been related to the presence of unknown additives in its composition and its high solubility [4,14].

Although specific properties of root canal sealers have been widely studied [1,3-5,7,12,13,15], limited data are available about their exact chemical composition [3,16]. The chemical characteristics of the constituents found in root canal sealers may define important physicochemical, mechanical and, above all, biological properties [4,5,7]. During root canal filling procedures, the sealer may extrude through the apical foramen, and its contact with periapical tissues may lead to undesirable effects [5,17]. Some sealers may release toxic substances after mixing, which may interfere with healing or cause adverse reactions [2,13,15]. Therefore, a study about their chemical characteristics may contribute to a better understanding of their clinical behavior and performance.

Different techniques have been used to study the chemical composition of dental materials: energy dispersive X-ray spectroscopy (EDX), Atomic Absorption Spectrometry (AAS), fluorescence spectrometer X-ray, X-ray diffraction analysis and inductively coupled plasma emission spectrometry [18-22]. EDX has been used in conjunction with Scanning Electron Microscopy (SEM) wherein SEM does the structural analysis and elemental analysis is done by EDX [23,24]. AAS has been used as an analytical tool to measure the concentration of a particular chemical in solutions or directly in solid samples [25,26]. The combination of the use of SEM/EDX and AAS is important to analyze the exact composition of RCS and to describe all trace elements and chemical elements that determine their properties. The aims of the present study were to identify the chemical elements in AH Plus and MTA Fillapex using SEM/EDX and to determine heavy metal contents in the two sealers using AAS.

Materials and Methods

Tested materials

The materials evaluated in this study and their compositions are described in *Table 1*. All materials were handled according to the manufacturer's instructions.

Scanning electron microscopy and energy-dispersive X-ray

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Table 1. *Composition of the materials and their manufacturer.*

Materials	Composition (MSDS data)	Manufactures
AH Plus	Component A: epoxy resin, calcium tungstate, zirconium oxide, aerosil, iron oxide; Component B: adamantane amine, N,N-Dibenzyl-5-oxanonane, TCD-Diamine, calcium tungstate, zirconium oxide, aerosol	Dentsply De Trey Gmbh, Konstanz, Germany
MTA Fillapex	Components after mixture: resins (salicylate, diluting, natural), radiopaque bismuth, nanoparticulated silica, mineral trioxide aggregate, pigments	Angelus, Londrina, PR, Brazil

analysis (SEM/EDX)

Six standard polyethylene tubes measuring 3 mm in its internal diameter and 3 mm long, were prepared using a digital caliper reading to 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan) and a number 11 disposable scalpel blade (Swann Morton, Sheffield, UK). Three tubes for each group were placed on a polished glass slab (75 x 25 x 1 mm) and filled with freshly mixed sealers. The specimens were placed in a chamber at 95% relative humidity and 37°C for 48 hours. The specimens were fixed to a metal stub, surface-sputtered with gold-palladium (Bal-Tec AG, Balzers, Germany) at 20 mA and examined using a Leo Stereoscan 420i scanning electron microscope (Leica Electron Optics, Cambridge instruments, Cambridge, UK) at 8-10 kV and 2-nm resolution, without any preparation. Images at 5000X magnification were captured.
EDX was performed using the NSS Spectral Analysis System 2.3 (Thermo Fischer Scientific, San Jose, CA) to determine the chemical composition of the materials under evaluation. One EDX spectrum was obtained from the central region of each specimen under the following conditions: 25 kV accelerating voltage, 110 µA beam current, 10^{-6} Torr pressure (high-vacuum), 130 x 130 µm area of analysis at 1000X magnification, 100 s acquisition time and 30-35% detector dead time. The elemental analysis (weight% and atomic %) of samples was conducted using nonstandard analysis mode and the Phi-Rho-Z (Proza) correction method. High-resolution elemental maps were created using the net count method and the same detection-analysis system (NSS Spectral Analyses System 2.3).

Atomic absorption spectrophotometry (AAS)

Total metal content was determined according to the ISO 9917-1 method [27]. The metals analyzed were: arsenic, bismuth, calcium, chromium, lead, copper, chrome, iron, manganese, nickel and zinc. Five samples were prepared for each material. One gram of each sample, to the nearest 0.001 g, was weighed and transferred to a 100-mL Teflon tube. A mixture of 7.0 mL of nitric acid (65% v/v) and 21 mL of hydrochloric acid (37% v/v) was added to the Teflon tube and allowed to stand for 2 hours. The tube was loosely capped and transferred to a hood with an exhaust vent, where it was heated to 80°C by placing it on a heating block and then allowed to equilibrate for 150 min. After the reaction tube was cooled to room temperature, the mixture was filtered to obtain a filtrate in 50 mL of distilled water. A blank test was performed in parallel using the same procedure and the same quantities of all the reagents but not the test sample. For each metal, specific patterns were determined from universal standards (Merck, Darmstadt, Germany). A Spectra A atomic absorbance spectrometer (Varian, Ind. e Com. Ltda, São Paulo, Brazil) with an aspiration rate of 2 mL/min and a specific cathode for each metal was used. The parameters for each element under analysis were determined according to the electric current, wavelength and slit aperture of the device.

For arsenic, the quantification was performed using an atomic absorption spectrophotometer (Spectra A, Varian Ind. e Com. Ltda, São Paulo, Brazil) equipped with a hydride generator at 3 psi of hydrogen pressure, nitrogen at 50 mL/min, HCL solution in 10 mol and aspiration rate of 1 mL/min and 1% sodium borohydride in 1% sodium hydroxide (Merck, Darmstadt, Germany) at an aspiration rate of 1 mL/min. The aspiration rate of the sample was 6 mL/min in 40 s of integration time. The analytical conditions were: arsenic hollow cathode lamp (Varian Ind. e Com. Ltda, São Paulo, Brazil) operating at 193.7-nm wavelength with a 10-mA lamp operation current and slit width of 0.5 nm. Arsenic patterns were created in concentrations of 0.1, 0.25, 0.5, 1 and 2 mg/L. All the measurements were performed in quintuplicate.

The data were statistically analyzed by one-way analysis of variance and the Tukey's test at 5% level of significance, with Kolmogorov-Smirnov and Levene tests (normality and variances homogeneity). The tests were performed using IBM SPSS statistical software, version 21.0 for Windows (IBM Corporation, Somers, NY, USA).

Results

SEM/EDX

Table 2 shows the quantitative results of the main components of the RCS under test. EDX profiles of MTA Fillapex revealed high peaks of silicon (Si), calcium (Ca), bismuth (Bi), carbon (C) and oxygen (O), whereas peaks of zirconium (Zr), tungsten (W), carbon (C) and oxygen were found in AH Plus (*Figure 1A and 1B*).

AAS

Table 3 shows the mean values of metal contents, standard deviations and the differences between the cements according to AAS.

The values of cadmium for both RCS were lower than the quantification limit of the device. The difference between RCS was significant ($P<0.05$). The levels of arsenic, copper, chromium, iron, nickel and zinc were higher in AH Plus. MTA Fillapex had high values of bismuth, calcium and lead. Manganese was detected only in MTA Fillapex.

Discussion

The standard reference of a RCS should provide a complete seal after setting, dimensional stability, a long setting time to ensure sufficient working time, insolubility to tissue fluids, adequate adhesion to root canal walls and biocompatibility [28]. Studies compared the biological and physicochemical properties of RCS [1,3,11,12,29], and various products have been suggested as innovative materials. However, the ideal RCS has yet to be found.

The chemical characteristics of dental materials used in close contact with periapical tissues are predictive factors of their physical, chemical and biological properties [4,16,22]. Their chemical composition is decisive in choosing the standard material for clinical practice [14]. EDX is a reliable, accurate and reproducible method to quantify the main constituents or components on the surface of different materials or mixtures [16,22,24]. Each element produces its own characteristic set of X-ray lines at precisely defined energies [19]. The measurement of these line energies indicates what elements are present [24,30,31]. However, by EDX may be difficult to determine whether the counts on the X-ray map come from the X-ray line of a certain element, from continuum radiation, or from overlapped elements [31]. Organic compounds, which have carbon, hydrogen and oxygen, may not be accurately quantified [30].

EDX revealed that MTA Fillapex was mostly composed of oxygen, carbon, silicon, calcium and bismuth, whereas AH Plus was essentially formed by carbon, zirconium, tungsten and oxygen.

Interestingly, traces of Ti were identified in MTA Fillapex. The MTA Fillapex manufacturer has not described this element, and its presence may be attributed to contamination during manufacture or to industrial secrets. EDX analysis was unable to detect iron (Fe), which is described in the AH Plus MSDS as iron oxide (*Tables 1 and 2*).

Table 2. *Main components of tested materials analyzed by using energy-dispersive X-ray spectroscopy.*

Element	Endodontic sealers			
	MTA Fillapex		AH Plus	
	at%*	wt%**	at%	wt%
Carbon (C)	34.01	17.79	67.22	34.98
Oxygen (O)	37.60	26.19	20.73	14.37
Aluminum (Al)	0.38	0.45	0.00	0.00
Bismuth (Bi)	2.02	18.37	0.00	0.00
Calcium (Ca)	8.52	14.87	1.96	3.40
Chlorine (Cl)	0.00	0.00	0.24	0.37
Sulfur (S)	3.33	4.66	0.00	0.00
Silicon (Si)	13.72	16.79	0.00	0.00
Titanium (Ti)	0.42	0.88	0.00	0.00
Tungsten (W)	0.00	0.00	1.97	15.72
Zirconium (Zr)	0.00	0.00	7.88	31.16
Total	100.00	100.00	100.00	100.00

*at% - atomic%; **wt% - weight%

Other studies [3,16,23] found slightly different chemical compositions of AH Plus and MTA Fillapex. Sampaio et al. [16] evaluated the chemical elemental composition of MTA Fillapex and AH Plus by using SEM/EDX and observed that MTA Fillapex was composed of aluminum, titanium, sulfur, silicon, calcium and bismuth, while AH Plus was formed by Al, Ca, iron (Fe), chlorine (Cl), zirconium (Zr), tungsten (W) and hafnium (Hf). Gandolfi & Prati [23] used SEM/EDX to verify the chemical characteristics of the root canal filling material in the dentine/sealer interface and on the surface of sealer samples. The root sections filled with calcium silicate MTA cements had aluminum (Al) and bismuth (Bi) and high peaks of calcium (Ca) and silicon (Si), whereas the samples filled with AH Plus had Si, tungsten (W) and platinum (Pt), as well as Ca, zinc (Zn) and zirconium (Zr). The EDX analysis of the outer surface of the sealers found the same chemical elements. In a recent study [3], SEM/EDX and AAS were used to evaluate the changes in the structure of the surface of endodontic sealers after a solubility test. EDX analysis of MTA Fillapex revealed that the external surface was composed of carbon (C), Zr, oxygen (O), W, Ca and Si initially, and after the solubility test, there was a reduction of C, Zr and Si values and an increase of Ca, W, O and titanium (Ti). In AH Plus, C, Zr, W, Bi and Ca were identified before the solubility test, and, after that, there was a reduction of C, Zr and Bi and an increase of O, W, Ca and Si. No bismuth was detected after the solubility test. The AAS analysis revealed a high level of Ca^{2+} in all materials, except AH Plus sealer. The differences between those studies might be explained by variations in experimental conditions. For the EDX analysis, five pieces of each sample were selected at random, and the result was based on their mean values, differently from the parameters used in our study.

Moreover, MTA Fillapex and AH Plus are paste-to-paste sealers, and some components may get deposited at the lower

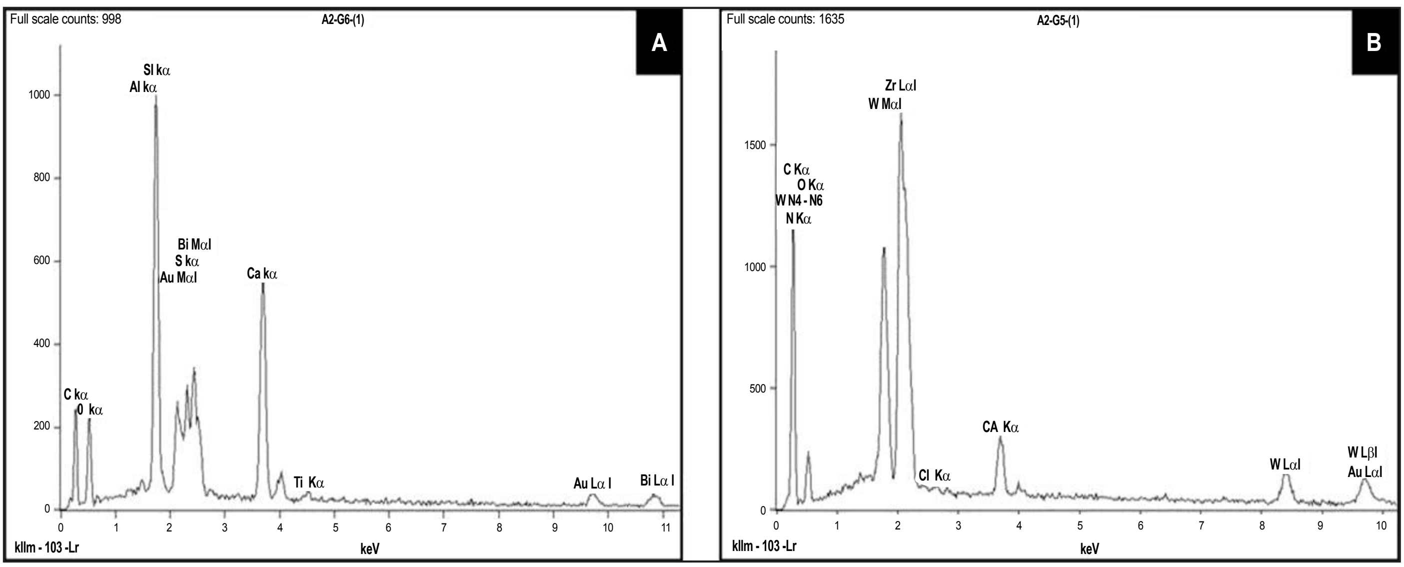

Figure 1. *Representative EDX spectrum of the tested endodontic sealers. A: MTA Fillapex; B: AH Plus.*

Table 3. *Means and standard deviations of metal contents (mg/kg) for the tested materials by using atomic absorption spectrophotometry.*

Metal	Endodontic sealers	
	MTA Fillapex	**AH Plus**
Arsenic (As)	0.0020 (0.00)[a]	0.0032 (0.00)[b]
Bismuth (Bi)	61.02553 (13.22382)[a]	0.017 (0.000816)[b]
Cadmium (Cd)	*<QL	*<QL
Calcium (Ca)	72.8668 (5.04399246)[a]	0.345033 (0.023163)[b]
Copper (Cu)	0.00023333 (0.0012472)[a]	0.006533 (0.001837)[b]
Chromium (Cr)	0.004067 (0.000471)[a]	0.036333 (0.002055)[b]
Iron (Fe)	0.0260667 (0.00124989)[a]	0.255667 (0.02655)[b]
Lead (Pb)	0.021467 (0.002347)[a]	0.014667 (0.001886)[b]
Manganese (Mn)	0.01763333 (0.00135728)	*<QL
Nickel (Ni)	0.002833 (0.000634)[a]	0.003667 (0.000471)[b]
Zinc (Zn)	0.0074667 (0.00073636)[a]	0.010933 (0.000624)[b]

*<QL – lower than the quantification limit of the device (0.002 mg/kg). On horizontal lines the different superscript represents statistically significant difference (P<0.05).

end of the tube. Therefore, depending on the portion of the tube from where the sealer was taken, results may differ from those found for the upper portions [13,14].

The bond strength to root dentin of MTA-based and epoxy resin-based sealers was evaluated [9]. The Endo-CPM sealer had the highest bond strength, and there were no significant differences between AH Plus and MTA Fillapex. The similarities between the bond strength of MTA Fillapex and AH Plus were expected and corroborated by the composition of the sealers, because MTA Fillapex has a combination of resin and silica. However, our study found clear differences in the chemical composition of these two materials.

The use of net counts (elemental maps) ruled out questions about the chemicals represented in each energy peak and provided a precise analysis of the chemical structure of the sealers. Moreover, the 2D observation provided information about basic morphology and a comparison of the elemental distribution on the surface of sealers. Some chemicals not detected using EDX, such as Ni and Mg in MTA Fillapex and Al and Fe in AH Plus, were then detected. The analysis of AH Plus revealed the presence of W, whose energy peak is very similar to that of Si. Thus, no conclusion was made about whether this sealer has Si in its composition [17,18]. The molecules released from the material and its surface structures are critical factors in material-cell interactions [17].

AAS is a powerful tool to determine the concentration of a particular metal in a sample. Atoms of different chemicals absorb light according to characteristic wavelengths, and the analysis of a sample to see if it contains a particular chemical means using light that corresponds to that element. The amount absorbed is compared with the calibration curve to calculate the concentration of that chemical in the unknown sample. The main advantages of AAS are: the decomposition step is omitted; no reagents are added; and only small sample amounts are required [25]. However, the sample is destroyed, and only one element can be determined at a time. In addition, the evaporation of the matrix may introduce systematic errors [20,25,26,32]. In dentistry, AAS has been used to study the concentration of heavy metals released from Portland and MTA-based cements [20] and to determine the components released from RCS during solubility tests [1,3,6,7,29].

The determination on the levels of heavy metals in commercially available RCS is very important. ISO 9917-1 [27] was adopted to ensure reproducibility and further comparisons. In the present study, other toxic heavy metals, such as antimony, beryllium, molybdenum, barium, uranium, cerium, mercury, cobalt, silver, strontium, titanium and indium, were not investigated Ours results showed that MTA Fillapex contained higher contents of calcium, bismuth and lead than AH Plus, which, in contrast, had higher concentrations of arsenic, copper, chromium, iron, nickel and zinc. Calcium oxide is one of the most important components of MTA-based cements [22]. Calcium ions, the most frequent in MTA Fillapex, are associated with calcium hydroxide formation during hydration. Calcium hydroxide increases RCS biocompatibility by stimulating periapical repair, collagenization and mineralization [33]. Bismuth has low toxicity as a result of its low absorbance by humans [34]. It can be found freely in nature, as well as in minerals such as bismuthine and in bismuth oxide. This element is added to improve the radiopacity of materials, but it may affect their physicochemical properties [8].

Arsenic is a metalloid encountered in nature in both inorganic and organic forms and in different stages of oxidation. It can inhibit cellular respiration, alter genetic expression of stress proteins and react with sulfhydryl groups to deactivate proteins and enzymes [34,35]. The maximum specified for arsenic in ISO 9917-1 [27] is 2 mg/kg, and both sealers meet the ISO standard. Iron is important for cellular homeostasis and, mainly, for oxygen transport by hemoglobin in blood vessels [34]. The maximum level of iron in drinking water should be 0.3 mg/L [35]. Iron in dental materials may cause discoloration of teeth [35], because Fe^{+3} is one of the strongest chromophores [18]. Chromatic changes in human tooth crowns induced by MTA Fillapex and Roth-811 was evaluated, and minimal color differences after the application of MTA Fillapex was found [36,37].

As important as calcium, bismuth, arsenic and iron are copper, zinc, chromium and nickel. Copper, found in nature mostly in impure mineral forms, participates in iron fixation to hemoglobin, the synthesis of neurotransmitters, cardiac and vascular integrity, bone resistance and neutrophil maturation. Copper has been known to be one of the chromospheres of MTA [18]. The minimum acceptable daily oral intake for copper is 0.020 mg/kg of body weight for adults. Zinc contributes to the functioning of the immune system and is essential to wound healing and ammonium dinitramide synthesis [34]. Metallic zinc is not toxic, but some zinc compounds, such as zinc oxide or sulfide, are harmful, and their excessive absorption may interfere with the organic absorption of iron and copper. Chromium is fundamental for human life, although excess chromium, as well as its oxidative form, may damage the respiratory system, mucosa, and skin and contribute to the development of lung cancer [35]. Nickel is an allergen and a possible carcinogen [34].

The results obtained using both methods complemented each other remarkably, which confirms that EDX and AAS are suitable for use in association as methods for the study of the chemical composition of different dental materials. The understanding of the physical, mechanical and chemical properties of RCS and the interactions between these materials and periapical tissues should contribute to develop new products. Further studies should be conducted to evaluate the effect of chemical composition on the physicochemical and biological characteristics of RCS.

Conclusion

The chemical composition of MTA Fillapex and AH Plus was in accordance to MSDS provided by manufacturers. Evidence of the presence of heavy metals was found in both RCS.

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References

1. Flores DSH, Rached-Júnior FJA, Versiani MA, Guedes DFC, Sousa-Neto MD, Pécora JD. Evaluation of physicochemical properties of four root canal sealers. *International Endodontic Journal*. 2011; **44**: 126-135.

2. Miletić I, Devcić N, Anić I, Borcić J, Karlović Z, Osmak M. The cytotoxicity of Roeko Seal and AH Plus compared during different setting times. *Journal of Endodontics*. 2005; **31**: 307-309.

3. Borges RP, Sousa-Neto MD, Versiani MA, Rached-Júnior FA, De-Deus G, Miranda CES. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. *International Endodontic Journal*. 2012; **45**: 419-428.

4. Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. *Journal of Endodontics*. 2013; **39**: 1281-1286.

5. Mc Michen FRS, Pearson G, Rahbaran S, Gulabivala K. A comparative study of selected physical properties of five root-canal sealers. *International Endodontic Journal*. 2003; **36**: 629-635.

6. Versiani MA, Carvalho-Junior JR, Padilha MIAF, Lacey S, Pascon EA, Sousa-Neto MD. A comparative study of physiochemical properties of AH Plus and Epiphany root canal sealants. *International Endodontic Journal*. 2006; **39**: 464-471.

7. Resende LM, Rached-Junior FJA, Versiani MA, Souza-Gabriel AE, Miranda CES, Silva-Sousa YTC. A comparative study of physicochemical properties of AH Plus, Epiphany, and Epiphany SE root canal sealers. *International Endodontic Journal*. 2009; **42**: 785-793.

8. Garrido ADB, Lia RCC, França SC, Da Silva JF, Astolfi-Filho S, Sousa-Neto MD. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on *Copaifera multijuga* oil-resin. *International Endodontic Journal*. 2010; **43**: 283-291.

9. Assmann E, Scarparo RK, Böttcher DE, Grecca FS. Dentin bond strength of two mineral trioxide aggregate-based and one epoxy resin-based sealers. *Journal of Endodontics*. 2012; **38**: 219-221.

10. Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. *International Endodontic Journal*. 2007; **40**: 329-337.

11. Scelza MZ, Linhares AB, Da Silva LE, Granjeiro JM, Alves GG. A multiparametric assay to compare the cytotoxicity of endodontic sealers with primary human osteoblasts. *International Endodontic Journal*. 2012; **45**: 12-18.

12. Silva EJNL, Rosa TP, Herrera DR, Jacinto RC, Gomes BPFA, Zaia AA. Evaluation of cytotoxicity and physicochemical properties of calcium silicate-based endodontic sealer MTA Fillapex. *Journal of Endodontics* 2013; **39**: 274-277.

13. Vitti RP, Prati C, Silva EJNL, Sinhoreti MAC, Zanchi CH, De Souza e Silva MG, Ogliari FA. Physical properties of MTA Fillapex sealer. *Journal of Endodontics*. 2013; **39**: 915-918.

14. Viapiana R, Flumignam DL, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M. Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Porltand cement-based experimental endodontic sealers. *International Endodontic Journal*. 2014; **47**: 437-448.

15. Bin CV, Valera MC, Camargo SE, Rabelo SB, Silva GO, Balducci I, Camargo CH. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. *Journal of Endodontics*. 2012; **38**: 495-500.

16. Sampaio FC, Alencar AH, Guedes OA, Veloso HH, Santos TO, Estrela C. Chemical elements characterization of root canal sealers using scanning electron microscopy and energy dispersive X-ray analysis. *Oral Health and Dental Management*. 2014; **13**: 27-34.

17. Güven EP, Yalvaç ME, Kayahan MB, Sunay H, Şahin GB. Human tooth germ stem cell response to calcium-silicate based endodontic cements. *Journal of Applied Oral Science*. 2013; **21**: 351-357.

18. Dammaschke T, Gerth HUV, Züchner H, Schäfer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. *Dental Materials*. 2005; **21**: 731-738.

19. Asgary S, Parirokh M, Eghbal MJ, Stowe S, Brink F. A qualitative X-ray analysis of white and grey mineral trioxide aggregate using compositional imaging. *Journal of Materials Science: Materials in Medicine*. 2006; **17**: 187-191.

20. Schembri M, Peplow G, Camilleri J. Analyses of heavy metals in mineral trioxide aggregate and Portland cement. *Journal of Endodontics*. 2010; **36**: 1210-1215.

21. Chang SW, Shon WJ, Lee W, Kum KY, Baek SH, Bae KS. Analysis of heavy metal contents in gray and white MTA and 2 kinds of Portland cement: a preliminary study. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology*. 2010; **109**: 642-646.

22. Estrela C, Sousa-Neto MD, Guedes OA, Alencar AHG, Duarte MAH, Pécora JD. Characterization of calcium oxide in root perforation sealers materials. *Brazilian Dental Journal*. 2012; **23**: 539-546.

23. Gandolfi MG, Prati C. MTA and F-doped MTA cements used as sealers with warm gutta-percha. Long-term study of sealing ability. *International Endodontic Journal*. 2010; **43**: 889-901.

24. Hegde MN, Devadiga D, Jemsily PA. Comparative evaluation of effect of acidic beverage on enamel surface pre-treated with various remineralizing agents: An *In vitro* study. *Journal of Conservative Dentistry*. 2012; **15**: 351-356.

25. Langmyhr FJ, Sundli A. Atomic absorption spectrometric determination of cadmium and lead in dental material atomization directly from the solid state. *Analytica Chimica Acta*. 1974; **73**: 81-85.

26. L'vov BV. Fifty years of atomic absorption spectrometry. *Journal of Analytical Chemistry*. 2005; **60**: 382-392.

27. International Standardization Organization. *Dentistry: Water-Based Cements-Part 1: Powder/liquid Acid-Base Cements*. Switzerland: ISO 9917-1; 2003: 1-22.

28. Grossman LI (Editor). Endodontic Practice (10th Edn.) Philadelphia, USA: *Henry Kimpton Publishers*. 1981: 297.

29. Marin-Bauza GA, Rached-Junior FJA, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE, Silva-Sousa YT. Physicochemical properties of methacrylate resin-based root canal sealers. *Journal of Endodontics*. 2010; **36**: 1531-1536.

30. Vaughan D. Energy-dispersive X-ray microanalysis: An introduction. Middleton, USA: NORAN Instruments. 1999.

31. Goldstein JI, Newbury DE, Echlin P, Joy DC, Lyman CE, Lifshin E, Swayer L, Micheal JR (Editors). Scanning electron microscopy and X-ray microanalysis (3rd Edn.). New York, USA: Springer Science + Business Media. 2003.

32. Welz B, Sperling M. Atomic absorption spectrometry. West Sussex, UK: John Wiley & Sons Ltd. 2008.

33. Huang FM, Tai KW, Chou MY, Chang YC. Cytotoxicity of resin, zinc oxide-eugenol, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. *International Endodontic Journal*. 2002; **35**: 153-158.

34. Masterton WL, Hurley CN. Chemistry: principles & reactions. Belmont: Brooks/Cole Cengage Learning, 2009.

35. Kum KY, Kim EC, Yoo YJ, Zhu Q, Safavi K, Bae KS, Chang SW. Trace metal contents of three tri calcium silicate materials: MTA Angelus, Micro Mega MTA and Bioaggregate. *International Endodontic Journal*. 2014; **47**: 704-710.

36. Ioannidis K, Mistakidis I, Beltes P, Karagiannis V. Spectrophotometric analysis of crown discoloration induced by MTA-and ZnOE-based sealers. *Journal of Applied Oral Science*. 2013; **21**: 138-144.

37. Lenherr P, Allgayer N, Weiger R, Fillipi A, Attin T, Kraste G. Tooth discoloration induced by endodontic materials: A laboratory study. *International Endodontic Journal*. 2012; **45**: 942-949.