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CIMENTO EXPERIMENTAL BIOATIVO À BASE DE GLICEROL SALICILATO CONTENDO α-FOSFATO TRICÁLCICO PARA USO ENDODÔNTICO

Fernando Freitas Portella

Tese apresentada como requisito obrigatório para obtenção do título de **Doutor em Odontologia** na área de concentração em Clínica Odontológica.

Susana Maria Werner Samuel

Orientadora

Porto Alegre, março de 2015

Porto Alegre, 24 de março de 2015

Ao PPGODO-ODO

Vimos por meio deste informar que as sugestões de alteração propostas pela Banca examinadora da Tese de Doutorado do aluno **Fernando Freitas Portella**, composta pelos professores Mario Tanomaru Filho (UNESP Araraquara), Antonio Shigueaki Takimi (UFPel) e Fabiana Soares Grecca (UFRGS), defendida às 8h30min do dia 23 de março de 2015, foram discutidas entre o orientador e o doutorando, e a Tese foi editada considerando os aspectos debatidos.

Sem mais para o momento.
Atenciosamente.

Prof. Dra. Susana Maria Werner Samuel

Ilmo.Prof. Dr. Cassiano Cassiano Kuchenbecker Rosing
Coordenador do Programa de Pós-Graduação em Odontologia
Nesta Faculdade

"A vontade de se preparar tem que ser maior do que a vontade de vencer. Vencer será consequência da boa preparação."

Bernardo Rezende

DEDICATÓRIA

Dedico este trabalho ao meu avô **Antônio Cândido de Freitas**, o impulsionador do estudo na minha família. De pouco estudo, mas de sabedoria infindável.

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NOTA PRELIMINAR

A presente Tese foi redigida segundo a Resolução nº 093/2007 do Conselho de Ensino, Pesquisa e Extensão da Universidade Federal do Rio Grande do Sul. Enquadra-se na forma descrita no item "c" do artigo 3º da Resolução: "Tese, Dissertação ou Trabalho de Conclusão de Curso que contenham artigo(s) já publicado(s)".

RESUMO

O tratamento de canal ideal compreende a desinfecção e selamento do sistema de canais radiculares, de forma a impedir a reinfecção e permitir o reparo dos tecidos periapicais. Para isso é necessário o emprego de materiais que promovam um perfeito selamento, o que até o presente momento não foi completamente alcançado. O objetivo do presente estudo *in vitro* foi desenvolver um cimento endodôntico à base de resina de glicerol salicilato e α-fosfato tricálcico (αTCP), que apresente potencial de estimular a deposição mineral. Os cimentos foram formulados a partir de 70% em massa de uma resina de glicerol salicilato, obtida a partir da reação de transesterificação do salicilato de metila com o glicerol, e 30% em massa de carga inorgânica, composta de hidróxido de cálcio e αTCP, em diferentes proporções: 30% $Ca(OH)_2$, 25% $Ca(OH)_2$ + 5% αTCP, 20% $Ca(OH)_2$ + 10% αTCP, 15% $Ca(OH)_2$ + 15% αTCP. A resina sintetizada foi caracterizada por cromatografia de exclusão por tamanho, ressonância magnética de prótons e espectroscopia Raman. Os cimentos foram caracterizados quanto ao tempo de presa, degradação *in vitro*, potencial de alcalinização do meio, citotoxicidade e capacidade de estimular a deposição mineral. A resina de glicerol salicilato foi obtida por meio da reação de transesterificação, com 72% de rendimento. Os grupos avaliados apresentaram um tempo de presa de 240 min a 405 min e capacidade de alcalinizar o meio. Além disso, o aumento na concentração de αTCP diminuiu a degradação dos cimentos. A viabilidade celular após 48 h de contato direto com os cimentos experimentais não apresentou diferença quando comparada a um cimento obturador comercial (AH Plus) usado como referência. Os cimentos contendo 10% e 15% de αTCP exibiram a presença de uma camada superficial rica em fosfatos de cálcio após a imersão em água e *simulated body fluid* (SBF) por sete dias, verificada por espectroscopia Raman e MEV-EDS. Os resultados mostraram êxito no desenvolvimento do cimento endodôntico bioativo à base de resina de glicerol salicilato e α-fosfato tricálcico.

Palavras-chave: Cimentos dentários. Fosfatos de cálcio. Hidróxido de cálcio. Obturação do canal radicular.

ABSTRACT

Dental root canal treatment comprises the disinfection and complete filling of root canals in order to prevent the reinfection and allows the healing of periapical tissues. The use of materials that promotes an effective sealing has no achieved at the moment. The aim of this *in vitro* study was to develop a root canal sealer based on a glycerol salicylate resin and α-tricalcium phosphate (αTCP) that presents potential to stimulate mineral deposition. Sealers were formulated based on 70wt% of a glycerol salicylate resin, obtained by transesterification of methyl salicylate and glycerol, and 30wt% of inorganic filler, composed by calcium hydroxide and αTCP, in different proportions: 30% $Ca(OH)_2$, 25% $Ca(OH)_2$ + 5% αTCP, 20% $Ca(OH)_2$ + 10% αTCP, 15% $Ca(OH)_2$ + 15% αTCP. Synthesized resin was characterized by proton nuclear magnetic resonance spectroscopy, size exclusion chromatography and Raman spectroscopy. Sealers were characterized based on setting time, *in vitro* degradation over time, pH, cytotoxicity and mineral deposition. The glycerol salicylate resin was obtained by transesterification reaction, with 72% yield. Sealers presented setting time ranging from 240 min to 405 min, and basic pH over 8.21 after 28 days. Higher αTCP concentration leads to sealers with low solubility. Cell viability after 48 h in direct contact with sealers was similar to a commercial sealer (AH Plus) used as reference. The 10% and 15% αTCP sealers exhibited a calcium-phosphate layer on its surface after immersion in water and simulated body fluid (SBF) for 7 days, verified by Raman spectroscopy and MEV-EDX. The bioactive root canal sealer based on glycerol salicylate resin containing αTCP was successfully developed.

Key-words: Calcium Hydroxide. Calcium Phosphates. Dental Cements. Root Canal Obturation.

SUMÁRIO

1. INTRODUÇÃO

O preenchimento dos canais radiculares com materiais obturadores, incluindo-se os cimentos endodônticos e materiais de corpo, como a guta-percha, visa tornar inviáveis as bactérias residuais, aprisionando-as nos canalículos e túbulos dentinários, e evitar a percolação de fluidos via canal radicular, estando a qualidade da obturação relacionada ao sucesso da terapia (GILLEN et al., 2011; NG et al., 2011).

Até o momento, que se tenha conhecimento, nenhum cimento endodôntico disponível no mercado, associado a qualquer técnica obturadora já desenvolvida, é capaz de selar completamente o ápice radicular. Diversos tipos de cimentos, com diferentes composições químicas, foram propostos para serem usados em combinação com a guta-percha, com a finalidade de melhorar o selamento, entretanto, até o momento, sem alcançar um vedamento completo (YUCEL et al., 2006).

Cimentos obturadores contendo resina de salicilato (WALLER; JANDOUREK, 1999) apresentam boa biocompatibilidade, propriedade antimicrobiana e desempenho clínico aceitável (LEONARDO et al., 1997; SILVA et al., 1997; BERBERT et al., 2002; WALTIMO et al., 2001; FARIA-JUNIOR et al., 2013), entretanto, apresentam elevada solubilidade (FARIA-JUNIOR et al., 2013), podendo levar a uma redução da longevidade da obturação. Visto isso, o desenvolvimento de uma resina de salicilato com estrutura química diferente das resinas de isobutil salicilato presente nos cimentos comerciais, poderia suplantar as limitações dos materiais disponíveis. As resinas de salicilato geralmente são uma blenda de polissalicilatos multifuncionais, sintetizadas a partir da transesterificação de ésteres, principalmente salicilato de metila, e alcoóis, como o neopentilglicol e o trimetilolpropano (WALLER; JANDOUREK, 1999). Uma alternativa inovadora seria a síntese da resina de salicilato a partir do glicerol, um álcool produzido em larga escala como subproduto da obtenção do biodiesel (SALVI; PANWAR, 2012). Para cada dez partes de biodiesel produzido, uma parte de glicerol bruto é gerada (KARINEN; KRAUSE, 2006; CHI et al., 2007), o que tem estimulado pesquisadores de todo mundo a procurar novos empregos ao glicerol. O glicerol, largamente

utilizado na indústria farmacêutica (FLUHR *et al.*, 2008) e de alimentos (HERNANDEZ-IZQUIERDO; KROCHTA, 2008) representa uma alternativa para a obtenção de resinas de salicilato, as quais podem ser utilizadas como matriz carreadora para íons ou outras substâncias na formulação de novos cimentos endodônticos. Ainda, o glicerol é um precursor para a síntese de triacilglicerol e fosfolipídeos no fígado e tecido adiposo, o que indica a biocompatibilidade do composto (MIGNECO *et al.* 2009).

Estudos demonstraram a viabilidade em se realizar a remineralização da superfície dentinária a partir da deposição mineral (KIM *et al.*, 2010; Mai *et al.*, 2010). Existem duas alternativas para desencadear esse tipo de remineralização, referidas na literatura como "bottom-up" e "top-down". Na primeira abordagem são utilizados análogos biomiméticos de proteínas da matriz, tais como os ácidos poliacrílico e polivinilfosfônico, que estabilizam os cristais de fosfato de cálcio nanoprecursores em núcleos de crescimento no interior da matriz de colágeno desmineralizada, de onde propaga-se a remineralização. O segundo método consiste em saturar o meio com íons cálcio e fosfato, de forma que os mesmos precipitem sobre a superfície a ser mineralizada. Em ambas as situações se faz necessária a presença de algum material solúvel que possa fornecer íons a um meio aquoso, sendo comumente utilizados cimentos de silicato de cálcio para esse fim (LIU *et al.*, 2011). Alani e colaboradores em 2009, utilizando um compósito experimental à base de policaprolactona e um vidro fosfatado, em uma abordagem "top-down", mostraram ausência de infiltração, aferida por corante, em raízes obturadas com o referido material. Os autores relacionam o selamento obtido à presença de deposições minerais na interface do material obturador com a dentina radicular.

A utilização de materiais que estimulem a formação de tecido mineralizado (cemento/osso) na região periapical, após o tratamento endodôntico radical é desejada. Diversos biomateriais tem sido empregados para reparo de defeitos ósseos, destacando-se os cimentos de silicato de cálcio e os cimentos de fosfato de cálcio. Os materiais utilizados para reparo devem possuir um conjunto de propriedade físicas, químicas e biológicas que permitam desempenhar a função desejada e propiciar uma resposta adequada dos tecidos (GUASTALDI; APARECIDA, 2010). Os dentes e ossos dos vertebrados são um compósito natural formado por fibras colágenas envoltas por um sólido inorgânico (GUASTALDI;

APARECIDA, 2010). Sabe-se que apesar de não possuir composição estritamente definida e apresentar variações entre os estágios de maturação e envelhecimento, a estrutura cristalina e a composição da fase inorgânica dos tecidos duros dos vertebrados assemelha-se à hidroxiapatita, e por essa razão, os cimentos de fosfato de cálcio apresentam-se como os principais biomateriais utilizados para a reposição e regeneração de tecido ósseo (DOROZHKIN, 2010; DOROZHKIN, 2013).

Diversos cimentos de fosfato de cálcio tem sido utilizados como biomateriais, apresentando diferentes razões cálcio/fósforo, conforme o quadro 1. Um dos fatores que pode predizer o comportamento *in vivo* desses cimentos é a solubilidade dos fosfatos de cálcio em água, que de forma geral, é inversa a relação Ca/P do composto. O fosfato tricálcico, que pode ser sintetizado em diferentes formas alotrópicas, apresenta boa osteocondutividade, potencial de osteoindução e capacidade de estimular a proliferação celular. Sendo essas propriedades maiores para o αTCP em comparação com o βTCP, provavelmente devido à sua estrutura cristalina, que possibilita uma maior troca de íons com o meio, além de sua maior solubilidade em pH fisiológico (GUASTALDI; APARECIDA, 2010; GANDOLFI *et al.*, 2010; LI *et al.*, 2012). Ainda, como resultado da reação do αTCP com água, há a precipitação de hidroxiapatita (GINEBRA *et al.*, 1997; MOREJON-ALONSO, *et al.*, 2012).

Quadro 1. Tipos de fosfatos de cálcio e suas solubilidades

Razão molar Ca/P	Nome e abreviação usual	Fórmula química	Solubilidade em água a 25ºC (g/l)
0,5	Fosfato monocálcico (MCP)	$Ca(H_2PO_4)_2$	~17
1,0	Mono-hidrogênio fosfato de cálcio (DCP)	$CaHPO_4$	~0,048
1,33	Fosfato octacálcico (OCP)	$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$	~0,0081
1,5	α-fosfato tricálcico (αTCP)	$\alpha\text{-}Ca_3(PO_4)_2$	~0,0025
1,5	β-fosfato tricálcico (βTCP)	$\beta\text{-}Ca_3(PO_4)_2$	~0,0005
1,2 - 2,2	Fosfatos de cálcio amorfo (ACP)	$Ca_xH_y(PO_4)_z.nH_2O$	*
1,67	Hidroxiapatita (HÁ)	$Ca_{10}(PO_4)_6(OH)_2$	~0,0003
1,67	Fluorapatita (FA)	$Ca_{10}(PO_4)_6F_2$	~0,0002
2,0	Fosfato tetracálcico (TTCP)	$Ca_4(PO_4)_2O$	~0,0007

Adaptado de Dorozhkin, 2013. * não pode ser determinada precisamente.

Sendo assim, o desenvolvimento de um cimento à base de resina de glicerol salicilato e α-fosfato tricálcico, que propicie as condições necessárias à precipitação mineral na interface obturação/dentina e o reparo dos tecidos periapicais, apresenta-se como uma alternativa promissora para otimização dos tratamentos endodônticos.

2. OBJETIVO

O objetivo do presente estudo foi desenvolver um cimento endodôntico à base de resina de glicerol salicilato e α-fosfato tricálcico, que apresente potencial de estimular a deposição mineral.

Dessa forma, em um primeiro momento objetivou-se:

- Sintetizar uma resina de glicerol salicilato;
- Caracterizar as propriedades da resina obtida e verificar a aplicabilidade para o uso em um cimento endodôntico.

A resina sintetizada foi utilizada em cimentos "modelo" contendo diferentes concentrações de α-fosfato tricálcico. Nesse momento objetivou-se caracterizar os cimentos quanto às:

- Propriedades químicas e físicas;
- Citotoxicidade;
- Capacidade de promover a deposição de fosfatos de cálcio.

3. ARTIGOS

O corpo da presente tese é composto por dois artigos:

- Artigo I: *Synthesis and characterization of a glycerol salicylate resin for bioactive root canal sealers.* Publicado no periódico International Endodontic Journal (doi: 10.1111/iej.12149) (PORTELLA *et al.*, 2014);
- Artigo II: *Glycerol salicylate-based containing α-tricalcium phosphate as a bioactive root canal sealer.* Publicado no periódico Journal of Biomedical Materials Research Part B: Applied Biomaterials (doi: 10.1002/jbm.b.33326) (PORTELLA *et al.*, 2015).

Os manuscritos, formatados de acordo com os requisitos dos periódicos aos quais foram submetidos, encontram-se a seguir.

3.1. Artigo I

Synthesis and characterization of a glycerol salicylate resin for bioactive root canal sealers

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Running title: Glycerol salicylate resin

Key-words: salicylate resin, salicylate, glycerol, bioactive sealers, RAMAN.

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Abstract

Aim To develop and characterize a salicylate resin with potential use in bioactive endodontic sealers.

Methodology Methyl salicylate, glycerol, and titanium isopropoxide were added in a closed system for the transesterification reaction. The resin obtained was characterized by proton nuclear magnetic resonance spectroscopy (1H NMR) and size exclusion chromatography (SEC). To verify the applicability of the resin to the development of endodontic sealers, experimental cements were prepared by mixing glycerol salicylate resin, calcium hydroxide, and methyl salicylate in ratios of 2:1:1, 1:2:1, 1:1:2, 1:1:1, 4:1:1, 1:4:1, and 1:1:4. Setting times were measured according to ISO 6876. Features of the hardening reaction were described by micro-RAMAN spectroscopy.

Results The transesterification reaction had a 72% efficiency. The 1H NMR analysis revealed the presence of the expected functional groups (hydroxyls and aromatic rings), and the SEC confirmed the molar mass of the resin produced. The setting times of experimental sealers ranged from 70 min (ratio 1:1:1) to 490 min (ratio 1:1:4). The conversion of the salicylic groups (1 613 cm-1) to salicylate salt (1 543 cm-1) and the reduction of calcium hydroxide peaks (1084 cm-1 and 682 cm-1) were confirmed by micro-RAMAN spectroscopy, which showed the calcium chelation by the resin.

Conclusion The new glycerol salicylate resin was successfully synthesized and revealed a potential application in the development of endodontic sealers.

Introduction

The main objective of root canal treatment is to heal and/or to prevent the contamination of periapical tissues. Filling of the canal system plays an important role in achieving this outcome, and the materials used as root canal sealers should not be cytotoxic. Additionally, they ideally should be biological stimulators, as the cements and bulk materials are in contact with dentine and periapical tissues (Leonardo *et al.* 1995, Tanomaru Filho *et al.* 1998). Therefore, root canal sealers that have ionic delivery have been proposed to enhance sealing of the apical region by mineral deposition to the canal walls (Alani *et al.* 2009).

Salicylate resin was introduced as a resin matrix for endodontic sealers in the early nineties (Waller & Jandourek 1999). Commercial sealers that contain salicylate resin have presented reliable biocompatibility (Leonardo *et al.* 1997, Silva *et al.* 1997), satisfactory antibacterial properties (Berbert *et al.* 2002), and acceptable clinical performances (Waltimo *et al.* 2001). However, commercial salicylate based sealers present high solubility (Faria-Júnior *et al.* 2013) leading to decreased treatment longevity. Synthesis of novel salicylate sealers with improved properties are required to overcome these limitations.

Resins based on salicylate are a blend of multifunctional polysalicylates, synthesized by the transesterification of esters (mainly methyl salicylate) and alcohols, such as neopentylglycol and trimethylolpropane (Waller & Jandourek 1999). An innovative alternative to salicylate resins is the use of glycerol, an alcohol that is produced on a large scale as a biodiesel byproduct (Salvi & Panwar 2012). For every ten parts of biodiesel produced, one part of crude glycerol is generated as a byproduct (Karinen & Krause 2006, Chi *et al.* 2007). Glycerol, widely used in the pharmaceutical (Fluhr *et al.* 2008) and food industries (Hernandez-Izquierdo & Krochta 2008), is an alternative to the production of salicylate resins, which can serve as a carrying matrix for ions or other substances in the formulation of endodontic sealers. Thus, the purpose of this study was to develop and characterize a salicylate resin to be used in bioactive endodontic sealers.

Material and Methods

Resin synthesis

The salicylate resin was synthesized by transesterification of glycerol (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil, 99.5%) and methyl salicylate (Vetec

Química Fina Ltda, Duque de Caxias, RJ, Brazil, 99%). The reagents were added in a ratio of 1:7.5 (w/w) in a closed vacuum (10^{-2} mmHg) system (a volumetric flask coupled to a vacuum pump and a condenser to simple distillation) and heated at 100°C for 1 hour. The vacuum was removed, the titanium isopropoxide (Sigma-Aldrich, St. Louis, MO, USA, 97%) was added as a catalyst agent (0.05% w/w) at 60°C, and the temperature of the system was increased to 204°C for 17 hours. The temperature was decreased to 178°C until methanol, obtained as byproduct, was eliminated by simple distillation. The vacuum was reapplied to remove the excess methyl salicylate. The distillation of methanol and vacuum application were used to purify the resin. Figure 1 shows the molecular structures of reagents and the expected products of the transesterification reaction.
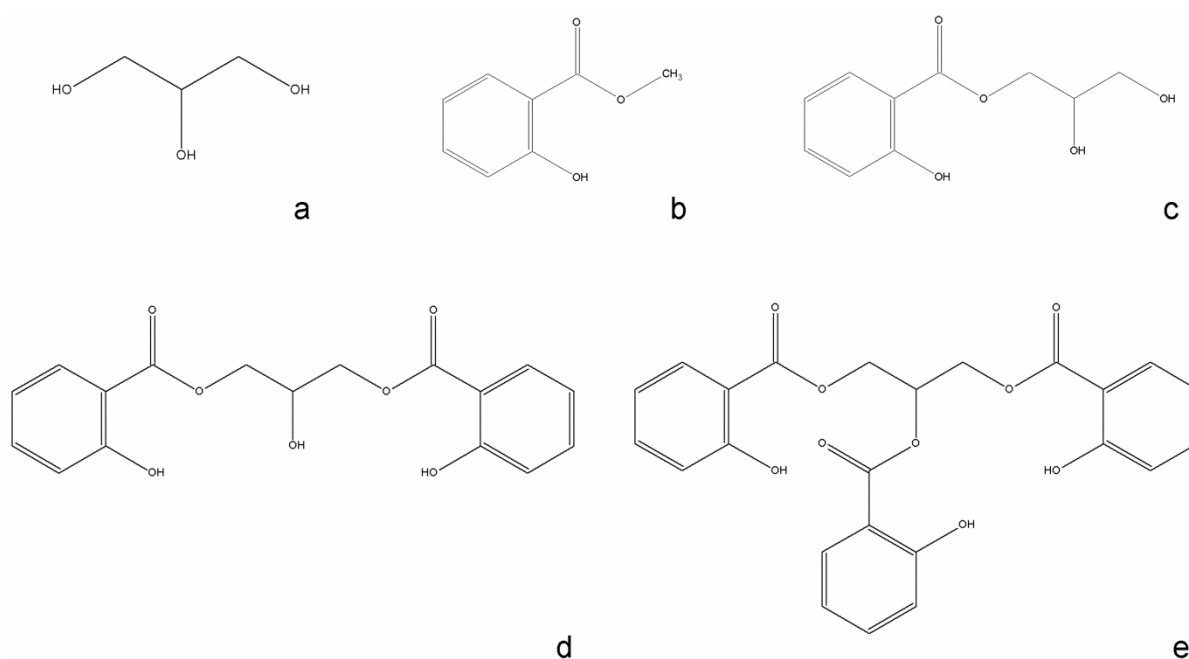

Figure 1. Molecular structure of (a) glycerol, (b) methyl salicylate, (c) glycerol monosalicylate (MW=212.20), (d) glycerol disalicylate (MW=332.30), and (e) glycerol trisalicylate (MW=452.41).

Characterization of the resin

The molecular weights of the products were determined with a size exclusion chromatography (SEC) composed of a tetra detector array (TDA 302, VISCOTEK VE2001, Houston, TX, USA) and a polystyrene column, THF was used as solvent with a flow rate of 1mL/min. The molecular structure was confirmed by proton nuclear

magnetic resonance spectroscopy (^{1}H NMR) with a VARIAN VNMRS 300 instrument (Palo Alto, CA, USA), using deuterated chloroform as a solvent and tetramethylsilane as an internal standard.

Experimental cements

Experimental cements were formulated and tested to confirm that the resin obtained was applicable to the development of root canal sealers. The applicability was verified by mixing resin, calcium hydroxide (Biodinâmica, Ibiporã, PR, Brazil, p.a.) and methyl salicylate in different proportions by weight: 1:1:1, 2:1:1, 1:2:1, 1:1:2, 4:1:1, and 1:1:4. Hardening and setting times of experimental cements were verified according to ISO 6876 (2001). The cements were manipulated and placed on cylindrical matrixes, 10 mm in diameter and 2 mm in height. After 120 seconds of mixing, indentations were formed on the surface of the specimens with a 100 g ± 0.5 g cylindrical indenter (Ø2 ± 0.1 mm). Indentations were made until no indentations could be visualized by operator naked eye, and the time of this occurrence was registered as the setting time. The test was repeated 3 times, and the setting time was calculated as the mean of the measures.

The chemical reaction between the resin and calcium hydroxide was characterized by micro-Raman spectroscopy (Senterra, Bruker Optics, Ettlingen, Germany). Figures 2 and 3 show the Raman spectrum of each reagent separately. The cement (proportion 1:1:1) was prepared, and the surface was excited by a 785 nm laser, with 100 mW of power, 10 seconds of integration time, and 2 co-additions. One spectrum was obtained immediately after mixing and after the setting time. The spectrums were submitted to a concave rubberband correction with one interaction and 64 baseline points on Opus 6.5 software (Bruker Optics, Ettlingen, Germany) and then the data was transferred to Microsoft Office Excel 2007 (Microsoft, Redmond, WA) to graph plotting. The chemical reaction was verified in a manner similar to that in a previous study (Mazinis *et al.* 2007).
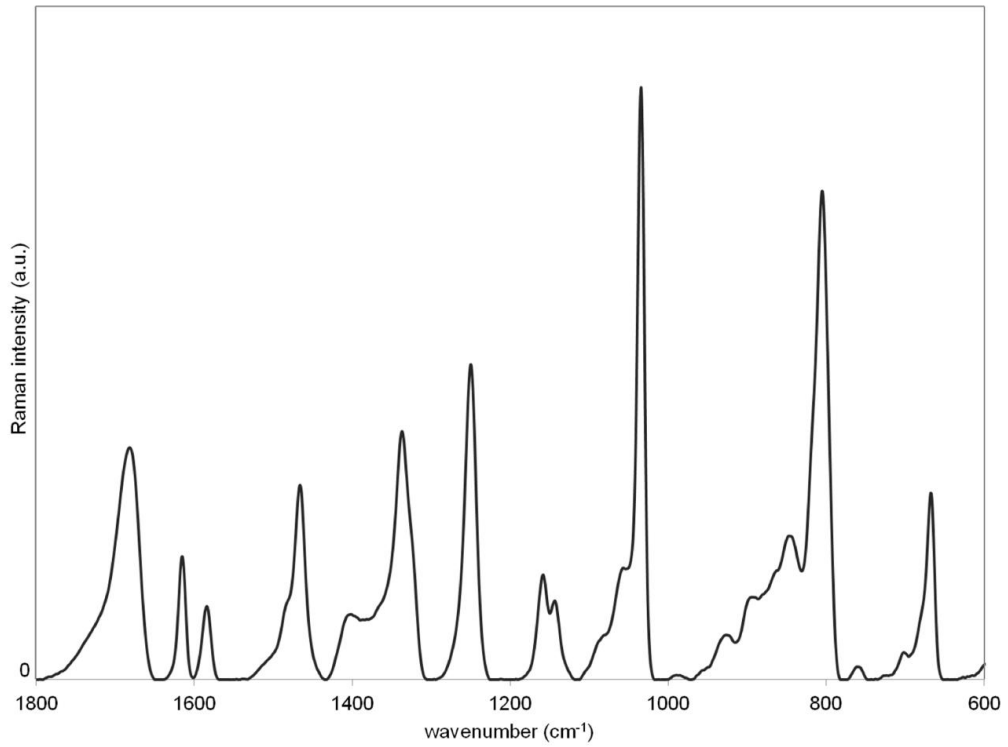
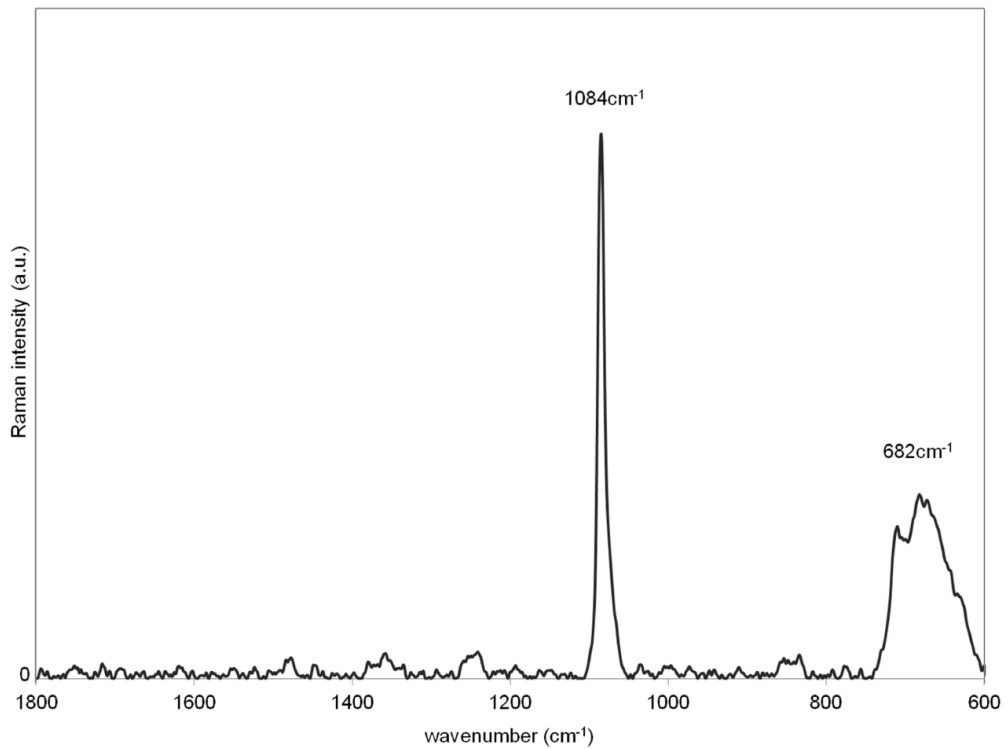**Figure 2.** Raman spectrum of the glycerol salicylate resin.

Figure 3. Raman spectrum of the commercial calcium hydroxide used to formulate the experimental cements.

Results

The yield of the transesterification reaction was 72%. The ^{1}H NMR analyses (Figure 4) revealed the presence of the expected proton signals of the functional groups for the proposed reaction: phenolic OH-protons appears at 10.5 ppm, the aromatic protons between 7 – 8 ppm, the methinic proton (-CH) of the glycerol moiety at 5.8 ppm and the methylenic protons (-CH$_2$) in the range of 4 – 5 ppm. The average molecular weight obtained from SEC (265 g/mol) indicated that the resin obtained composed of a mixture of 50-60% glycerol monosalicylate and 40-50% glycerol disalicylate, as this proportion, by weight, present a molecular weight range of 260.00 to 272.25g/mol. Only traces of glycerol trisalicylate were expected, since the central hydroxyl of the triol is less reactive than the extremities hydroxyls.
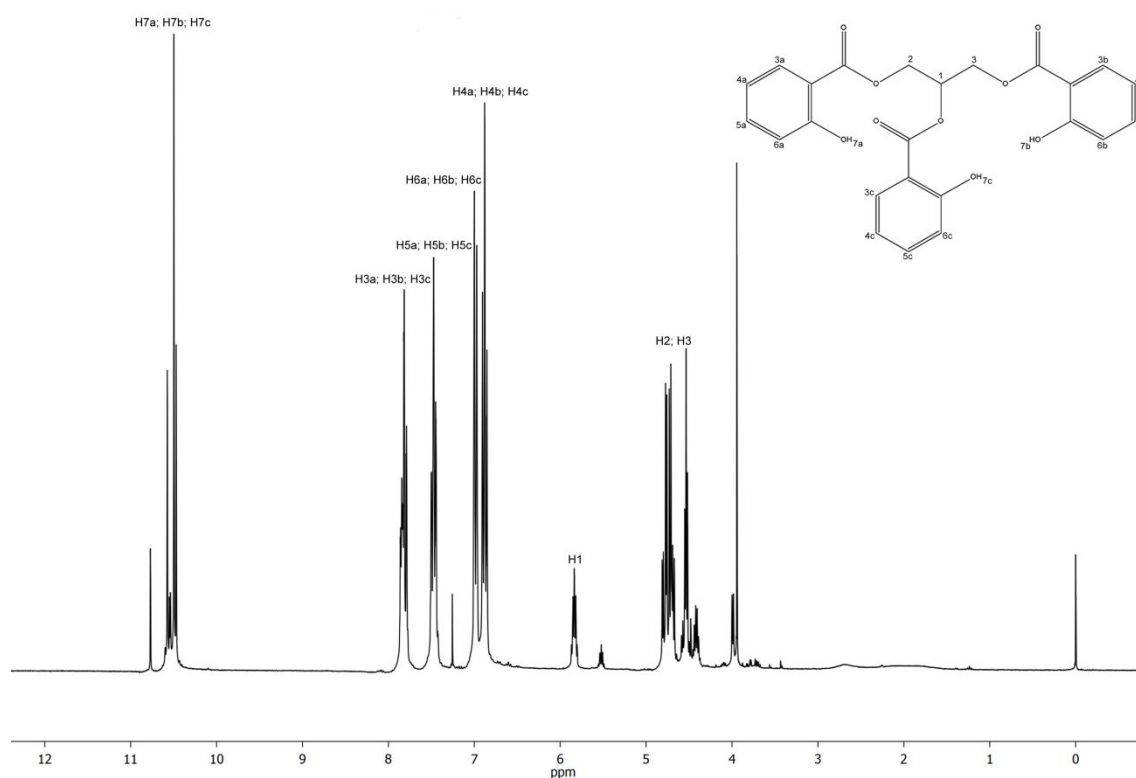

Figure 4. ^{1}H NMR spectrum of the resin and respective hydrogen assignments.

The setting time of the experimental cements ranged from 70 min for the 1:1:1 proportion to 490 min for the 1:1:4 proportion, as shown in Table 1. A 50% addition of methyl salicylate to the mixture produced an increase of the setting time, reaching 490 min.

Table 1. Setting time of experimental sealers as function of components proportion (in weigth)

resin:Ca(OH)$_2$:methyl salicylate	setting time (min)
1 : 1 : 1	70
2 : 1 : 1	135
1 : 2 : 1	*
1 : 1 : 2	95
4 : 1 : 1	360
1 : 1 : 4	490

*without handling condition

Micro-Raman spectroscopy (Figure 5) revealed the chelation of calcium by the hydroxyl resin salicylate groups, promoting the hardening of cements. The spectrum peaks of calcium hydroxide (1084 cm^{-1} and 682 cm^{-1} (Chaix-Pluchery *et al.* 1984)) decreased and a group of three absorptions was observed for the region between 1520 and 1640 cm^{-1} which is characteristic of a structure that features the ester carbonyl group of the salicylate ligand and calcium-coordinated phenoxide as shown in Figure 6 (Dain *et al.* 2011). The highest frequency stretch (at 1613 cm^{-1}) corresponds to a vibrational mode that involves C–C stretching of the aromatic ring coupled to stretching of the phenoxy and carboxylic acid C–O groups. A second absorption, observed at 1576 cm^{-1} is focused on C=O stretching of the ester carbonyl group, with coupling to an acid C-O-H wag and ring C–C stretching. The third (1543 cm^{-1}) corresponds to C_{ring}–C_{acid}–O stretching coupled to ring C–C stretches. A fourth prominent absorption is observed at 1153 cm^{-1} corresponding to acid C-O-H wag coupled to ring C–C stretches. The Raman spectrum of methyl salicylate non-metal coordinated showed only two bands in the region around 1600cm^{-1} (Varghese *et al.* 2007) as the glycerol salicylate resin (Figure 2).
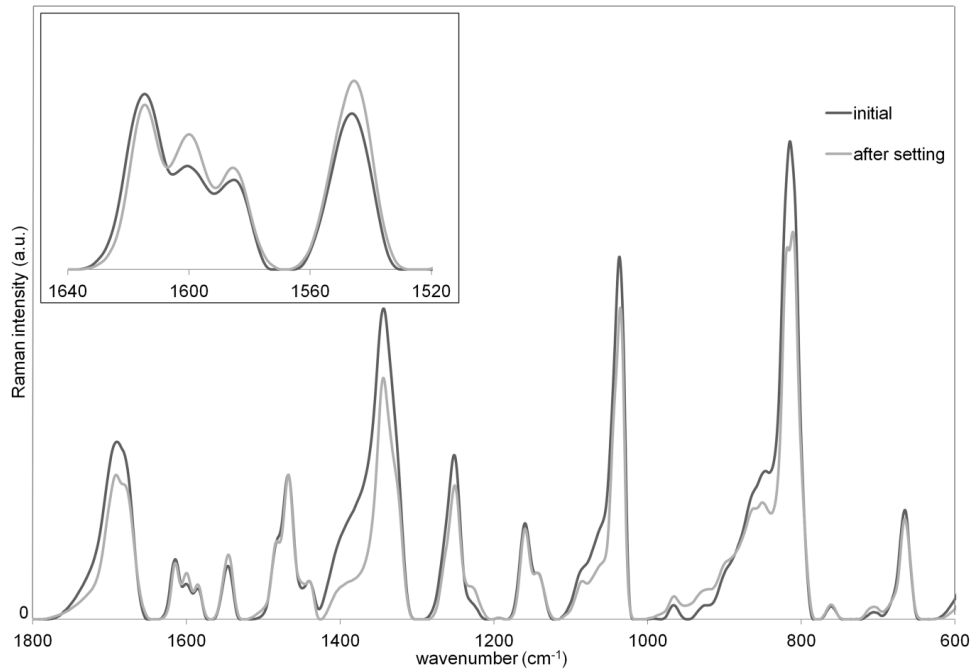
Figure 5. Raman spectrum of the setting reaction of the experimental sealer. The region of salicylic group peaks was enlarged to show the production of the salicylate salt.
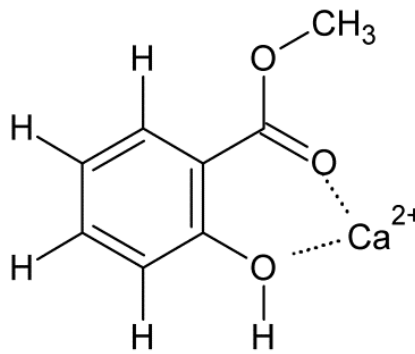

Figure 6. Representative scheme of a structure that features a neutral carboxylic acid group of the salicylate ligand and calcium-coordinated phenoxide.

Discussion

Root canal treatment is comprised of the cleaning and shaping of the root canals by following the filling of the canal system. However, the sealing of the root canal system is still a concern in endodontics (Kopper *et al.* 2006, Kim *et al.* 2010). The use of bioactive materials to fill the root canal system could be a viable method to achieve effective sealing, obliterating the gaps between the filling material and the root canal walls, sealing dentinal tubules and forming a mineralized barrier at the

apex, as these materials could interact with periapical tissues (Collares *et al.* 2012). Alani *et al.* (2009) proposed a polycaprolactone–iron phosphate glass composite as a root canal filling material, which releases ions and achieves an appropriate seal via mineral precipitation on the filling/dentin interface, completely preventing fluid percolation through the root canal and filling material. Bioactive materials should be composed of active components, such as drugs and inorganic compounds, along with a carrier matrix with high biocompatibility and a controlled release of the active components. These biomaterials raise the possibility of potentially controlling the relative benefits of ion release to induce antibacterial effects and to regenerate periapical tissues (Gandolfi *et al.* 2011a, Gandolfi *et al.* 2011b). In this study, a glycerol salicylate resin was *synthesized* that could be adequate as a bioactive sealer.

The chemical groups noted during the NMR analysis and the molecular weight of the product obtained on SEC confirmed the presence of glycerol salicylate, which was the expected product of the transesterification reaction of glycerol and methyl salicylate. The applicability of the salicylate resin was verified by the formulation of experimental sealers that contained different concentrations of calcium hydroxide and methyl salicylate. Methyl salicylate was used to adjust the rheological properties of sealers and to reduce the viscosity of the glycerol salicylate resin. Setting times were registered; all formulations presented setting times, according to ISO 6876, except for the formulation containing 50% calcium hydroxide. This formulation did not present handling characteristics (impossible to achieve a homogeneous mixture), most likely due to a high filler concentration.

The hardening of cements occurs through calcium chelation by the hydroxyl of the salicylate groups of the resin, as characterized by micro-RAMAN spectroscopy (Figure 5). This correlates with the results of a previous study, in which this interaction was demonstrated by Fourier Transformed Infrared Spectroscopy (Mazinis *et al.* 2007). The groups with low concentrations of calcium hydroxide showed longer setting times. These results could be due to the presence of excess salicylate groups, which cause the failure of reaction stoichiometry. Theoretically, each mol of calcium hydroxide can react with 2 mol of the salicylate group. The molecular weight-average of the resin obtained on SEC was 265g/mol, indicating that glycerol monosalicylate (MW=212.20 g/mol) and glycerol disalicylate (MW=332.30 g/mol) are most likely the major components. Therefore, in a blend composed of 50-

50 mono and disalicylate, for each one part by mass of calcium hydroxide, approximately five parts by mass of resin will be necessary to fit the stoichiometric reaction.

The reaction could generate different products, according to the number of hydroxyl groups of glycerol that participates in the reaction. The resin obtained could be a mixture of mono-, di-, or trisalicylate glycerol, presenting molecular weights of 212.20, 322.30, and 452.41g/mol, respectively. The major yield of the reaction will be achieved when one glycerol molecule reacts with three methyl salicylate molecules; for this to take place, for each part of glycerol, five parts, in mass, of methyl salicylate will be necessary. Methyl salicylate was added in excess of 50% to ensure that the entire amount of glycerol reacted. This feature of the synthesis process could explain its 72% efficiency because part of the total mass of reagents was converted to methanol (byproduct) and probably some methyl salicylate remained unreacted.

The characteristics of synthesized salicylate resin could be used as a promising carrier agent for the development of bioactive endodontic sealers. Glycerol has excellent biocompatibility (it is a precursor for the synthesis of triacylglycerols and of phospholipids in the liver and adipose tissue) (Migneco *et al.* 2009) that could be used in calcium based sealers to promote remineralization when in contact with connective tissues. Moreover, salicylates are anti-inflammatory agents (Duthie & Wood 2011) that are widely used in the pharmacological industry (Amann & Peskar 2002). To combine the outstanding properties of glycerol and salicylate in a resin seems to be a promising approach in the development of endodontic sealers, especially if those materials are in contact with periapical tissues.

Conclusion

The salicylate resin synthesized in this study was successfully generated, and it exhibited applicability in bioactive endodontic sealers that contained inorganic components, that permit its incorporation into the matrix resin by chelation.

Acknowledgments

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3.2. Artigo II

Glycerol salicylate-based containing α-tricalcium phosphate as a bioactive root canal sealer

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ABSTRACT

The use of bioactive materials instead of inert materials to fill the root canal space could be an effective approach to achieve a hermetic seal and stimulate the healing of periapical tissues. The purpose of this study was to develop and characterize an endodontic sealer based on a glycerol salicylate resin and α-tricalcium phosphate (αTCP) at physical and chemical properties. Different sealers were formulated using 70% of a glycerol salicylate resin and 30% of a mixture of calcium hydroxide and αTCP (0, 5, 10 or 15%, in weight). Sealers formulated were characterized based on setting time, *in vitro* degradation over time, pH, cytotoxicity and mineral deposition. Sealers presented setting time ranging from 240 to 405 min, and basic pH over 8.21 after 28 days. Higher αTCP concentration leads to sealers with low solubility. Cell viability after 48 h in direct contact with sealers was similar to a commercial sealer used as reference. The 10% and 15% αTCP sealers exhibited a calcium-phosphate layer on the surface after immersion in water and SBF for 7 days. Glycerol salicylate sealers with 10% and 15% α-tricalcium phosphate showed reliable physical chemical properties and apatite-forming ability.

Keywords: α-tricalcium phosphate, bioactive sealers, glycerol, salicylate, Raman.

Running Head: *αTCP bioactive root canal sealer*

INTRODUCTION

Dental root canal treatment comprises the disinfection and complete filling of root canals in order to heal the periapical tissues. The quality of sealing promoted by endodontic sealers is one of the main reasons for clinical success[1]. Recently, resin-based sealers (e.g., epoxy, methacrylate and salicylate based) were introduced for endodontic treatment to improve sealing and resistance to degradation[2].

The salicylate resins present a reliable clinical performance[3] due to the anti-inflammatory[4] and biocompatibility[5] properties with a high solubility observed[6]. Portella *et al.*[7] synthesized a trifunctional salicylate resin with 72% efficiency in transesterification reactions at a clinically acceptable setting time. To increase mechanical properties of root canal sealers, bioactive inorganic fillers are incorporated in a resin matrix, such as calcium phosphates. Calcium phosphate-based sealers induce a favorable cell response[8], release of calcium and hydroxyl ions[9] and stimulated deposition of apatite[10]. However, commercial available calcium phosphate-based sealers present high solubility, decreasing the clinical success of root canal treatment[11].

In this way, combining glycerol salicylate resin and αTCP seems to be a promising approach to increase the quality of endodontic sealers. The purpose of this study was to evaluate the influence of α-tricalcium phosphate (αTCP) addition to a glycerol salicylate resin at physical and chemical properties.

MATERIAL AND METHODS

Experimental sealers were obtained by mixing a glycerol salicylate resin, synthesized according to Portella *et al.*[7], calcium hydroxide and α-tricalcium phosphate. All sealers were composed by 70wt% resin and 30wt% inorganic components. The inorganic portion was a mixture of calcium hydroxide and αTCP. The content of αTCP used was 0, 5, 10 and 15 wt% in designed experimental groups. The calcium hydroxide ($Ca(OH)_2$ p.a., Biodinâmica, Ibiporã, PR, Brazil, batch no. 86812) and αTCP, synthesized according to Santos *et al.*[12], had a particle size analyzed by laser diffraction analyzer (CILAS 1180 Particle-Size-Analyzer, Compagnie Industrielle des Lasers, Orleans, Loiret, France). The reagents were hand mixed, inserted in a silicon matrix and covered by polyester film to obtain samples for each of the following tests.

Setting time

Time required to harden the sealers was assessed according to a previous study[7]. The sealers were manipulated, placed on cylindrical matrixes and stored in a humid cabinet at 36⁰ C. Indentations were formed on the surface of the specimens with a 100-g and 2-mm diameter needle, as specified on ISO 6876[13], until no indentations could be visualized by the operator's naked eye, and the time of this occurrence, it was registered as the setting time. The setting time was calculated as the mean of three measures.

In vitro degradation and pH analysis

The weight loss of experimental sealers was determined after 24 hours, 7 days and 28 days of storage in water and in simulated body fluid (SBF). The SBF preparation was described elsewhere[14]. Three discs 10±0.1 mm in diameter and 1.0±0.1 mm thick were immersed in 10 ml of distillated water or SBF and stored at 36 ⁰C for each period. The dried mass of specimens was determined before and after the immersion. To obtain the dried mass, the specimens were placed in desiccators containing silica gel and stored at 36 ⁰C. Each specimen was weighed repeatedly at 24-h intervals until a constant mass was obtained (until the specimen mass's decrease did not differ more than 0.1 mg in a 24-h period). The weight loss (WL) percentage was calculated according to the following equation[15]:

$$WL (\%) = (W_f - W_0)/W_0 \times 100$$

Where W_f is the weight after immersion and W_0 is the weight before immersion. The mean weight loss of three specimens was recorded for each experimental sealer. Two-way ANOVA and Tukey's test were used to assess differences between groups submitted to the same storage solution. Comparisons were made at 5% significance.

The pH of the storage water and SBF were evaluated using a digital pHmeter (pHmetro DM-23, Digicrom Analytical Ltda, São Paulo, SP, Brazil). Three measurements were performed for each period: 24 hours, 7 days and 28 days. Changes in pH were assessed by two-way ANOVA followed by Tukey's multiple comparison test.

Bioactivity assay: Raman and SEM-EDX surface characterization

Glycerol salicylate resin and inorganic reagents were mixed, inserted in a cylindrical matrix, and covered by a polyester coverslip to form disks 6.5 ± 0.1 mm in diameter and 1.5 ± 0.1 mm thick. After preparation, samples were stored in a humid cabinet until hardening. Three disks of each experimental sealer were produced and the surfaces were characterized by Raman spectroscopy and by SEM-EDX immediately after hardening and after storage in 10 ml of distilled water or SBF for 7 days at 36 ºC[14]. Before analysis the groups submitted to immersion were washed with 10 ml of distilled water for 20 s and stored in a desiccator. The SBF used had the following ion concentration, in mol/m³: Na^+ (213.0), K^+ (7.5), Mg^{2+} (2.3), Ca^{2+} (3.8), Cl^- (221.7), HCO^{3-} (6.3), HPO_4^{3-} (1.5) and SO_4^{2-} (0.8). The pH was adjusted to 7.4.

A 200x200-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. Spectra were obtained at the region between 1800 and 440 cm^{-1} in a backscattering mode. The integral peaks, 962 cm^{-1} and 1613 cm^{-1}, corresponding to the symmetric stretching υ_1 mode of phosphate[16] and vibration mode of carbon-to-carbon stretching of the aromatic ring of salicylic group[17], respectively, were calculated using Opus software (Opus 7.5, Bruker Optics, Ettlingen, Germany). To standardize the integral calculation peak areas, the entire region below peaks from 980 to 949 cm^{-1} and 1624 to 1605 cm^{-1} were considered. Changes on the ratio between the integral intensity of 962 cm^{-1} and 1613 cm^{-1} bands ($A_{962cm^{-1}}/A_{1613cm^{-1}}$) were used to analyze the formation of a apatite-like layer on the sealer surface after immersion in distilled water and SBF[16].

The sample was carbon-sputtered and examined using scanning electron microscopy (JSM 5800, Jeol Ltd, Tokyo Japan) at 10 kV, followed by energy dispersive X-ray analysis to assess the elemental composition.

Cytotoxicity test

Cell viability was assessed by direct contact test using fibroblast cells (L929) (BCRJ batch no. 000604, Rio de Janeiro, Brazil) according to procedures described in ISO 10993-5[18]. The specimens used were 3.0 ± 0.1 mm in diameter and 1.0 ± 0.1 mm thick and were previously sterilized in hydrogen peroxide plasma. The MTT test

was performed in triplicate and the cell viability was read at 540 nm absorbance (Multiskan EX Microplate Reader, MTX Lab Systems Inc, VA, USA) after 24 or 48 h in direct contact with the sealers. Cells without any sealer were considered to be controlled for cytotoxicity, and samples of a widely used commercial sealer (AH Plus, Dentsply DeTrey GmbH, Konstanz, Germany) were used for comparison of the results. Data were transformed to log scale and analyzed by two-way ANOVA and Tukey's test.

RESULTS

Mean particle size of calcium hydroxide and α-tricalcium phosphate was 6.77 μm ($D_{10\%}$=1.24 μm, $D_{50\%}$=5.87 μm and $D_{90\%}$=13.75 μm) and 6.03 μm ($D_{10\%}$=1.03 μm, $D_{50\%}$=5.43 μm and $D_{90\%}$=12.00 μm), respectively. Setting time of sealers and changes in pH of SBF of the degradation test are shown in Table 1. Setting time ranged from 240 (0 αTCP) to 405 min (15% αTCP). The pH of water turns basic in all groups after 24 h, the highest value (11.42±0.03) measured for 0 αTCP at 7 days. All sealers were able to increase the pH of SBF over time, reaching 8.94±0.01 for 0 αTCP and 8.21±0.04 for 15% αTCP groups, the highest and lowest pH after 28 days, respectively.

Immersion in distillated water and SBF reveals that all sealers had mass loss after soaking that increased with time, as shown in Figure 1. The sealers containing 10% and 15% αTCP showed less weight loss after 28 days of water or SBF immersion (p<0.05). Sealers without αTCP ranged from 72.49% to 60.63%. Groups with 15% αTCP showed a mass loss of 41.15% and 31.86% in water and SBF immersion, respectively.

Table 1. Setting time and pH of water and SBF according to experimental sealers.

Experimental sealer	Setting time (min)	Water pH			SBF pH		
		24h	7 days	28 days	24h	7 days	28 days
0 αTCP	240	7.62 (0.10)^Ac	11.42 (0.03)^Aa	9.72 (0.03)^Ab	7.68 (0.02)^Ac	7.94 (0.05)^Ab	8.94 (0.01)^Aa
5% αTCP	240	7.53 (0.01)^Ac	7.99 (0.18)^Ba	9.78 (0.01)^Ab	7.65 (0.02)^Ac	7.83 (0.04)^Bb	8.65 (0.02)^Ba
10% αTCP	315	7.72 (0.02)^Ab	7.78 (0.13)^Bb	8.63 (0.10)^Ba	7.64 (0.03)^Ac	7.74 (0.00)^Cb	8.37 (0.01)^Ca
15% αTCP	405	7.59 (0.01)^Aa	7.53 (0.10)^Ca	7.55 (0.15)^Ca	7.55 (0.01)^Bc	7.77 (0.02)^Cb	8.21 (0.04)^Da

Initial pH of water and SBF is 5.78 (0.12) and 7.40 (0.01), respectively. Means followed by distinct capital letters indicates significant difference (p<0.05) for the same storage period. Means followed by distinct lowercase letters indicates significant difference (p<0.05) between pH measured in different times.
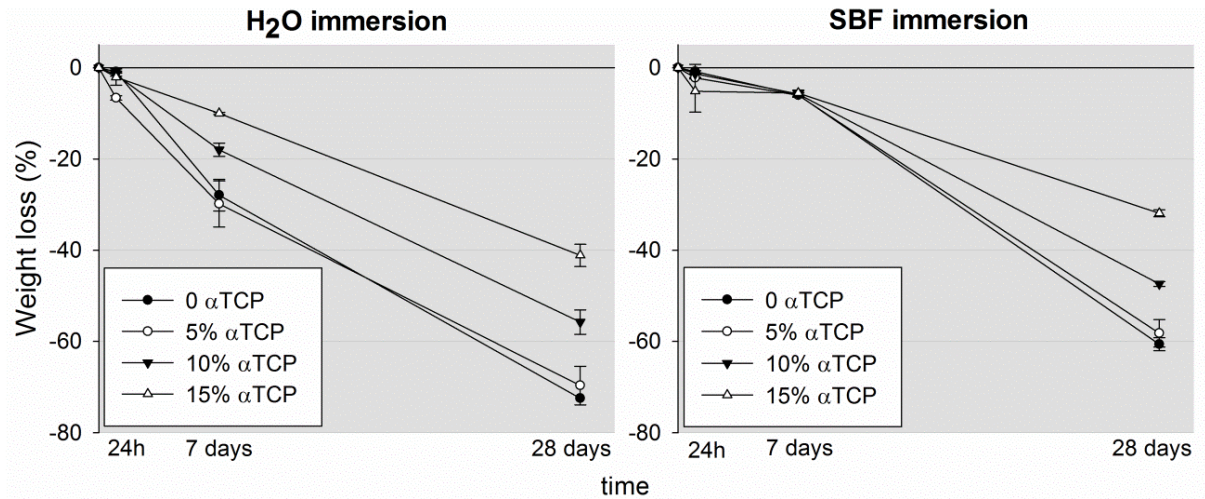
Figure 1. Weight loss (%) of the sealers after soaking in water or SBF for different periods.
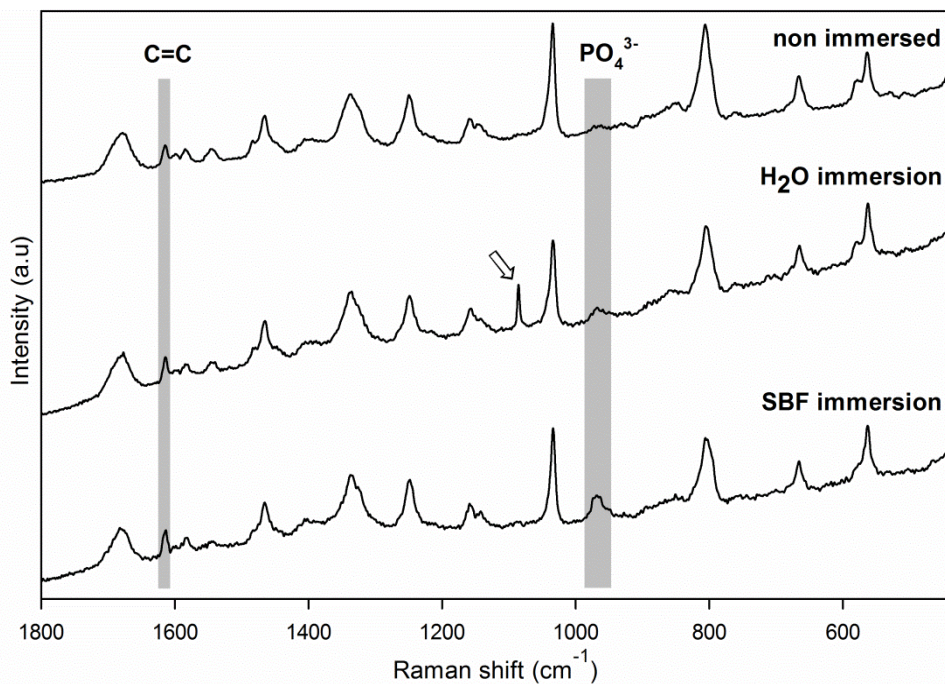

Figure 2. Representative Raman spectra of 15% αTCP sealer after setting and after 7 days of water or SBF immersion. The 1613- and 962-cm^{-1} regions in vibration mode, carbon-to-carbon stretching of the aromatic ring of salicylic group, and a symmetric stretching mode of phosphate, respectively, were highlighted. Note the presence of a peak at 1084 cm^{-1} (open arrow) on the spectra of a sealer after water immersion. This peak corresponds to the $Ca(OH)_2$ chemical bonds and could repeatedly be viewed for all groups after soaking specimens in water.

A representative Raman spectrum is shown in Figure 2. The peak corresponding to the stretching mode of phosphate increases after seven days of immersion in distillated water and SBF. A peak at 1084 cm^{-1} was noticed for specimens immersed in water. Figure 3 shows a mapping characterization of the specimen's surface regarding the changes in ratio of the intensity of 962-cm^{-1} and 1613-cm^{-1} Raman bands ($A_{962cm^{-1}}/A_{1613cm^{-1}}$). In the non-immersed specimens, the ratio $A_{962cm^{-1}}/A_{1613cm^{-1}}$ increased with the increased amount of αTCP in the sealer's composition. After water and SBF immersion, no changes were seen in the 0%and 5% αTCP groups, while for 10% and 15% αTCP compositions, changes occurred, with an increase in the $A_{962cm^{-1}}/A_{1613cm^{-1}}$ ratio.
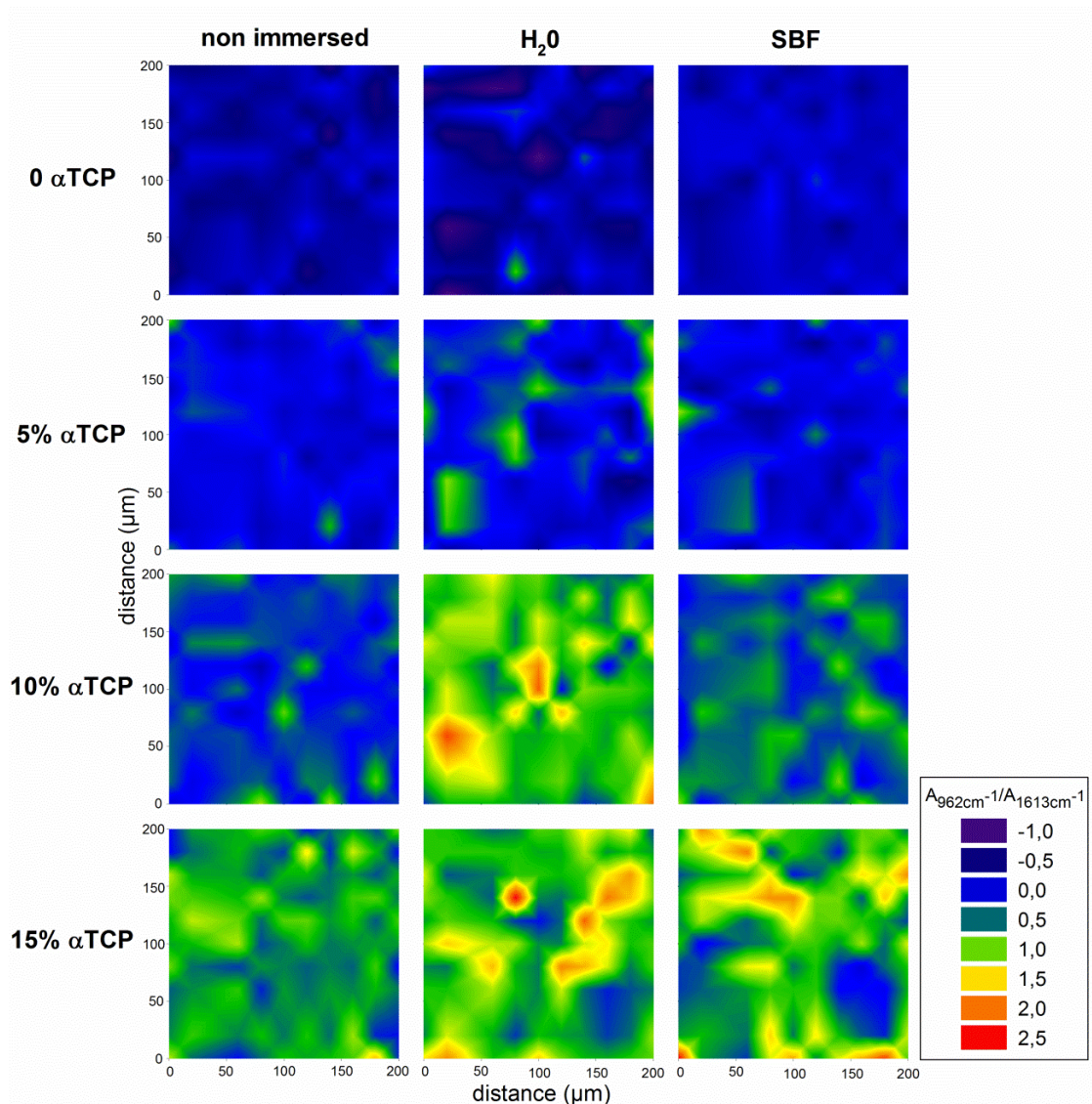

Figure 3. Raman mapping of the intensity of the ratio of the 962-cm^{-1} peak area to the 1613-cm^{-1} peak area measured on a 200x200-μm area of sample surfaces.

The values of the $A_{962cm^{-1}}/A_{1613cm^{-1}}$ ratios are presented in Figure 4. In the 10% αTCP sealer, the initial value of 0.32 increased to 0.88 for specimen immersion in water and 0.45 for SBF. The 15% αTCP sealer ratio ranged from 0.66 to 0.90 and 0.87, after water and SBF immersion, respectively.
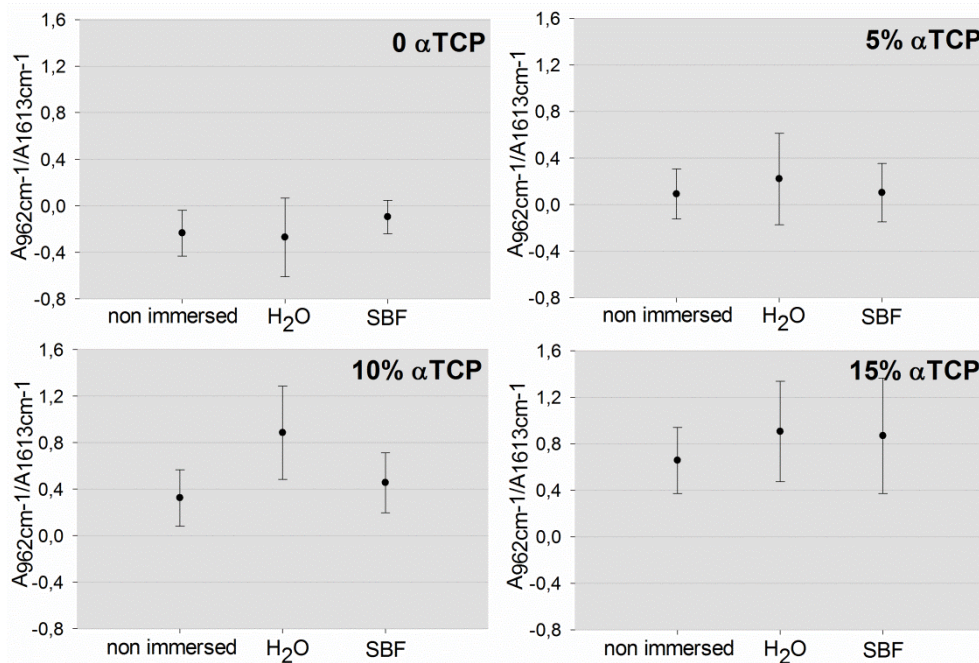

Figure 4. Variation of the ratio of the 962-cm^{-1} peak area to the 1613-cm^{-1} peak area measured on Raman spectra in function of specimen treatment (non-immersed, after water storage and after SBF storage) for each sealer. The circle represents the mean value of 100 points, and the bars represent the standard deviation.

Figure 5 presented the microstructure of sealers after 7 days of immersion in distillated water and SBF. SEM images revealed that all sealers present similar surface morphology before immersion. The EDX analysis after water immersion showed an increase in calcium and phosphorus on the specimen surface. The immersion in SBF produced irregular surfaces with calcium and phosphorous at elevated concentrations, except for the 15% αTCP. A representative image of a needle-like structure found around calcium and phosphorous deposition is shown in Figure 6. Figure 7 shows fibroblast viability after 24 or 48 h in direct contact with sealers. The sealers with αTCP additionally presented lower cell viability than commercial sealer AHPlus at 24 h ($p<0.05$). However, the incorporation of αTCP showed no difference from the commercial sealer ($p>0.05$).
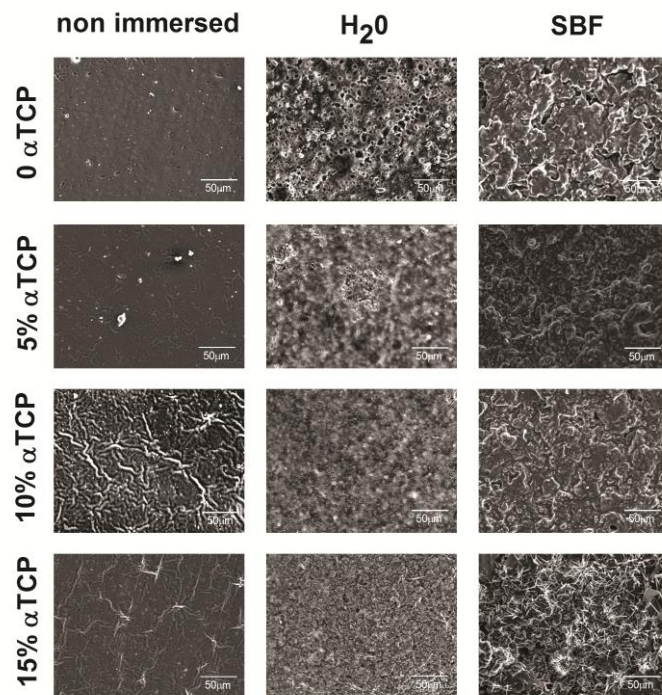
Figure 5. SEM (500x magnification) of the surface of each sealer after different treatments.
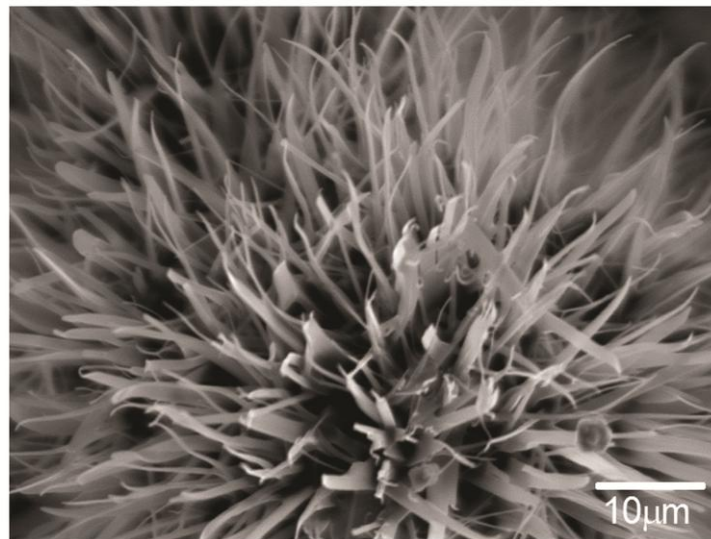

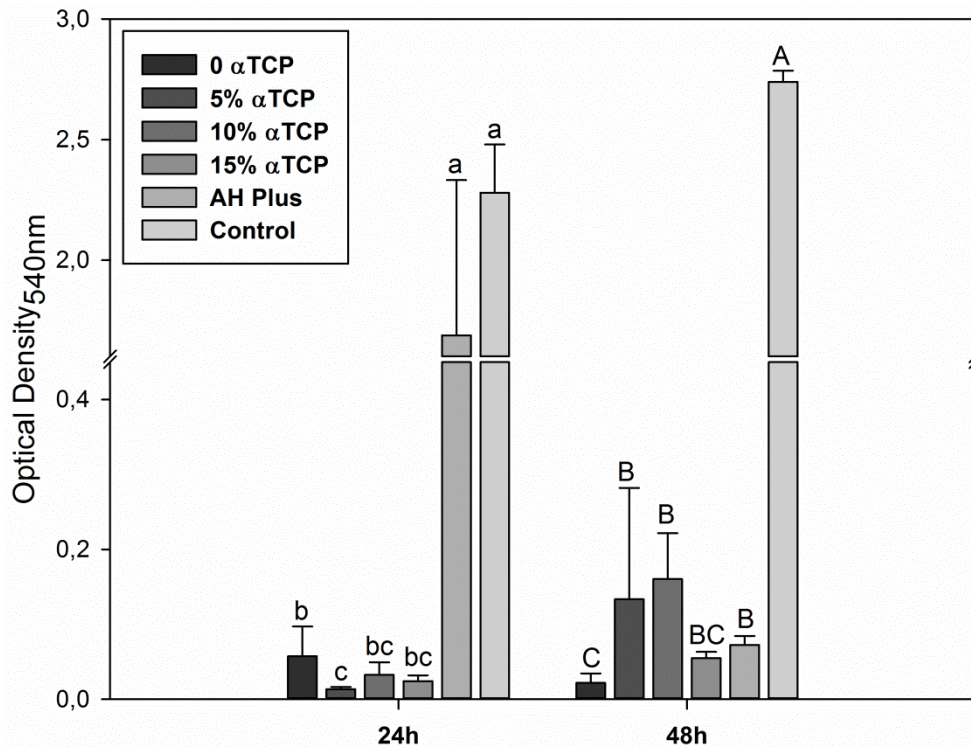**Figure 6.** SEM (2000x magnification) of 15% αTCP sealer after SBF immersion for 7 days.

Figure 7. Cell viability (±standard deviation) of L929 upon direct contact with sealers after culturing for 24 h and 48 h. Distinct lowercase letters indicate a significant difference (p<0.05) at 24 h, and distinct capital letters indicate a difference at 48 h.

DISCUSSION

Grossman's concept[19] posits that inert materials are preferred to root canal sealers (discussed recently). New bioactive root canal sealers introduced in clinical use accelerate healing of apical tissues and promote effective sealing of the root canal system[20, 21]. The α-tricalcium phosphate sealers engineered in this study presented bioactive potential to be used in periapical teeth tissues. The addition of αTCP increased the experimental glycerol salicylate root canal sealer properties.

Hardening of the salicylate sealers is promoted by calcium chelation achieved by a 1:5 ratio of calcium hydroxide to resin[7]. To ensure correct stoichiometry of the chemical reaction, 30%wt of inorganic compounds with at least 15% calcium hydroxide was used for the development of sealers. The setting reaction time ranged from 240 to 405 min, similar to commercial sealers[22]. The faster setting time was recorded at 0% and 5% αTCP groups (30% and 25% calcium hydroxide, respectively). The high reactivity and availability of calcium hydroxide in comparison to αTCP may explain the faster reaction obtained. Further, the conversion of αTCP

into a calcium-deficient hydroxyapatite (CDHA) by hydration[15, 23] could retard the sealer setting, as in the following equation:

$$3\ \alpha Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH.$$
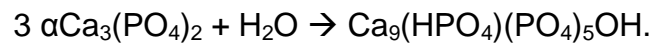
Groups with 0% and 5% αTCP presented the same setting time and increased pH, probably due to a fraction of calcium hydroxide not reacting that was quickly released. The pH increased for all groups after 28 days. As the concentration of αTCP decreased, an increase in pH was observed due to decreased hydroxyl ion release. The optimum pH of alkaline phosphatase activity in bone formation is 8.5[24]. In this study, the pH achieved by αTCP sealers was approximately 60% of enzymatic activity[25]. The alkalization potential shown by the experimental sealers could lead to an increased rate of healing process, antimicrobial properties and biocompatibility. The cytotoxicity of experimental sealers was higher than that of the commercial sealer in 24 h, reaching no significant difference after 48 h. These results could be due to an increased setting after 48 h and to a regeneration process of fibroblast cells. The cell viability values measured are in accordance with other studies evaluating several root canal sealers[26, 27] via direct contact with fibroblast cells.

Root canal sealer specimens immersed in SBF presented less weight loss than in water. The SBF ion-saturated medium could have promoted the conversion of αTCP in CDHA, decreasing the calcium phosphate compound solubility[28], which could be observed by the decreased weight loss with increased αTCP concentration. After 28 days in SBF, the 15% αTCP sealers exhibited 31.86% loss in mass in contrast with 60.63% of the 0 αTCP sealer in the same period. Faria-Júnior *et al.*[6] evaluated the solubility of salicylate-based sealers after 15 h of water immersion with values ranging from 9.97% to 15.03%. The authors showed that higher solubility leads to increased pH and efficacy against *Enterococcus faecalis* biofilm.

A requirement for biomaterials is the capacity to promote the deposition of bone-like apatite on the surface when implanted in a living body[29]. Interaction between the bioactive sealer and fluid tissues has been viewed as a promising approach to heal a compromised seal[21]. In this study, sealers with 10% and 15% αTCP showed a noticeable phosphate content on the surface independent of immersion media. Water presented in a medium leads to the transformation of αTCP into CDHA[15], and a release of Ca^{2+} and PO_4^{3-} could lead to nucleation and crystallization as apatite. In water-immersed specimens, an erosion process could be observed (Fig. 5)[30] as the calcium hydroxide concentration increased. Groups with

15% αTCP showed no surface erosion due to a decreased content of calcium hydroxide. The peak of calcium hydroxide (1084cm^{-1}) was present in the Raman spectra of water immersed specimens[7]. After SBF immersion, specimens presented an irregular surface with grains with higher calcium and phosphate concentrations than the non-immersed samples, indicating an apatite layer growth. Szuber *et al.*[16] noted similar change patterns for α-tricalcium phosphate cement after SBF immersion. The SEM-EDX showed the early formation of a calcium-phosphate layer on a sealer's surface. The calcium phosphate formed on a sample surface suggests the bioactivity of αTCP sealers. *In vivo*, the clastic cells of periapical tissue could partially dissolve this calcium-phosphate layer, releasing Ca^{2+} and PO_4^{3-}, leading to the precipitation of carbonated hydroxyapatite due to supersaturation. The precipitation of stains and crystallites of calcium phosphate at voids between core materials and sealer could enhance the sealing. A study showed that calcium silicate–based sealers are capable of promoting the precipitation of calcium-phosphate deposits on interfacial voids of obturation materials, reducing the empty spaces and improving the sealing ability in comparison to traditionally used sealers[31]. Furthermore, the apatite layer formed could facilitate protein adsorption and osteprogenitor cell attachment. The calcium and phosphate ions released trigger the osteoblastic proliferation and differentiation to achieve biomineralization[32, 33], leading to a biological seal.

Therefore, the experimental root canal sealer obtained from the mixture of glycerol salicylate resin, calcium hydroxide and α-tricalcium phosphate showed solubility, hydroxyl ion release and surface changes in the formation of an apatite layer suitable to be used as a bioactive sealer for root canal filling. Thus, glycerol salicylate sealers with 10% and 15% α-tricalcium phosphate showed reliable physical chemical properties and apatite-forming ability.

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4. CONSIDERAÇÕES FINAIS

Esforços visando ao desenvolvimento de novos cimentos obturadores que propiciem um melhor vedamento em comparação aos materiais disponíveis no mercado, são necessários, uma vez que o selamento do sistema de canais radiculares tem papel fundamental no sucesso da terapia endodôntica. Nesse estudo foi proposto um cimento obturador à base de uma resina de glicerol salicilato e α-fosfato tricálcico. Espera-se que o emprego da resina de glicerol salicilato produza um material com menor solubilidade, quando comparado aos cimentos comerciais contendo resina de isobutil salicilato, e que a adição de α-fosfato tricálcico confira bioatividade ao cimento, de forma que a interação com os tecidos periapicais leve a uma deposição mineral da superfície do material, melhorando o selamento e estimulando o reparo tecidual.

Os objetivos propostos na presente tese foram alcançados e estão contemplados em dois artigos, de forma que o Artigo I mostra o processo de obtenção da nova resina de salicilato e confirma a sua aplicabilidade para uso em cimentos que contenham íons cálcio disponíveis para reagir com os grupos salicilato da resina, auferindo presa química. No Artigo II foram formulados cimentos a partir da resina sintetizada e da adição de hidróxido de cálcio e α-fosfato tricálcico, em diferentes concentrações. Observou-se que maiores concentrações de α-fosfato tricálcico (10% e 15%) produziram cimentos mais resistentes à degradação em água e com potencial de estimular a deposição de fosfatos de cálcio sobre a sua superfície.

Espera-se que a precipitação mineral, observada *in vitro* na superfície dos discos obtidos com o cimento desenvolvido, quando empregado para obturação de canais radiculares também ocorra na interface obturação/dentina e na região periapical, otimizando o selamento apical, e consequentemente a taxa de sucesso da terapia. Os resultados favoráveis obtidos nestes trabalhos justificam plenamente mais investimentos na avaliação e ajuste, se necessário, das propriedades de escoamento, espessura de película e radiopacidade, de acordo com a ISO 6876 (Dental root canal sealing materials), para viabilizar o uso clínico desse material. Além disso, as propriedades físicas e químicas do cimento podem ser ajustadas

para que o mesmo possa ser, utilizado também para proteção pulpar, haja vista que a alcalinização e a deposição mineral promovidas pelo material são desejadas tanto para a formação de dentina reacional quanto para a remineralização dentinária, nos casos de remoção parcial de tecido cariado.

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