UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

RAQUELI VIAPIANA

PROPRIEDADES FÍSICO-QUÍMICAS E MECÂNICAS, POTENCIAL BIOATIVO E CARACTERIZAÇÃO DA INTERFACE DENTINA-CIMENTO DE CIMENTOS ENDODÔNTICOS EXPERIMENTAIS À BASE DE CIMENTO PORTLAND ASSOCIADOS À RADIOPACIFICADORES MICRO E NANOPARTICULADOS

[*PHYSICOCHEMICAL AND MECHANICAL PROPERTIES, BIOACTIVITY POTENTIAL AND CHARACTERIZATION OF THE DENTIN-SEALER INTERFACE OF EXPERIMENTAL ROOT SEALERS BASED ON PORTLAND CEMENT WITH MICRO AND NANO PARTICULATE RADIOPACIFIERS*]

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Orientador [*Supervisor*]: Prof. Dr. Mário Tanomaru Filho

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Eu tenho um sonho
[*I have a dream*]
Marthin Luther King

Dedicatória

[Dedication]

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Viapiana R. Physicochemical and mechanical properties, bioactivity potential and characterization of the dentin-sealer interface of experimental root sealers based on Portland cement with micro and nano radiopacifiers [tese de Doutorado]. Araraquara: Faculdade de Odontologia da Unesp; 2014.

Abstract

Portland Cement is composed by calcium silicate and the association with additives or vehicles, may confer characteristics to enable the use of this cement as root canal sealer. However, Portland cement lacks in radiopacity which requires the addition of a radiopacifying agent to the mixture to be used as dental material. The purpose of this study was to assess the physicochemical and mechanical properties, the bioactivy potential and to characterize the dentin-sealers interfaces of Portland-based experimental root canal sealers (ES) containing nano or micro particles of zirconium oxide or niobium oxide. Setting time, compressive strength, flow ability, film thickness, radiopacity, solubility and dimensional stability were evaluated according to ISO 6876:2012 standards, whereas formaldehyde realease was investigated using gas-cromatography. Dentin bond strength was evaluated by push-out test and the sealer's microestruture and bioactivity potential were perfomed using X-ray energy espectroscopy, X-ray diffractometry and infrared spectroscopy. Dentin-sealers interface was assessed with respect to fluorescent microspheres penetration and it was also examined using confocal microscope and scanning electron microscope coupled to X-ray energy dispersive line scans. Data were analyzed by ANOVA and Tukey post-hoc test ($p < 0.05$). With the exception of radiopacity, ES showed physicochemical properties according to ISO 6876:2012 specifications, adequate dentin bond strength, great bioactivity potential and promoted coronal sealing and chemical interaction with dentin.

Keywords: Endodontics; Dental Materials; Physical and Chemical Properties; Calcium Silicicum

Viapiana R. Propriedades físico-químicas e mecânicas, potencial bioativo e caracterização da interface dentina-cimento de cimentos endodônticos experimentais à base de cimento Portland associados à radiopacificadores micro e nanoparticulados [tese de doutorado]. Araraquara: Faculdade de Odontologia da Unesp; 2014.

Resumo

O cimento Portland é constituído por silicato de cálcio e a associação com aditivos e veículos conferem características que podem viabilizar seu uso como cimento endodôntico. No entanto, o cimento Portland não apresenta radiopacidade própria, o que torna necessário a adição de um agente radiopacificador à mistura para ser utilizado como material dentário. O objetivo deste estudo foi avaliar as propriedades físico-químicas e mecânicas, o potencial bioativo e caracterizar a interface dentina-cimento de cimentos endodônticos experimentas à base de cimento Portland (ES) contendo nano ou micro partículas de óxido de zircônio ou óxido de nióbio. O tempo de presa, resistência à compressão, escoamento, espessura de filme, radiopacidade, solubilidade e estabilidade dimensional foram avaliadas de acordo com a norma ISO 6876:2012, enquanto que a liberação de fomaldeído foi avaliada por meio de cromatografia gasosa. A resistência de união dentinária foi avaliada por meio de teste push-out e tanto a caracterização da microestrutura dos cimento quanto a análise do potencial bioativo foram realizadas utilizando energia dispersiva de raios-x, difractometria e espectroscopia infravermelha. A interface dentina-cimento foi avaliada com relação à penetração de microesferas fluorescentes e examinada em microscopia confocal e microscopia eletrônica de varredura associada à escaneamento por energia dispersiva de raios-x linear. Os dados foram submetidos aos testes ANOVA e Tukey ($p < 0,05$). Com exceção da radiopacidade, os ES apresentaram propriedades físico-químicas de acordo com as especificações ISO 6876, adequada resistência de união à dentina, potencial bioativo e promoveram selamento coronário e interação química com a dentina.

Palavras-chave: Endodontia; Materiais Dentários; Propriedades físicas e químicas; Silicato de cálcio

Summary

1 Introduction

The success in endodontic therapy is directly related to the careful completion of all phases involved in the treatment, which is comprised by coronal access, biomechanical preparation and obturation of the root canals. Root canal obturation aims to completely fill root canal system with materials biologically inert and/or biocompatible to prevent reinfection and to allow periapical tissues healing (Ørstavik[47], 2005). This procedure is usually performed using a solid core (gutta-percha/resilon points) in association to endodontic sealers, which essentially fill voids and irregularities between the solid material and dentin walls (Gatewood[21], 2007). Root canal sealers present different formulations and, in general, they can be classified as: zinc oxide and eugenol-based sealers (Chandrasekhar et al.[13], 2011); calcium hydroxide-based sealers; glass-ionomer-based sealers (Tanomaru-Filho et al.[56], 2008), silicon-based sealers (Barbizam et al.[3], 2007), resin-based sealers (Neelakantan et al.[44], 2011) and, more recently, mineral trioxide aggregate-based sealers (Scarparo et al.[53], 2010), also denominated calcium silicate-based sealers (Sagsen et al.[50], 2011).

AH Plus is an epoxy resin-based sealer recognized for having physicochemical properties in accordance to nº 57 ANSI/ADA specifications regarding setting time, flow ability and radiopacity (Resende et al.[49], 2009) beyond low solubility and disintegration degree (Schafer, Zandbiglari[54], 2003), great dentin bond strength (Nunes et al.[45], 2008), antimicrobial activity (Nawal et al.[43], 2011) and satisphactory biological properties (Onay et al.[46], 2007).

With respect to root canal sealers with calcium hydroxide in the composition, the use of Sealapex in obturations widespread due to the biocompatibility and biological activity induced by this material on periapical tissues (Desai, Chandler[18], 2009; Veloso et al.[59], 2006). The biological property of this sealer has been related to the material's ability to release calcium ions and to maintain a high pH over long periods (Eldeniz et al.[20], 2007). However, the first studies with Sealapex demonstrated radiopacity levels inferior to 3 mm Al; thus, a new version of this sealer was proposed with satisfactory radiopacity (Guerreiro-Tanomaru et al.[30], 2009).

The use of mineral trioxide aggregate (MTA) as reparative material in Dentistry is well established as well. MTA is basically composed by Portland cement plus bismuth oxide as radiopacifier. Studies suggest that bismuth oxide negatively affect the hydration process of MTA and, consequently, the formation of calcium silicate hydrate, which is the final product of the reaction (Asgary et al.[2], 2008; Viana Viola et al.[60], 2012; Parirokh, Torabinejad[48], 1995). Consequently, the structure produced by this process will be porous and with lower mechanical strength in comparison to pure Portland. Moreover, bismuth oxide may be also related to an increase on MTA's citotoxity (Gomes Cornélio et al.[23], 2011).

Considering bismuth oxide replacement, zirconium oxide and niobium oxide have already exhibited great ability to promote radiopacity to endodontic cements (Camilleri[7],

2007; Coomaraswamy et al.[14], 2007; Camilleri et al.[9], 2011), likely due to the fact that they are transition metals of high atomic number. Moreover, studies had observed biocompatibility in dental implants covered with these chemical elements (Cutajar et al.[15], 2011; Leitune et al.[36], 2013).

MTA acceptance is related to the excellent biological response induce by this cement on living tissues, such as biocompatibility and bioactivity, (Bernarbe et al.[4], 2005; Sarkar et al.[52], 2005; Tanomaru-Filho et al.[57], 2006; Gorduysus et al.[28], 2007; Gomes-Filho et al.[26], 2009). On the other hand, the lack of flow ability of this material precludes its use as sealer in orthograde root canal fillings. Thus, endodontic sealers based on MTA or containing the main MTA' compounds, such as tricalcium or dicalcium silicate, have been investigated. For example, the CER sealer (named Fast Endodontic Cement) (Santos et al.[51], 2005; Gomes-Filho et al.[25], 2009) exhibited shorter setting time and increased flow ability in comparison to conventional MTA besides biocompatibility and ability to stimulate the deposition of mineralized tissue. The New Endodontic Cement (NEC) was another prototype that displayed shorter setting time and improved flow ability in comparison to MTA (Asgary et al.[1], 2009; Asgary et al.[2], 2008). Some commercial versions of sealers based on MTA have already been studied as well, such as EndoBinder Sealer, which showed similar bacterial sealing ability to MTA (Jacobovitz et al.[34], 2009). Other sealers developed to be used in root canal filling were the MTA Obtura (Bernardes et al.[5], 2010), the Endo CPM Sealer (Gomes-Filho et al.[24], 2012) and, recently, the MTA Fillapex (Gomes-Filho et al.[27], 2012). The Araraquara Dental School, UNESP – Univ Estadual Paulista (SP, Brazil) has dedicated some researches attempting to develop a Portland cement-based root canal sealer. Initially, this prototype sealer was denominated MTA Sealer and it was composed by white Portland cement, a radiopacifying agent (zirconium oxide), additives (calcium chloride) and a resinous vehicle. The preliminary tests with this material demonstrated that it showed high calcium release, great increase in pH up to 48 hours after mixing and long setting time, which are desirable features for a root canal sealer (Massi et al.[39], 2011).

Another aspect that have stimulated researchers is the incorporation of nanoparticles in dental products aiming to favour the interaction of the material's particles with surrounding environment as result of the improvement on material's physico-chemical, mechanical or biological properties. Accordingly, this study was subdivided in four chapters. In the first and second chapters, the physicochemical and mechanical properties of Portland-based root canal sealers containing nano or micro particles of zirconium oxide or niobium oxide as radiopacifiers, also denominated experimental sealers (ES), were compared to commercially available sealers (AH Plus, MTA Fillapex and Sealapex). The third chapter was dedicated to

compare the bioactivity potential of ES, MTA Fillapex and AH Plus; and the fourth chapter, to evaluate the interface between these sealers and dentin.

2 Chapter 1

Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Portland cement-based experimental endodontic sealers*

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ABSTRACT

Aim To evaluate the physicochemical and mechanical properties of Portland cement-based experimental sealers (ES) with different radiopacifying agents (zirconium oxide and niobium oxide micro- and nanoparticles) in comparison with the following conventional sealers: AH Plus, MTA Fillapex and Sealapex.

Methodology The materials were tested for setting time, compressive strength, flow, film thickness, radiopacity, solubility, dimensional stability and formaldehyde release. Data were subjected to ANOVA and Tukey tests ($P < 0.05$).

Results MTA Fillapex had the shortest setting time and lowest compressive strength values ($P < 0.05$) compared with the other materials. The ES had flow values similar to the conventional materials, but higher film thickness ($P < 0.05$) and lower radiopacity ($P < 0.05$). Similarly to AH Plus, the ES were associated with dimensional expansion ($P > 0.05$) and lower solubility when compared with MTA Fillapex and Sealapex ($P < 0.05$). None of the endodontic sealers evaluated released formaldehyde after mixing.

Conclusion With the exception of radiopacity, the Portland cement-based experimental endodontic sealers presented physicochemical properties according to the specifications no 57 ANSI/ADA (ADA Professional Product Review, 2008) and ISO 6876 (Dentistry — Root Canal Sealing Materials, 2012, British Standards Institution, London, UK). The sealers had setting times and flow ability that was adequate for clinical use, satisfactory compressive strength and low solubility. Additional studies should be carried out with the purpose of decreasing the film thickness and to determine the ideal ratio of radiopacifying agents in Portland cement-based root canal sealers.

Keywords: Endodontics; Endodontic sealers; Physicochemical properties.

Introduction

Newly proposed root filling materials should have their physicochemical and biological properties tested. The American National Institute/American Dental Association and the International Organization for Standardization define norms and standardized tests for evaluating, amongst other parameters, the setting time, flow, film thickness, solubility, radiopacity, dimensional stability and compressive strength of endodontic sealers (ANSI/ADA, 2008, ISO 2012). The chemical properties of the sealer must also be evaluated as one of the main purposes of the endodontic treatment is to promote repair of the periapical tissues. It has been reported that some root canal sealers release toxic substances after mixing, such as formaldehyde, which is responsible for the cytotoxicity of endodontic sealers (Bin et al. 2012) and may hinder tissue repair processes (Leonardo et al. 1999). Amongst the available epoxy-based endodontic materials, AH Plus (Dentsply De Trey, Konstanz, Germany) has been used as the gold standard for comparisons with other endodontic sealers (Garrido et al. 2010). Other sealers such as Sealapex (Kerr, Romulus, MI, USA) contain salicylate resin and calcium particles, promoting favourable biological response (Waltimo et al. 2001).

Mineral trioxide aggregate (MTA) has been widely used in dentistry as root-end filling material due to its excellent biological properties (Wälivaara et al. 2012). However, the handling and the properties of the freshly mixed material after manipulation with distilled water do not allow the use of MTA as a root canal sealer. Thus, experimental sealers (ES) based on mixing tricalcium silicate cement and radiopacifier using a vehicle other than water have been investigated. MTA-based sealers include the endodontic cement (CER) that seems to be biocompatible and to induce hard tissue deposition (Gomes-Filho et al. 2009); the new endodontic cement (NEC) exhibited lower setting time and higher flow ability than MTA (Asgary et al. 2009); Endobinder (Jacobovitz et al. 2009) had a microbial sealing ability comparable with MTA; MTA Obtura had greater flow ability than the minimum recommended in the ADA 57 specification (Bernardes et al. 2010), and Endo CPM Sealer, which failed to prevent apical leakage (Gomes-Filho et al. 2012). MTA has also been mixed with a water

soluble polymer, which resulted in a material with improved properties and was suitable for use as endodontic sealer cement with radiopacity greater than the 3 mm thickness of Al specified by the international standard (Camilleri 2009) and good dimensional stability (Camilleri & Mallia 2011). MTA Fillapex® is another MTA-based root canal sealer that was developed in 2010 by Angelus Indústria de Produtos Odontológico S/A (Londrina, PR, Brazil). This material is composed of salicylate resin, diluent resin, bismuth oxide, silica nanoparticles, MTA and pigments, and according to the manufacturer, its physicochemical properties are in compliance with the ISO 6876 (ISO 2001). Several properties of MTA Fillapex such as biocompatibility (Gomes-Filho et al. 2011, Zmener et al. 2012), bioactivity (Salles et al. 2012), cytotoxicity (Scelza et al. 2011), solubility (Borges et al. 2012), antibacterial effect (Morgental et al. 2011) and sealing ability (Gomes-Filho et al. 2012) have been investigated, but there is still a lack of information about the physicochemical and mechanical properties of MTA Fillapex.

Other prototype sealers based on the MTA system of a mixture of cement and radiopacifier have been proposed as bismuth oxide may alter the mechanical properties (Coomaraswamy et al. 2007), enhance the cytotoxicity of MTA (Gomes Cornelio et al. 2011) and promote teeth discoloration (Camilleri 2014). Alternative radiopacifiers such as zirconium oxide has been tested in prototype root-end filling materials (Camilleri et al. 2011, Hungaro Duarte et al. 2012). Niobium oxide is another material that has shown ability to increase radiopacity of methacrylate-based root canal sealers (Leitune et al. 2013), and it has been used in titanium alloys of osseo-integrated implants due to its excellent biocompatibility, resistance to corrosion and disintegration (Denry et al. 2005) and ability to promote apatite formation (Karlinsey et al. 2006). The goal of the present study was to evaluate the physicochemical and mechanical properties of Portland cement-based experimental endodontic sealers containing different radiopacifying agents and compare these materials with the conventional sealers AH Plus, Sealapex and MTA Fillapex. The null hypothesis is that radiopacifiers particle size does not exert an influence on the physical and chemical properties of the ES.

Materials and Methods

The physicochemical properties of the materials were evaluated by assessing their setting time, compressive strength, flow, film thickness, radiopacity, solubility, dimensional stability and formaldehyde release (Table 1) according to the ANSI/ADA Standard No.57 (2008) and ISO 6876 (2012).

Table 1: Composition and manufacturers of the endodontic sealers evaluated.

Material	Composition	Manufacturer
AH Plus	Paste A: epoxy resin; calcium tungstate; zirconium oxide; aerosil; iron oxide.	Dentsply, De Trey, Konstanz, Germany
	Paste B: adamantane amine; N, N-diberncil-5-diamine oxanone-1,9; TCD-diamine; calcium tungstate; zirconium oxide; aerosil; silicon oil	
Sealapex	Catalyzer paste: isobutyl salicylate resin; silicon dioxide; bismuth trioxide; titanium dioxide; pigments	SybronEndo – Sybron Dental Specialties, Glendona, CA, USA
	Base paste: sulphonamide resin; N-ethyl toluene; silicon dioxide; zinc oxide, calcium oxide	
MTA Fillapex	Salicylic resin, diluent resin, natural resin, bismuth oxide, silica nanoparticles, mineral trioxide aggregate; pigments (presented as paste-paste)	Angelus, Londrina, PR, Brazil
ES* Zr micro	Portland cement, zirconium oxide microparticles, additives (calcium chloride); resinous vehicle.	FOAr-Unesp, Araraquara, SP, Brazil
ES* Zr nano	Portland cement, zirconium oxide nanoparticles, additives (calcium chloride); resinous vehicle.	FOAr-Unesp, Araraquara, SP, Brazil
ES* Nb micro	Portland cement, niobium oxide microparticles, additives (calcium chloride); resinous vehicle.	FOAr-Unesp, Araraquara, SP, Brazil
ES* Nb nano	Portland cement, niobium oxide nanoparticles, additives (calcium chloride); resinous vehicle.	FOAr-Unesp, Araraquara, SP, Brazil

*Experimental Portland cement-based root canal sealer.

The following ES were prepared based on white Portland cement (Portland Cement; CPB-40; Votorantin cimentos, Camargo Correa S.A., Pedro Leopoldo, MG, Brazil) added to a radiopacifying agent (30% by weight):

1. micro-zirconium oxide (ZrO2; Sigma Aldrich, St Louis, MO, USA) - ES-Zr-micro;

2. nano-zirconium oxide (Institute of Physics of São Carlos, University of São Paulo, São Carlos,

Brazil) - ES-Zr-nano;

3. micro-niobium oxide (CBMM, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) - ES-Nb micro;

4. nano-zirconium oxide (Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil) - ES-Nb nano;

The nanoparticles of Nb_2O_5 and ZrO_2 were prepared by the polymeric precursor method. The ZrO_2 supports were prepared from the precursor salt $ZrO(NO_3)_2 \cdot xH_2O$ (Alfa Aesar). Aqueous solutions of this salt were prepared, mixed and added to an aqueous solution of citric acid (held at 60 °C), with constant stirring. Subsequently, ethylene glycol ($HOCH_2CH_2OH$) was added to polymerize the citrate by a polyesterification reaction (at 120 °C). The citric acid: metal molar ratio was 3:1, while the citric acid: ethylene glycol mass ratio was 60:40. The resulting polymer resin was then calcined at 300° C for 4h, and then after 600ºC/2h to produce ZrO2 crystalline particles. Whereas to produce Nb_2O_5 nanoparticles, an aqueous solution of niobium ammonium oxalate $\{NH_4[NbO(C_2O_4)_2(H_2O)](H_2O)N$ (CBMM, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) was prepared and ammonium hydroxide was dropped upon thereafter. The niobium hydroxide precipitated was filtered and washed to eliminate oxalate ions and dissolved into a citric acid (CA) aqueous solution ([CA]/[Nb] = 3) and filtered. The niobium content in the solution was precisely determined by gravimetric analysis. The solution was stirred for 2h at 70ºC to promote the complex reaction. Ethylene glycol was added to the mixture with mass ratio was 60:40. The translucent solution was heated and stirred over several hours. A polymerization process started during the water evaporation, resulting in a highly viscous solution. This resin was heated in an electric furnace at 300ºC for 4h. The resulting black and soft mass was milled and calcined in an electric furnace for 2h over alumina slabs at 700ºC/2h.

The ES were manipulated with an epoxy resin composed of equal amounts of catalyzer and base pastes which were mixed in a powder/liquid ratio of 5:3 (mass) for the materials containing micro-particles (ES-Zr-micro and ES-Nb-micro) (Massi *et al.* 2011) and 5:4 for those containing nanoparticles (ES-Zr-nano and ES-Nb-nano). The powder/liquid ratio of the sealers containing nanoparticles was determined by a previous pilot study. AH Plus (Dentsply, De Trey, Konstanz, Germany), Sealapex (Kerr, Romulus, MI, USA) and MTA Fillapex

(Angelus, Londrina, PR, Brazil) were used as controls. Based on the finding of Baldi et al. (2012), in the present study the initial 18 cm of the AH Plus tube were discarded prior to submitting this sealer to physicochemical testing.

Setting Time

The sealers were mixed and inserted in plastic moulds measuring 10 mm (inner diameter) and 1 mm in height (n = 8). Determination of setting time was performed using an indentation technique using Gilmore needle with a head weight of 100 g as suggested by ISO 6876 (2012). During the tests, the moulds containing the sealer samples were kept in an incubator with temperature set at 37°C and 95% humidity. The setting time of each sealer was established by calculating the mean time elapsed from mixing until the Gilmore needle failed to leave an indentation on the surface of the specimens.

Compressive strength

Six specimens measuring 12 mm in height and 6 mm in diameter were made by filling metallic moulds with samples of the materials and placing them in an oven set at 37ºC and 95% relative humidity for up to 3 times the setting time. After removing the specimens from the moulds, their lengths were confirmed using a digital caliper (Mitutoyo, Suzano, SP, Brazil). The flat surfaces of each specimen were polished with a wet 600-grit sandpaper and specimens were returned to the oven at 37ºC and 95% relative humidity for 24 h and 21 days. The compressive strength of the materials was tested in an Emic DL 2000 testing machine (Emic Equipamentos e Sistemas de Ensaio, São José dos Pinhais, PR, Brazil) with a load cell of 5 kN and speed of 0.5 mm/min. The maximum stress that each material was able to sustain was expressed in MPa (N/mm^2), which was calculated considering the maximum loads in relation to the diameter of the specimens.

Flow

After mixing, 0.05 mL of sealer was dispensed on a glass slab (n = 10); 180 s after mixing started, a second glass slab plus a weigh of 100 g was positioned over the sealer, resulting in a 120 g load over the mixed material. Ten minutes after start of mixing the load was removed, and linear measurements of the largest and smallest diameters formed by the compressed mixture were obtained by means of a digital micrometer (Mitutoyo, Suzano, SP, Brazil). The mean diameters were calculated. Samples where the difference between the largest and the smallest diameter was greater than 1 mm were discarded. Flow was further analyzed by photographing each glass slab with the sealer sample next to a millimeter ruler. The images obtained were evaluated by using the UTHSCSA Image Tool for Windows Version 3.00, and the area occupied by the sealer was expressed in mm^2.

Film thickness

The thickness of the assembly consisting of the superimposed glass slabs, with surface area of 200 mm^2 and 5 mm thickness was measured using a digital micrometer with 1 μm increments (L.S. Starrett® Company, Athol, MA, USA). Following that, 0.05 mL of the mixed sealer was placed between the slabs and 180 s after the start of mixing, a 150 N load (Emic DL 2000 testing machine) was applied to the surface of the assembly. Finally, 10 min after the start of mixing, the thickness of the assembly was measured in order to determine the difference between the thickness of the superimposed slabs with and without the sealer samples. Measurements were repeated six times (n = 6) for each material evaluated.

Radiopacity

Standardized specimens (n = 5) measuring 10 mm in diameter and 1 mm thick were fabricated from each material. Samples had their radiopacity tested as previously described by Tanomaru-Filho *et al.* (2007). Each specimen was positioned on an occlusal radiographic film (Insight – Kodak Comp, Rochester, NY, USA) along with an aluminum step-wedge and the

assembly was radiographed using a Focus 50540 X-ray unit (Instrumentarium Dental, Tuusula, Finland) operating at 60 kV, 7 mA, with 0.32 pulses per second, and at a film-focus distance of 33 cm. The exposed films were processed in an automatic film processor (A/T 2000®XR, Air Techniques Inc., Hicksville, NY, USA), digitized, and evaluated using the Image J Launcher software for Windows, in order to determine the radiopacity of each sealer in relation to the aluminum step-wedge (mm Al).

Solubility

The specimens had standardized dimensions (1.5 mm in thickness and 7.75 mm in diameter) and a nylon thread was embedded into the material (n = 10). The initial mass of each specimen was determined by using a precision balance with an accuracy of 0.0001 g (HM-200 A & D Engineering, Inc., Bradford, MA, USA). Following that, specimens were immersed two at a time in 7.5 mL distilled and deionized water. The specimens were secured to the lid of the containers by means of the attached nylon threads, and the assembly was kept in an oven at 37 °C for seven days. After that period, the specimens were removed from the container, washed in distilled water, and placed in a dehumidifier for 24 h. Subsequently, the final mass of each specimen was determined and the loss was expressed as the percentage of the original mass.

Dimensional Stability

The dimensional stability of the materials was evaluated as previously described by Carvalho-Júnior *et al.* (2007). Eight specimens measuring 3.58 mm in height and 3 mm in diameter were fabricated from each material. Their flat surfaces were polished with a 600-grit wet sandpaper. The initial length of each specimen was measured with a digital caliper (Mitutoyo, Suzano, SP, Brazil). Specimens were then stored in flasks containing 2.24 mL distilled water at 37 ºC for 30 days. After that, the specimens were removed from the flasks, dried with absorbent paper, and their final lengths were determined. The percentage of

dimensional change was calculated as follows:

$$[(L30 – L) /L] X 100$$

where L is the initial length of the specimen and L30, the length after 30 days.

Analysis of formaldehyde release

Formaldehyde release was tested for each material at 1h and 24 h after mixing. Mixed sealer samples (0.05 g) were diluted in 20 mL of methanol for 10 min (*n*=5). The liquid was filtered using a teflon membrane filter with pore size of 0.22 µm and analyzed in an automated gas chromatograph-mass spectrometer (GCMS-QP2010 Plus, Shimadzu CG-EM, Kyoto, Japan), in positive mode, with ionizing energy of 70 eV and DB 5 MS column (30 m x 0.25 mm x 0.25 mm). All analyses were carried out by electron impact for acquisition of mass (20 to 500 m/z). The initial oven ramp temperature was 30ºC and gradually increased by 10 °C/min until the temperature reached 200ºC. Helium was used as the carrier gas, at a constant flux of 1.36 mL/min^{-1} and sample aliquots (1 µL) were injected in split mode (1:200 ratio). The injector and detector temperatures were maintained at 280 and 300 ºC, respectively.

Statistical analysis

The data were evaluated using the software BioEstat 5.0 (CNPq, Brasília, DF, Brazil) which presented a normal distribution, thus it was subjected to ANOVA and Tukey post-hoc test to perform multiple comparison, with the significance level set at 5% (α= 0.05).

Results

Setting time

The longest setting time were observed for AH Plus sealer ($P < 0.05$), whilst MTA Fillapex had the shortest setting time ($P < 0.05$). The final setting time for MTA Fillapex was statistically similar to those of ESZr- micro and ES-Nb-nano ES ($P > 0.05$), as shown in Table 2.

Compressive strength

AH Plus and the ES Zr-nano and Nb-micro and Nbnano had the highest compressive strength values at 24 h ($P > 0.05$). After 21 days, AH Plus had the highest strength ($P < 0.05$), followed by ES-Zr-nano and ES-Nb-nano ($P > 0.05$). ES-Zr-nano and ES-Zrmicro presented similar values. MTA Fillapex had the lowest compressive strength at both time intervals ($P < 0.05$). Sealapex was not subjected to compressive strength testing, because the specimens failed to set (Table 2).

Flow

The flow of the different sealers was based on the mean diameters (mm) and areas (mm2) of the materials evaluated. Considering the mean diameter values, the greatest flow was observed for AH Plus, MTA Fillapex and the ESs Zr-micro, Zr-nano and Nb-nano ($P > 0.05$). According to the area, the highest flow was observed for AH Plus, MTA Fillapex, ES-Zr-micro and ES-Nb-nano ($P > 0.05$). Sealapex presented the lowest flow values amongst the materials evaluated ($P < 0.05$). All the sealer were in accordance with the ISO 6876 (2012) patterns, which request at least 17 mm of flow ability from root canal sealer (Table 2).

Film thickness

All materials evaluated had film thickness values higher than the value indicated by ISO 6876 (2012) (at maximum 50 μm). ESs had the highest film thickness values. ES-Nb-nano had the lowest film thickness of the ESs tested, and values were comparable with AH Plus and MTA Fillapex ($P > 0.05$) (Table 2).

Radiopacity

AH Plus was the sealer with the highest radiopacity ($P < 0.05$) followed by MTA Fillapex and Sealapex ($P > 0.05$). The lowest radiopacity values were identified in the ESs,

which were similar amongst themselves ($P > 0.05$) but statistically different from the controls ($P < 0.05$). The radiopacity values verified for the prototypes were lower than the 3 mm thickness of Al specified by ISO 6876 (2012) (Table 2).

Solubility

The materials with the highest solubility were Sealapex and MTA Fillapex ($P > 0.05$). The ESs had intermediate values. AH Plus was the only material to have an increase in mass ($P < 0.05$) (Table 2).

Dimensional stability

The epoxy-based materials (AH Plus and ESs) showed linear expansion, with AH Plus and ESs Zr-micro, Zrnano and Nb-micro presenting statistically similar values ($P > 0.05$). MTA Fillapex and Sealapex had linear contraction ($P > 0.05$). Comparing the prototypes, there was no statistically significant difference amongst them, but ES Nb-nano was the most stable prototype and statistically similar to Sealapex, which was the material that had the lowest values of dimensional changes (Table 2).

Table 2: Means and standard deviations of the physicochemical and mechanical properties evaluated in the different sealers.

	Setting Time (min)	Compressive strength (MPa)		Flow		Film thickness (μm)	Radiopacity (mmAl)	Solubility (%)	Dimensional Stability (%)
	Initial	24 h	21 d	mm	mm^2				
AH Plus	1345±16[a]	76.33±7.22[a]	74.08±5.99[a]	23[a]	482±53[ab]	85±8[b]	15.74±0.25[a]	-0.25±0.10[d]	0.59±0.38[ad]
Sealapex	911±154[b]	-	-	19[d]	349±36[e]	64±12[c]	7.43±0.89[b]	14.07±2.19[a]	-0.07±0.28[cd]
MTA Fillapex	66±4[e]	5.34±1.41[c]	3.10±1.49[e]	22[ab]	433±25[ad]	75±12[bc]	7.11±0.35[b]	14.94±0.93[a]	-1.65±0.82[e]
ES Zr micro	288±38[d]	59.4±4.39[b]	46.48±2.99[c][d]	23[a]	488±107[a]	120±10[a]	2.64±0.15[c]	3.42±0.80[b]	1.26±0.63[a]
ES Zr nano	564±162[c]	76.98±6.69[a]	50.96±2.44[b][c]	22[ac]	418±34[bcde]	133±8[a]	2.42±0.26[c]	2.24±0.46[bc]	1.02±0.50[ab]
ES Nb micro	248±25[d]	70.6±9.09[ab]	42.17±3.98[d]	21[bcd]	350±16[e]	122±4[a]	2.46±0.40[c]	1.89±0.61[c]	0.87±0.83[ac]
ES Nb nano	632±107[c]	69.93±6.68[ab]	53.91±2.91[b]	23[a]	461±27[ac]	102±10[b]	2.31±0.41[c]	1.92±0.53[c]	0.21±0.88[bca]

* Different letters in the same column indicate statistically significant differences ($p<0.05$)

Analysis of formaldehyde release

Chromatographic analysis did not show formaldehyde release in any of the materials evaluated, at both experimental periods (1 and 24 h) (Figs 1 and 2).

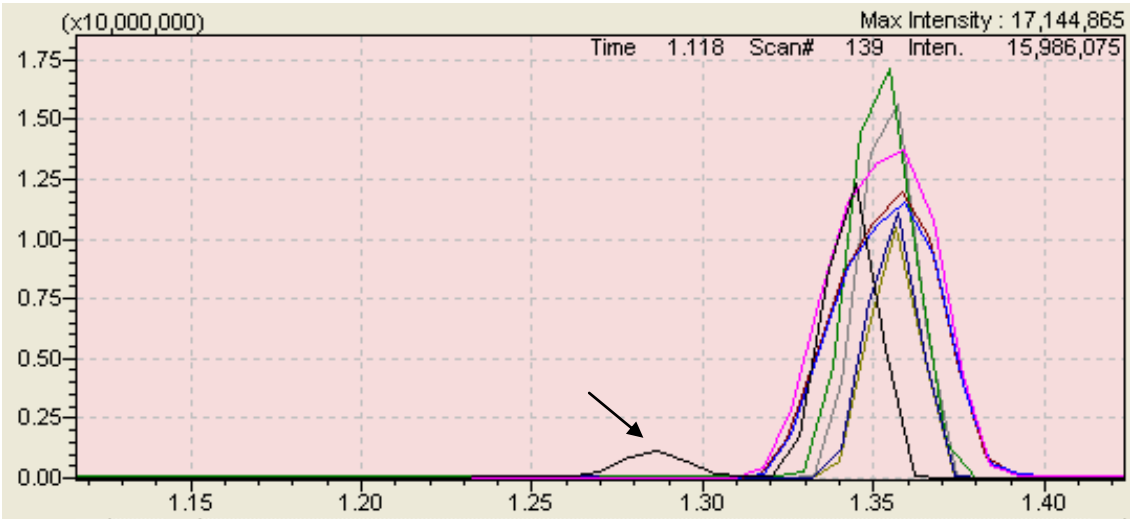

Figure 1 Chromatographic analysis of the different sealers at 1 h after mixing and comparison with the formaldehyde standard curve (1.27 min) (arrow).

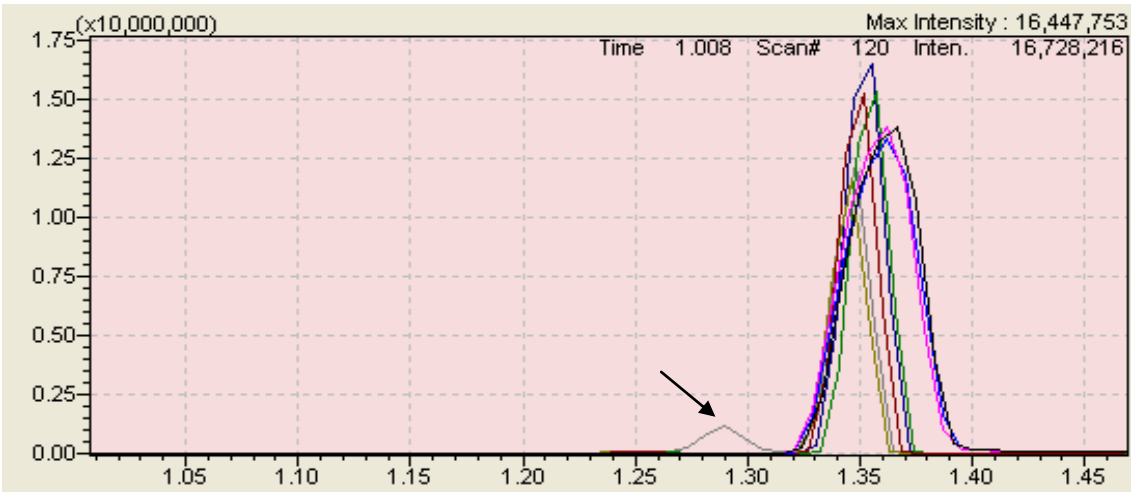

Figure 2 Chromatographic analysis of the different sealers at 24 h after mixing and comparison with the formaldehyde standard curve (1.27 min) (arrow).

Discussion

The incorporation of nanoparticles in dental materials mainly aims at improving the biological properties, mostly the antibacterial effects (Cheng et al. 2012), and to avoid genotoxic risks (Opačić-Galić *et al.* 2012). The effects of incorporation of nanoparticles within root canal sealers have not been investigated. Thus, the purpose of this study was to investigate

the influence of the nanoparticles of different radiopacifying agents, such as zirconium and niobium oxide, on the physicochemical properties of Portland-based root canal sealers. To produce nanoparticles of Nb_2O_5 and ZrO_2, the polymeric precursor method was used. This method is based on a metal citrate polymerization with EG. A hydroxycarboxylic acid such as citric acid is commonly used as a chelating agent for the cations in an aqueous solution. The addition of a polyalcohol such as EG leads to the formation of an organic ester. The polymerization is promoted by heating at around 120 °C resulting in a homogeneous resin in which the cations are distributed evenly throughout the organic matrix. The resin is then calcined to produce the desired oxides.

The physicochemical tests performed in this study followed the specification n°. 57 ANSI/ADA (2008) and ISO 6876 (2012), which allows reproducibility and further comparison amongst studies. However, some of the standardized tests are unsuitable and time consuming. The setting time assessment is based on the visual inspection only, thus it can be subjective. The ISO 6876 (2012) suggests the use of an indenter with a particular head weight and the use of moulds. This method was adopted, and a Gillmore needle was the preferred indenter. ASTM C 266 (2008) specifies testing of Portland cements with a Gillmore needle. This method uses two needles with a different weight to determine both the initial and the final setting times of the material. This method was not considered appropriate for the current tests conducted as ASTM C 266 (2008) does not use a mould but rather a cement pat of dimensions that cannot be reproduced with dental materials. The quantity of material tested affects the results of setting time testing.

The setting time of an endodontic sealer should be extended, thus allowing adequate time for filling of the root canal (Massi et al. 2011). In the present study, the ESs presented adequate setting times for clinical use. Particularly, the ESs containing radiopacifiers in nano scale size had longer setting times when compared with the micro scale. The reduction

in particle size with an increase in specific surface area of the powder should not be expected to affect the setting time of the test material. However, the increase in specific surface resulted in the need of more resin vehicle to obtain a sealer with similar consistency to the one using microsized particles. The higher quantity of vehicle added during manipulation was very likely responsible for the longer setting time presented by sealers containing nanoparticles. The higher proportion of monomer to interact during the polymerization reaction would cause the retardation in setting. The setting time observed for AH Plus was longer than that reported by Garrido et al. (2010) and Massi et al. (2011). Polymerization of AH Plus sealer involves a reaction between the amines in the epoxy resin (Bisphenol A and Bisphenol F) (Flores et al. 2011). The setting time for this material varies according to the portion of the tube (initial, middle or final) that the paste is dispensed from (Baldi et al. 2012). Following their methodology, in the present study, the initial 18 cm of the AH Plus tube was also discarded prior to submitting this sealer to physicochemical testing.

MTA Fillapex had the lowest mechanical resistance in both periods of analysis and it can be related to composition of this material which is not only composed of MTA, but it also contains additives such as resins and pigments, which could decrease compressive strength resistance. Besides, the low compressive strength resistance obtained for MTA Fillapex after 21 days can be related to its high solubility as reported by other studies. It has been suggested that this increased solubility was responsible for the loss of matrix, which was confirmed by EDX analysis that revealed a carbon element decrease probably related with polymer degradation (Borges et al. 2012) and, consequently, a decrease in mechanical resistance. AH Plus and the ESs had high compressive strength values within the first 24 h, probably due to the strong bond between the resin monomers (Borges et al. 2012). The compressive strength test at 21 days after mixing showed that AH Plus maintained its strength, whilst the ESs were associated with a reduction of approximately 20–40% in compressive strength in comparison with the results at 24 h. This may be explained by the lack of hydration of Portland compound in the ESs as a

resinous vehicle was used to mix this sealer instead of water (Formosa et al. 2013). The particle size of the radiopacifiers affected the compressive strength of the ESs in different ways. The analysis performed 24 h after complete set of the sealers showed that ES containing nanoparticles of zirconium oxide were more resistant to failure in compression ($P < 0.05$) when compared with the microparticulate sealers. Nb-nano and Nb-micro had a similar pattern of compressive strength in the first hours. On the other hand, the compressive strength of the sealer containing Nb nanoparticles was less affected by storing at 95% of humidity for 21 days when compared with its analogous containing microparticles. Compressive tests performed on ESs containing zirconium oxide showed that both versions of the ESs, containing nano- or microparticles of the radiopacifier, were similarly affected by storage. It was not possible to conduct compressive strength testing for Sealapex, as this material did not achieve complete setting even after one week at 37 °C and 95% relative humidity, as previously described (Gettleman et al. 1991).

Recently, the ISO 6876 specification was revised and new patterns were established for flow ability (ISO 6876, 2012). Changes in the specifications included the way the flow values should be presented, which means that it must be rounded to the nearest integer in millimetres and the minimum value required for flow ability decreased from 20 mm to 17 mm. According to the results of the present study, all the sealers are in compliance with the actual ISO specification, as all of them presented flow ability greater than 17 mm. On the other hand, it is important to note that an excessive flow is not desirable, as this may increase the risk of apical extrusion especially in opened apex teeth. None of the ESs had flow values higher than AH Plus, which means that prototypes are as safe as AH Plus with respect to apical extrusion. Sealapex had the thinnest film that could be related to a high flow ability of the sealer; however, Sealapex had the lowest values of flow ability. The inconsistency between the results can be related to the size particles that comprise this sealer as small particles can promote high viscosity in resinous materials because of the resulting increase in surface area (Hannemann

2008). This phenomenon also explains why the ES containing nanoparticles of zirconium oxide had lower flow ability in comparison with the micro version of the sealer. However, this concept cannot be applicable to the ESs containing nanoparticles of niobium oxide as this material had greater flow ability when compared with analogous containing microparticles, and this is more likely associated with the greater quantity of vehicle used for mixing this sealer.

Film thickness tests provide information about the volume occupied by the sealer in the root canal after filling. Considering that some root canal sealers can be dissolved by oral fluids or suffer shrinkage after setting, a thin film thickness is recommended (Pane et al. 2012). ISO 6876 (2012) recommends that a root canal sealer should not have more than 50 μm of film thickness. Therefore, none of the root canal sealers tested was in accordance with this specification. The resinous component or manipulation with a resinous vehicle can promote high viscosity to the sealers. MTA Fillapex had a thin film thickness, but the mean value obtained for this sealer in the present study (75 μm) was higher than the thickness reported by the manufacturer (39.6 μm). The ESs had a high film thickness when compared with the conventional endodontic sealers (AH Plus, MTA Fillapex and Sealapex), which is most likely related to the high viscosity presented by these materials, and the comparison amongst experimental materials showed that the sealer containing nanoparticles of niobium oxide had the lowest film thickness.

In the present study, AH Plus had the highest radiopacity values, corroborating previous studies (Marciano et al. 2011, Baldi et al. 2012). The radiopacifying agents in AH Plus are zirconium oxide, iron oxide and calcium tungstate (Tanomaru-Filho et al. 2007). Sealapex and MTA Fillapex had intermediate and statistically similar results, probably due to the presence of bismuth trioxide in both sealers (Guerreiro- Tanomaru et al. 2009). It has been demonstrated that zirconium oxide (Cutajar et al. 2011) and niobium oxide (Leitune et al. 2013) confer radiopacity to endodontic sealers. Thus, the concentration of zirconium oxide in the ESs

was based on the findings of Cutajar et al. (2011), which revealed that Portland cement replaced with 30% zirconium oxide exhibited excellent radiopacity property whilst the niobium oxide was added at the same concentration to allow further comparison amongst ESs. However, all the ESs evaluated in the present work had values below the minimum recommended by the ISO and ANSI/ADA (3 mm Al). Thus, further studies are necessary to determine the ideal ratios of radiopacifying agents for these materials, as these elements (zirconium and niobium) have lower atomic mass than bismuth and tungsten, the radiopacifying agents in MTA Fillapex, Sealapex and AH Plus, respectively.

The solubility of a specific material is the loss of mass during a period of immersion in water (Carvalho- Junior et al. 2007). Taking this into consideration, the solubility testing in the current study was devised according to methodologies reported previously (Carvalho-Junior et al. 2007, Garrido et al. 2010, Vivan et al. 2010, Lee et al. 2011, Marciano et al. 2011, Borges et al. 2012). This methodology is based on the measurement of the mass loss of the specimen before and after immersing the sample in distilled water for 7 days. The guidelines published in ISO 6876 are based only on measurement of the mass of the residues released by the samples obtained after evaporating the liquid in which the sample was immersed. Thus, ISO solubility tests do not consider the possibility that specimens could absorb water from the environment (95% RH) during setting, which are significant for Portland or MTA-based sealers as this materials have a high water uptake ability (Cutajar et al. 2011); also, all the readings are calculated from the solution used to immerse the sample and it does not take into consideration the evaporation rate of relative humidity, which can play a significant role in the results (Fridland & Rosado 2005). Therefore, in this study, the samples were kept in a desiccator for 24 h before weighing, to stabilize the weight. Another shortcoming in the solubility protocol suggested by ISO is that the liquid, from which the residues are obtained after evaporation to calculate the solubility, is poured together with the specimens into a fluted filter which is discarded, thus part of the residues are lost, consequently the results are not reliable. Another

way to make the solubility reading still more realistic beyond the calculation of the percentage mass loss, could be obtaining a constant mass for each sample before and after the immersion in solution.

The solubility guidelines for endodontic sealers (ISO 6876, 2001) establish a maximum loss of 3% of the initial mass. Materials with higher solubility may release irritants and increase the risk of leakage and bacterial colonization (Flores et al. 2011). In the present work, the epoxy resin-based sealers (AH Plus and experimental materials) had the lowest solubility values. Epoxy resin is a polymer with strong cross-links (Borges et al. 2012) and low solubility (Flores et al. 2011, Marciano et al. 2011). Sealers based on niobium oxide were less soluble than the relative zirconium oxide-based sealers. Furthermore, the nano-zirconium was less soluble than the nano version of the material. Conversely, Sealapex and MTA Fillapex had high solubility in water, surpassing the recommended limits. These results are in agreement with Borges et al. (2012), who attributed the high solubility of Sealapex to the complex and nonhomogeneous setting reaction of this material, resulting in a fragile matrix (Schafer & Zandbiglari 2003). MTA Fillapex had high solubility in the present study, which is probably related to the additives that are incorporated in the composition of the sealer and destabilize its matrix. Also, MTA Fillapex has MTA in the composition, which is more soluble than Portland cement (Bodanezi et al. 2008, Vivan et al. 2010) and bismuth trioxide that could had exert a negative effect as other studies affirmed that it can lower the molecular stability of MTA-based materials (Coomaraswamy et al. 2007). Moreover, MTA Fillapex had a solubility similar to that of Sealapex, which may be justified by their similar formulations (Borges et al. 2012).

Although the dimensional change test was removed from the last published ISO 6876 guideline (2012), this test was performed to investigate the behaviour of the ESs in a humid environment. Therefore, the ISO 6876 (2002) was taken as reference and it recommended that endodontic sealers should not have linear contraction values greater than 1%

or expansion of more than 0.1%. To perform the dimensional change tests in this study, the methodology described by Carvalho-Junior et al. (2007) was used. The methodology proposed by these authors uses samples with smaller dimension. This made testing more difficult as samples were difficult to handle. The main limitation of the dimensional test suggested by ISO 6876 specification is that the dimensional changes are measured only in the vertical direction and the material can expand or shrink in all directions, which makes it difficult to measure small changes. Therefore, other authors proposed various methodologies by restraining dimensional changes horizontally through the use of cylindrical metal moulds and also the use of sensitive recording apparatus such as a linear variable differential transducer (LVDT) (Storm et al. 2008, Camilleri & Mallia 2011). Regardless of the problems in dimensional stability methodology, AH Plus and the ESs expanded, corroborating previous studies on epoxy resin-based sealers (Carvalho-Junior et al. 2007). Although epoxy resin-based endodontic sealers are regarded as 'contraction-free' (Marciano et al. 2011), their expansion is still possible because epoxy resins are capable of absorbing water (Flores et al. 2011). With this in mind, the expansion that occurs in these materials probably compensates the polymerization contraction of resin-based materials (Carvalho-Junior et al. 2007, Garrido et al. 2010, Flores et al. 2011). On the other hand, MTA Fillapex had smaller dimensions after 30 days in distilled water. These results may be related to the high solubility of this sealer. Sealapex had less dimensional variation. Despite its high solubility, MTA Fillapex had a greater ability to absorb water, which may lead to volumetric expansion (Schafer & Zandbiglari 2003, Desai & Chandler 2009).

In addition, endodontic sealers may release toxic substances such as formaldehyde, which can negatively interfere with the repair process (Leonardo et al. 1999). Formaldehyde may be originally present in the material's composition or arise during the setting reaction. Its presence in endodontic materials has been linked to genotoxic and mutagenic effects (Tai et al. 2002, Huang et al. 2010). In the present work, chromatographic analyses were conducted 1 and 24 h after the sealers were mixed to detect the presence of formaldehyde during

the setting reaction. Leonardo et al. (1999) used infrared spectrophotometry to detect formaldehyde; whilst in the current study, gas chromatography was employed. The latter testing methodology is more precise for detection of this compound, with the ability to detect formaldehyde at levels as low as 0.25 ppm (Cohen et al. 1998). In the present work, formaldehyde was not detected at any level in the endodontic sealers evaluated, both at 1 and 24 h after mixing.

According to the results observed in the present study, the null hypothesis was rejected as some of the physicochemical properties, such as setting time, compressive strength, flow ability and film thickness seem to be affected by the particle size (nano- or microparticles) of the tested radiopacifiers (niobium or zirconium oxide). Further studies need to be conducted in order to provide a better understanding of the particle size of radiopacifiers on the physicochemical properties of Portland-based root canal sealers. Amongst the ESs, the Portland-based root canal sealer containing nanoparticles of niobium oxide showed the most promising results.

Conclusions

With the exception of radiopacity, the Portland cement-based experimental endodontic sealers had physicochemical properties according to the specifications no 57 ANSI/ADA (2008) and ISO 6876 (2012). These sealers had setting times and flow ability that were adequate for clinical use, satisfactory compressive strength and low solubility. Additional studies should be carried out with the purpose of decreasing the film thickness and to determine the ideal ratio of radiopacifying agents in Portland cement-based root canal sealers.

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3 Chapter 2

Dentin bond strength of Portland-based root canal sealers*

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Abstract

Introduction: This study aimed to assess the dentin bond strength of experimental root canal sealers (ES) based on Portland cement with different radiopacifying agents (nano or micro particles of niobium oxide or zirconium oxide), in comparison to AH Plus and MTA Fillapex. **Methods:** Human canines were transversally sectioned into slices of 2 mm thickness at 2, 6 and 10 mm from apex. Drills with different tapers were used to enlarge the root canal at each radicular third (#701 for apical samples, #703 for mid-root samples and #710 for coronal samples). Dentin was treated with 2.5% NaOCl (10 min), 17% EDTA (3 min). Samples were dried-out and distributed (n=24) in groups: AH Plus, MTA Fillapex, ES micro Zr, ES nano Zr, ES micro Nb and ES nano Nb. Sealers were mixed, inserted into the root canal space and the samples were stored at 37 ^{o}C and 95% humidity for 7 days. Push-out test was performed using punches activated at a crosshead of 0.5 mm/min and compatible with the canal space at each third (1.4 mm, 1.1 mm and 0.8 mm). Failures mode (cohesive, mixed or adhesive) were determined under stereomicroscope. Data were statistically analysed by Anova and Tukey's test. **Results:** AH Plus displayed the highest bond strength values at all root canal thirds ($p < 0.05$), while MTA Fillapex exhibited the lowest bond strength values ($p < 0.05$). All ES demonstrated statistically similar bond strength values at cervical and apical thirds compared to each other ($p > 0.05$). AH Plus exhibited cohesive and mixed failures, while ESs demonstrated a predominance of cohesive failure at all radicular thirds. MTA Fillapex exhibited mainly adhesive failures. **Conclusion:** Portland-based root canal sealers exhibited bond strength to radicular dentin regardless the type and particle size of the radiopacifier agent.

Key words: Endodontics; Endodontic sealer; bond strength; push-out test; Root canal filling

Introduction

Sealers used to perform endodontic obturations have to fill up spaces or voids between the solid core (usually represented by gutta-percha or resilon) and dentin walls (1). Ideally, the root canal sealers should adhere to intraradicular dentin to maintain the integrity of sealer-dentin interface to prevent reinfection of the root canal space, and to resist displacement under mechanical stresses or operative procedures (2).

Endodontic sealers can physically or chemically interact with dentine. The physical interaction usually is established through the penetration of the material into dentine tubules which creates micromechanical retention. Alternatively, the chemical interaction occurs when dentine uptakes silicon and calcium ions from root canal sealers with time (3).

In Dentistry, the use of mineral trioxide aggregate (MTA) cements widespread due to the excellent biological properties, such as biocompatibility and bioactivity, displayed by this material (4, 5, 6). MTA is mainly composed by Portland cement (PC) (7), which represents 75% of the mixture and PC itself consists of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite according to manufacturer. Regarding composition, the only difference between MTA and Portland cements is the presence of bismuth oxide as radiopacifier in MTA (7, 8) and this component has been responsible for mechanical properties depredation (9) and cytotoxicity (10).

The inconvenient of using MTA as sealers in orthograde fillings is related to the granular consistency and lack of flow ability exhibited by the material when it is mixed with distilled water (11). Therefore, researches have been testing alternative vehicles or additives aiming to develop a MTA-based sealer (11). A great example of these efforts is the endodontic sealer MTA Fillapex produced by Angelus Dental Solutions (Londrina, SP, Brazil). This material can be considered calcium silicate-based since it contains MTA in the composition. In addition, this sealer had demonstrated suitable physicochemical properties (12, 13). However, scientific reports are controversial regarding MTA Fillapex's dentin bond strength (14-17).

Dicalcium and tricalcium silicates are the main compounds of Portland cements (18). Consequently, root canal sealers with Portland cement in the composition can be considered calcium silicate-based sealers as well. Experimental sealers based on Portland cement with alternative radiopacifiers to bismuth oxide, such as zirconium oxide or niobium oxide, had been investigated and demonstrated suitable setting time, flow ability, solubility and compressive strength, regardless radiopacifier particle size (19). Furthermore, the interface created by these sealers and dentin had been analysed and elemental migration was identified, suggesting material interaction with dentine (20). However, if the interaction of the experimental sealers with dentin improves material's bond strength is still unknown. Thus, the purpose of this study was to assess the dentin bond strength of experimental sealers (ES) based on Portland cement containing different radiopacifying agents (nano or micro particles of niobium oxide or zirconium oxide) in comparison to AH Plus and MTA Fillapex.

Materials and Methods

This project was submitted and approved by the Research Ethics Committee of the Araraquara Dental School (São Paulo State University, Araraquara, SP, Brazil) with protocol number 87/11*. Forty-eight human canines extracted for periodontal reasons with complete root formation, no accentuated root curvature or calcifications were included in this study. The teeth were decoronated standardizing the root length in 15 mm and the root canals were pre-enlarged using K-files up to size #25 (Destsply-Maillefer, Ballaigues, Switzerland) to remove pulp tissue. The roots were centred into polyvinyl chloride cylindrical moulds (20 mm high X 16.7 mm internal diameter) with the apexes fixed at the bottom with dental wax and they were embedded in polyester resin (Maxi Rubber Ind. Químicas Ltda, Diadema, SP, Brazil). To ensure a parallel position of the roots relative to the mould walls, a #25 K-file was kept within the root canal throughout the embedding procedure, in order to check the parallelism between them. The specimens were removed from the moulds 24 hours after the complete setting of the polyester resin. Then, they were longitudinally sectioned at 2, 6, and 10 mm from the apex

Cf. (Annex) p.139

using a cutting machine (Isomet 1000-Buehler - Lake Bulff, IL) under water cooling, which resulted in three slices with 2 mm thickness from coronal, middle and apical root thirds.

Each slice was positioned in a specifically designed metallic sample holder (Figure 1) which was endowed with screws to hold the samples horizontally. The canals were enlarged using conic diamond drills connected to a handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil) in low speed and with diameter and taper compatible with the root canal space at cervical, middle and coronal thirds. The handpiece was kept attached to the arm of a dental surveyor (B2 Delineator, Bio Art, São Carlos, SP, Brazil), which restrained lateral movements to only vertical direction throughout the enlargement procedure, and kept the alignment between drill and specimen. Samples from coronal third were enlarged using a #710 conic drill introduced into the canal up to 2 mm shorter to the tip end. Conic drills #703 and #701 were used to enlarge the canals of the samples from mid-root and apical thirds, respectively, which were introduced into the canal up to the alignment of the coronal surface of the specimen with the end of the drill tip. The use of these drills produced standardized conic cavities in the samples and assured the involvement of all canal walls. Previously the placement of the tested sealers, the dentin walls were treated with of 2.5% NaOCl (2 mL) followed by 17% EDTA (3 min). A final irrigation with distillate water (5 mL) was performed to remove any trace of chemical solutions. The samples were randomly distributed in five groups (n=24) according to the evaluated root canal sealer and each group was subdivided in three (n=8) according to the root canal third (cervical, mid-root and apical).

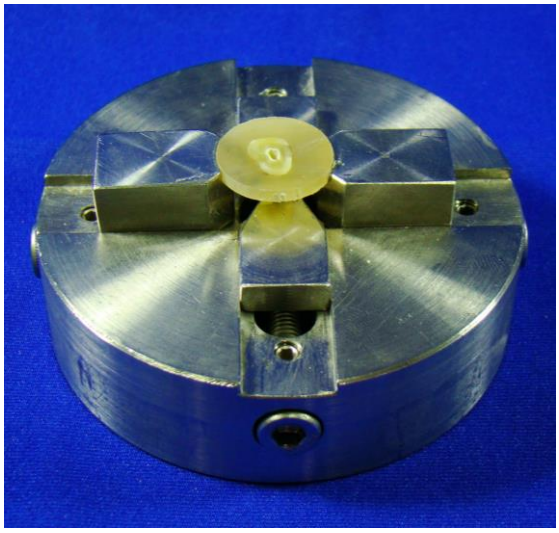

Figure 1: Metalic dispositive used to hold the samples during root canal enlargement and push-out test.

A range of commercial and experimental (ES) root canal sealers were included in this study:

- AH Plus (Dentsply, De Trey, Konstanz, Germany);
- MTA Fillapex (Angelus Dental Solutions, Londrina, SP, Brazil);
- ES micro Zr (Araraquara Dental School, São Paulo State University, Brazil) composed by Portland cement, micro-zirconium oxide (ZrO_2; Sigma Aldrich, St Louis, MO, USA) and epoxy resin (vehicle);
- ES nano Zr (Araraquara Dental School, São Paulo State University, Brazil) composed by nano-zirconium oxide (Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil) as radiopacifier and epoxy resin (vehicle);
- ES micro Nb (Araraquara Dental School, São Paulo State University, Brazil) composed by Portland cement, micro-sized niobium oxide (CBMM, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) and epoxy resin (vehicle);
- ES nano Nb (Araraquara Dental School, São Paulo State University, Brazil) composed by Portland cement, nano-sized niobium oxide (Institute of

Physics of São Carlos, University of São Paulo, São Carlos, Brazil) and epoxy resin (vehicle);

Commercially disposed sealers were mixed following manufacturer's instructions while experimental sealers were mixed with an epoxy resin composed of equal amounts of catalyser and base pastes in a powder/liquid ratio of 5:3 (mass) for the sealer with micro-Nb particles and 5:4 for the sealer with nano-Nb particles (19). The root canal spaces were dried-out using paper points (Dentsply-Herpo, Petropolis, RJ, Brazil) and the samples were placed over a glass slab covered by polyester film. The canal spaces were filled with tested sealers using a plastic syringes (1 mL) with a #22 gauge needle coupled. The excess of sealers over samples' surfaces was removed and the samples were stored at 37ºC and 95% of humidity.

After 7 days of storage, the samples' surface were slightly polished with #600 grit silicon carbide sandpaper (Norton, Lorena, SP, Brazil) to remove excess of sealers and submitted to push-out test. The same metallic device was used to hold the sample during essay, which were positioned with the smaller diameter of the canals facing upward. Push-out test was performed using an Emic DL 2000 testing machine (Emic Equipamentos e Sistemas de Ensaio, São José dos Pinhais, PR, Brazil) with a load cell of 1 kN. The machine was calibrated at a constant speed of 0.5 mm/min and stainless steel cylindrical punches with 0.8, 1.1 and 1.4 mm of diameter were used for apical, middle and coronal samples, respectively. Constant load was applied up to sealer dislodgment and the maximum load in N was converted to MPa.

In order to evaluate the sealer's failure mode, two longitudinal grooves were confectioned on the buccal and lingual aspect of each specimen using a double-faced diamond disc (KG Sorensen, Cotia, SP, Brazil) at a low-speed (Dabi Atlante, Ribeirão Preto, Brazil) and the specimens were separated in two halves using a double-bevel chisel to expose the internal surface of the dentin walls. The specimens were examined under a stereomicroscope (Tecnival SQF-F) with 3.5 x of magnification and failure was classified in adhesive (when dentin walls were devoid of sealer), cohesive (when sealer's remains were observed on dentin walls) or mixed (when both patterns of failure were observed).

The preliminary statistical test indicated normal sampling distribution. Thus, push-out data were submitted to ANOVA and Tukey-Krammer post-hoc test with 5% of significance.

Results

AH Plus displayed the highest bond strength values at all root canal thirds ($p < 0.05$), while MTA Fillapex exhibited the lowest ones ($p < 0.05$). Intermediate values were verified for ES sealers with no statistical difference among themselves at cervical and apical thirds ($p > 0.05$). At mid-root third, ES nano Zr was the ES that demonstrated the greatest bond strength values, followed by ES micro Zr and ES micro Nb ($p > 0.05$). ES nano Nb showed the lowest bond strength in comparison to the others ES at mid-root third, however these values were statistically similar to ES micro Nb and ES micro Nb ($p > 0.05$). Original bond strength values verified with tested sealers at apical, mid-root and coronal thirds can be observed in Table 1.

Table 1. Mean and standard deviation of bond strength values (MPa) obtained at cervical, middle and apical root canal thirds of samples filled with different root canal sealers.

Root canal third	Root canal sealers					
	AH Plus	*MTA Fillapex*	*ES Zr Micro*	*ES Zr Nano*	*ES Nb Micro*	*ES Nb Nano*
Cervical	17.23±3.81^{a}	0.07±0.04^{c}	8.70±1.56^{b}	8.56±2.76^{b}	6.78±3.34^{b}	8.82±2.11^{b}
Mid-root	22.34±2.51^{a}	0.06±0.02^{d}	9.23±2.58^{bc}	10.27±1.77^{b}	7.47±3.19^{bc}	6.41±1.54^{c}
Apical	20.80±6.12^{a}	0.46±0.26^{c}	11.36±3.43^{b}	11.16±5.26^{b}	13.65±4.79^{b}	9.13±2.74^{b}

Different letters in the same row indicate statistically significant differences ($P < 0.05$).

With respect to failure modes (Table 2), AH Plus and ES demonstrated a predominance of cohesive or mixed failure at all root thirds, except ES micro Nb which showed

Table 2. Original values and percentage (%) of failure modes of each tested group after the push-out test.

Sealers	Root canal thirds								
	Cervical			Mid-root			Apical		
	Adhesive	Cohesive	Mixed	Adhesive	Cohesive	Mixed	Adhesive	Cohesive	Mixed
AH Plus	2 (25)	3 (37.5)	3 (37.5)	1 (12.5)	4 (50)	3 (37.5)	2 (25)	3 (37.5)	3 (37.5)
MTA Fillapex	7 (87.5)	-	1 (12.5)	8 (100)	-	-	4 (50)	1 (12.5)	3 (37.5)
ES micro Zr	1 (12.5)	7 (87,5)	-	-	5 (62.5)	3 (37.5)	3 (37.5)	5 (62.5)	-
ES nano Zr	1 (12.5)	5 (62.5)	2 (25)	1 (12.5)	4 (50)	3 (37.5)	-	1 (12.5)	7 (87.5)
ES micro Nb	3 (37.5)	2 (25)	3 (37.5)	6 (75)	2 (25)	-	1 (12.5)	3 (37.5)	4 (50)
ES nano Nb	-	8 (100)	-	1 (12.5)	2 (25)	5 (62.5)	-	3 (37.5)	5 (62.5)

high percentage of adhesive failures at mid-root third. MTA Fillapex exhibited mainly adhesive failure at all radicular thirds.

Discussion

The association of a root canal sealer to gutta-percha or resilon points aims to provide a three-dimensional sealing for root canal system, since core materials do not promote any bond ability to dentin. Therefore, the sealers used to perform obturations should adhere to dentin and resist to the challenges imposed by the oral environment such as mechanical stress, moisture and bacterial penetration.

In this study, the dentin bond strength of sealers with experimental formulations based on Portland cement and containing different radiopacifiers (micro or nano niobium oxide and micro or nano zirconium oxide) were investigated and compared to commercially available sealers (AH Plus and MTA Fillapex) due to the similar composition of these materials. All experimental sealers and also AH Plus presented epoxy resin in the composition. Since ES are mainly composed by tricalcium silicate and dicalcium silicate, it is reasonable the comparison with a calcium silicate-based sealer, which in this case, it was represented by MTA Fillapex. With respect to the formulation of the experimental sealers, Portland cement was selected to participate in the composition as inorganic phase due to its biocompatibility (21) and ability to produce biologically compatible carbonated apatite (22). All the experimental sealers were mixed with an epoxy resin vehicle, which provided flowability to the sealer (19). The only variables amongst experimental sealers were the type and the particle size of the radiopacifying agents added to material's composition. Both niobium oxide and zirconium oxide had already shown ability to provide radiopacity to endodontic sealers (23) and Portland cements (24). An advantage of using these substances as radiopacifiers lies in the fact that the oxidized forms of niobium and zirconium may demonstrate ability to enucleate hydroxyapatite crystals (25, 26) and, consequently, it may favour hard tissue healing.

More than tensile and shear tests (27), push-out assay has been widely accepted to evaluate dentin bond strength of root canal sealers (14-16, 28, 29, 30). This study opted to use push-out test to assess dentin bond strength of the experimental sealers because conditions comparable to clinical situation can be provided to the test (30), in which the materials are placed directly into prepared canals with a natural canal shape and tubule arrangement (29,31). Furthermore, this assay is relatively simple, reproducible and it allows the identification of minor bond strength values (32, 33).

Minimal variations were proposed to push-out methodology performed in this study. The samples were transversally sectioned before filling the root canal spaces with the tested sealers to avoid sealer's displacement due to blade vibration during cutting. Therefore, the enlarged canals were filled solely with the tested sealers without any solid core (gutta-percha or resilon) since the literature reports that push-out strength might be affected by core material stiffness (34). Additionally, drills with different diameter were used to enlarge the canals at each root third, which produced standardized preparations with known diameter. Consequently, punches with different diameters and that covered 90% of sealer's surface were used to apply load at each third (34).

The results of this study indicated that AH Plus presented the greatest bond strength values. This finding may be explained by the difference on sealer's setting time. AH Plus presents longer setting time in comparison to the other sealers (19) and, consequently, this sealer disposes of more time to mechanically interlock with dentin (33). In comparison to MTA Fillapex, AH Plus and experimental sealers presented greater dentin bond strength ($p < 0.05$) and these results are likely related to presence of an epoxy compound in AH Plus and ES composition, which is responsible by materials' flow ability and, consequently, penetration of the sealers into dentin wall's irregularities (33). Furthermore, great bond strength values can be related to the cohesion amongst sealer molecules, which increases the resistance of the sealers to dislodgment (31). Failure mode analysis indicated that AH Plus exhibited cohesive and mixed failures, which corroborates the findings of other studies (29). Whereas ES displayed mainly

cohesive failures, except for ES micro Nb, which had a high level of adhesive failures at mid-root third. Different hypotheses may justify the high incidence of cohesive failures for ES. Cohesive failure of sealers has been related to great dentin bond strength, in which the load applied exceeds the cohesion strength existing amongst sealer's molecules. In the case of experimental sealers, parallel studies about the setting reaction have been conducted and the results demonstrated that the use of a resinous vehicle to mix the sealers instead of water, resulted in lack of hydration reaction of the calcium silicate particles, which may had decreased experimental sealers' cohesion.

At all radicular thirds, the ESs presented intermediate bond strength values in comparison to AH Plus and MTA Fillapex. When experimental sealers were compared among themselves, it could be observed that only samples from mid-root third displayed statistical difference. At this region, ES micro Zr revealed the greatest dentin bond strength and ES micro Nb the lowest one. ES micro Zr and ES micro Nb ($p > 0.05$) exhibited intermediate values, which were sometimes similar to ES nano Zr and other times similar to ES nano Nb. Despite the similarity between radiopacifiers particle size (both nanoparticles), the difference on bond strength values verified for ES nano Zr and ES micro Nb may be explained by differences on chemical properties and reactivity exhibited by niobium and zirconium.

On the other hand, bond strength values verified for MTA Fillapex were extremely low at all root thirds and failures mainly adhesives. Other studies also demonstrated low dentin bond strength for MTA Fillapex (14-16) and they had attributed the lack of adhesion of this sealer to the ability that the MTA compound has to induce apatite crystals deposition, which acts as interface and compromise sealer's adhesion (14). However, the samples from this study remained stored at 95% of humidity and not immersed in synthetic body fluid, such as phosphate buffered saline (PBS) or Hank Balanced Salt Solution (HBSS) after setting. Thus, the formation of this interfacial layer was not expected in MTA Fillapex samples and neither in Portland-based sealer's samples. On the other hand, the sealing ability displayed by MTA Fillapex has been attributed to the expansion experienced by the MTA compound after setting

(35), but if MTA Fillapex suffered any expansion in this study, the results suggested that it did not improved dentin bond strength.

As mentioned before, the formation of apatite crystals at sealers-dentin interface was not expected on calcium silicate-based sealers since there were no phosphates available to interact with materials in the medium used to store the samples. Studies had correlated the ability to induce apatite deposition at sealer-dentin interface to great dentin bond strength (36) due to the formation of an interfacial layer with tag-like structures which, theoretically, could promote micromechanical retention (37). Further studies must be conducted to investigate the influence of synthetic body fluids on dentin bond strength of calcium silicate based sealers, such as MTA Fillapex and Portland-based sealers.

Conclusion

Portland-based sealers exhibited bond strength to radicular dentin regardless the type and particle size of the radiopacifier agent.

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4 Chapter 3

Chemical characterization and bioactivity of epoxy resin and Portland cement-based sealers with niobium and zirconium oxide radiopacifiers*

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Abstract

Objective: The purpose of this study was to characterize and to evaluate the bioactivity potential of experimental root canal sealers (ES) based on Portland cement, epoxy resin with nano- and micro- particles of niobium or zirconium oxide used as radiopacifiers in comparison to AH Plus and MTA Fillapex.

Methods: Specimens of the sealers (10 mm in diameter x 1 mm thick) were prepared and the radiopacity was evaluated according to ISO 6876 (2012) specifications. Characterization of the sealers was performed under the scanning electron microscope (SEM) immediately after setting and after immersion for 28 days in Hank's balanced salt solution (HBSS). In addition X-ray energy dispersive (EDX), X-ray diffraction (XRD) and infrared spectroscopy (FT-IR) analysis were also performed. The pH and calcium ion release were measured at periods of 1, 7, 14, 21 and 28 days using a digital pH meter and an atomic absorption spectrophotometer, respectively.

Results: The experimental sealers exhibited an average radiopacity of 2.5 mm thickness of aluminum which was similar to MTA Fillapex ($p > 0.05$) and inferior to AH Plus ($p < 0.05$). AH Plus did not show bioactivity while MTA Fillapex displayed deposition of calcium phosphate. Although the experimental sealers did not exhibit the formation of hydration product, they encouraged the deposition of crystalline spherical structures of calcium deficient phosphate. The highest pH and calcium release values were observed with the experimental sealers ($p < 0.01$). ES-Nb-micro was the only sealer to present hexagonal shaped crystal deposition.

Significance: Novel root canal sealers based on a mixture of Portland cement, epoxy resin and radiopacifier exhibited bioactivity comparable to MTA Fillapex and radiopacifier particle size had limited effect on the sealer's microstructure and chemical properties.

Key words: Characterization; Chemical properties; Dental materials; Mineral trioxide aggregate; Portland cement; Physical properties; Root canal sealers

1. Introduction

Bioactivity can be defined as a beneficial effect produced by some materials when they are implanted in living tissues and, through biochemical and biophysical reaction, interacts with them leading the formation of carbonated apatite crystals which are the main mineral phase of hard tissues, such as bone, dentin and cement [1]. Ideally, root canal sealers and root-end filling materials should act as biomaterials since they are in directly contact with periapical tissues through the radicular apex of the tooth.

Mineral trioxide aggregate (MTA) is used extensively in Dentistry mainly as reparative cement in cases of perforation [2], pulpotomies [3] and root-end fillings [4] due to the excellent biological properties exhibited when in contact with connective tissues. MTA induces hard tissue deposition, and is thus bioactive [1,5-7]. The bioactivity results from the reaction of the calcium hydroxide produced during the hydration of the Portland cement component with phosphates present in tissue fluids [8]. Most root canal sealer cements do not possess any bioactivity. MTA Fillapex (Angelus, Londrina, Brazil) which is a sealer based on MTA, exhibits bioactivity when in contact with simulated tissue fluids in human cell culture [9].

Mixtures of MTA and Portland cement mixed with water result in a granular and sandy paste with unfavourable handling characteristics that precludes the use of MTA as root canal sealer cement [10]. Therefore, many studies have been performed attempting to develop a root canal sealer based on MTA [11-20] or calcium silicate [21-23], which is the main compound in MTA. MTA Fillapex is composed of MTA and other compounds, such as resins, that result in an endodontic material with adequate physicochemical properties to be used as sealer [24]. Since MTA and Portland present similar chemical composition [25] and biological response [26], an experimental root canal sealer (MTA Sealer), containing white Portland cement, a radiopacifying agent (zirconium oxide), an additive (calcium chloride) and a resinous vehicle, which conferred viscosity to the sealer, have been developed and its physicochemical and biological properties showed promising results [17,20].

The role played by radiopacifying agents in the bioactivity of the endodontic materials is still not well reported. Bismuth oxide is the radiopacifying agent present in MTA Fillapex and some studies have shown that this compound, in general, negatively affects the physico-chemical [27,28] and biological properties [29,30] of MTA cements. To avoid the side effects caused by bismuth oxide, alternative radiopacifying agents have been proposed [31-36]. Replacement of bismuth oxide with zirconium oxide resulted in a material with physicochemical properties comparable to the commercial version that contained bismuth oxide [37,38]. Furthermore, this association resulted in a bioactive material since it induced the deposition of precipitates that precedes apatite formation when in contact with simulated body fluid [38].

Niobium is a transition metal that can also be added to root canal sealers to enhance radiopacity. Niobium oxide increased the radiopacity of methacrylate-based root canal sealers [39]. Also, the oxidized form exhibited biocompatibility and ability to enucleate hydroxyapatite when it was used to cover dental implants [40].

The purpose of this study was to characterize and evaluate the bioactivity potential of experimental root canal sealers based on Portland cement and an epoxy resin incorporating nano or micro particles of niobium or zirconium oxide radiopacifiers and compare these novel sealers to AH Plus and MTA Fillapex.

2. Methodology

The materials used in this study included a range of conventional and experimental (ES) root canal sealers:

- AH Plus (Dentsply International, Addlestone, UK);
- MTA Fillapex (Angelus Dental Solutions, Londrina, SP, Brazil);
- ES-Zr-micro (Araraquara Dental School, São Paulo State University, Brazil) composed of a mixture of Portland cement, micro-sized zirconium oxide (Sigma Aldrich, St Louis, MO) and an epoxy resin;

- ES-Zr-nano (Araraquara Dental School, São Paulo State University, Brazil) composed of a mixture of Portland cement, nano-sized zirconium oxide (Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil) and an epoxy resin;
- ES-Nb-micro (Araraquara Dental School, São Paulo State University, Brazil) composed of a mixture of Portland cement, micro-sized niobium oxide (CBMM, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) and an epoxy resin;
- ES-Nb-nano (Araraquara Dental School, São Paulo State University, Brazil) composed of a mixture of Portland cement, nano-sized niobium oxide (Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil) and an epoxy resin;

The radiopacifiers replaced 30% by weight of white Portland cement (Portland Cement; CPB-40; Votorantin Cimentos, Camargo Correa S.A., Pedro Leopoldo, MG, Brazil). The micro-particles of niobium and zirconium oxide were purchased from companies manufacturing chemicals. The nanoparticles of niobium and zirconium oxide were prepared by the polymeric precursor method. The zirconium oxide supports were prepared from the precursor salt $ZrO(NO_3)_2 \cdot xH_2O$ (Alfa Aesar). Aqueous solutions of this salt were prepared, mixed and added to an aqueous solution of citric acid (held at 60°C), with constant stirring. Subsequently, ethylene glycol ($HOCH_2CH_2OH$) was added to polymerize the citrate by a polyesterification reaction (at 120°C). The citric acid: metal molar ratio was 3:1, while the citric acid: ethylene glycol mass ratio was 60:40. The resulting polymer resin was then calcined at 300° C for 4h, and the after 600ºC/2h to produce ZrO_2 crystalline particles. Whereas to produce niobium oxide nanoparticles, an aqueous solution of niobium ammonium oxalate $\{NH_4[NbO(C_2O_4)_2(H_2O)](H_2O)N$ (CBMM, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) was prepared and ammonium hydroxide was dropped upon thereafter. The niobium hydroxide precipitated was filtered and washed to eliminate oxalate ions and dissolved

into a citric acid (CA) aqueous solution ([CA]/[Nb] = 3) and filtered. The niobium content in the solution was precisely determined by gravimetric analysis. The solution was stirred for 2h at 70ºC to promote the complex reaction. Ethylene glycol (EG) was added to the mixture with mass ratio was 60:40. The translucent solution was heated and stirred over several hours. A polymerization process started during the water evaporation, resulting in a highly viscous solution. This resin was heated in an electric furnace at 300°C for 4h. The resulting black and soft mass was milled and calcined in an electric furnace for 2h over alumina slabs at 700°C/2h.

The experimental sealers were manipulated with an epoxy resin composed of equal amounts of catalyzer and base pastes which were mixed in a powder/liquid ratio of 5:3 (mass) for the materials containing micro-particles (ES-Zr-micro and ES-Nb-micro) [17] and 5:4 for those containing nanoparticles (ES-Zr-nano and ES-Nb-nano). The powder/liquid ratio of the sealers containing nanoparticles was determined by a previous pilot study. AH Plus (Dentsply, De Trey, Konstanz, Germany) and MTA Fillapex (Angelus, Londrina, PR, Brazil) were used as controls and manipulated according to manufacturer's instructions.

2.1 *Evaluation of radiopacity*

Radiopacity evaluation of the set sealers and the raw materials making up the prototype sealers was performed using ISO 6876:2012 recommendations [41]. Three specimens 10 ± 1 mm in diameter and 1 ± 0.1 mm thick were used. The powders were compacted in circular molds of similar dimensions. The specimens were radiographed by placing them directly on a photo-stimulable phosphor (PSP) plate adjacent to a calibrated aluminium step wedge (Everything X-ray, High Wycombe, UK) with 3 mm increments. A standard X-ray machine (GEC Medical Equipment Ltd., Middlesex, UK) was used to irradiate X-rays onto the specimens using an exposure time of 1.60 seconds at 10 mA, tube voltage at 65 ± 5 kV and a cathode-target film distance of 300 ± 10 mm. The radiographs were processed (Clarimat 300, Gendex Dental Systems, Medivance Instruments Ltd., London, UK) and a digital image of the radiograph was obtained. The grey pixel value on the radiograph, of each step in the step-wedge

was determined using an imaging program, Microsoft Paint (Microsoft Corp., Redmond, WA, USA) as a number between 0 and 255 with 0 representing pure black and 255 pure white. A graph of thickness of aluminum vs. grey pixel value on the radiograph was then plotted and the best-fit logarithmic trend line was plotted through the points. The equation of the trend line gave the grey pixel value of an object on the image as a function of the object's thickness in mm of aluminum. This equation was inverted so as to express the object's thickness as a function of its grey pixel value on the radiograph. The grey pixel values of the cement specimens were then determined using the imaging program, and plugged into this equation to calculate the equivalent radiopacity of the cement sample, expressed in mm of aluminium.

2.2 Characterization of raw materials

The Portland cement, micro zirconium and niobium oxide were characterized using a combination of scanning electron microscopy (SEM) and X-ray energy dispersive analysis (EDX), and X-ray diffraction (XRD) analysis.

Scanning electron microscopy and X-ray energy dispersive analysis

The powders were impregnated in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark) under vacuum. The resin blocks were then ground using progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens were mounted on aluminum stubs, carbon coating and viewing under the scanning electron microscope (SEM; Leo 1430 Oxford, Cambridge, UK). Scanning electron micrographs of the different material microstructural components at different magnifications in back-scatter electron mode were captured and X-ray energy dispersive analysis (EDX) of the different phases was carried out.

X-ray diffraction analysis

Phase analysis of unreacted powders was carried out using X-ray diffraction. The diffractometer (Bruker D8 Advance, Bruker Corp., Billerica, MA, USA) used Cu Kα radiation at 40 mA and 45 kV. Samples were presented in powder form and the detector was rotated between 15-45°. A step of 0.02°2θ and a step time of 1 s were used. For the powdered specimens the sample holder was spun at 15 rpm. Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

2.3 Characterization of set materials

The set sealers were characterized using a combination of scanning electron microscopy (SEM) and X-ray energy dispersive analysis (EDX), X-ray diffraction (XRD) analysis and Fourier transform infrared spectroscopy (FT-IR). The characterization was performed on freshly prepared materials and also on sealers that had been immersed in 7 mL of Hank's balanced salt solution (HBSS; H6648, Sigma Aldrich, St. Louis, MO, USA) at 37°C for 28 days. In addition, the surface morphology of the set sealers after 28-day of immersion in HBSS was assessed by scanning electron microscopy.

Scanning electron microscopy and X-ray energy dispersive analysis

Cylindrical specimens (10 mm in diameter and 2 mm thick) were prepared for the set cements. They were divided into three groups. Group 1 was allowed to set for 24 hours at 37 ± 1°C. Group 2 and 3 were allowed to set for 24 hours at 37 ± 1°C after which they were immersed in HBSS for 28 days. Both group 1 and 2 were impregnated in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark) under vacuum. The resin blocks (groups 1 and 2) were then ground using progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens in Group 3 were not included in resin and they were only dried in a desiccator with soda lime to avoid surface

carbonation. All specimens were mounted on aluminum stubs, carbon coating and viewing under the scanning electron microscope (SEM; Leo 1430 Oxford, Cambridge, UK). Scanning electron micrographs of the different material microstructural components at different magnifications in back-scatter electron mode for Groups 1 and 2 were captured and X-ray energy dispersive analysis (EDX) of the different phases was carried out. Group 3 was viewed using secondary electron imaging.

X-ray diffraction analysis

The set sealers immediately and after 28 days of immersion in HBSS were crushed using a mortar and pestle prior to testing. X-ray diffraction analysis was performed using the same parameters set for the raw materials.

Fourier transform infra-red spectroscopy

The compositions of set sealers was investigated immediately after setting and after 28 days of immersion in HBSS using Fourier transform infrared (FT-IR) spectroscopy. To obtain the FT-IR spectrums from powder compounds and from the mixed material immediately after setting or after 28 days in HBSS, 2-5 mg of each powder component or crushed mixed samples of the sealers was added to 100 mg potassium bromide and analyzed in the IR spectrophotometer (Shimadzu IRAffinity-1; Shimadzu Corp., Kyoto, Japan) using transmitted infrared spectroscopy.

2.4 pH and calcium ion release

Polyethylene tubes (n = 10) measuring 10 mm in length and 1 mm of internal diameter were filled with freshly prepared sealers and immersed in 10 mL distilled water. The specimens were stored at 37 °C throughout the experiment. Distilled water (10 mL) served as negative control (pH=7). pH and calcium ion release were performed after 1, 7, 14, 21 and 28 days. At each time point the elution was collected for testing and solutions were replaced by

fresh distilled water. The pH was measured using a previously calibrated digital pH meter (Digimed, Santo Amaro, SP, Brazil). The calcium ion release was assessed using an atomic absorption spectrophotometer (H1170 Hilger & Watts; Rank Precision Industries Ltd. Analytical Division, London, UK). The concentration of calcium ions released from the materials was quantified using a calcium hollow cathode lamp (422.7-nm wavelength and 0.7-nm window) operated at 20 mA. The readings of calcium ion release were compared with a standard curve obtained from multiple dilutions of pure calcium in ultrapure water.

2.5 Statistical Analysis

The data were evaluated using SPSS (Statistical Package for the Social Sciences) software (PASW Statistics 18; SPSS Inc., Chicago Illinois, USA). Parametric tests were performed as K-S tests on the results indicated that the data were normally distributed. Analysis of variance (ANOVA) with $p = 0.05$ and Tukey post-hoc test were used to perform multiple comparison tests.

3. Results

3.1 Evaluation of radiopacity

The radiopacity of the powders is shown in Figure 1a and for the set sealers in Figure 1b. The white Portland powder exhibited radiopacity values which were < 3 mm thickness of aluminium which is recommended by ISO 6876. Both radiopacifiers namely niobium oxide and zirconium oxide had higher radiopacity values compared to Portland cement ($P < 0.001$). Zirconium oxide had double the radiopacity value to that of niobium oxide ($P < 0.001$). The experimental sealers exhibited an average radiopacity of 2.5 mm thickness of aluminium which was similar to MTA Fillapex. AH Plus exhibited a higher radiopacity ($P < 0.001$).

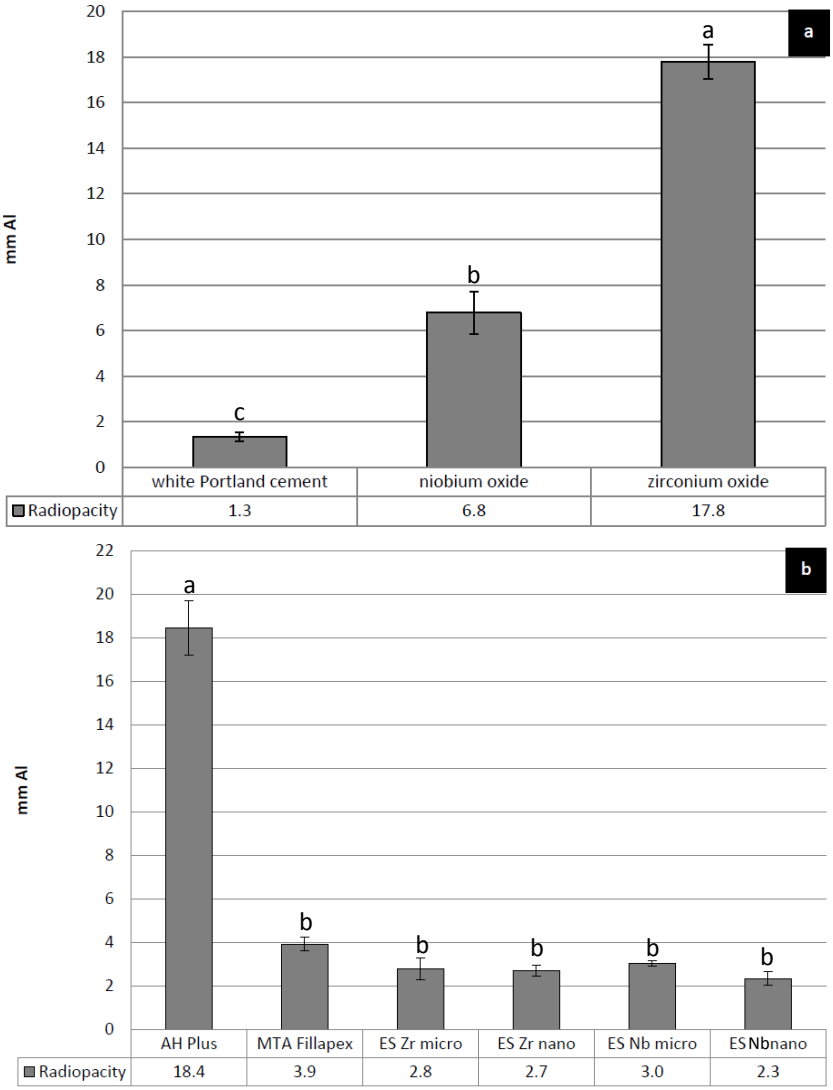

Figure 1. Mean radiopacity of (a) raw materials used to make up the sealers and (b) sealers under study.

3.2 Characterization of raw materials

The scanning electron micrographs of the un-reacted powders are shown in Figure 2a. The EDX analysis and XRD plots of the powders is shown in Figure 2b. The Portland cement was composed of elongated particles about 15 µm long (Figure 2aA) which exhibited main elemental peaks for calcium and silicon and some minor elemental peaks for aluminium and magnesium (Figure 2bA). The XRD plot for Portland cement exhibited peaks for the tricalcium silicate phase (ICDD: 31-0301) which were present at 29.348, 29.504, 32.187, 32.501, 34.256, 41.157° 2θ (Figure 2b). The zirconium oxide (ICDD: 83-0939) displayed peaks at 17.437, 24.054, 24.451, 28.169, 31.47, 34.143, 34.414, 35.294, 40.72° 2θ. The niobium oxide

displayed a particular microstructure with agglomeration of particles into particular shapes (Figure 2aC). The niobium oxide was mostly pure as indicated by both EDX and X-ray diffraction analysis (Figure 2b). The XRD plot showed the presence of two crystalline structures namely a pentoxide (ICDD: 37-1468) with major peaks at 23.746, 24.429, 25.51, 31.537, 31.626, 32.232, 33.062, 35.227, 38.863° 2θ and $Nb_{16.8}O_{42}$ (ICDD: 71-0336) with the main peaks at 22.602, 28.391, 28.903, 36.608, 36.995° 2θ. The phase $Nb_{16.8}O_{42}$ is similar to the niobium pentoxide but having an orthorhombic crystalline structure.

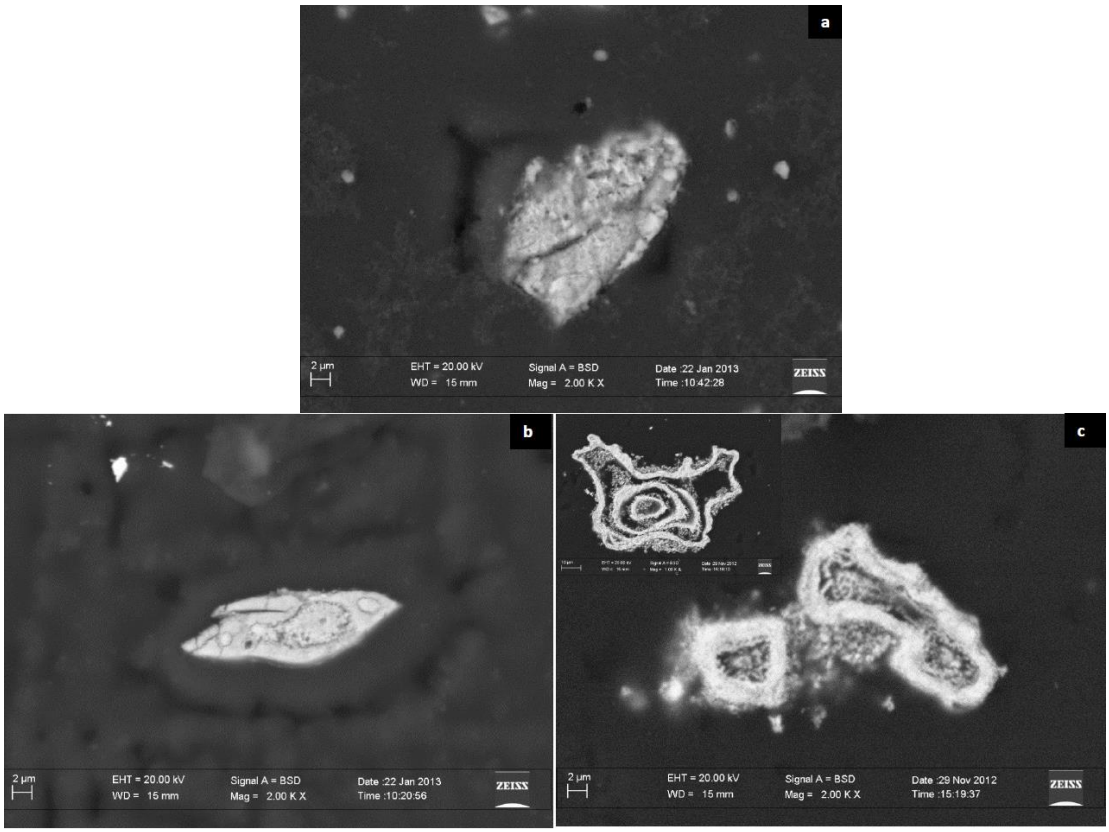

Figure 2a: Back scatter scanning electron image of (A) un-hydrated Portland cement (B) zirconium oxide (C) niobium oxide powder showing their micro-structural features.

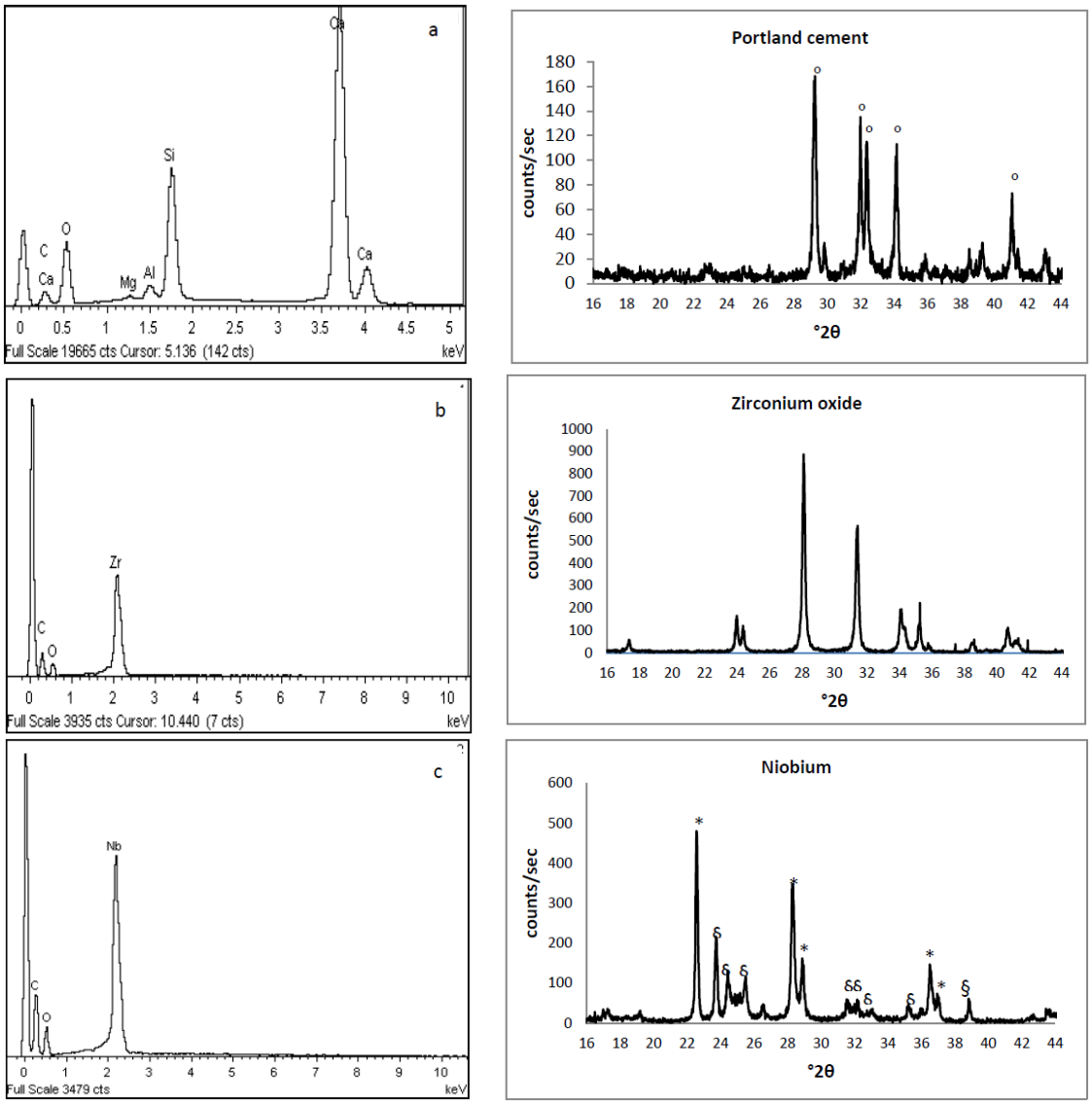

Figure 2b: X-ray energy dispersive analysis and X-ray diffractograms of (A) un-hydrated Portland cement, (B) zirconium oxide, (C) Niobium oxide; (°: Tricalcium silicate; §:Nb2O5; *: $Nb_{16.8}O_{42}$).

3.3 Characterization of set materials

The scanning electron micrographs and EDX analysis of the polished sections of the freshly prepared set sealers and sealers immersed in HBSS for 28 days are shown in Figure 3a and 3b respectively. The XRD and FTIR plots of both the freshly mixed and aged sealers immersed in HBSS are shown in Figure 4. The AH Plus sealer was composed of a resin matrix into which were interspersed shiny particles ~10 μm in diameter which were rich in calcium and tungsten. Smaller particles rich in zirconium were also present. There were no visible changes in microstructure and surface morphology after immersion in HBSS for 28 days. The surface

microstructure was completely flat and no surface deposits were visible (Figure 3b). The XRD plots also indicate no changes in material composition after immersion in physiological solution. The XRD plot of AH Plus exhibited very definite peaks for calcium tungstate (ICDD: 41-1431) at 18.608, 28.729, 34.178, 39.201° 2θ and zirconium oxide (ICDD: 83-0939) at 28.169 and 31.47° 2θ (Figure 4).

The MTA Fillapex was composed of particles rich in calcium and silicon interspersed in a matrix with smaller particles composed of bismuth. The freshly prepared MTA Fillapex exhibited a high degree of porosity and the different particles were clearly identified (Figure 3aB). After immersion for 28 days in HBSS a denser microstructure was displayed (Figure 3bB1). Surface deposits of spherical particles with a typical microstructure of hydroxyapatite were evident and coated the entire material surface (Figure 3bB2). The XRD plots exhibited peaks for bismuth oxide (ICDD: 41-1449) at 26.923, 27.378, 33.040° 2θ and minor peaks for tricalcium silicate (ICDD: 31-0301) at 29.504, 32.187, 32.501, 34.256° 2θ. There were no changes in peak intensity as the material aged in HBSS.

The zirconium-based experimental sealers although having a similar chemical composition as indicated by the EDX (Figure 3a) and XRD (Figure 4C) analysis exhibited a different microstructure which did not change when the materials were aged for 28 days in HBSS (Figure 3b). The sealer containing the micro-zirconium oxide particles displayed porosity and the cement particles were easily discernible in the resin matrix (Figure 3aC). The porosity was still evident after aging for 28 days in HBSS. No reaction rims were visible around the cement particles in the aged material (Figure 3bC1). The sealer containing the nano-zirconium oxide exhibited a more homogenous microstructure where the cement particles were not evident and no porosity was observed (Figure 3aD). The microstructure did not change with material aging (Figure 3bD1). The micro-zirconium sealer demonstrated a higher bioactivity than the nano-zirconium as more spherical crystalline deposits were present on the micro-zirconium variety. The XRD analysis of the ES zirconium oxide displayed the lack of Portlandite (calcium hydroxide) peak usually evident at 18° 2θ. This corroborated the lack of reaction rims present

around the cement particles after aging for 28 days (Figure 3bC, D) thus showing the lack of reaction of tricalcium silicate. The peak intensity of tricalcium silicate did not reduce on aging as it was not converted to a less crystalline calcium silicate hydrate (Figure 4C).

The niobium oxide-based experimental sealers (Figure 3a, b E, F) although once again having a similar composition presented a different microstructure. The micro-niobium oxide was composed of two types of niobium oxide which had a different crystalline structure. This feature was evident in XRD analysis of both niobium oxide (Figure 2b) and the niobium-based sealers (Figure 4D). The micro niobium oxide-based sealer exhibited a very porous microstructure where large areas were filled only by the resin matrix. The niobium oxide particles were segregated in particular areas and tended to clump together forming very irregular shapes which occasionally were several microns large (Figure 3aE). On the other hand the nano-variant was composed of a pentoxide only (Figure 4D). The nano-niobium oxide particles were more regular in shape and were evenly distributed within the resin matrix (Figure 3aF). Aging in HBSS did not alter the sealer microstructure or its composition as was evident in the scanning electron micrographs (Figure 3bF1) and XRD analysis (Figure 4D). There was no evidence of hydration products i.e. deposition of calcium hydroxide and formation of calcium silicate hydrate. The surface microstructure was on the other hand altered considerably (Figure 3bE2, F2). Both niobium based sealers exhibited spherical deposits on the sealer surface. The extent of coating was similar in both sealer types independent on the particle size of niobium oxide. The micro-niobium based sealer in addition, exhibited hexagonal shaped crystals which are the typical microstructure of calcium hydroxide.

The FT-IR spectroscopy of the materials performed immediately after setting and after immersion in HBSS for 28 days is shown in Figure 4. The band in the 2350 cm^{-1} region present in all spectra was an artifact produced by the instrument. AH Plus sealer exhibited variations in the high frequency part of the spectrum (4000 - 1300 cm^{-1}). The stretching vibration of the N-H group present at 2900 cm^{-1} was less distinct after storage of AH Plus for 28 days in HBSS. AH Plus contains dibenzyldiamine, aminoadamantane and tricyclodecane-

diamine in paste B. These organic components seem to be affected by storage. MTA Fillapex did not show any changes when immersed in HBSS for 28 days. On the other hand the prototype sealers exhibited changes in the low frequency region of the IR spectrum (900 and 650 cm^{-1}) and also in the middle region. Peaks in the region of 650 cm^{-1} and in 1400-1500 cm^{-1} region were observed. The former were attributed to PO_4^{3-} and the latter to CO_3. These bands indicate the presence of a phosphate phase which was calcium deficient thus showing the presence of carbonate.

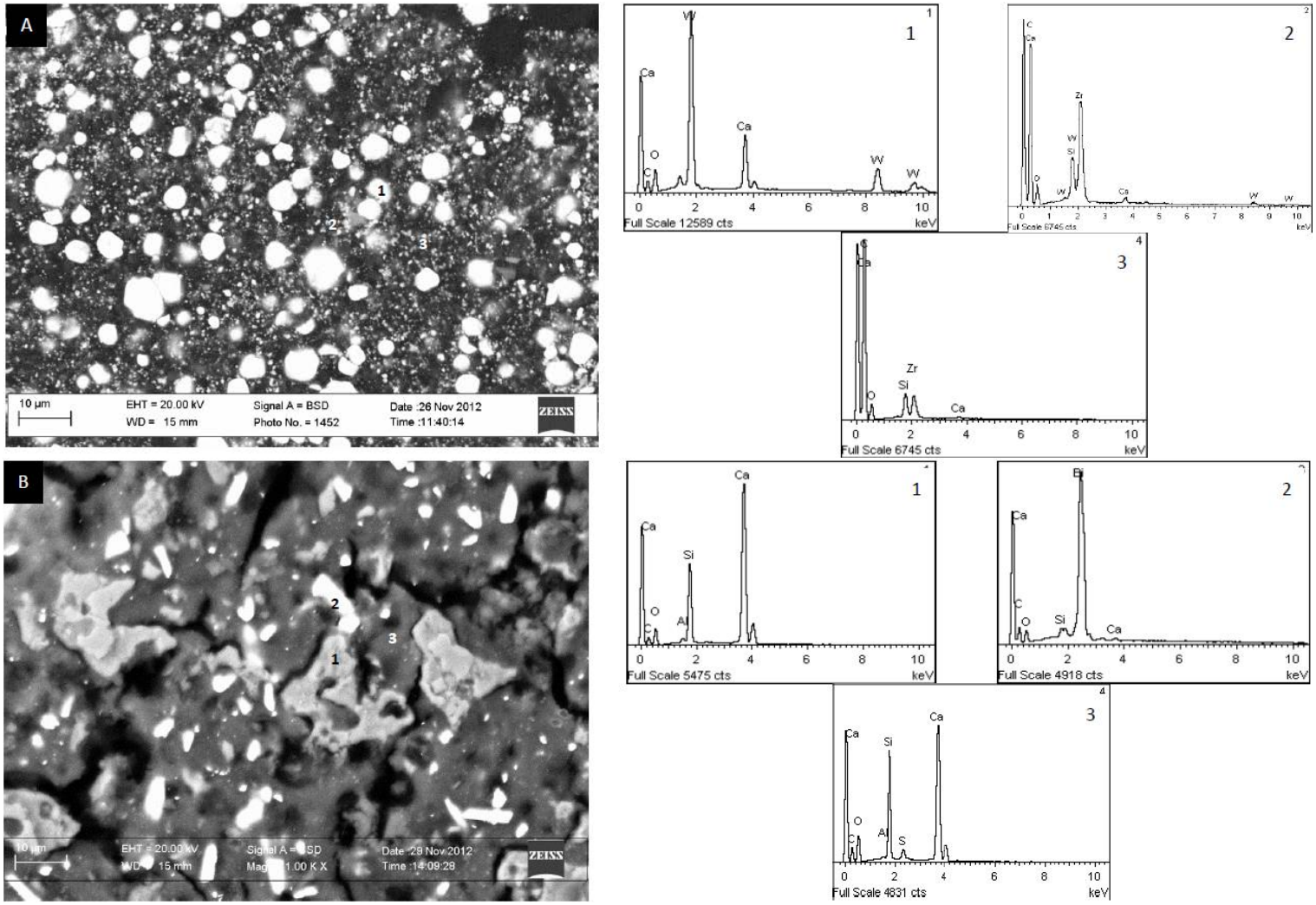

Figure 3a: Back scatter scanning electron micrographs and EDX analysis of polished sections of the freshly prepared set sealers (A) AH Plus, (B) MTA Fillapex, (C) ES-Zr-micro, (D) ES-Zr-nano, (E) ES-Nb-micro, (F) ES-Nb-nano.

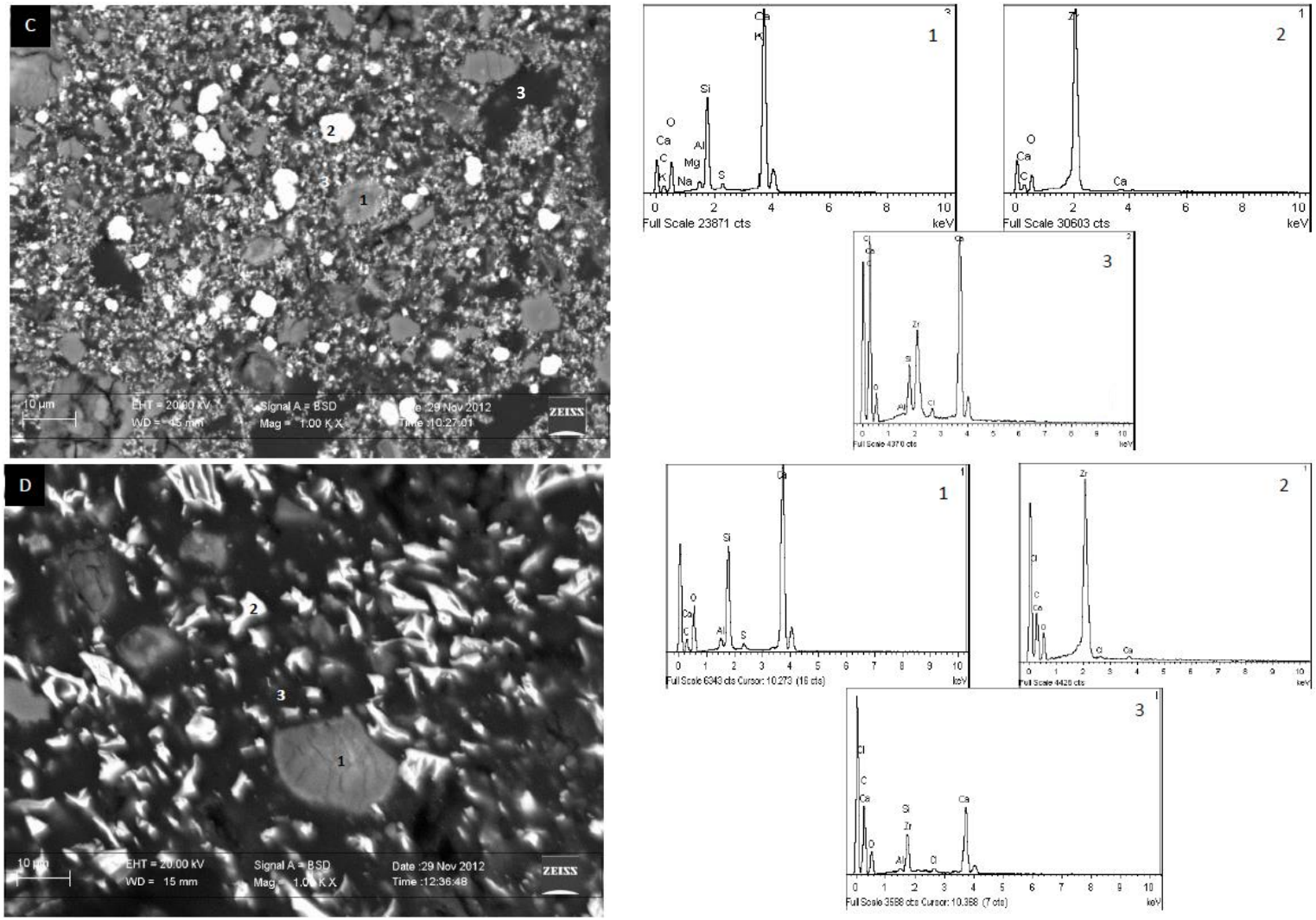

Figure 3[a] (continued)

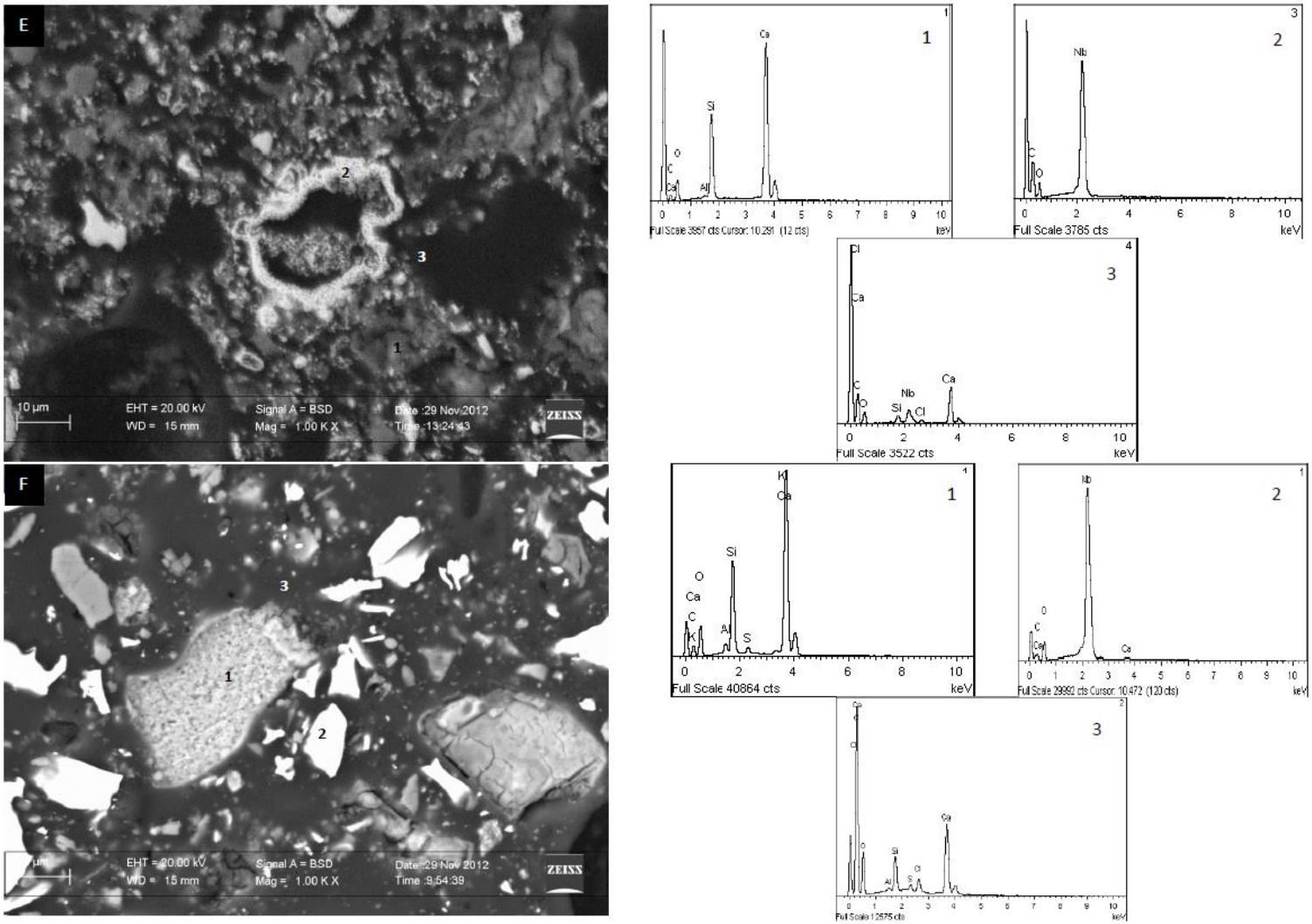

Figure 3[a] (continued)

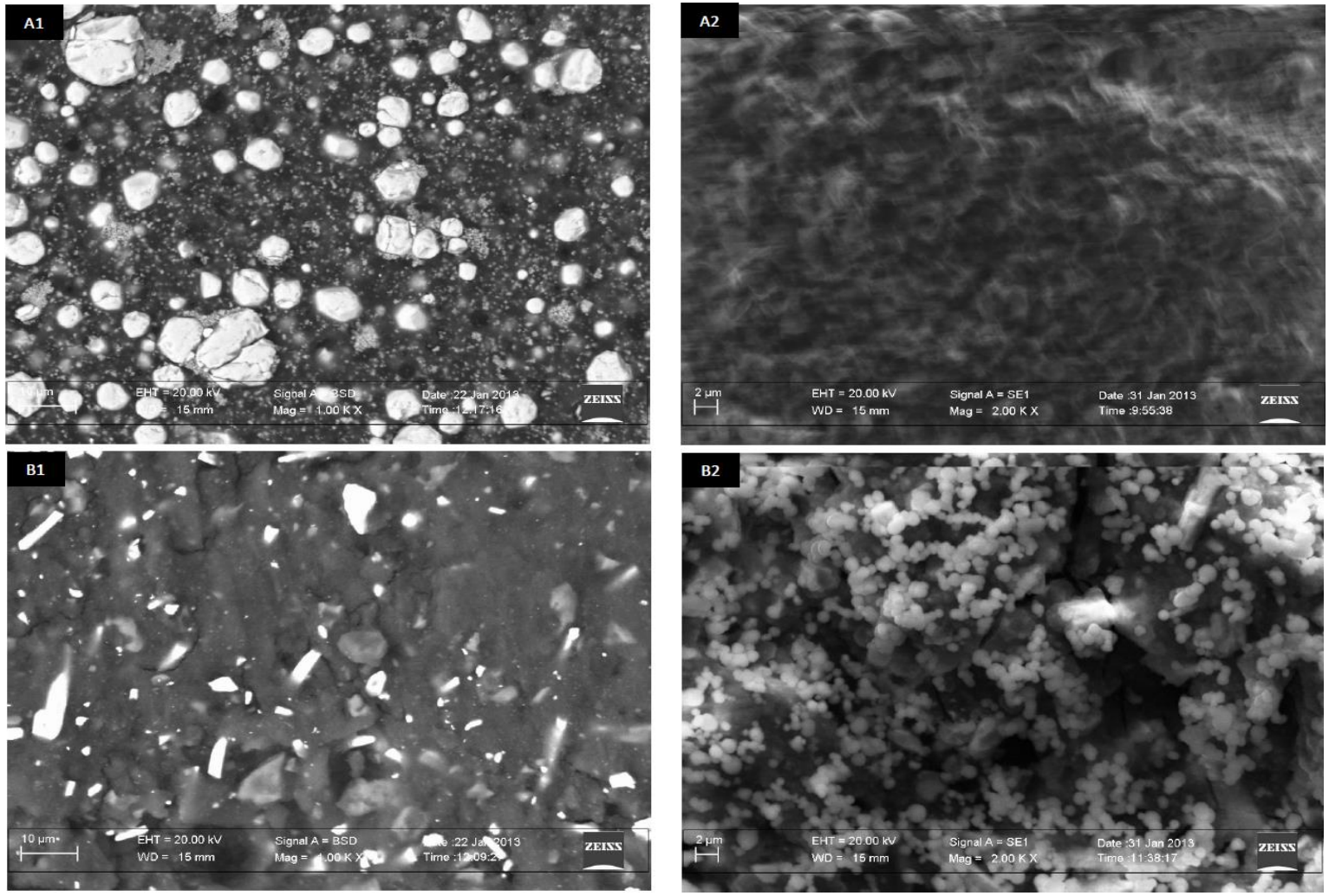

Figure 3b: (1) Back scatter scanning electron micrographs of polished sections and (2) secondary electron scanning electron micrographs of set sealers aged in Hank's balanced salt solution (HBSS) for 28 days (A) AH Plus, (B) MTA Fillapex, (C) ES-Zr-micro, (D) ES-Zr-nano, (E) ES-Nb-micro, (F) ES-Nb-nano.

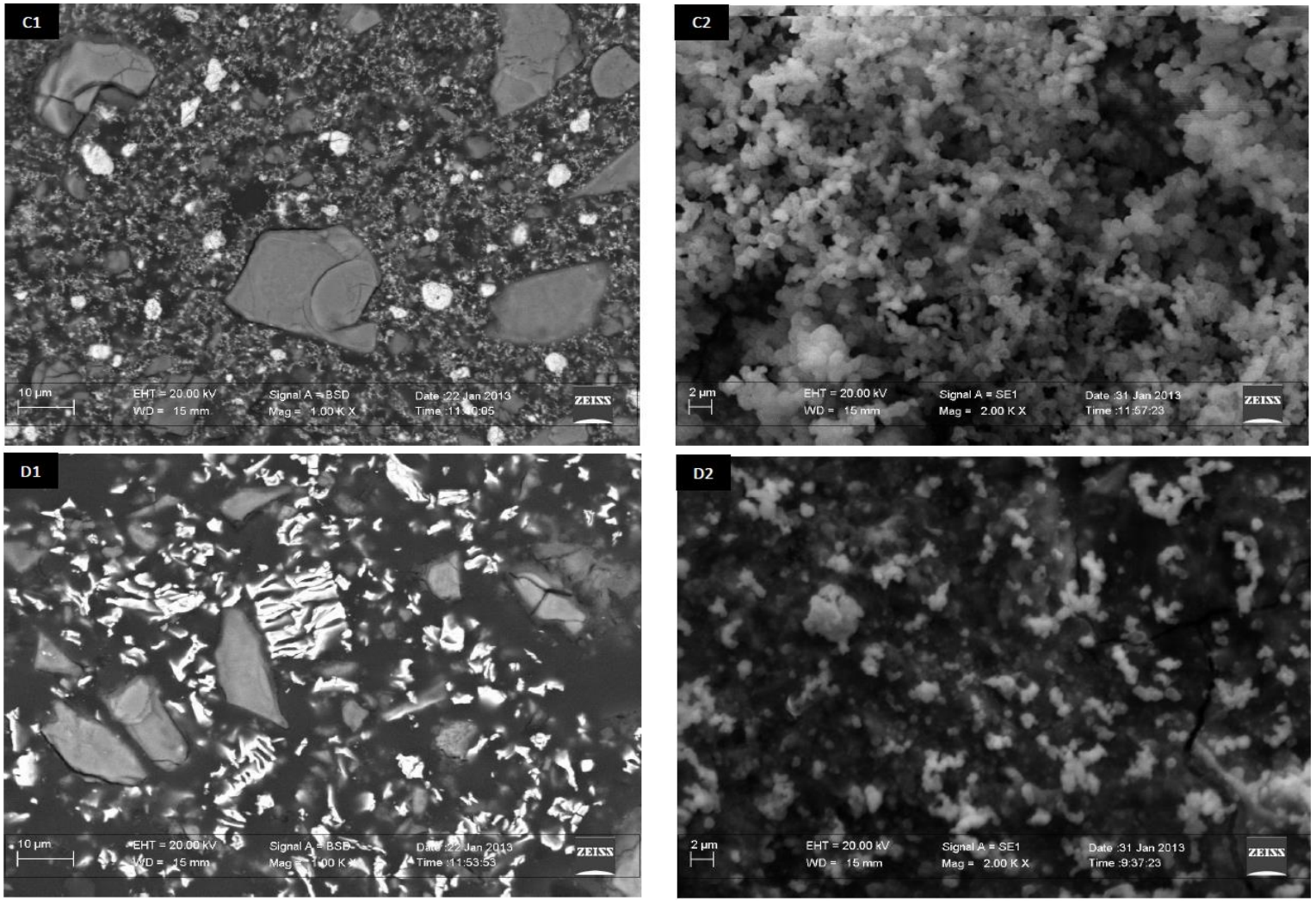

Figure 3b: (continued)

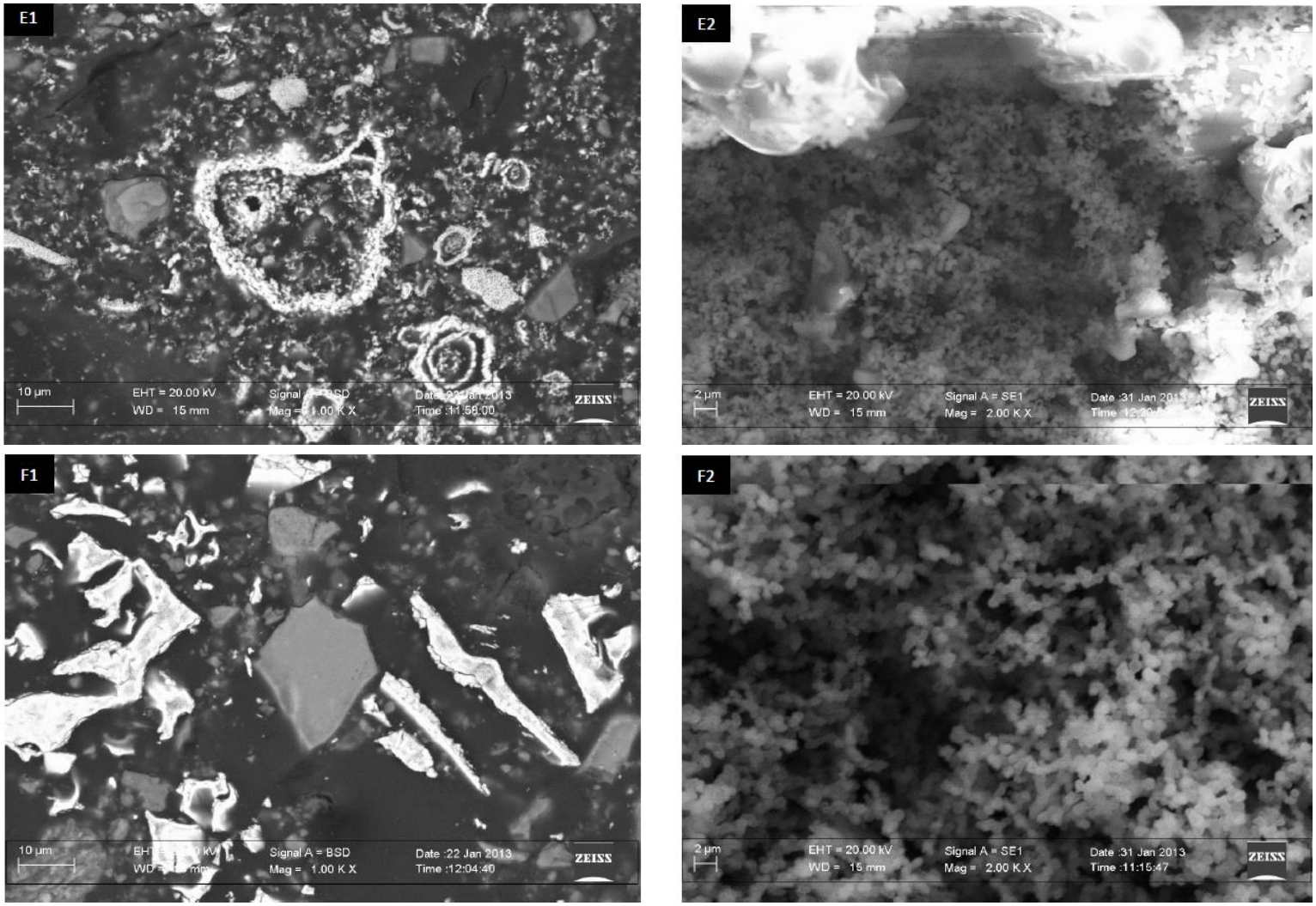

Figure 3b. (continued)

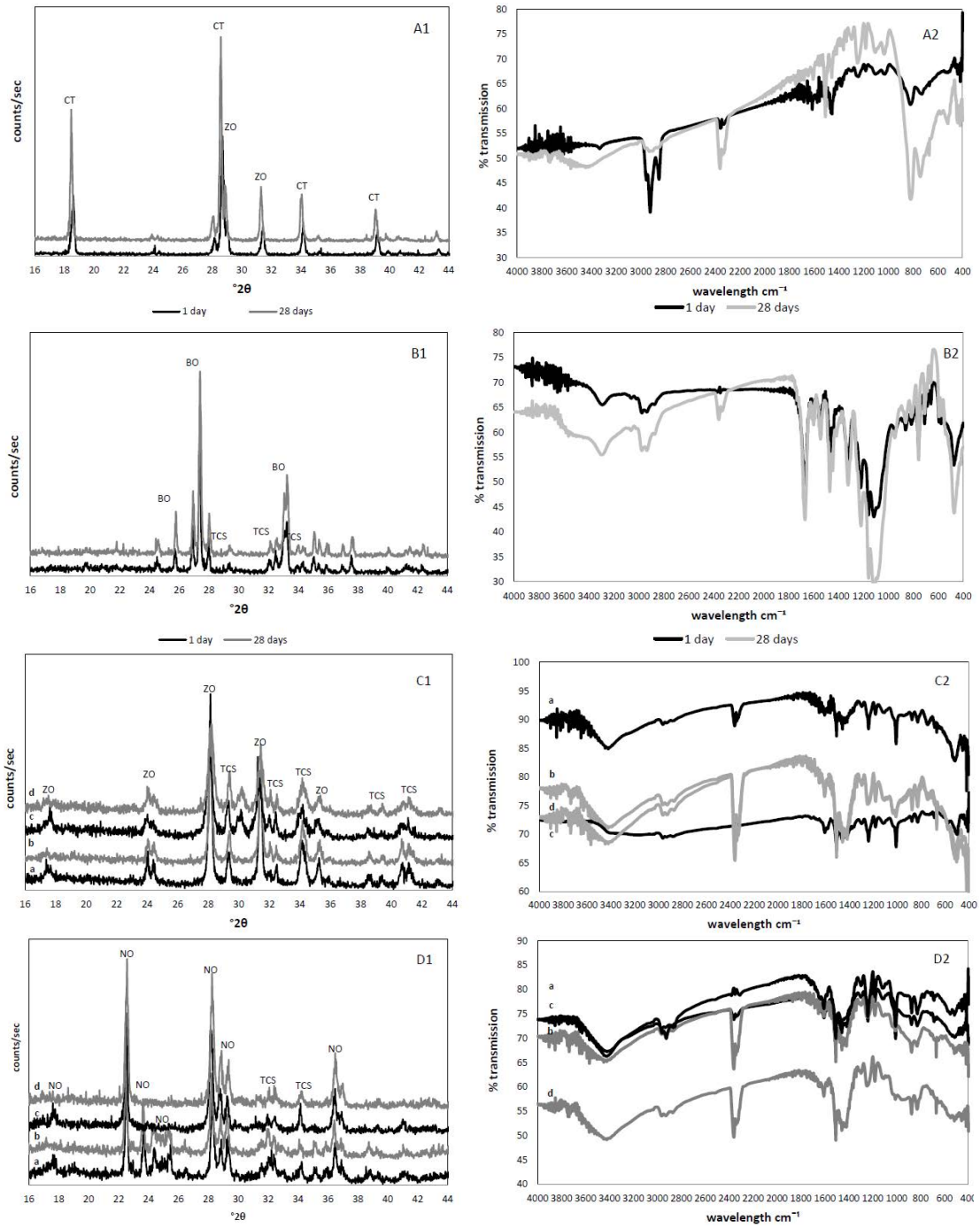

Figure 4: (1) X-ray diffraction plots and (2) FT-IR spectroscopy scans of (A) AH Plus, (B) MTA Fillapex, (C) a: ES-Zr-micro 1 day; b: ES-Zr-micro 28 days; c: ES-Zr-nano 1 day; d: ES-Zr-nano 28 days; (D) a: ES-Nb-micro 1 day; b: ES-Nb-micro 28 days; c: ES-Nb-nano 1 day; d: ES-Nb-nano 28 days (CT: calcium tungstate, ZO: zirconium oxide, BO: bismuth oxide, TCS: tricalcium silicate, NO: niobium oxide)

3.4 pH and calcium ion release

The results for pH of the storage solution of the different sealers under study are shown in Figure 5. All the sealers tested exhibited a mildly alkaline pH. AH Plus displayed a pH

of 8 which was maintained throughout the testing period. The pH of MTA Fillapex increased steadily to a maximum of 10 at 28 days of testing. The experimental sealers all displayed an immediate rise in pH which was maintained initially but reduced to less alkaline by the end of the 28 day testing period. There was no difference between the pH values of the different experimental sealers ($P > 0.05$) thus suggesting that the particle size and type of radiopacifier did not have any effect on the pH of the storage solution.

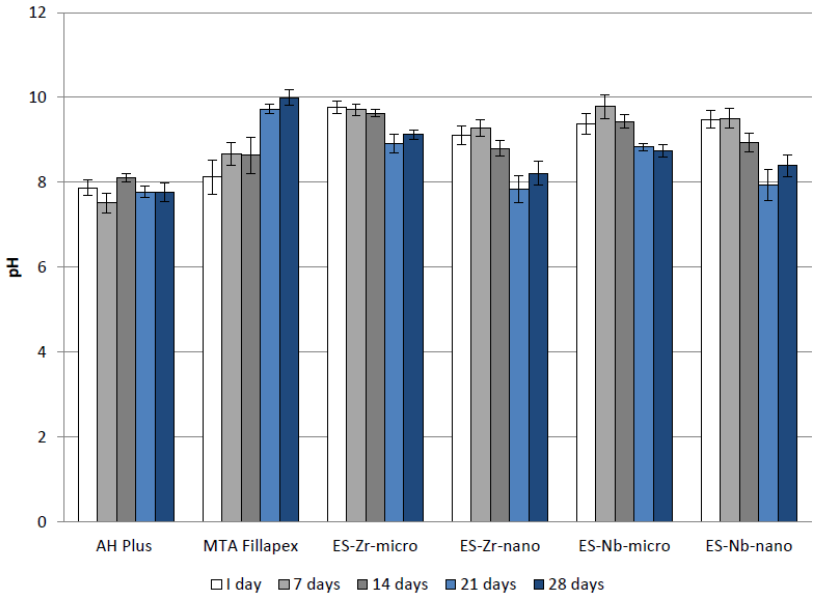

Figure 5: pH of storage solution of different sealers measured weekly over a period of 28 days (± SD).

The results of the calcium ion release of the different sealers in distilled water are shown in Figure 6. The MTA Fillapex and AH Plus sealers exhibited low calcium ion release in solution. All the experimental sealers released a higher level of calcium ions in solution when compared to AH Plus and MTA Fillapex ($P < 0.001$). The calcium ion release decreased over the 28-day testing period. There was no particular trend for the calcium ion release for the experimental sealers with regards the type and particle size of the radiopacifier. The sealer which included micro-zirconium oxide particles exhibited higher calcium ion release than the other experimental sealers at all-time intervals ($P < 0.001$).

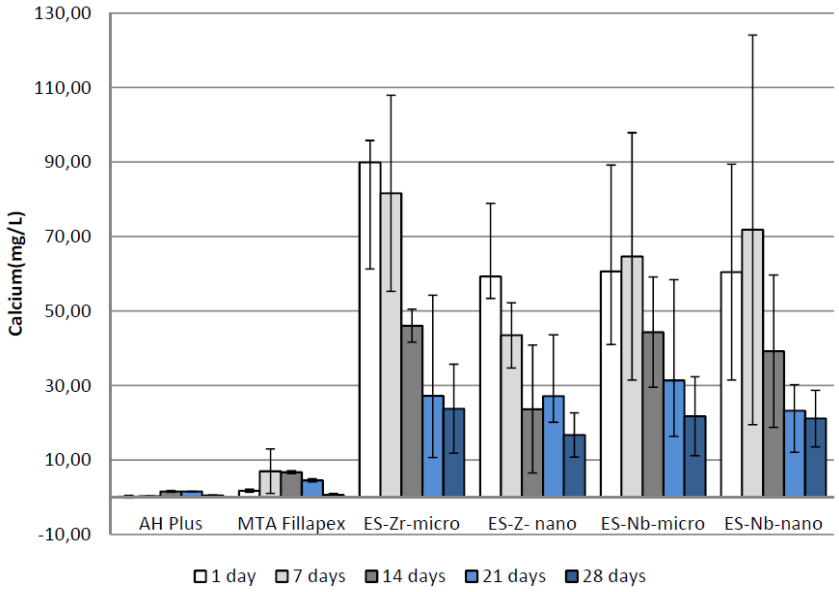

Figure 6: Calcium ion concentration (mg/L) of storage solution of different sealers measured weekly over a period of 28 days (± SD).

4. Discussion

Root canal sealers can be inert or able to induce hard tissue deposition. In general, the bioactivity of a root canal sealer is directly related to the chemical constitution and it depends on the interaction between it and surrounding environment [42].

Clinical evidence of MTA bioactivity can be partially attributed to the mineralization ability of the Portland component [8]. Hydration of Portland cement results in the deposition of calcium hydroxide on the material surface which in turn produces hydroxyapatite in the presence of fluids containing phosphate [5,43]. Based on these biological events promoted by Portland cement and MTA, experimental root canal sealers were developed containing white Portland mixed with a resinous vehicle, which provided adequate handling properties and viscosity and since Portland cement is not able to confer enough radiopacity to the sealer, radiopacifiers were introduced in the mixture. MTA includes 20% bismuth oxide which confers the necessary radiopacity to the material.

In the current study zirconium and niobium oxide were used as radiopacifiers in combination to Portland cement which was mixed with a resin vehicle to enhance sealer manipulation. AH Plus was used as a control since it is an epoxy resin-based root canal sealer

which contains zirconium oxide as radiopacifier in addition to calcium tungstate while MTA Fillapex is a MTA-based root canal sealer that contains bismuth oxide as radiopacifying agent.

Root canal sealers' radiopacity is an important physical property as root canal filling materials need to be distinguished from adjacent anatomic structures [44] and it allows investigation of the quality of the obturations during follow-up. In the present study, although zirconium oxide exhibited twice the radiopacity of niobium oxide no difference in radiopacity was demonstrated when both compounds were incorporated in the Portland-based root canal sealer system. In addition the particle size of the radiopacifier did not affect the radiopacity value. In general, the experimental sealers showed lower values of radiopacity when compared to AH Plus which exhibited very high values similar to previous reports [45]. MTA Fillapex exhibited similar radiopacity values to the experimental sealers which was ~3 mm aluminium which is the limit recommended by ISO 6876:2012 [41].

In this study, a number of techniques have been employed in order to investigate the material microstructure and also determine the chemical composition of both the raw materials and the set sealers. The X-ray diffraction (XRD) is based on intrinsic properties of crystalline solids whereby the angle of radiation is unique for each crystal form. Thus XRD analysis only detects regular structures (crystalline) in the composition of the test materials or precipitates while amorphous structures cannot be identified [46]. On the other hand, the Fourier transform infrared spectroscopy (FT-IR) is an unspecific technique that only identifies functional chemical groups of the chemical components and each functional group absorbs a specific wavelength of radiation in the infrared region. Consequently, the graph of radiation intensity versus frequency (spectrogram) allows the characterization of the functional groups of standard or unknown material.

Assessment of the microstructure of the materials after 24 hours exhibited the typical morphology of Portland-like materials. Cement particles were interspersed in a matrix containing radiopacifier material. After the sealers were immersed in HBSS for 28 days no hydration rims signifying the reaction of cement and deposition of calcium silicate hydrate were

detected. This is in contrast to what has been observed in MTA and calcium silicate-based cements mixed with water (27,47,48). The epoxy resin thus affected the material hydration and acted as a barrier preventing the diffusion of the HBSS into the sealer's matrix and the interaction with Portland particles to promote hydration. Long term investigation of the hydration of such system is necessary because with cement aging in a physiological solution it would be expected to allow the penetration of aqueous substances in the epoxy-resin. Thus, the Portland cement hydration would be expected to proceed. Although the MTA Fillapex is reported to be a MTA-like system, reaction rims were not observed around the reacting cement particles. The XRD plots showed the absence of calcium hydroxide in the set materials for all materials tested.

The microstructure of both the zirconium oxide and niobium oxide-based sealers was different when the particle size of the radiopacifier was varied. The micro- and the nano-particles for both zirconium oxide and niobium oxide exhibited a different microstructure. This was more marked in the niobium oxide whereby the micro-niobium oxide tended to exhibit agglomerated shapes which could be several microns wide while the nano-particles were dispersed. Furthermore, the micro-niobium oxide exhibited two phases (monoclinic and orthorhombic) while the nano-niobium oxide one phase. This was evident in XRD analysis. Polymorphism is a result of variations in the temperature and pressure of a material during its manufacture. The different crystalline phases present are responsible for the altered physic-chemical properties that a solid material can present, such as solubility, melting point, density, hardness, crystal configuration, optical and electrical properties. Zirconium oxide can be present in three crystalline forms (monoclinic, tetragonal or cubic) depending on the temperature and environment, which have particular properties. In the current study only one crystalline form was demonstrated for the zirconium oxide.

Although the formation of calcium hydroxide during material hydration was not evident for the experimental sealers, calcium ions were released in solution. This was shown by the alkaline pH and chemical analysis of leachates in contact with the materials which suggests

a strong correlation between the pH induced by the sealer and its ability to release calcium ions. Furthermore despite the absence of hydration, when mineral deposition ability was evaluated after 28 days of immersion in HBSS, it could be verified that the experimental sealers and the MTA Fillapex presented bioactivity. The results observed in the current study are in accordance with the previous research evaluating the bioactivity of MTA based root canal sealers [49,50]. With respect to MTA Fillapex's bioactivity, the micrographs showed that this sealer presents a certain ability to induce mineral deposition, which corroborates previous research investigating the bioactivity of MTA Fillapex [9]. In both periods of analysis, AH Plus did not present any bioactivity potential probably because mostly calcium present in this material are not in the ionized form.

The spectroscopic analyses of the materials after immersion in HBSS for 28 days exhibited the presence of phosphate phases in addition to a carbonate phase. This signifies the presence of a calcium deficient phosphate phase which is in accordance to previous research investigating the bioactivity of MTA and related materials [6]. The alkaline pH induces the substitution of the phosphate groups with carbonate. These changes were not observed in MTA Fillapex.

The experimental root canal sealers showed high pH value up to 14 days after mixing, which decreased to more neutral levels in the following periods. The opposite occurred to MTA Fillapex, which showed an increase in pH with time and it presented the highest values only after 21 days. Conversely, studies that evaluated pH of the medium in the first hours up to 7 days observed high pH values during these periods for this sealer [51,52].

The influence of radiopacifying agents on root canal sealers bioactivity remains unclear. Zirconium oxide acts as inert filler and did not participate in the hydration reaction of the Portland cements [38]. Scientific literature reports that both zirconium oxide and niobium oxide have been used to coat dental implants to favour bone deposition [53,54]. Niobium oxide had already showed ability to enucleate minerals over the surface in others studies [40] and it is important to note that the addition of niobium oxide to Portland-based root canal sealers seemed

to improve the bioactive potential of these materials. Therefore, it is possible that the radiopacifying agents did not exert directly influence on bioactivity, but could have favour ionic interaction.

Further studies must be conduct to evaluate the long-term hydration of the experimental sealers and also to investigate whether the radiopacity can be improved using different concentration or association of radiopacifying agents.

5. Conclusion

Novel root canal sealers based on a mixture of Portland cement, epoxy resin and radiopacifier exhibited bioactivity comparable to MTA Fillapex. Radiopacifier particle size had limited effect on the sealer's microstructure, chemical properties and it did not enhance the ES's radiopacity to attend ISO specification.

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5 Chapter 4

Interface of dentine to root canal sealers*

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ABSTRACT

Objective: Root canal sealers can interact physically or chemically with dentine. The aim of this study was to characterize the dentine-root canal sealer interface of experimental sealers based on Portland cement using an epoxy-based vehicle in comparison to an epoxy resin sealer, AH Plus.

Methods: Root canals were biomechanically prepared and filled with any one of the four experimental epoxy sealers containing Portland cement with micro- and nano-particles of either zirconium oxide or niobium oxide radiopacifers, or AH Plus. The dentine-sealer's interfaces were assessed by coronal penetration of fluorescent microspheres, the penetration of sealers labelled with Rhodamine B inside the dentine tubules (following obturation with gutta–percha and sealers using System B technique) assessed by confocal laser scanning microscopy, and the chemical characterization of dentine-sealers interface by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) line scans.

Results: No penetration of fluorescent microspheres at the root-dentine to sealer interface was recorded for all test materials. Sealers presented greater ability to penetrate within the dentinal tubules at the coronal and mid-root thirds. The experimental sealers containing radiopacifier nano-particles exhibited a more homogeneous microstructure along the whole length of the canal. EDS-line scans results showed a migration of silicon and niobium into dentine. Peak overlap between zirconium and the phosphorous compromised the identi- fication of the migration of the zirconium oxide into dentine.

Conclusions: All five sealers promoted coronal sealing. The experimental sealers exhibited promising characteristics and were comparable to AH Plus sealer. Elemental migration of the experimental sealers suggests material interaction with dentine which was not displayed by AH Plus

Keywords: Root canal obturation; Characterization; Root dentine to sealer interface; Fluorescent microspheres; Rhodamine B; Portland cement; AH Plus

1. Introduction

Root canal obturation is usually performed using solid cones such as gutta–percha or Resilon™ to fill the root canals in conjunction with a sealer that fills the spaces between the solid material and dentine,[1] to create an impermeable seal of the root canal system and prevent bacterial entry. Endodontic materials can physically or chemically interact with dentine. The physical interaction usually is established through the penetration of the material into dentine tubules to create micromechanical retention. The formation of ‘‘tags” along the material-dentine interface have been reported with tricalcium silicate-based materials, resulting in an increase of the push-out strength of this material.[2,3] These ‘‘tags’’ are related to the interfibrillar precipitation of hydroxyapatite crystals resulting from the interaction of the material with phosphates from the surrounding environment.[2–6] Further- more, dentine can interact with the material to uptake ions, such as, silicon and calcium.[7] This interfacial layer has been described as the ‘‘mineral infiltration zone’’.[8] The formation of the mineralized zone is related to the alkaline caustic effect of the calcium silicate cement’s hydration products that degrades the collagenous component of the interfacial dentine resulting in a porous structure that can be easily permeate by ions which mineralizes the adjacent dentine. This interfacial layer had been already observed with calcium silicate-based materials, such as ProRoot MTA[1] and Portland cements.[4] The use of these materials as root canal sealers is precluded due to their sandy consistency arising from the particle size distribution of the MTA.[9] Some studies had proposed the use of different vehicles and additives to improve the consistency and the flowability of these materials and to enable their application in orthograde procedures, to take advantage of their biocompatibility and ability to induce hard tissue deposition.[10–13] Thus, a new class of root canal sealers based on tricalcium silicate has been developed.[11,14–19] The interaction of the new class of tricalcium silicate sealers with dentine is worthy of investigation.

The purpose of the present study was to characterize the dentine-root canal sealer interface of different experimental root canal sealers based on Portland cement and epoxy resin vehicle in comparison to an epoxy resin-based sealer, AH Plus®.

2. Materials and methods

The materials used in this study included four experimental (ES) root canal sealers and one based on epoxy resin (AH Plus, Dentsply, De Trey, Konstanz, Germany). The ES were Portland cement-based (Portland Cement; CPB-40; Votorantin cimentos, Camargo Correa S.A., Pedro Leopoldo, MG, Brazil) replaced by radiopacifying agents (30% by weight):

- Micro-zirconium oxide (ZrO2; Sigma–Aldrich, St Louis, MO, USA) – ES-Zr-micro.
- Nano-zirconium oxide (Institute of Physics of Sa˜o Carlos, University of São Paulo, São Carlos, Brazil) – ES-Zr-nano.
- Micro-niobium oxide (CBMM, Companhia Brasileira de Metalurgia e Minerção, Araxá, MG, Brazil) – ES-Nb-micro.
- Nano-zirconium oxide (Institute of Physics of São Carlos, University of São Paulo) – ES-Nb-nano.

The micro-particles of niobium oxide and zirconium oxide were purchased from companies manufacturing chemicals. The nanoparticles of niobium oxide and zirconium oxide were prepared by the polymeric precursor method. The experimental radiopaque cements containing micro-particles (ES-Zr-micro and ES-Nb-micro)[10] were mixed with 37.5% of an epoxy resin composed of equal amounts of catalyser and base pastes, while for those containing nanoparticles (ES-Zr-nano and ES-Nb-nano) 44.4% of epoxy resin were used. The powder/ liquid ratio of the sealers containing nanoparticles was determined by a previous pilot study. AH Plus (Dentsply, De Trey, Konstanz, Germany) was used as control and manipulated according to manufacturer's instructions.

2.1 Sample preparation

Forty five single rooted teeth extracted for periodontal reasons were used. No ethical approval or patient consent was sought as the country legislation did not restrict the collection of extracted teeth. Only teeth with complete root formation and no accentuated root curvature were included in the study. The teeth were cleaned of soft tissue and calculus using an

ultra-sonic device and decoronated to a standard root length of 15 mm. The root canals were instrumented with ProFile rotary nickel–titanium instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to size 35 (0.06 taper), 1 mm shorter than the standardized root length (14 mm), using 10 mL of 5% NaOCl irrigation between the changes of the rotary files. The final rinse was performed with 2 mL of 17% EDTA, which remained for 5 min inside the root canals, followed by saline (2 mL) to remove any traces of chemical solutions. The teeth were assorted in 3 groups according to the methodology performed to evaluate dentine-sealer interfaces.

2.2 Assessment of the root dentine-sealer interface with fluorescent markers

The root canals (n = 3) were dried and filled only with the test sealers using a lentulo spiral drill. The root apices were blocked with flowable composite and teeth were completely coated with two layers of nail varnish except for the coronal orifice. A dye was used to assess the dentine to material interfacial characteristics of the obturated roots. Carboxylate-modified fluorescent micro- spheres (FluoSpheres1 Invitrogen, Life Technologies Grand Island, NY, USA) 0.5 mm diameter conjugated with a red dye and diluted in 1:10 concentration with water were employed. The obturated roots were immersed in the dye immediately after completion of obturations for 24 h at 37 ^{o}C after which they were removed from the dye solution, washed with water and embedded in epoxy resin (EpoFix, Struers, Ballerup, Denmark). The roots were then sectioned longitudinally (Accutom 50, Struers, Ballerup, Denmark) under copious water irrigation along the long axis of the root canal. The root dentine–cement interface was examined using a light microscope with a fluorescence attachment (Axio Scope A1, Carl Zeiss, Göttingen, Germany) with a dry 20x magnification objective lens. The carboxylate-modified fluorescent microspheres had an excitation/emission wavelengths of 580/605 nm. Images of the root dentine-cement interface at different levels along the root canal for every material type were captured digitally.

2.3 Assessment of the root dentine-sealer interface by confocal laser scanning microscopy

One gram of the test sealers were mixed with 0.001 g of Rhodamine B (0.1%) and the sealers were placed inside the root canals of the specimens (n = 3) using a lentulo drill. All the root canals were obturated with gutta-percha using the continuous wave condensation technique with System B for both the back-packing and down-packing stages of the obturation. The roots surfaces were coated with nail varnish except for the apical foramen, which was exposed to Hank's balanced salt solution (HBSS; H6648, Sigma–Aldrich, St. Louis, MO, USA) solution for 24 h and 28 days. After these periods of time, the roots were embedded in epoxy resin and longitudinally sectioned along the long axis using a diamond wafering blade (Buehler, Lake Buff, Wisconsin, USA) attached to the cutting machine (Accutom 50, Struers, Ballerup, Denmark). The sectioned roots were then ground and polished using progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens were examined using confocal laser scanning microscopy (Nikon Eclipse, Tokyo, Japan) with a 60x oil immersion objective to investigate the sealers penetration inside the dentine tubules at different root thirds (coronal, mid root and apical regions).

2.4 Assessment of the root dentine-sealer interface by scanning electron microscopy and line scans

Additional human single rooted teeth (n = 3) were filled only with the test sealers and had their coronal access restored with glass ionomer cement. The root surfaces were coated with two layers of nail varnish leaving only the apical foramen exposed to HBSS at 37 C^{o}. After 28 days, the roots were removed from HBSS, embedded in epoxy resin, sectioned and polished as described above. The specimens were mounted on aluminium stubs, carbon coated and viewed with the scanning electron microscope (Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany). The back-scatter electron mode was used and Energy Dispersive Spectroscopy (EDS) was performed along the length of the root canal. Energy

dispersive spectroscopy (EDS) line analysis was also performed across the interface with the dentine.

3. Results

3.1 Assessment of the root dentine-sealer interface with fluorescent markers

The light micrographs of the test sealers used to obturate the root canals are shown in Fig. 1. The sealers obturated the entire length of the root canal well. The experimental sealers had less porosity than AH Plus in the bulk of the materials. Despite the porosity, the sealers were well compacted and the contact of the sealer to the root canal walls was adequate and no voids or gaps in the dentine-sealers interface was observed. There was no penetration of fluorescent microspheres along the root dentine to sealer interface. The fluorescent microspheres were only visible in the coronal region along the sealer surface.

3.2 Assessment of the root dentine-sealer interface by confocal laser scanning microscopy.

The confocal images obtained 24 h (Fig. 2a) and 28 days (Fig. 2b) after obturation showed a common pattern of sealer penetration inside dentine tubules. The sealers presented more ability to fill dentine tubules mainly at the coronal and mid-root thirds while none of the sealers penetrated inside dentine tubules at the apical region in the samples analysed 28 days after obturation. The only sealers that presented some degree of penetration at the apical third were the nano-zirconium oxide and AH Plus. The former exhibited a higher intensity of penetration than the latter. Micro-niobium oxide-based sealer was the only sealer that did not exhibit any degree of penetration at the coronal third.

3.3 Assessment of the root dentine-sealer interface by scanning electron microscopy and line scans.

The scanning electron micrographs and EDS analysis of the test sealers are shown in Fig. 3. All the sealers were composed of a resin matrix with radiopacifier material interspersed in the matrix. The EDS analysis of AH Plus exhibited the presence of calcium, tungsten and zirconium. Cement particles were evident in the experimental sealers. Both the zirconium oxide and the niobium oxide exhibited a different microstructure dependent on the material processing used to produce the radiopacifier agent's particles. The micro- zirconium oxide particles were circular while the nano ones were elongated. The micro-niobium oxide particles showed the tendency to coalesce into very complex shapes. The EDS analysis of the experimental sealers exhibited peaks for calcium, silicon, aluminium, magnesium and sodium, which are the constituents of cement in combination with peaks for zirconium or niobium depending on which radiopacifier was used. Only the sealers incorporating niobium oxide as radiopacifier exhibited the presence of a phosphorus peak related to the material.

The scanning electron micrographs of the dentine-sealer interface are shown in Fig. 4. In all sections along the root the AH Plus exhibited areas which were devoid of radiopacifier particles. AH Plus exhibited good contact with the walls of the root canal. The micro-zirconium oxide and micro-niobium oxide based sealers exhibited an irregular distribution of components in the microstructure. The coronal region of both micro-based sealers had more areas which were devoid of radiopacifier particles thus composed mostly of resin. On the other hand the nano-zirconium oxide sealer exhibited a uniform homogenous microstructure along the whole length of the root canal with the radiopacifier particles interspersed within the resin matrix in contrast to the nano-niobium oxide based sealer which exhibited a high proportion of resin matrix devoid of radiopacifier all along the length of the root. Sealer penetration in the dentinal tubules of the root obturated with micro-niobium sealer was evident.

The line scans for the different elements are shown superimposed on scanning electron micrographs in Fig. 5. The calcium was present in both root dentine and all sealers tested as evident in the line scans for calcium. The EDS analysis of the interfacial area is shown in Fig. 6. Phosphorus peaks overlap zirconium peaks on EDS analysis (Fig. 6) which explains the similarity of the line scans of zirconium and phosphorus in AH Plus, and the zirconium oxide-based experimental root canal sealers. In niobium oxide-based root canal sealers the presence of phosphorus was detected in the material analysis (Fig. 3), in the EDS line scans (Fig. 5) and also in the EDS analysis of the interfacial layer (Fig. 6). The tungsten in AH Plus did not move across the interface. In contrast the silicon which is part of the cement component in the experimental sealers tended to be present at the dentine-sealers interface and also into the root dentine and across the interface being mostly evident with the nano-particulate sealers. The zirconium was present in the AH Plus and zirconium-based experimental sealers. Evidence of zirconium at the interface and into the root dentine could not be verified because of the peak overlap with phosphorous. On the other hand niobium was present at both the interface and into the dentine of specimens obturated with niobium oxide-based sealers. No carbon was present in the root dentine of the roots obturated with the experimental sealers showing lack of penetration of the resin component into dentine. A more intense carbon peak could be identified in the roots obturated with AH Plus sealer suggesting the penetration of the resin component into dentine.

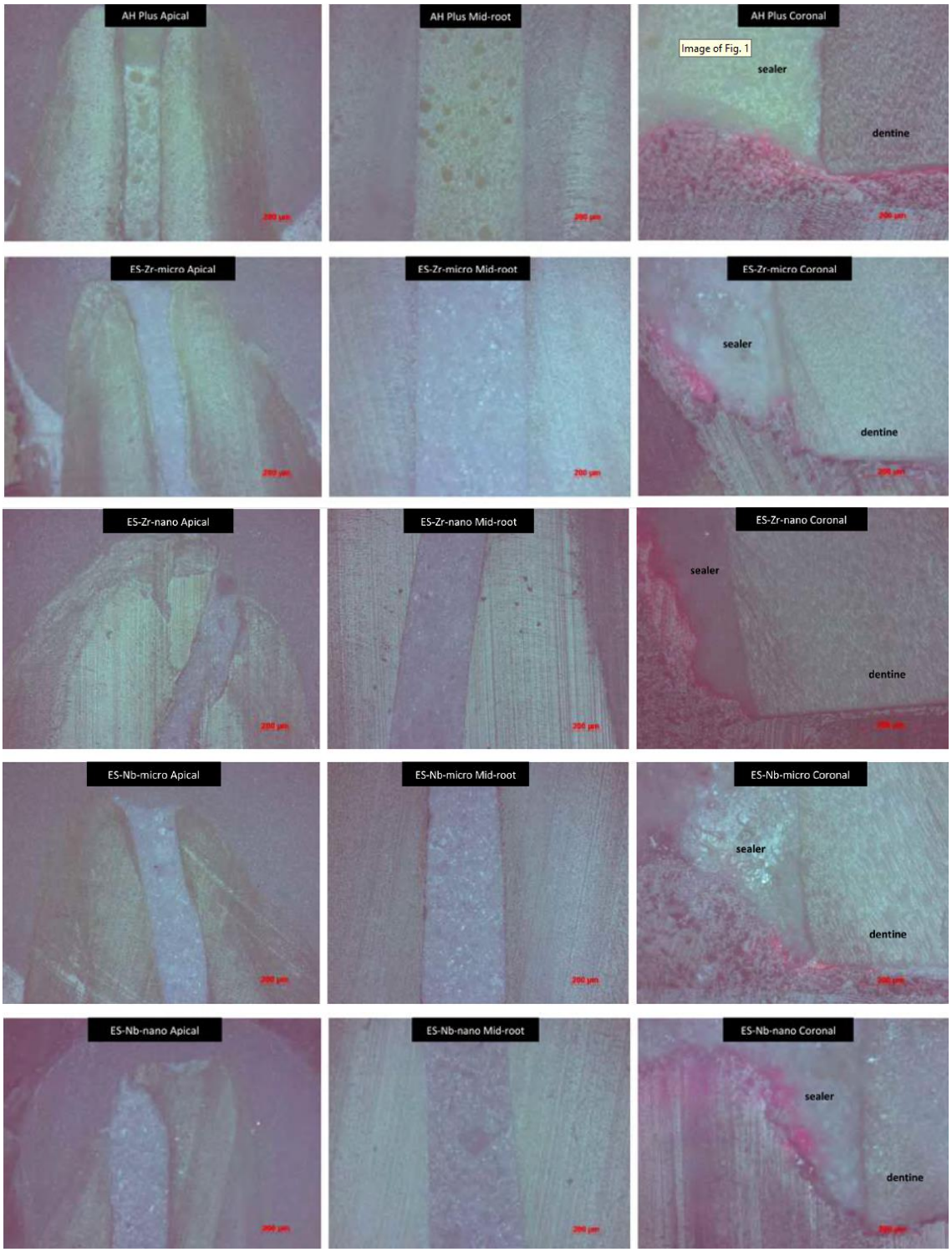

Fig. 1 – Light micrographs of root apices at different locations along the root canals obturated with test sealers subjected to fluorescent, carboxylate modified microspheres 0.5 mm in diameter conjugated with a red dye. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

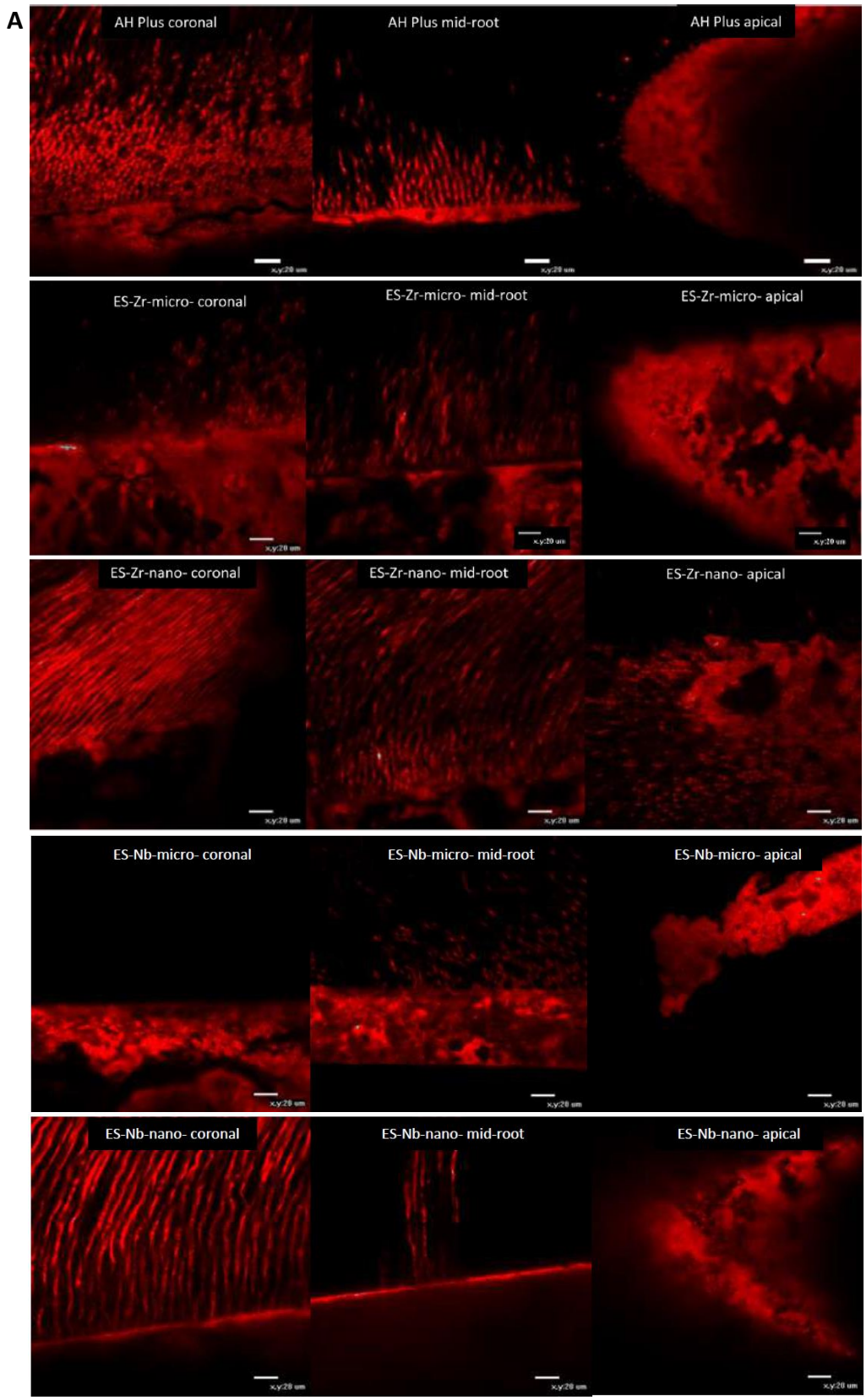

Fig. 2 – (a) Root dentine to sealer interfacial characteristics of different test sealers after 1 day of, placement viewed under confocal microscope tracing the presence of sealer inside the dentinal tubules and (b) root dentine to sealer interfacial characteristics of different test sealers after 28 days, immersion in Hank's balanced salt solution viewed under confocal microscope tracing the presence of, sealer inside the dentinal tubules.

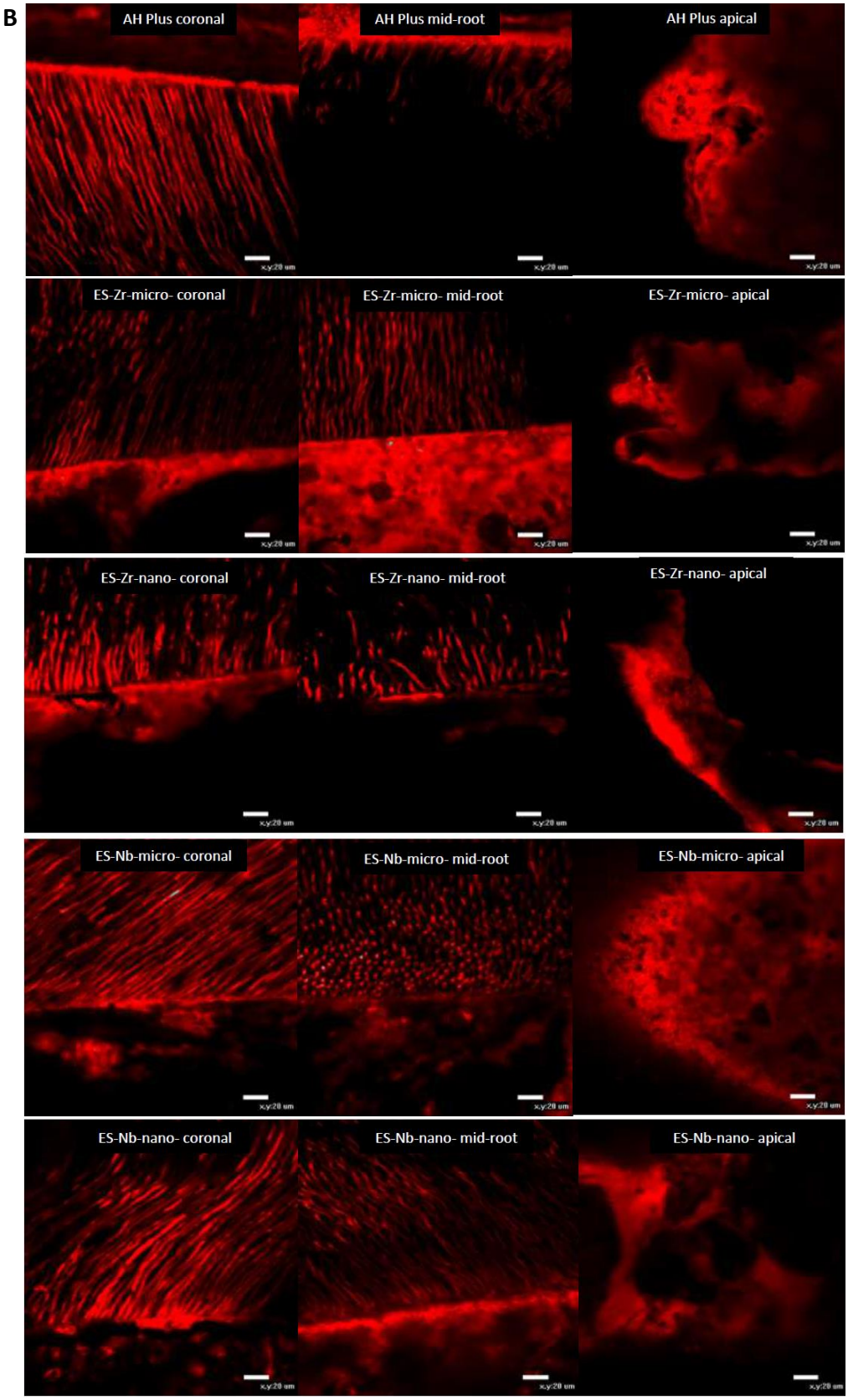

Fig.2 – (continued)

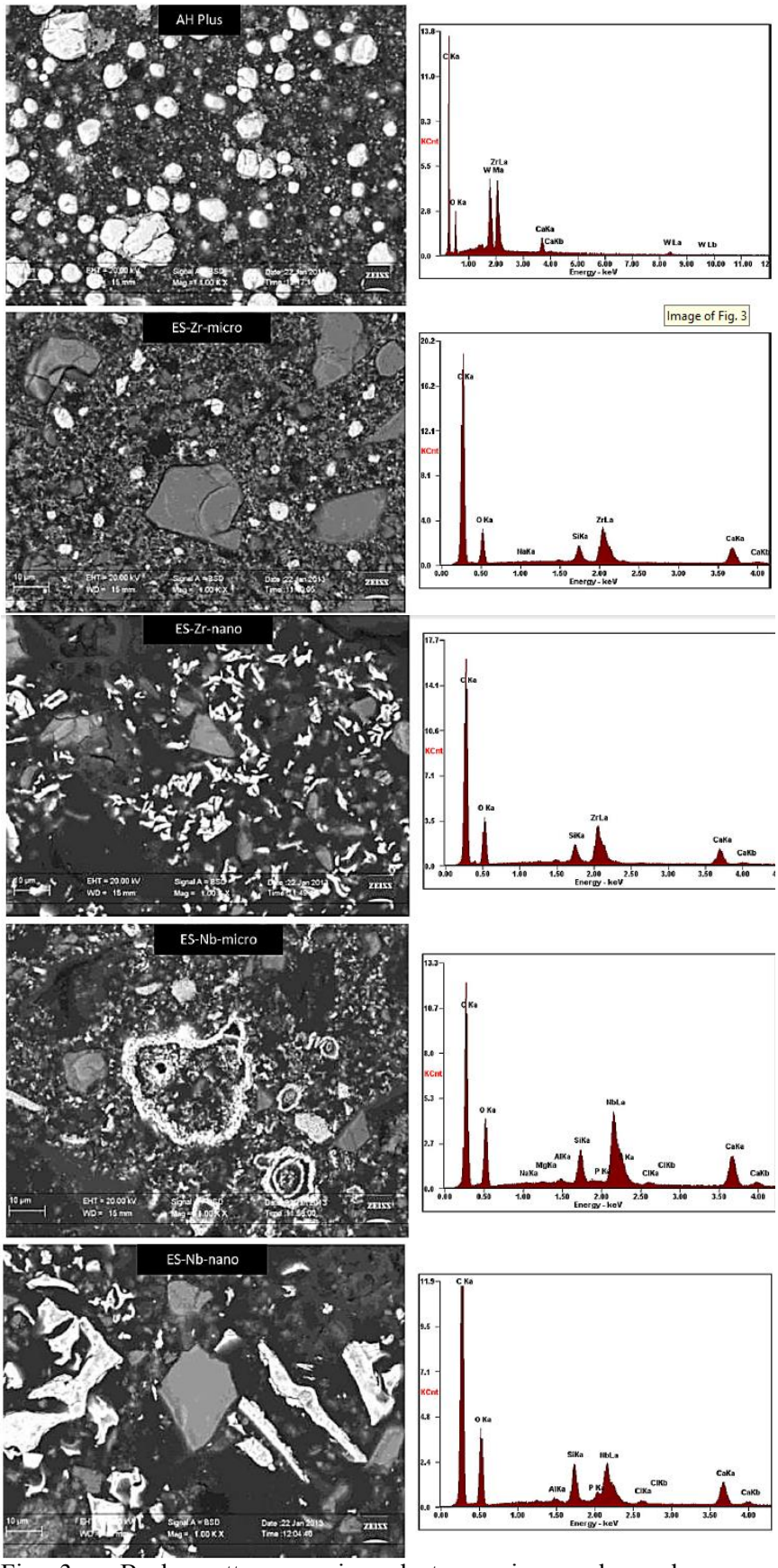

Fig. 3 – Back scatter scanning electron micrographs and energy dispersive X-ray analysis of test sealers, showing general microstructure and elemental composition.

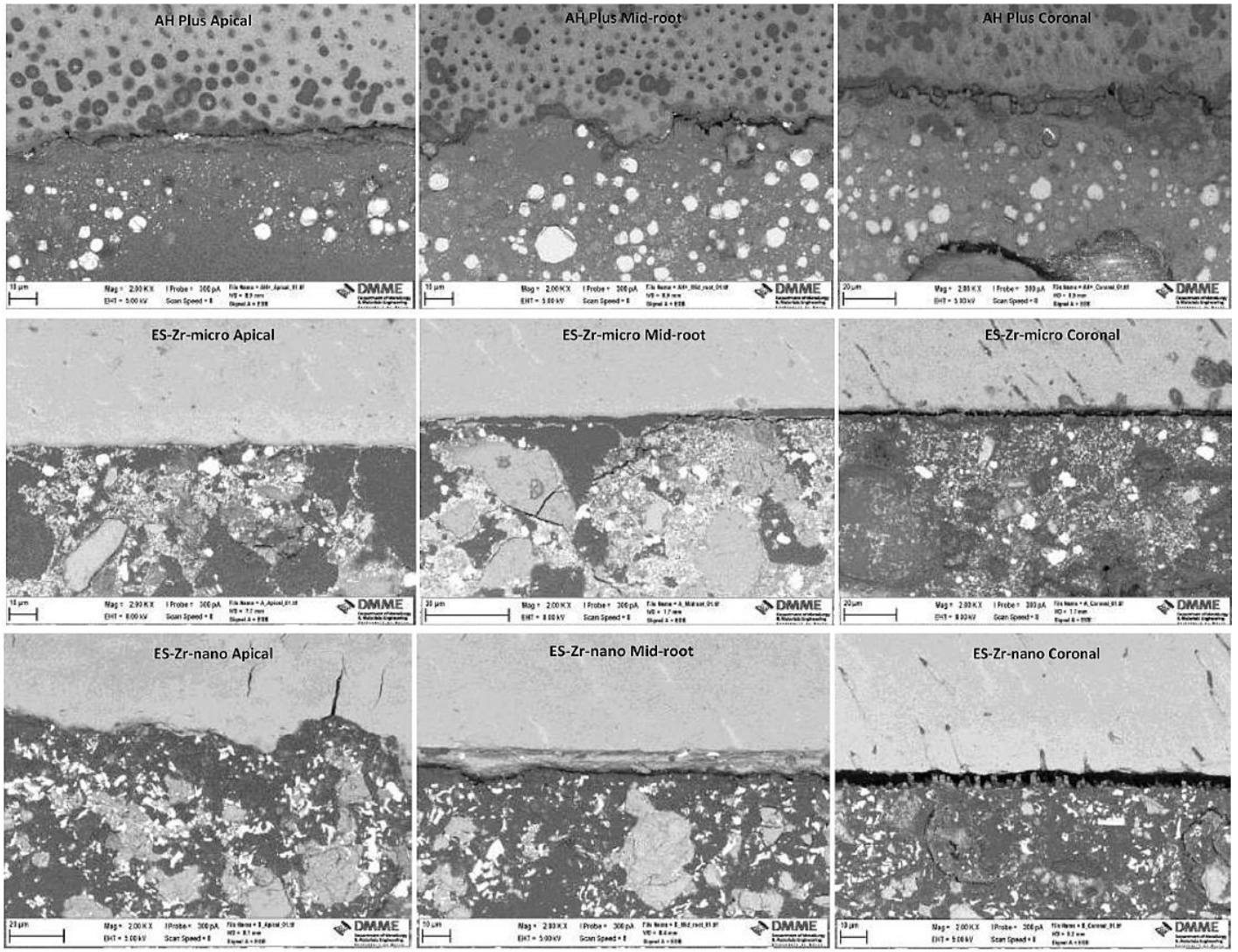

Fig. 4 – Back scatter scanning electron micrographs of different test sealers showing interfacial characteristics.

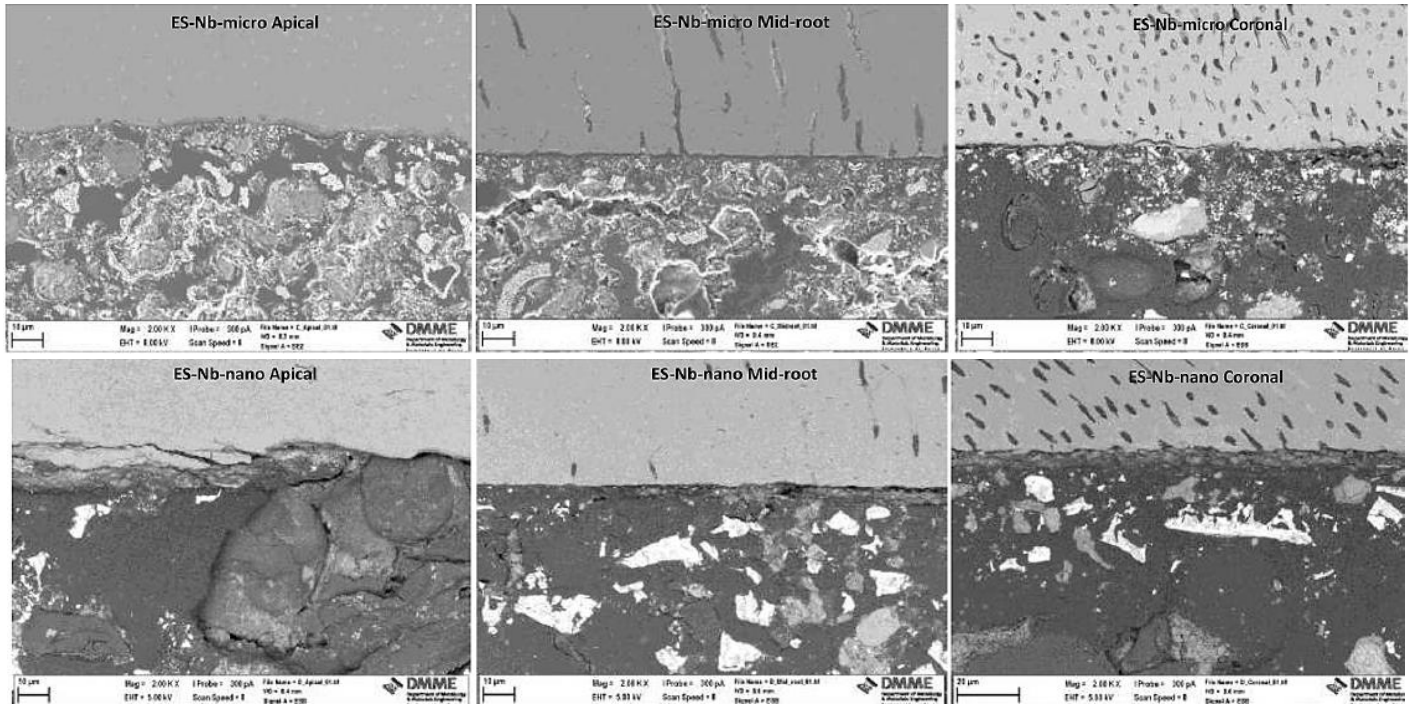

Fig. 4 – (Continued)

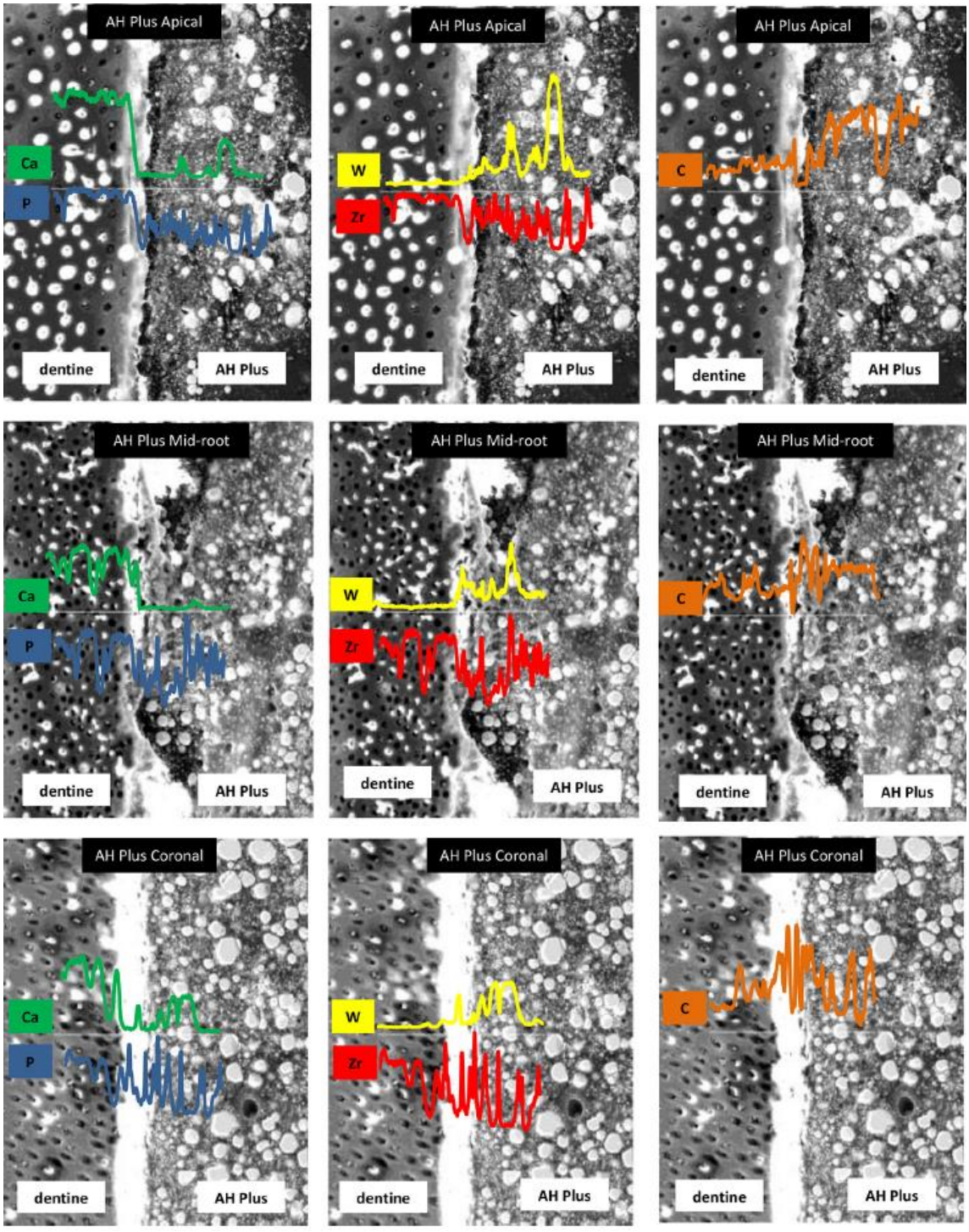

Fig. 5 – Line scans of various elements and their interaction at the interface between root dentine and, the root canal sealers in the apical, mid-root and coronal portions of the root canal (2K X mag).

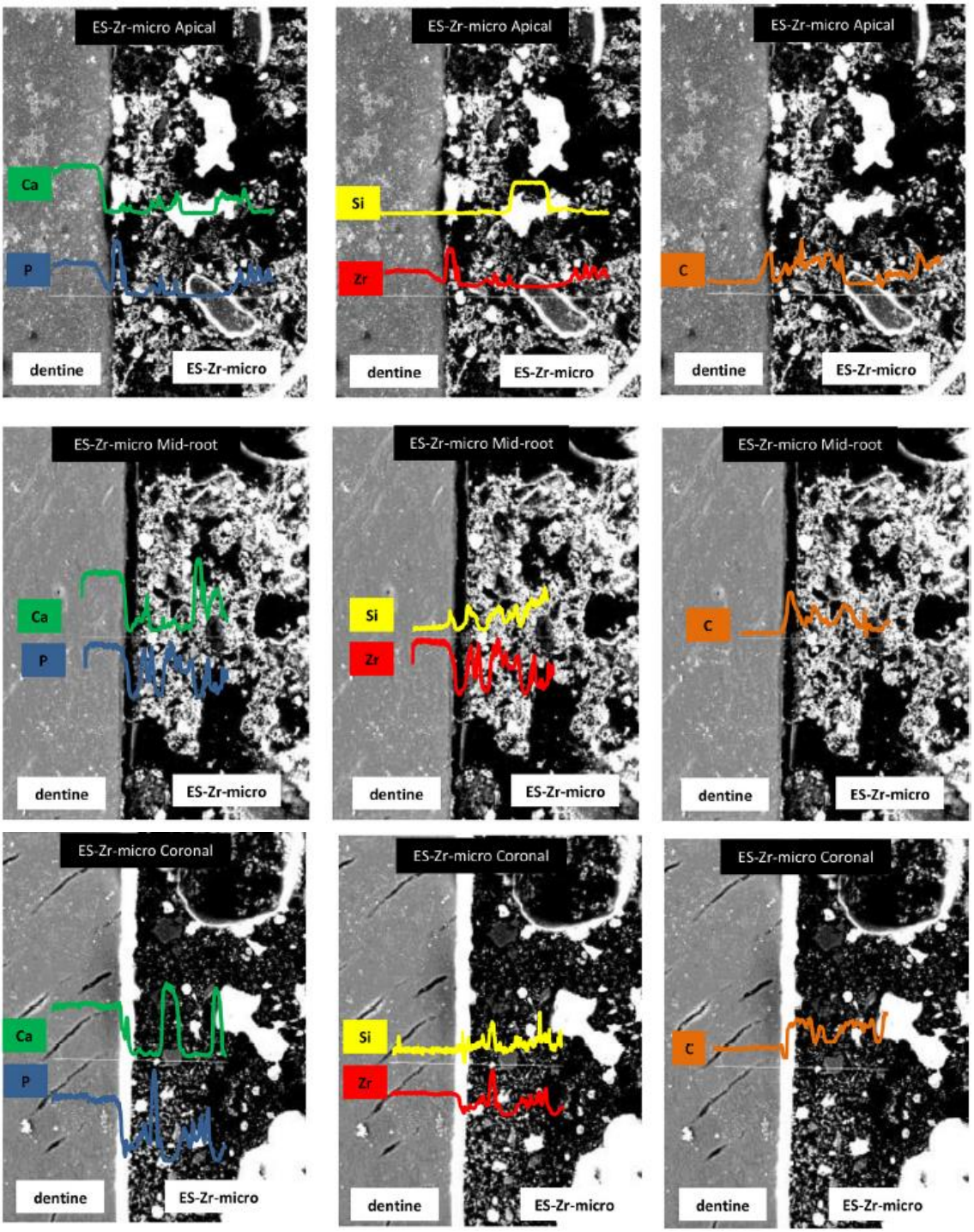

Fig. 5 – (Continued).

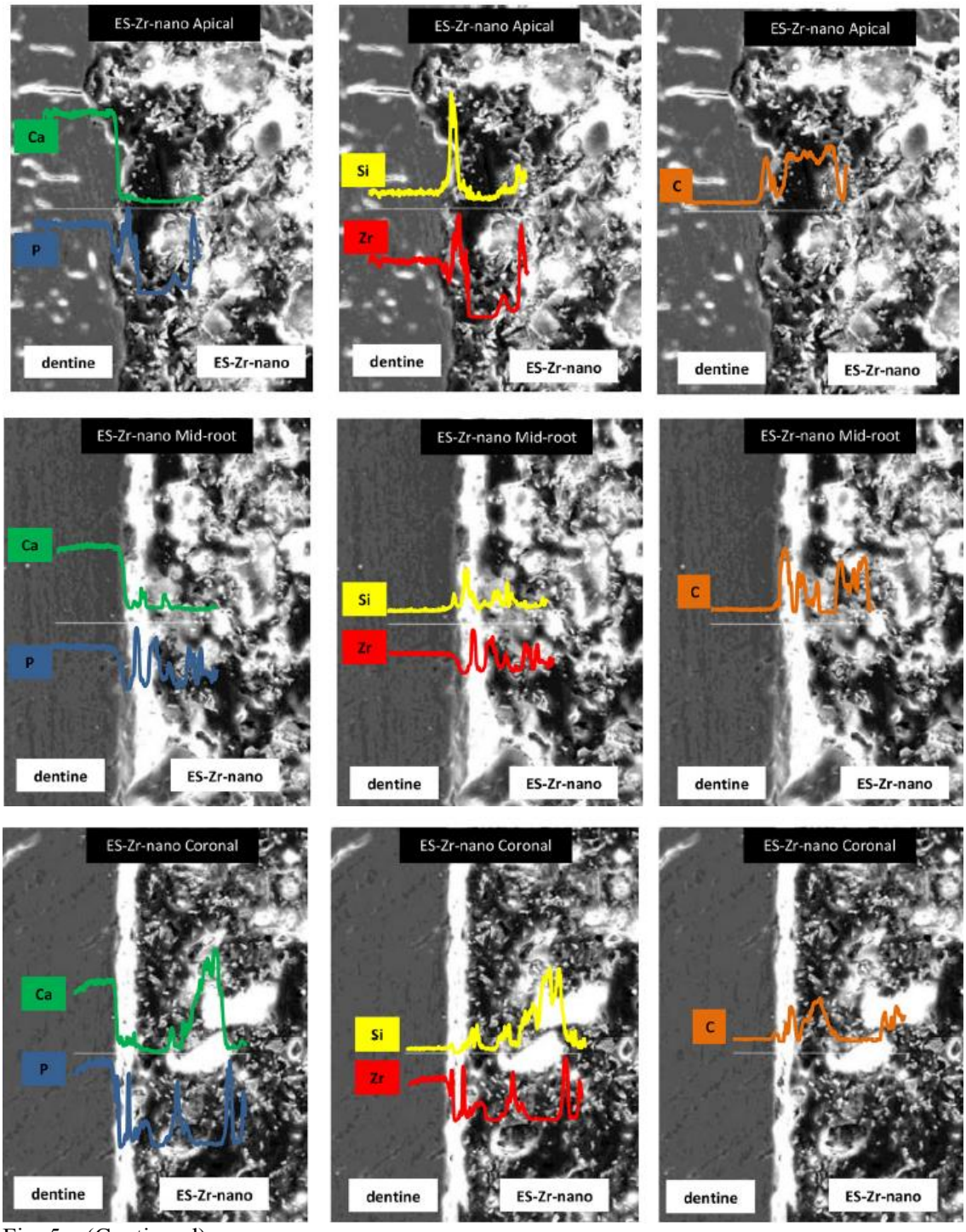

Fig. 5 – (Continued).

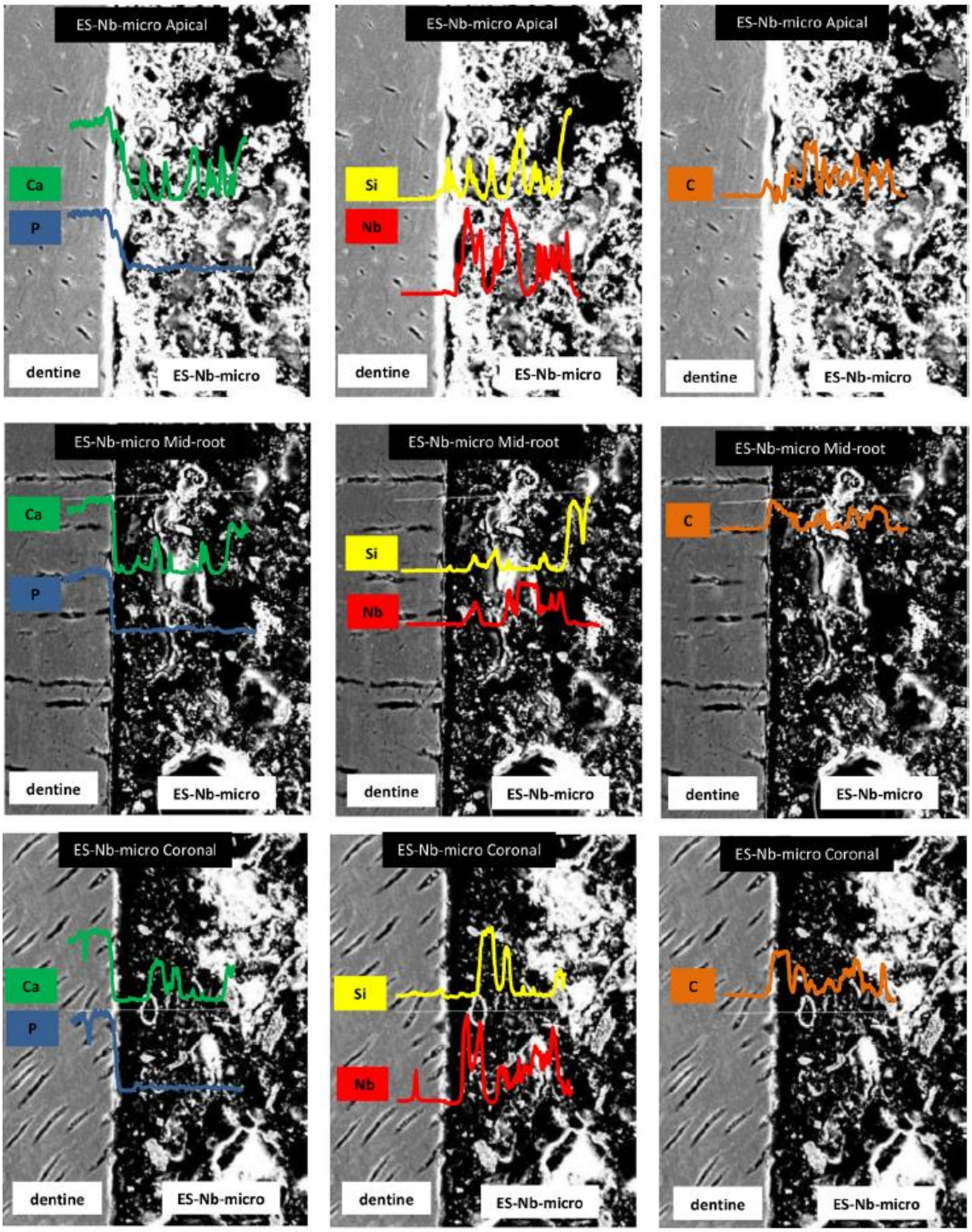

Fig. 5 – (Continued).

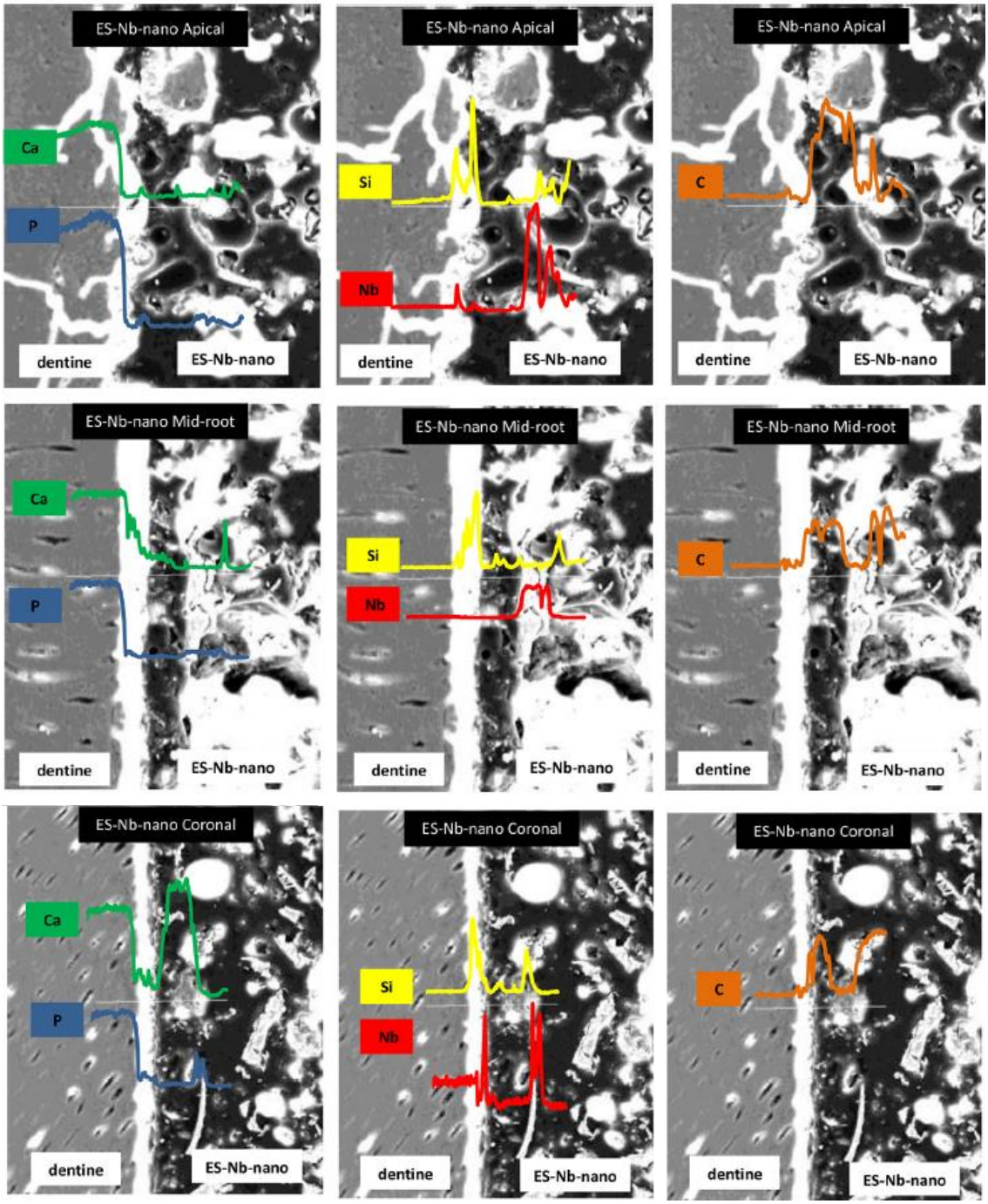

Fig. 5 – (Continued).

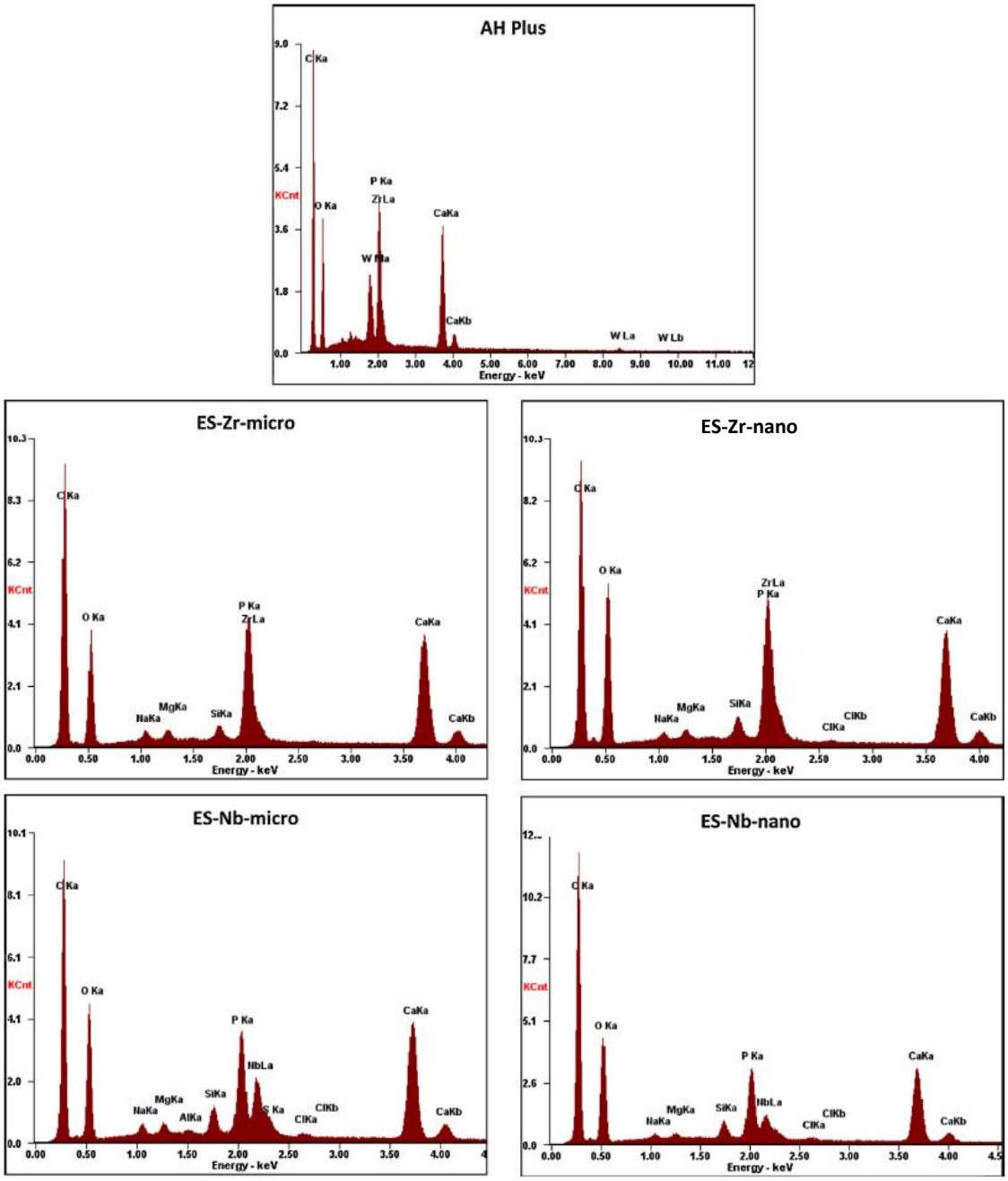

Fig. 6 – Energy dispersive X-ray spectroscopy of the tooth to material interface showing the peak, overlap.

4. Discussion

During root canal filling procedures it is difficult to predict or avoid the formation of voids or gaps inside the obturation mass or at the dentine-sealer interface. Even when thermoplasticized obturation techniques are associated to adhesive sealers with satisfactory physico-chemical properties, hermetic seal is still not guaranteed.[20] Indeed, the microorganisms from the oral environment can infiltrate the obturation to reach these free spaces along the obturation to restart proliferation which can be responsible for endodontic failure with time.[21]

Root canal sealers play an important role in obturations since they complement the filling and remain interposed between gutta and percha or Resilon™ cones and dentine. The experimental sealers investigated in this study were composed of epoxy resin, Portland cement and a radiopacifier which was either zirconium oxide or niobium oxide in either nano- or micro-size particles. These radiopacifiers were used because they exhibited promising radiopacity in previous research.[22–24] The incorporation of nanoparticles in dental materials mainly aims at improving the biological properties mostly the antibacterial effects, and to avoid genotoxic risks.[25,26] The nano-particles were produced from micro-particles by a chemical process. The resultant nano-particles exhibited a different shape modifying the physical and chemical properties of the materials.[27] Since the experimental sealers were mixed with an epoxy resin-based vehicle, AH Plus was used as a control for comparison purposes. Carboxylate-modified conjugated fluorescent micro-spheres were used to predict whether the spaces present at the interface of the root dentine and the material compro-mised the sealing ability of the obturation. Fluorescent microspheres have been employed to assess the interfacial characteristics of Biodentine used as a dentine replacement material.[28] The microspheres used in the present research are similar to the average size of the streptococci (0.5–0.7 μm).[29] The penetration of the microspheres along the dentine-material interface or through the bulk of the sealer mimics a clinical condition where an appropriate coronal seal could not be provided immediately after obturation and the filling is directly exposed to a humid and contaminated environment. Thus, the sealer's ability to prevent the immediate penetration of contaminated fluids is significant. A

study comparing the percolation ability between tracers and bacteria verified that inert particles mimic bacterial percolation into the marginal hiatus and the authors suggested that this model can be used to establish a relative scale of the behaviour of various bacterias during percolation.[30]

Energy dispersive spectroscopy was used to investigate the dentine-material interface and to identify penetration of the sealers. The scanning electron microscopy provides information about the morphology of the sealer's particles while the analysis by energy dispersive spectroscopy displays the elemental composition of the test materials and radiopacifier. The operator can calibrate the equipment to analyse the surface by selecting specific points, scan the whole surface area, or use linear scans. In this study, the line scans provided information about the chemical composition not only of the dentine and sealers itself but also enable to identify the migration of ions between the two substrates and to chemical characterize the interface between them. This technique had already been used to assess the chemical exchange between titanium dental implant and hydroxyapatite coatings and a diffusion of titanium ions into the hydroxyapatite coating layer was observed.[31] Furthermore the interaction of Biodentine and Bioaggregate with dentine when used as root-end filling materials has also been evaluated using this method.[32]

All five root canal sealers prevented the immediate ingress of microspheres regardless the presence of porosity within the materials. The size of the radiopacifiers particles (micro- or nano-scale) did not affect the porosity in the bulk of the materials, the microsphere penetration at the interface nor the sealer's ability to ingress into the dentine tubules. The confocal microscopy revealed very good bonding of the sealers to the adjacent dentine with formation of resin tags within the dentine tubules. This prevented the ingress of the micro-spheres at the root dentine to sealer interface. The penetration of the root canal sealers into the dentine tubules is desirable because it will improve the sealing ability and the retention of the material, and it may entomb any residual bacteria and the chemical components of the sealers may exert antibacterial effect.[33] The penetration ability of the sealers depends on several factors

including: smear layer removal[34]; filling technique[35,36]; number and size of the dentine tubules[37]; physicochemical properties of the root canal sealer and particle size of the components[38,39]; and also it can be enhanced by the products, such as hydroxyapatite, produced during the setting reaction of the materials.[37] There is a greater number of tubules in the cervical dentine, which decreases in density in the radicular dentine thereby the apical dentine displays less tubule density when compared to coronal and middle root thirds, with some areas completely devoid of tubules.[40] In the current study greater penetration at the coronal and mid-root dentine compared to apical dentine was exhibited. The root canals obturated with nano-zirconium oxide Portland-based sealer and AH Plus also exhibited some ability to penetrate in dentine tubules at the apical third. The greater penetration of the sealer at the coronal and mid-root thirds might also be related to the lateral pushing effect promoted by the System B plugger during obturation, and the limited action of EDTA at the apex of the root canal as verified by other researchers.[41]

The unequal distribution of the inorganic compounds was probably a result of the use of the lentulo drill to place the sealers inside the root canals, which caused particle flocculation in certain areas. The Portland particles and the radiopacifiers' micro-particles are bigger and heavier in comparison to nano-particles; consequently, the greater surface area of these particles improved the contact between them and the lentulo drill increasing the pushing action exerted by the drill.

The SEM and confocal images of the dentine-sealers interface suggested that the sealers were able to penetrate inside the dentine tubules and these findings were supported by the EDS line scans since it allowed the identification of the elements which were able to cross the dentine-material interface and spread into dentine structure. Silicon and niobium were the main elements that were observed in the dentine. The migration of zirconium is doubtful due to the peak overlap of zirconium and phosphorous observed in the EDS analysis. Other important observation was the intensification of the carbon peak into the dentine of the AH Plus samples. This observation suggests that the penetration of AH Plus into dentine tubules showed by other

studies is mainly related to the penetration of the resin matrix and not of the inorganic compounds.[42]

The formation of an interfacial layer at the dentine-material interface had been attributed to the apatite-deposition ability of the silicate-based materials in the presence of phosphate solutions. When these materials hydrate, calcium hydroxide (Portlandite) is produced and the dissociation of this by-product promotes an increase in the environment pH and provides calcium ions to interact with the phosphates of the surrounding tissues to induce apatite deposition.[4] Scientific studies have suggested that dentine may uptake the elements released by bioactive materials, such as calcium and silicon, resulting in increased mineralization in the adjacent dentine.[6] The only limitation of the line scan methodology used to investigate dentine-sealer interface relies in the fact that the identification of calcium ion migration and the penetration of phosphorous ions could not be precisely measured since dentine is mainly composed of the same elements. Therefore, it was not possible to determine if the test materials were able to induce some mineralization in the adjacent dentine. However, phosphorous peaks were verified in the samples obturated with sealers containing either nano-or micro-particles of niobium oxide. This phosphorous likely is derived from the HBSS in which the samples were immersed; thus the synthetic solution was able to penetrate through the dentine-sealer interface and either through the bulk of niobium oxide-based experimental sealers. On the other hand, the investigation of the penetration of phosphorous in zirconium oxide-based experimental sealer was limited due to the overlap of zirconium and phosphorous peaks identified on EDS analysis. Differently from experimental sealers, AH Plus did not show any trace of phosphorous along the interface and neither inside the bulk of the sealer.

3.4 Conclusion

Sealers based on Portland cement and epoxy resin did not allow the entry of bacteria sized particles coronally. The experimental sealers exhibited promising characteristics

and were comparable to AH Plus sealer. Elemental migration of the experimental sealers suggests material interaction with dentine which was not displayed by AH Plus.

Acknowledgements

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6 Discussion

The main reason that motivates researches with endodontic sealers till nowadays lie in the fact that there is no available sealer that meets all the requirements described for root canal sealers[29], particularly with respect to biological properties. Portland cement had exhibited similar biocompatibility to MTA[37], which is well recognized by the excellent biological properties, such as biocompatibility and bioactivity. The experimental sealers proposed in the present study were formulated with Portland in the composition aiming to develop a new root canal sealer with optimal tissue response and with physical, chemical and mechanical properties in accordance to ISO 6876:2012[33] and to attend Grossmann's requirements. The disadvantage of using Portland as main compound of the experimental sealers is related to lack of radiopacity exhibited by this material, which makes the association with a radiopacifying agent indispensable to become this material suitable for Dentistry purposes. Zirconium oxide and niobium oxide were the radiopacifiers of choice in this study. Bismuth oxide is the component that confers radiopacity to MTA cement[10]. This substance was not tested as radiopacifier in this study because bismuth oxide has been responsible for mechanical strength depredation of Portland cements[14] and cytotoxicity[23]. The addition of zirconium oxide to Portland-based or tricalcium silicate-based cements had demonstrated ability to promote radiopacity[15]. This association had also proved to be bioactive[12] and it produced a structure with appropriate physical and mechanical properties[15].

On the other hand, niobium oxide has been broadly investigated as part of titanium alloys in implants for biomedical or dental applications[55]. The prestige by niobium oxide as component of dental materials increased because it is a non-cytotoxic substance with high corrosion resistance[19,40,42]. Moreover, niobium oxide had improved the radiopacity ability of endodontic sealers[36] and had demonstrated mineral tissue deposition ability[35].

Nanotechnology has great potential to improve the properties of the dental materials. Nanomaterials can decrease biofilm accumulation, inhibit the demineralization process, promote remineralization, exert antibacterial effect[41] and improve material's fracture resistance[58]. Therefore, the influences of zirconium oxide and niobium oxide nanoparticles on physico-chemical and mechanical properties; on dentin-sealers interface; and, on Portland cement-based root canal sealers adhesiveness were investigated. The main concern regarding the use of nanoparticles in biomaterials is related to genotoxicity. According to the literature, zirconium oxide nanoparticles films exhibit favourable bioactivity and cytocompatibility[38] and do not offer genotoxic risks to human cells[17]. The effects produced by the incorporation of

niobium oxide nanoparticles on biomaterials' properties have not been investigated so far and neither the influence of any type of nanoparticles on root canal sealers properties.

In addition, it is important to discuss others aspects involved in the present study. During physico-chemical properties'analysis, it could be observed that Sealapex exhibited difficult to achieve complete setting even after one week at 37 °C and 95% relative humidity. This event has already been described by other studies[22] and it was crucial to result in the suppression of this sealer in subsequent studies.

Portland cements were not specifically designed to be used as dental material. Originally, Portland cements are used as concrete for civil construction purposes[6] and this is why some impurities, such as sodium and potassium[12] plus a wide range of particle size[16] can be observed in the composition of Portland cements. The wide range on particle size of Portland likely justify the long time required to properly mix the experimental sealers with the epoxy vehicle. It could be also observed that radiopacifiers' nanoparticles did not improve the handling properties of the experimental sealers. In fact, the incorporation of nanoparticles in the composition required greater vehicle volume to promote satisfactory physical properties such as flow ability, which consequently resulted in longer setting time. To overcome this problem, a solution could be the formulation of experimental sealers only composed by primary Portland compounds, such as tricalcium or dicalcium silicates. According to the results of this study, some of the physicochemical properties, such as setting time, compressive strength, flow ability and film thickness seemed to be affected by radiopacifiers' particle size. However, nano-particulate sealers did not exhibited greater advantages over micro-particulate ones, since differences observed between them were very slight and had limited effect on sealer's microstructure, bioactivity ability, and dentin bond strength values.

In terms of radiopacity, the radiopacifiers tested in the present study (nano or micro particulate forms of zirconium oxide or niobium oxide) conferred limited radiopacity to the experimental sealers, suggesting that radiopacifiers proportion (30% by weight) was not sufficient to promote radiopacity values above to the minimum recommended by ISO 6876:2012[33] (3 mm Al). An alternative to improve radiopacity ability of the experimental sealers would be the increase of radiopacifier ratio or the incorporation of another radiopacifying agent composed by chemical elements of higher atomic mass like calcium tungsten[32] or tantalum oxide[31].

It is important to note that experimental sealers exhibited lack of Portland particles hydration. This event is likely consequence of the type of vehicle used do mix experimental sealers. Unlike the mixture of pure Portland with water, epoxy resin vehicle is not

absorbed by Portland particles (calcium silicate particles). In water, calcium silicate particles undergo hydrolysis producing calcium hydroxide and calcium silicate hydrate gel[7]. Since experimental sealers exhibited high levels of calcium ions release and increased environmental pH, it is suspected that external Portland particles of experimental sealers reacted with the water from the surrounding environment to produce calcium hydroxide. However, epoxy resin acted isolating calcium silicate particles in the bulk of the material, preventing the hydration of the inner particles. The lack of hydration did not affect experimental sealers' bioactivity, but it may have been responsible for the high incidence of cohesive failures verified for experimental sealers, since the union between Portland grains are attained through reactions with water and the decrease on compressive strength has been related to the addition of admixtures[8]. Therefore, the use an epoxy resin as vehicle of experimental sealers did not affect bioactivity potential but it may have been responsible by the lack of hydration and cohesion of the Portland particles

AH Plus is considered the gold standard for comparison of endodontic sealers. Although this material exhibits great physico-chemical properties[49], it could be observed that this sealer did not show any ability to induce mineral tissue deposition. Regardless the lack of hydration, experimental sealers exhibited bioactivity potential. However, if the type of radiopacifying agent exerted any influence on bioactivity potential or if MTA Fillapex showed greater bioactivity in comparison to experimental sealers it is still unclear since quantitative analisys was not performed in this study.

In general, experimental sealers containing nano/micro particles of zirconium oxide or nano/micro particles of niobium oxide had similar behaviours with respect to physico-chemical and mechanical properties, dentin bond strength and interaction with dentin.

7 Conclusions

With the exception of radiopacity and film thickness, ES showed physicochemical properties according to ISO 6876:2012 specifications, adequate dentin bond strength, bioactivity potential and they chemically interact with dentin besides promoting coronal sealing.

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*De acordo com o manual da FOAr/UNESP, adaptadas das normas Vancouver. Disponível no site: http://www.foar.unesp.br/#!/biblioteca/manual

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Annex

UNIVERSIDADE ESTADUAL PAULISTA " JÚLIO DE MESQUITA FILHO"

FACULDADE DE ODONTOLOGIA DE ARARAQUARA

Comitê de Ética em Pesquisa

Comitê de Ética em Pesquisa

Certificado

Certificamos que o projeto de pesquisa intitulado ***"AVALIAÇÃO FÍSICO-QUÍMICA E MECÂNICA DE CIMENTOS ENDODÔNTICOS À BASE DE AGREGADO TRIÓXIDO MINERAL EM COMPARAÇÃO AO AH PLUS E SEALAPEX"*** sob o protocolo nº **87/11**, de responsabilidade do Pesquisador (a) ***MARIO TANOMARU FILHO*** está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa–FOAr, com validade de 01 (um) ano, quando será avaliado o relatório final da pesquisa.

Certify that the research project titled ***"PHYSICO-CHEMICAL AND MECHANICAL EVALUATION OF MINERAL TRIOXIDE AGGREGATE BASED ROOT CANAL SEALERS IN COMPARISON TO AH PLUS AND SEALAPEX",*** protocol number **87/11**, under Dr ***MARIO TANOMARU FILHO*** responsability, is under the terms of Conselho Nacional de Saúde/MS resolution # 196/96, published on May 10, 1996. This research has been approved by Research Ethic Committee, FOAr-UNESP. Approval is granted for 01 (one) year when the final review of this study will occur.

Araraquara, 6 de março de 2012.

Prof. Dr. Maurício Meirelles Nagle

Coordenador