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AVALIAÇÃO DE CIMENTOS ENDODÔNTICOS A BASE DE METACRILATO CONTENDO α-FOSFATO TRICÁLCICO, FOSFATO OCTACÁLCICO OU HIDROXIAPATITA

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Porto Alegre, 2015

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RESUMO

Novos biomateriais para obturação do sistema de canais radiculares têm sido introduzidos no mercado ao longo dos últimos anos com o objetivo de melhorar o selamento desse sistema, bem como estimular o seu reparo, em uma tentativa de proporcionar melhores propriedades físico-químicas e bioatividade. O objetivo do presente estudo *in vitro* foi desenvolver e caracterizar cimentos endodônticos resinosos à base de metacrilato contendo três diferentes fosfatos de cálcio: α-fosfato tricálcio, fosfato octacálcico e hidroxiapatita nanoestruturada. Os cimentos foram formulados a partir da obtençao de uma resina base utilizando-se 70% de uretano dimetilmetacrilato (UDMA), 15% de glicerol 1,3 dimetilmetacrilato (GDMA) e 15% de etoxilado bisfenol A glicol dimetacrilato (BISEMA 6), em peso. Para produzir um cimento de cura dual, foram adicionados 0,5% mol de canforoquinona (CQ), 1% mol de di-hidroxietil p- toluidina (DHEPT), 1% mol de peróxido de benzoila (BP) e 1% mol de etil 4-dimethilaminobenzoato (EDAB). Para conferir radiopacidade aos cimentos, foram adicionados 40% de trifluoreto de itérbio (YbF3), em peso, previamente silanizado. À resina base, adicionou-se, em peso, 10% de α-fosfato tricálcico (α- TCP), fosfato octacálcico (OCP) ou hidroxiapatita (HAp), constituindo-se 3 grupos experimentais, havendo ainda um grupo ao qual não foram adicionados os fosfatos de cálcio, sendo este o grupo controle e um grupo composto pelo ccimento comercial AH Plus. Os cimentos endodônticos formados foram avaliados pelos testes de grau de conversão, radiopacidade, escoamento, espessura de filme, deposição de apatita, presença de íons cálcio em solução que simula os fluidos corporais "simulated body fluid (SBF) através de espectrometria de absorção atômica, pH, caracterização da interface cimento/dentina quanto à penetração dos cimentos nos túbulos dentinários e resistência de união. Todos grupos apresentaram radiopacidade, escoamento e espessura de filme de acordo com a ISO 6876. O grau de conversão de todos os cimentos aumentou ao longo de um período de 14 dias. Os cimentos apresentaram deposição mineral na ordem de G_{HAp}>$G_{\alpha\text{-}TCP}$>G_{OCP}, com excessão dos grupos $G_{Control}$ e G_{AHPlus}. Após 28 dias de imersão em SBF, todos os cimentos testados apresentaram maiores valores de resistência ao deslocamento comparados aos valores iniciais. Este estudo mostrou que cimentos endodônticos experimentais à base de metacrilato com adição de hidroxiapatita, α-fosfato tricálcico e fosfato octacálcico apresentaram deposição mineral após imersão em SBF sugerindo a bioatividade destes materiais. Os fosfatos de cálcio testados apresentaram propriedades físico-químicas adequadas.

Palavras-chave: Fosfatos de Cálcio. Cimento Endodôntico. Mineralização.

ABSTRACT

New biomaterials for filling the root canal system have been introduced to the market over the past few years in order to improve the sealing of the system and stimulate its repair, in an attempt to provide better physicochemical properties and bioactivity. The aim of this in vitro study was to develop and characterize methacrilate-based sealers containing three different calcium phosphates: tricalcium phosphate, octacalcium phosphate and nanostructured hydroxyapatite. Cements were formulated from obtaining a base resin using 70% of dimetilmetacrilato urethane (UDMA), 15% glycerol 1.3 dimetilmetacrilato (GDMA) and 15% ethoxylated bisphenol A glycol dimethacrylate (BISEMA 6), weight. To produce a dual cure cement, were added 0.5 mol% camphorquinone (CQ), 1 mol% dihydroxyethyl p-toluidine (DHEPT), 1 mol% of benzoyl peroxide (BP) and 1 mol% ethyl 4- dimethilaminobenzoato (EDAB). To impart radiopacity to the sealers, were added 40% ytterbium trifluoride (YbF3) by weight silanized previously. In the base resin, is added by weight 10% of α-tricalcium phosphate (TCP α-), octacalcium phosphate (OCP) or hydroxyapatite (HAp), constituting 3 experimental groups, there is still a group that were not added calcium phosphate, which is the control group and a group composed of the commercial sealer AH Plus.
Experimental sealers were evaluated by the degree of conversion testing, radiopacity, flow, film thickness, deposition of apatite, the presence of calcium ions in simulated body fluid solution (SBF) by atomic absorption spectrometry, pH, characterization of the interface cement/dentin and the penetration the cement in the dentinal tubules, and bond strength. All groups exhibited radiopacity, flow and film thickness in accordance with ISO 6876. The degree of conversion of all sealers increased over a period of 14 days. The sealers showed mineral deposition in order G_{HAp}>$G_{\alpha\text{-}TCP}$>G_{OCP}, except for $G_{Control}$ and G_{AHPlus} groups. After 28 days of immersion in SBF, all tested sealers presented greater values for resistance to dislodgement from root dentin. This study showed that experimental methacrylate-based root canal sealers with hydroxyapatite, α-tricalcium phosphate and octacalcium phosphate addition demonstrated mineral deposition after immersion in SBF, suggesting the bioactivity of these materials. All calcium phosphates tested presented suitable physicochemical properties.

Keywords: Calcium phosphates, root canal sealer, mineralization.

SUMÁRIO

1 INTRODUÇÃO

O objetivo de um tratamento endodôntico é a obtenção de um sistema de canais radiculares desinfetado, livre de microbiota e detritos, pronto para então ser selado com um material inerte. O sucesso do tratamento endodôntico depende de múltiplos fatores (ØRSTAVIK *et al.*, 2004) e alguns estudos mostraram que, em casos onde não é possível se obter a completa desinfecção do sistema de canais radiculares, o cimento endodôntico apresenta um importante papel na cicatrização dos tecidos periapicais (KATEBZADEH *et al.*, 1999). Dessa forma, a obturação deve selar o mais hermeticamente possível o sistema de canais radiculares, após sua limpeza e modelagem. Mesmo em casos onde haja uma imprópria desinfecção do sistema de canais radiculares, mantendo-se assim um biofilme cultivável em seu interior, se poderia, teoricamente, sepultar esses microorganismos através de uma obturação perfeita desse sistema (SALEH *et al.* 2004). Logo, alcançaria-se ainda, o sucesso clínico (PETERS, WESSELINK, 2002).

Entretanto, a obturação do sistema de canais radiculares apresenta alguns desafios, principalmente devido à grande dificuldade de adesão entre os materiais obturadores propostos e as paredes do canal. Assim sendo, os cimentos endodônticos devem prevenir a infiltração e apresentar estabilidade dimensional (BERGMANS *et al*., 2005). Estudos *in vitro* demonstraram que os cimentos resinosos à base de metacrilato apresentam redução na micro-infiltração, em comparação com os cimentos endodônticos já amplamente utilizados, como os de óxido de zinco e eugenol (WONG *et al*., 2013). Além disso, se espera que estes materiais apresentem biocompatibilidade (ONAY, OZDEMIR, 2007) e propriedades antimicrobianas e físico-químicas adequadas (RESENDE *et al*., 2009).

Recentemente, tem havido um crescente interesse na aplicação do conceito de engenharia de tecidos para endodontia (MURRAY *et al*., 2007) e as pesquisas avançam no sentido do desenvolvimento de materiais que apresentem, além das propriedades físico-químicas já bem estabelecidas, alguma bioatividade que auxilie na promoção do reparo biológico. Devido a sua grande semelhança química com o tecido ósseo, vários ortofosfatos de cálcio apresentam ótima biocompatibilidade e bioatividade, o que possibilita o seu emprego na produção ou revestimento superficial de enxertos ósseos (DOROZHKIN, 2009). Em um estudo utilizando um cimento endodôntico experimental, Alani e colaboradores (2009) mostraram ausência de infiltração, aferida por corante, nos espécimes obturados com o referido material. Os autores relacionam o selamento obtido à presença de deposições

minerais na interface do material obturador com a dentina radicular. Além da liberação de íons cálcio, outros autores também é demonstram a precipitação de cristais de apatita (SHOKOUHINEJAD *et al., 2012*), sugerindo a bioatividade destes materiais. IMAMURA e colaboradores (2010) demonstraram a transformação de HA carbonatada semelhante à presente em dentina a partir do uso de um cimento com incorporação de fosfato octacálcico (OCP) para a obturação de canais radiculares de dentes decíduos, melhorando a sua capacidade de selamento.

Outros exemplos são os fosfatos tricálcicos α-TCP e β-TCP, que embora tenham exatamente a mesma composição química, diferem na estrutura cristalina e solubilidade, conforme se observa na Tabela 1. A fase β-TCP é mais estável que a fase α-TCP. Assim, o α-TCP é mais reativo em sistemas aquosos, tem maior energia específica e pode ser hidrolisado a uma mistura de outros fosfatos de cálcio (DOROZHKIN, 2009).

A hidroxiapatita é o segundo ortofosfato de cálcio mais estável, depois da Fluorapatita (FA) e o menos solúvel. A hidroxiapatita sintética possui propriedades semelhantes às da hidroxiapatita biológica, e sua utilização nos materiais odontológicos já foi proposta como carga para resinas compostas (DOMINGO *et al*., 2003), sistemas adesivos (LEITUNE *et al*., 2013) e cimentos endodônticos (COLLARES *et al.,* 2012).

Tabela 1. Diferentes fosfatos de cálcio e suas propriedades.

Razão molar Ca/P	**Componentes e suas abreviações usuais**	**Fórmula química**	**Solubilidade a 25 °C, log(Ks)**	**Solubilidade a 25 °C, g/L**
1,33	Fosfato octacálcico (OCP)	$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$	96,6	~0,0081
1,5	α- Fosfato tricálcico (α-TCP)	α-$Ca_3(PO_4)_2$	25,5	~0,0025
1,5	β - Fosfato tricálcico (β -TCP)	β - $Ca_3(PO_4)_2$	28,9	~0,0005
1,67	Hidroxiapatita (HA, HAp ou OHAp)	$Ca_{10}(PO_4)_6(OH)_2$	116,8	~0,0003
1,67	Fluorapatita (FA ou FAp)	$Ca_{10}(PO_4)_6F_2$	120,0	~0,0002

Fonte: Dorozhkin, 2013

Diante disso, o desenvolvimento de cimentos endodônticos resinosos contendo fosfatos de cálcio e apresentando a capacidade de deposição mineral pode ser uma promissora

alternativa para a estimulação do reparo dos tecidos apicais bem como para a promoção de melhores resultados na terapia endodôntica.

2 OBJETIVOS

Este trabalho teve como objetivo desenvolver cimentos endodônticos à base de metacrilato contendo α-fosfato tricálcico, fosfato octacálcico ou hidroxiapatita e caracterizá-los quantos às suas propriedades físico-químicas e deposição mineral.

3 MANUSCRITOS

Esta dissertação de mestrado se apresenta na forma de dois manuscritos, escritos na língua inglesa e que seguem as normas referentes ao periódico *Journal of Endodontics,* para o qual serão submetidos.

3.1 MANUSCRIPT 1

Apatite formation and mechanical properties of calcium phosphate methacrylate-based root canal sealers

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ABSTRACT

Introduction: The aim of this study was to evaluate the physicochemical properties and apatite formation of three experimental endodontic root canal sealers containing three different calcium phosphates (CP): α-tricalcium phosphate ($G_{\alpha\text{-TCP}}$), octacalcium phosphate (G_{OCP}) or hydroxyapatite (G_{HAp}).

Methods: Radiopacity, flow, film thickness, degree of conversion (DC), apatite formation, calcium on simulated body fluid (SBF) solution and pH were analyzed. Radiopacity, flow and film thickness were conducted according to ISO 6876/2001 standards. The DC was measured immediately, 7 and 14 days after photocuring. Apatite formation was analized by soaking the experimental sealers in SBF for 7 and 28 days. The Ca^{2+} on SBF solution and pH were measured at periods of 7 and 28 days with spectrophotometer and pH meter, respectively.

Results: All sealers presented radiopacity values over 3 Al mm ($p>0.05$). The flow ranged from 14,63 (±0.88) to 21,61 (±1.73) and the $G_{Control}$ was significant different from other groups ($p<0.05$). All groups presented film thickness values according with ISO 6876 and no statistical significant increase in the degree of conversion over time. An increase on CP deposition was observed on the surface of all experimental sealers in an order $G_{HAp}>G_{\alpha\text{-TCP}}>G_{OCP}$, except on the $G_{Control}$ and AH Plus. No difference was found among groups on the pH results.

Conclusions: This study showed that experimental methacrylate-based root canal sealers with addition of hydroxyapatite, α-tricalcium phosphate and octacalcium phosphate demonstrated mineral deposition after immersion in SBF and suitable physicochemical properties.

KEYWORDS

Hydroxyapatite, root canal sealer, mineralization, pH

INTRODUCTION

New obturation biomaterials have been introduced over the past years to improve the seal of the root canal system and its repair, in an attempt to provide improved physicochemical properties and bioactivity (1-2). Studies with sealers containing calcium phosphates (CP) have shown ion release (3) and precipitation of apatite crystals (4), suggesting bioactivity and presenting suitable radiopacity, flow and film thickness (5).

CP are materials that can promote the dissolution and precipitation of Ca^{2+} and PO_4^{3-} ions (6). There are several types of CP, which can be classified by the molar ratio between the calcium and phosphorous atoms Ca/P, ranging from 0.5 (monocalcium phosphate monohydrate) to 2.0 (tetracalcium phosphate) (7). Because of its chemical similarity to the inorganic part of human bones and teeth, calcium orthophosphates have some specific advantages over other types of bioceramics. Hydroxyapatite (HAp) is a type of calcium orthophosphate, being the main component of bone and having a similar structure presented in the mineral phase of teeth. HAp is more stable in aqueous solutions, and it has been added in the compositions of root canal sealers to improve its properties (5). Alpha tricalcium phospate (α-TCP) is more reactive in aqueous systems, has a higher specific energy and it can be hydrolyzed to a mixture of other CP. The octacalcium phosphate (OCP) structure displays apatitic layers with atomic arrangements of Ca^{2+} and PO_4^{3-} ions similar to those of HAp, playing an important role in *in vivo* formation of apatitic biominerals.

However, there is a lack of studies that evaluate de influence of the CP addition on the physicochemical and bioactivity properties. Thus, the aim of this study was to evaluate the physicochemical properties and apatite formation of experimental methacrylate-based root canal sealers containing three different CP.

MATERIALS AND METHODS

The monomers used to formulate an experimental root canal sealer were urethane dimethacrylate (UDMA), glycerol-1,3-dimethacrylate (GDMA), ethoxylated bisphenol A glycol dimethacrylate (BISEMA6), camphorquinone (CQ), N,N-dihydroxyethyl-para-toluidine (DHEPT) and benzoil-peroxyde (BP), (Esstech Inc, Essington, PA, USA). Hydroxyapatite (HA), octacalcium phosphate (OCP) and α-tricalcium phosphate (α-TCP) were used with a mean particle size of 26.8 nm, 4.94 μm and 6.03 μm respectively. The experimental root canal sealers were formulated by mixing 70 wt.% UDMA, 15 wt.% BISEMA6 and 15 wt.% GDMA. CQ, DHEPT and BP were added to produce a dual-cure root canal sealer. The filler particles used were a mixture of YbF_3 in a weight ratio of 40% (8) and HA, OCP, α-TCP in a weight ratio of 10%. The filler particle mixture was added at 100 wt.% to the monomer blend. To perform sealer photoactivation, a light-emitting diode activation unit (Radii; SDI, Bayswater, Australia) was used. An irradiation value of 1200 mW cm^2 was confirmed with a digital power meter (Ophir Optronics, Danvers, MA, USA). AH Plus® (Dentsply) sealer was manipulated according to manufacturers' instructions.

RADIOPACITY

The radiopacity was evaluated using five specimens per group. The specimens were 10.0 mm (±0.5 mm) in diameter and 1.0 mm (±0.2 mm) thick. Radiographic images were obtained using a phosphor plate digital system (VistaScan; Durr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) at 70 kV and 8 mA, with 0.2 s of exposure time and a focus-film distance of 400 mm. For each film, the 5 specimens from each group were positioned. An aluminum step-wedge meeting ISO 6876/2001 was exposed simultaneously with the specimens in all images. The images were saved in TIFF format and analyzed using ImageJ 1.48d software (Wayne Rasband, National Institutes of Health, USA). The means and

standard deviations of the grey levels (pixel density) of the aluminum step-wedge and the specimens were obtained in a standardized area of 1.5 mm^2.

DEGREE OF CONVERSION

The degree of conversion (DC) was evaluated using Fourier transform infrared spectroscopy (FTIR) with a Vetrex 70 (Bruker Optics, Ettlingen, Germany) spectrometer equipped with an attenuated total reflectance device composed of a horizontal diamond crystal with a mirror angle of 45 degrees. Opus software (Bruker Optics) was used in the monitoring scan mode, with Blackman-Harris 3-Term apodization in a range of 4000–400 cm^1 and resolution of 4 cm^{-1}. With this setup, one spectrum was obtained prior to photocuring and one immediately after photocuring. The samples (3 μL) were dispensed into a polyvinyl siloxane mould placed on the diamond crystal and light-activated for 40 s (n = 3). The same samples were analyzed after 1 and 7 days. For the experimental groups and the control group, the degree of conversion was calculated considering the intensity of carbon-carbon double bond stretching vibration (peak height) at 1635 cm^{-1}, and using the carbonyl group at 1720 cm^{-1} from the polymerized and unpolymerized samples as an internal standard (9). To calculate the AH Plus degree of conversion, for each spectrum it was determined the height of the oxirane ring peak absorption at 915 cm^{-1} and the p-phenylene group peak absorption at 830 cm^{-1}. The p-phenylene group absorbance the intensity of which does not vary during the curing process is used as internal standard (10). Considering that the time of cure of AH Plus is 24 hours, the immediate and 1 day analysis were taken by using the same values.

FILM THICKNESS

The film thickness evaluation was conducted according to ISO 6876/2001. Two glass plates (10 x 10 x 5 mm) were placed together and their combined thickness was measured. At the center of one of the plates, 0.05 mL of experimental sealer was placed, and a second plate

was then placed on top of the material. At 180 ± 5 s after the start of mixing, a load of 150 N was applied vertically onto the top glass plate. Ten minutes after the start of mixing, the thickness of the two glass plates and the interposed sealer film was measured using a digital caliper. The difference in the thickness of the two glass plates, with and without sealer, was recorded. The mean value of three measurements for each sealer was recorded.

FLOW

The flow test was conducted by placing 0.05 mL of each experimental sealer on a glass plate (40 x 40 x 5 mm) with a graduated 1.5 mL syringe. At 180 ± 5 s after the start of mixing, another equal glass plate and a load of 100 g were applied on top of the material. Ten minutes after the start of mixing, the load was removed, and the maximum and minimum diameters of the compressed disc of sealer were measured using a digital caliper. For each experimental group, the test was conducted three times.

MINERAL DEPOSITION

One disc of 10.0 mm (±0.5 mm) in diameter and 1.0 mm (±0.2 mm) thick of each experimental sealer was immersed in 20 mL of an acellular and protein free simulated body fluid (SBF) solution with pH (7.42) for 7 and 28 days at 37ºC. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, NaH-CO3, KCl, K_2HPO_4. $3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$ and Na_2SO_4 into distilled water and buffered until pH 7.42 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_3$) and 1.0M hydrochloric acid at 37°C (11). One disc of each group was kept non-immersed and used as a control.

The micro-Raman spectroscopy analysis was performed using Senterra (BrukerOptik GmbH, Ettlingen, Germany) to investigate the mineral deposition on the experimental sealers surfaces. A 100 x 100-μm area of the specimen's surface was analyzed by Raman spectroscopy (Senterra, Bruker Optics, Ettlingen, Germany). The sample surface was

irradiated by a 785-nm laser at 50 mW on 100 points equally distributed. Each point was irradiated 3 times for 5 s. Post-processing of spectra was performed using the Opus Spectroscopy Software version 6.5 (BrukerOptik GmbH, Ettlingen, Baden-Württemberg, Germany) and consisted of analyzing the spectral components of the experimental sealers surface. For phosphate, a correspondent peak of 960 cm^{-1} (PO_4^{-1}) was used for integration.

CALCIUM ON SBF SOLUTION AND pH ANALYSIS

The SBF where the experimental sealers were immersed for 7 and 28 days at 37ºC, were analyzed for the release of calcium ions (Ca^{2+}) and pH of the solution. The release of Ca^{2+} was measured by an atomic absorption spectrophotometer (3300; Perkin Elmer, Waltham, USA) using a wavelength of 422.70 nm, a gap of 0.7 nm and a light current of 10 mA. A strontium chloride solution at concentration of 1 g/L was used to eliminate the interference of phosphates and sulfates and the possibility refractory oxides formation.

The pH values of the SBF solution pure and after the immersion of the experimental sealers and the AH Plus sealer after 7 and 28 days were measured at room temperature (20°C) by using a digital pH meter (HAANA - pH 21).

STATISTICAL ANALYSIS

The normality of the data was evaluated using the Kolmogorov–Smirnov test. Statistical analysis was performed using one-way ANOVA for radiopacity, flow, film thickness, two-way repeated measures ANOVA for degree of conversion (sealer and time) and Tukey's post hoc test at a 5% level of significance. For pH analysis, Kruskal-Wallis and Dunn's post hoc test at a 5% level of significance.

RESULTS

The radiopacity data are shown in Table 1. All groups presented radiopacity values over 3 Al mm and the AH Plus presented statistical significant higher values than other groups.

No statistical significant difference among groups was observed on the degree of conversion values (Table 1)($p>0.05$). G_{HAp} showed statistical significant increase on the DC values between immediate and 7 days results ($p<0.05$).

All experimental sealers presented film thickness values below 50 μm according with ISO 6876, and the no statistical significant difference was found ($p>0.05$).

The flow of experimental sealers ranged from 14.63 (±0.88) to 23.03 (±0.11). Control and AH Plus differed from other groups ($p<0.05$) however there was no significant difference between them both.

An increase on the CP deposition was observed on the surface of all experimental groups in an order $G_{HAp}>G_{\alpha\text{-}TCP}>G_{OCP}$. There was neither CP deposition on the $G_{Control}$ nor on the AH Plus. After 7 days, the SBF where G_{OCP} was immersed presented an increase of 11.20% in the Ca^{2+} values, which decreased in the same rate after 28 days of immersion. The SBF from G_{HAp} presented the lowest Ca^{2+} values in both immersion times tested. All groups, except for AH Plus, presented a decrease in the pH values along the time. In the same time of immersion in SBF, there was no statistical significant difference among the groups.

DISCUSSION

The pursuit for materials with the ability to stimulate an appropriate response in the host for a specific application is one of the goals to achieve on the material development field. The CP used in this study demonstrated suitable physicochemical properties and HA and α-TCP sealers presented apatite formation.

All radiopacity values were in accordance with ISO 6876. The experimental sealers showed lower values of radiopacity when compared to AH Plus, which exhibited high values similar to previous reports (12). AH Plus sealer contains zirconium oxide and iron oxide,

besides calcium tungstate, which contribute to its greater radiopacity. In the four experimental groups, the same radiopacifier was used, ytterbium trifluoride. Ytterbium is an element of the lanthanide series, with a high atomic number (z=71) and a refractive index of approximately 1.5 when forming a fluoride glass. It can promote increased radiopacity inside the root canal and increased polymerization in the middle and apical thirds of the canal (8). The DC of all groups tested increased with time ($p>0.05$) and G_{HAp} presented an increase when compared immediate and 7 days results ($p<0.05$). Absorption and scatter within the material are the major factors associated with light attenuation, depending on the formulation of the material, particularly the filler size, type and content (13). The refraction index of HAp (1.62) is higher than the initial refractive index of the monomer mixture (≈1.48)(14) and so the initial change in light transmission versus time may be explained by the mismatch between refractive index of the monomer and filler (15).

All groups presented film thickness values according to ISO 6876. The incorporation of fillers promotes high viscosity in the resin-based composite as the superficial area increases (16). In the present study, nanosized particles of HAp with a diameter of 26.8 nm were used, as well as OCP and α-TCP particles with a mean size of 4.94 μm and 6.03 μm respectively. However, even with the addition of CP, the viscosity and film thickness did not present statistical differences from the G_0 or the AH plus. The experimental sealers presented flow values ranged from 14.63 (±0.88) for G_{HAp} to 23.03 (±0.11) for AH Plus (Table 1). The ISO 6876/2001 standard requires a minimal flow of 20 mm for sealers. A decrease on the flow can be expected since the incorporation of fillers, due to its particle size and increase on the superficial area (17). Moreover, a high flow might increase the chance of the material extrusion toward the periapical region and provide periapical damage, since almost all resin-based sealers currently used in endodontics exhibit a variable degree of toxicity, especially when freshly mixed (18).

The mechanism of repair stimulation by deposition of mineralized tissue depends on pH and ability to release Ca^{2+} (19). The G_{HAp} presented higher mineral deposition on the specimens' surface (Figure 1). The solubility of HAp (≈0.0003) is lower than any other calcium phosphates in water, except from fluorapatite (≈0.0002)(7), which makes it thermodynamically more stable than OCP and α-TCP. As the HAp precipitates, HAp crystal nuclei are formed, once formed in SBF, the apatite grows spontaneously. It was expected to happen since this experimental sealer group had HAp as filler. The α-TCP transformation into apatite occurs similar to HAp, through CP clusters, which constitute the HAp. The G_{OCP} presented the lowest apatite formation, among the CP groups. As the OCP and the HAp have very similar structures, and as the HAp is more stable, HAp precipitates preferentially. Moreover, Ban et al. (20) and Yokoi et al. (21) reported that OCP did not transform to HAp in SBF, while several researchers (22-23) reported the *in vivo* transformation OCP into HAp under physiological conditions. The SBF used in this study was an acellular and protein free solution. An *in vivo* environment present cells and blood proteins, antibodies, fibronectin, fibrinogen and high molecular weight kininogen, which could enhance apatite formation (24). AH Plus did not present any mineral deposition probably because mostly calcium present in this material are not in the ionized form ($CaWO_4$). A decrease on Ca^{2+} ions on SBF and on pH values throughout the time was observed (Table 1). After 7 days of immersion in SBF, G_{OCP} showed higher concentration on Ca^{2+} than SBF. OCP is more soluble (≈0.0081)(7) than HAp and α-TCP, and it could explain the initial increase on Ca^{2+} values. As HAp and α-TCP are more stable CP, the Ca^{2+} ions presented on the SBF solution adsorbed the experimental sealers specimens, which occurred on the G_{OCP} after 28 days of immersion. The pH decreased its values after 7 and 28 days of immersion. The formation of HAp seems to decrease the solution pH because HAp incorporates OH^- ions (25), which leads to an acidification of the media. Besides of that, as there was no renewal on the SBF, there was no fresh ions available,

including H^+ ions. As shown in Table 1, AH Plus increased SBF pH values after 7 days of immersion, followed by a decrease after 28 days of immersion, as previously reported by other studies (26-27).

A possible limitation of this study would be the use of SBF to predict the bioactivity of the experimental sealers with the addition of CP. Regular refreshing of the SBF or the use of dynamic SBF incubation system could minimize the effects of the supersaturation of the solution, thus leading to an increase on the apatite formation by the G_{OCP} and possibly on the G_{HAp} and $G_{\alpha\text{-}TCP}$ as well. Nevertheless, the SBF immersion tests have been successful in predicting the relative performance of biomaterials in vivo, simulating the actual physiological conditions experienced by biomaterials within the human body (24). This study showed that addition of CP demonstrated suitable physicochemical properties. HAp and α-TCP sealers presented apatite formation, suggesting the bioactivity of these materials and being promising of use to root canal sealing improvement.

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The authors deny any conflicts of interest related to this study.

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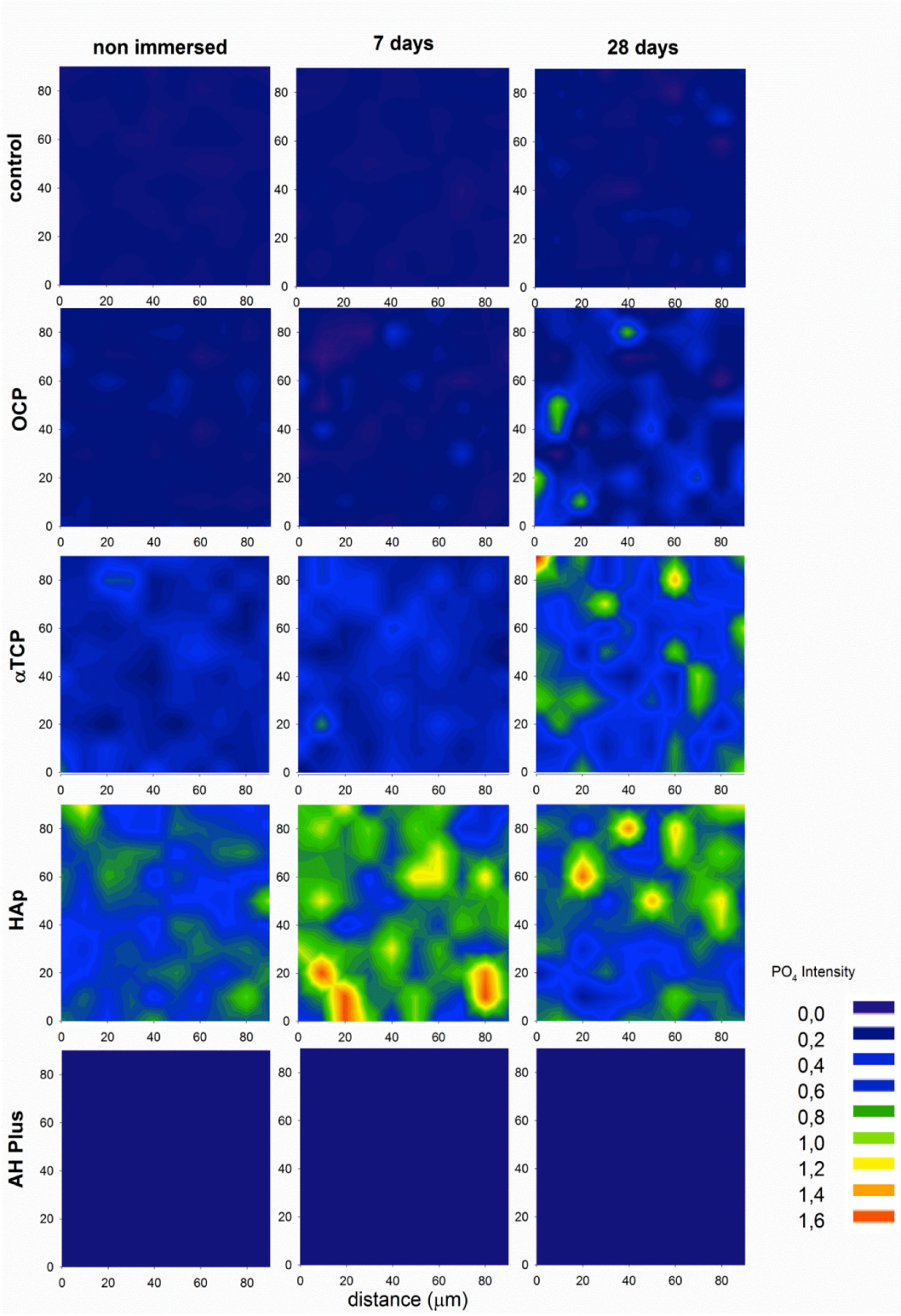
Table 1. Means and standard deviations for radiopacity (Al mm), degree of conversion (%), film thickness (μm), flow (mm), calcium (%) and pH of analyzed root canal sealers.

- Different lowercase letters indicate difference on the same line.

- Different capital letters indicate difference on the same column.

	$G_{Control}$	G_{OCP}	$G_{\alpha TCP}$	G_{HAp}	AH Plus
Radiopacity *(Al mm)*	5.03 ±0.29^{a}	5.05 ±0.45^{a}	4.98 ±0.35^{a}	4.87 ±0.1^{a}	10.33 ±0.23^{b}
Degree of conversion (%)					
Immediate	45.30 ±1.23^{Aa}	41.58 ±2.49^{Aa}	43.87 ±1.61^{Aa}	38.67 ±0.67^{Ba}	38.53 ±6.56^{Aa}
24 hours	46.23 ±1.01^{Aa}	42.81 ±8.89^{Aa}	44.67 ±1.55^{Aa}	40.26 ±3.78^{ABa}	38.53 ±6.56^{Aa}
7 days	50.74 ±5.87^{Aa}	44.69 ±3.04^{Aa}	50.55 ±2.86^{Aa}	47.39 ±1.36^{Aa}	46.74 ±4.98^{Aa}
Film thickness *(µm)*	36.66 ±5.77^{a}	43.33 ±5.77^{a}	46.66 ±5.77^{a}	46.66 ±5.77^{a}	36.66 ±5.77^{a}
Flow *(mm)*	21.61 ±1.73^{a}	16.63 ±1.31^{b}	16.26 ±0.63^{b}	14.63 ±0.88^{b}	23.03 ±0.11^{a}
Calcium on SBF solution *(%)*					
7 days	-4.31	11.20	-4.31	-14.13	-1.19
28 days	-12.93	-11.20	-32.67	-40.86	-7.78
pH					
7 days	7.3 ±0.02A^{a}	7.3 ±0.01A^{a}	7.3 ±0.01A^{a}	7.3 ±0.15A^{a}	7.6 ±0.09A^{a}
28 days	7.2 ±0.01A^{a}	7.1 ±0.01A^{a}	7.1 ±0.01A^{a}	7.1 ±0.01A^{a}	7.4 ±0.01A^{a}

Figure 1. Mineral deposition (peak 960cm^{-1}) at root canal sealers surface as function of immersion period and calcium phosphate rate.



3.2 MANUSCRIPT 2

Resistance to dislodgement of bioactive experimental endodontic sealers containing different calcium phosphates from root dentin after immersion in simulated body fluid

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ABSTRACT

Introduction: The aim of this study was to evaluate the adhesion to human root dentin of three experimental endodontic root canal sealers containing α-tricalcium phosphate, octacalcium phosphate and hydroxyapatite.

Methods: Resistance to dislocation from dentin by push-out test and interface sealer/dentin were analyzed, immediately and after soaking the experimental sealers in simulated body fluid (SBF) solution for 28 days. Data were analyzed by two-way repeated measures ANOVA and Tukey's post hoc test at a 5% level of significance.

Results: All sealers tested presented an increase on the bond strength values after immersion in SBF for 28 days ($p<0.05$). The micro-Raman analysis of the interfaces showed penetration of AH Plus and experimental sealers into root dentin tubules.

Conclusions: This *in vitro* study showed that after 28 days of immersion in SBF, sealers with the incorporation of α-tricalcium phosphate, octacalcium phosphate and hydroxyapatite had its push-out strength values increased, without compromising the interface sealer/dentin or the penetration of the sealers into the dentin tubules, being a suitable alternative for use in root canal system obturation.

KEYWORDS

Calcium phosphates, push-out, mineralization

INTRODUCTION

The filling of the root canal system present some challenges, mainly due to the great difficulty of seal between the proposed filling materials and the root canal walls. The literature suggests that the ability of root canal sealers to adhere to core material and to dentin may result in superior sealing ability, which could reduce coronal and apical leakage (1). Therefore, sealers should prevent infiltration and provide dimensional stability (2).

Differences in the adhesive properties of sealers to dentin may be expected for several reasons, including differences of root dentin between specimens, or even in different sites of the same root (3-4). The most common root canal sealers include zinc oxide eugenol, calcium hydroxide, and resin-based sealers. Although these sealers have been effective, there is still a search for a sealer with better properties (5). *In vitro* studies showed that the resin cements based on methacrylate show a reduction in the infiltration, in comparison with the sealers already widely used, such as zinc oxide and eugenol and the epoxy resin based (6).

In order to enhance sealing of the apical region, materials presenting ionic delivery have been proposed (7). Calcium phosphates are materials that can promote the dissolution and precipitation of calcium and phosphate ions (8), presenting excellent biocompatibility and osteoconductivity properties. Some studies have demonstrated that calcium phosphates cements are useful in the composition of sealers used in root canal treatment (9-11), improving the biological properties of the materials.

However, little information is available on the sealing ability of calcium phosphates sealers, especially after immersion in simulated body fluid (SBF) solution. Thus, the aim of this study was to evaluate the sealing ability of four experimental endodontic root canal sealers containing calcium phosphates before and after immersion in SBF. The commercial sealer AH Plus was used as a reference comparison.

MATERIALS AND METHODS

Experimental sealers formulation

The monomers used to formulate an experimental root canal sealer were urethane dimethacrylate (UDMA), glycerol-1,3-dimethacrylate (GDMA), ethoxylated bisphenol A glycol dimethacrylate (BISEMA6), camphorquinone (CQ), N,N-dihydroxyethyl-para-toluidine (DHEPT) and benzoil-peroxyde (BP), (Esstech Inc, Essington, PA, USA). Hydroxyapatite (HA), octacalcium phosphate (OCP) and α-tricalcium phosphate (α-TCP) were used with a mean particle size of 26.8 nm, 4.94 μm and 6.03 μm respectively. The experimental root canal sealers were formulated by mixing 70 wt.% UDMA, 15 wt.% BISEMA6 and 15 wt.% GDMA. CQ, DHEPT and BP were added to produce a dual-cure root canal sealer. The filler particles used were a mixture of Yb_3F in a weight ratio of 40% (12) and HA, OCP, α-TCP in a weight ratio of 10%. The filler particle mixture was added at 100 wt.% to the monomer blend. To perform sealer photoactivation, a light-emitting diode activation unit (Radii; SDI, Bayswater, Australia) was used. An irradiation value of 1200 mW cm^2 was confirmed with a digital power meter (Ophir Optronics, Danvers, MA, USA).

Specimens Preparation

Fifty extracted, single straight root human premolars, stored in distilled water at 4°C before use, were selected. The teeth were decoronated to a standardized root length of 15 mm. To standardize the working length, a #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was inserted into the root canal until it could be visualized at the apical foramen. The working length was determined by subtracting 1 mm from this length.

After measurement, the length of all roots was standardized to 14 mm. After working lengths were established, the root canals were instrumented using LA Axxess burs (SybronEndo) #1, small 0.20 tip/ 0.6 taper and instrumentation was completed by using Reciproc reciprocating nickel-titanium instruments (VDW, Munich, Germany), size R40, 0.06 taper. The root canals were irrigated using 10 mL of NaOCl 1% and final rinse was performed with 3 mL EDTA 17%. The root canals were dried using standardized paper points R40 (VDW, Munich, Germany). Obturation procedures were performed by using the single gutta-percha cone technique. The master cone was coated with a thin layer of sealer and slowly inserted to the working length.

Following instrumentation, 50 prepared root canals were randomly divided into 5

experimental groups (n=10), and filled with the correspondent sealer using a single cone of 0.06 taper gutta-percha (VDW, Munich, Germany) as follow:

$G_{Control}$: Root canals were filled with experimental sealer without any calcium phosphate addition

$G_{AH\ Plus}$: Root canals were filled with commercial sealer AH Plus

G_{HAp}: Root canals were filled with experimental sealer with addition of HA

$G_{\alpha\text{-}TCP}$: Root canals were filled with experimental sealer with addition of α-TCP

G_{OCP}: Root canals were filled with experimental sealer with addition of OCP

The teeth were stored at 37 °C and 100% humidity for 7 days to allow setting of the sealers. Each specimen was then sectioned perpendicular to the longitudinal axis of the root using a low-speed diamond-coated saw (Buehler, Lake Bluff, IL) under water cooling.

INTERFACE SEALER/DENTIN ANALYSIS BY MICRO-RAMAN

The first 0.7 mm slice of each third from each tooth was analysed by micro-Raman spectroscopy (Senterra, Bruker Inc., Karlshure, Germany), using these parameters: a 100 mW diode laser with 785 nm wavelength and spectral resolution of ~ 3-5 cm^{-1}. Accumulation time per spectrum was 5 s with 3 co-additions. For phosphate, a correspondent peak of 960 cm^{-1} (PO_4^{-1}) was used for integration. For resin base analysis of experimental sealers and control group, the peak 1630 cm^{-1} (corresponding to C=C of methacrylate) was used. For the AH Plus resin analysis, the peak 1550 cm^{-1} (corresponding to angular deformation of -NH- bonds of dibenzylamine resin) was used for integration. After the initial analysis, each slice was immersed in an acellular and protein free SBF with pH (7.42) for 28 days at 37ºC. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, NaH-CO3, KCl, K_2HPO_4. $3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$ and Na_2SO_4 into distilled water and buffered until pH 7.42 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_3$) and 1.0M hydrochloric acid at 37°C (13). Past 28 days, the slices were again submmited to a micro-Raman spectroscopy using the same parameters as described before.

PUSH-OUT TEST

The second 0.7 mm slice of each third from each root was immersed in an acellular and protein free SBF with pH (7.42) for 28 days at 37ºC for further push-out bond strength test. The SBF was prepared as previously described for the micro-Raman analysis.

The third 0.7 mm slice of each third from each tooth was subjected to a immediate push-out bond strength test using a universal testing machine (Shimadzu EZ-SX, Japan). The loading speed was 1 mm/min^{-1}) until dislodgement of the filling material occurred. The values at the time of dislodgement were recorded in Newton for each specimen.

STATISTICAL ANALYSIS

The normality of data was evaluated using the Kolmogorov–Smirnov test. Statistical analysis was performed using two-way repeated measures ANOVA and Tukey's post hoc tests at a 5% level of significance.

RESULTS

For the push-out test, no statistical significant difference was found among the groups before immersion in SBF ($p>0.05$) and the values ranged from 0.87 (± 1.36) for the G_{OCP} to 2.80 (± 1.88) for the AH Plus. After 28 days of immersion in SBF, all sealers significantly increased their values for resistance to dislocation from dentin and the values ranged from 2.90 (± 1.41) G_{OCP} to 4.91 (± 2.38) for the AH Plus.

The micro-Raman analysis showed that after 28 days of immersion in SBF, there were no changes along the interface sealer/dentin for the $G_{Control}$ (Figure 1). The sealer with Hap addition presented higher peaks in the interface sealer/dentin region after immersion period.

DISCUSSION

Success in root canal treatment is assigned to canal debridement, effective disinfection and obturation of the root canal system (14). Historically, a significant share of these steps had been allocated to obturation of the canal space. Obturation of the root canals has been described as a critical component of root canal treatment for sealing and isolating the canal

space from irritants that remain after appropriate shaping and cleaning, and for eliminating subsequent leakage from the perirradicular tissues or oral cavity into the filled canal space (15).

In this study, three different calcium phosphates were tested in an attempt to improve the sealing ability between the experimental methacylate-based sealer and the root dentin walls. After 28 days of immersion in SBF solution, all groups presented an increase on the push-out strength values, and no statistical significant difference was found among the calcium phosphates experimental groups. The only calcium phosphate that differed statistical significantly from the AH Plus sealer was the OCP. OCP is more soluble (≈0.0081)(16) than HAp and α-TCP and some authors (17-18) reported that this calcium phosphate did not transform to HAp in SBF. The SBF used in this study was an acellular and protein free solution. An *in vivo* environment present cells and blood proteins, could enhance apatite formation (19). The results found in this study are similar to previous studies that compared the push-out bond strength to root dentin of different endodontic sealers (20-21), in which the strength values for the AH Plus sealer were higher or presented no statistical significant difference from the methacrylate-based sealers.

In order to simulate a conventional endodontic treatment, as an irrigation protocol, 1% NaOCl was used during instrumentation and 17% EDTA used as final flushing, agents with the capacity to dissolve organic tissues and chelate calcium ions, respectively, with no use of saline solution. If, however, the final flushing after EDTA use is inadequate, some EDTA may remain in the root canal system. The residual EDTA in the root canal system may chelate calcium ions released from the experimental sealers and disturb the precipitation of hydrated products (22). This could have inhibited a greater presence of PO_4^{3-} ions in the root dentin surface, thus leading to non significant different results among experimental calcium phosphates groups and AH Plus sealer.

In a representative image of the interfaces sealer/dentin (Figure 1), the phosphate rate appears to be similar among the groups. The addition of calcium phosphates to experimental root canal sealers did not impair the penetration of sealers into the dentin tubules (Figure 2).

This study showed that after 28 days of immersion in SBF, sealers with the incorporation of HAp, α-TCP and OCP had its push-out strength values increased, without compromising the interface sealer/dentin or the penetration of the sealers into the dentin tubules, being a suitable alternative for use in root canal system obturation.

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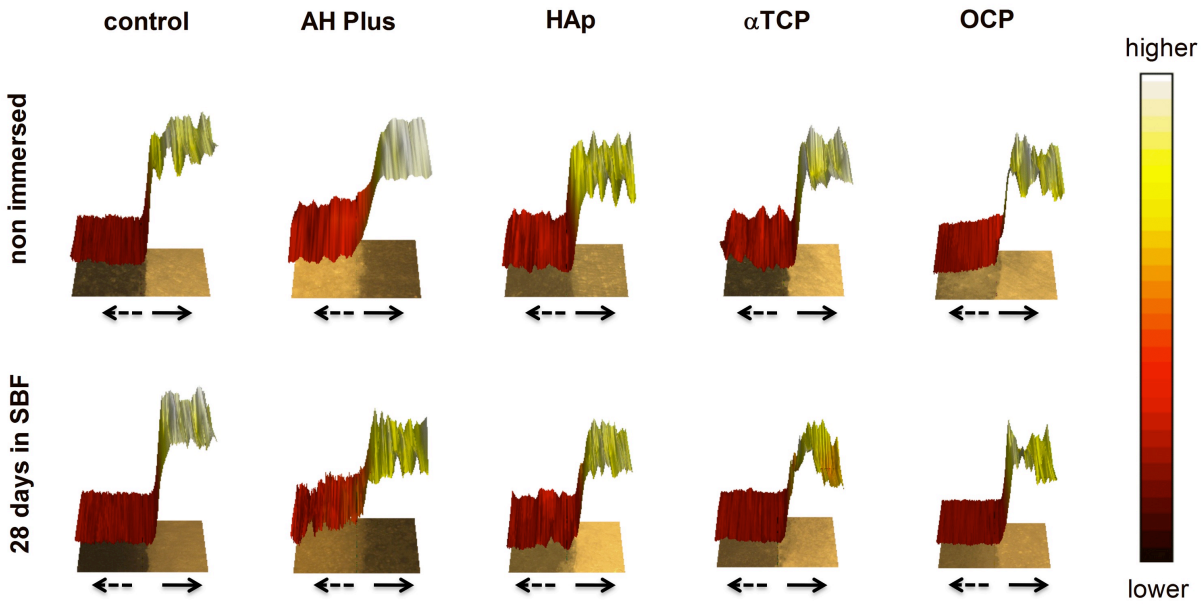
Table 1. Means, standard deviations and statistical analysis of push-out strength values (MPa) of different groups:

	Immediate	**After 28 days of immersion in SBF**
$G_{Control}$	$1,79 \pm 0,99^{Ab}$	$2,96 \pm 1,78^{Ba}$
$G_{AH\ Plus}$	$2,80 \pm 1,88^{Ab}$	$4,91 \pm 2,38^{Aa}$
G_{HAp}	$2,65 \pm 2,31^{Ab}$	$4,10 \pm 2,11^{ABa}$
$G_{\alpha TCP}$	$2,38 \pm 2,12^{Ab}$	$4,26 \pm 2,47^{ABa}$
G_{OCP}	$0,87 \pm 1,36^{Ab}$	$2,90 \pm 1,41^{Ba}$

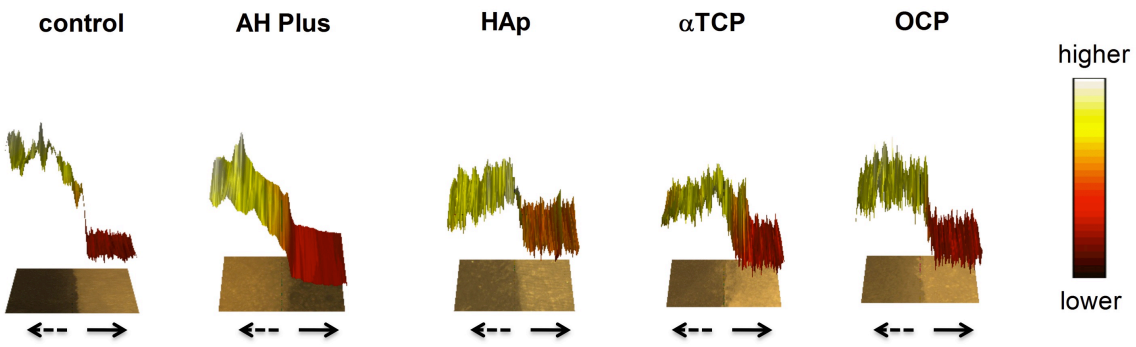
- Different lowercase letters indicate difference on the same line.
- Different capital letters indicate difference on the same column.

Figure 1. Interfaces sealer/dentin (peak 960cm^{-1}) as function of immersion period and calcium phosphate rate.



Arrows to the right indicate the root canal dentin region. Arrows to the left indicate the sealer region.

Figure 2. Interfaces sealer/dentin (integrated for resin base sealers*).



Arrows to the right indicate the root canal dentin region. Arrows to the left indicate the sealer region.

4 CONSIDERAÇÕES FINAIS

Novos biomateriais para obturação do sistema de canais radiculares têm sido introduzidos no mercado ao longo dos últimos anos com o objetivo de melhorar o selamento desse sistema, bem como estimular o seu reparo, em uma tentativa de proporcionar melhores propriedades físico-químicas e bioatividade. Neste trabalho propôs-se a avaliação destas propriedades. Formulou-se cimentos endodônticos resinosos experimentais com a adição de α-fosfato tricálcico, fosfato octacálcico e hidroxiapatita, desejando-se obter materiais com adequadas propriedades físico-químicas como radiopacidade, espessura de filme, escoamento, grau de conversão, e resistência ao deslocamento da dentina radicular, além de avaliar a capacidade desses fosfatos inferirem aos cimentos a capacidade de deposição mineral e troca de íons com o meio, levando a interação desses materiais com os tecidos, e estimulando o seu reparo.

A adição de fosfatos de cálcio aos cimentos endodônticos resinosos experimentais demonstrou propriedades físico-químicas favoráveis neste estudo. Os cimentos com a incorporação de hidroxiapatita e α-fosfato tricálcico apresentaram deposição mineral, sugerindo a bioatividade destes materiais. Para a continuidade dessa investigação e viabilização de uso desses materiais, testes para avaliação da citotoxicidade são sugeridos.

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