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ANÁLISE FÍSICO-QUÍMICA E HISTOLÓGICA DE UM CIMENTO DE REPARO ENDODÔNTICO EXPERIMENTAL À BASE DE BIOGLASS 45S5®.

SÃO LUÍS
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MICHAEL RANNIERY GARCIA RIBEIRO

ANÁLISE FÍSICO-QUÍMICA E HISTOLÓGICA DE UM CIMENTO DE REPARO ENDODÔNTICO EXPERIMENTAL À BASE DE BIOGLASS 45S5®.

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Orientadora: Profª Drª Soraia de Fátima Carvalho Souza.

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MICHAEL RANNIERY GARCIA RIBEIRO

ANÁLISE FÍSICO-QUÍMICA E HISTOLÓGICA DE UM CIMENTO DE REPARO ENDODÔNTICO EXPERIMENTAL À BASE DE BIOGLASS 45S5®.

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Orientadora- Profª Drª Soraia de Fátima Carvalho Souza

São Luís, 26 de fevereiro de 2018

Ao meu avô materno, Américo Oliveira Garcia (in memorian
que fez o "desejo de estudar" atravessar
gerações em nossa família.

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"Não vale a pena mergulhar nos sonhos
e esquecer de viver".

Alvo Dumbledore em Harry Potter e a Pedra Filosofal – *J. K. Rowling*

LISTA DE ABREVIATURAS

µL= Microlitro

µm= Micrômetro

ADD= Água destilada e deionizada

Al= Alumínio

BG= Bioglass 45S5®

BioG= Grupo com 40% de BG e 60% de OZn

Cp= Corpo-de-prova

Ca= Cálcio

E= Módulo de elasticidade

g= gramas

GPa= Gigapascal

HA= Hidroxiapatita

HC= Hidróxido de Cálcio

kV= Kilovoltagem

mA= miliamper

MEV= Microscopia eletrônica de varredura

mg= miligramas

min= Minuto

mL= Mililitro

mm= Milímetro

MPa= Megapascal

MTA= Agregado Trióxido Mineral

N= Newton

nm= nanômetro

Ø= Diâmetro

ºC= Graus Celsius

OZn= Óxido de zinco

PBS= Solução tampão de fosfato

pH= Potencial hidrogeniônico

PO_4= Fosfato

Rd= Radiopacidade

RMC= Resistência máxima à compressão

RPM= Rotações por minuto

RRE= Reabsorção radicular inflamatória externa

SCR= Sistema de canais radiculares

ANÁLISE FÍSICO-QUÍMICA E HISTOLÓGICA DE UM CIMENTO DE REPARO ENDODÔNTICO EXPERIMENTAL À BASE DE BIOGLASS 45S5®.

RESUMO

Foi conduzido um estudo in viro e in vivo com um cimento experimental de reparo endodôntico contendo vidro bioativo 45S5. Foram testados três grupos de cimento de reparo endodôntico: À base de vidro bioativo 45S5 (BioG), à base de óxido de zinco (ZnO) e agregado de trióxido mineral (MTA). Testes in vitro foram utilizados para avaliar suas propriedades físico-químicas: resistência à compressão, módulo de elasticidade, radiopacidade, variação do pH e liberação iônica de Ca^{+} e PO_4. Um modelo animal foi usado para avaliar a resposta do tecido ósseo ao cimento de reparo endodôntico. Empregou-se na avaliação estatística o teste t não pareado ou ANOVA e teste de Tukey (α= 5%). O BioG apresentou a menor resistência à compressão e o ZnO a maior radiopacidade entre os grupos, respectivamente ($p < 0,05$). Não houve diferenças significativas no módulo de elasticidade entre os grupos. BioG e MTA mantiveram pH alcalino durante os 7 dias de avaliação, tanto em pH 4 quanto em solução tamponada de pH 7. A liberação de PO_4 foi elevado no BioG, com pico em 7 dias ($p < 0,05$). A análise histológica mostrou reações inflamatórias menos intensas e neoformação óssea no MTA. O BioG mostrou reações inflamatórias que diminuíram com o tempo. O cimento experimental BioG apresentou boas características físico-químicas e biocompatibilidade necessária para o cimento endodôntico de reparo bioativo.

Palavras-chave: Biovidro. Endodontia. Histológico. MTA. Reabsorção Radicular.

PHYSICOCHEMICAL AND HISTOLOGICAL ANALYSIS OF AN EXPERIMENTAL ENDODONTIC REPAIR CEMENT CONTAINING 45S5 BIOACTIVE GLASS

ABSTRACT

An in vitro and in vivo study with an experimental endodontic repair cement containing 45S5 bioactive glass was conducted. There were three endodontic repair cement groups: 45S5 bioactive glass-based (BioG), zinc oxide-based (ZnO), and Mineral Trioxide Aggregate (MTA). In vitro tests were used to evaluate their physicochemical properties: compressive strength, modulus of elasticity, radiopacity, pH variation, and the ionic release of Ca^{+} and PO_4. An animal model was used to evaluate the bone tissue response to endodontic repair cement. Comparisons employed an unpaired t-test or ANOVA and Tukey's test (α = 5%). BioG showed the lowest compressive strength and ZnO showed the highest radiopacity among the groups, respectively ($p < 0.05$). There were no significant differences in the modulus of elasticity among the groups. BioG and MTA maintained an alkaline pH during the 7 days of evaluation, both at pH 4 and in a pH 7 buffered solutions. PO_4 was elevated in BioG, peaking at 7 days ($p < 0.05$). Histological analysis showed less intense inflammatory reactions and new bone formation in MTA. BioG showed inflammatory reactions that decreased over time. The BioG experimental cement had good physicochemical characteristics and biocompatibility required for bioactive endodontic repair cement.

Keywords: Bioglass. Endodontics. Histological. MTA. Root resorption

SUMÁRIO

1. INTRODUÇÃO

1.1 Reabsorções radiculares

Os tecidos dentários podem sofrer processos reabsortivos de maneira fisiológica ou patológica (DARCEY e QUALTROUGH, 2013). As reabsorções dentárias resultam da interação coordenada e complexa entre cementoblastos, cementoclastos, odontoblastos e odontoclastos (IGLESIAS-LINARES e HARTSFIELD, 2017).

Citocinas, hormônios esteróides sexuais, paratormônio e calcitonina são alguns dos inúmeros fatores regulatórios sistêmicos envolvidos no mecanismo das reabsorções dentárias (VAANANEN, 2005). As reabsorções dentárias patológicas podem estar associadas a doenças sistêmicas, tais como distúrbios endócrinos (CHAVES NETTO e colab., 2009), hipoparatireoidismo (ROBINSON e HARVEY, 1989), Síndrome de Turner (FILIPSSON e colab., 1965) e Doença de Paget (ANBINDER e colab., 2007). O selamento deficiente do sistema de canais radiculares, os traumas dentoalveolares, tratamentos ortodônticos, infecções bacterianas e os processos neoplásicos são considerados fatores etiológicos locais das reabsorções radiculares (BAKLAND, 1992).

As reabsorções radiculares podem ser classificadas quanto à localização da lesão, sendo interna ou externa, com um variado número de subclassificações (DARCEY e QUALTROUGH, 2013; TRONSTAD, 1988). As reabsorções internas são desencadeadas por traumas ou pulpite crônica e ocorrem pela ativação de dentinoclastos que reabsorvem as paredes internas dentinárias. Geralmente, apenas o tratamento endodôntico é suficiente para conseguir a paralisação desse tipo de reabsorção (DARCEY e QUALTROUGH, 2013).

As reabsorções externas têm múltiplos fatores etiológicos e podem ser classificadas como substitutivas, cervicais ou inflamatórias (HEGDE e HEGDE, 2013). Dentre os subtipos das reabsorções externas, as reabsorções radiculares inflamatórias externas (RRE) são o foco deste trabalho. Geralmente subdiagnosticadas, as RRE são sequelas comuns após injúrias traumáticas ou infecções periodontais, incluindo as periodontites apicais (FERNANDES e colab., 2013). Originam-se a partir de lesões que alteram a camada protetora radicular externa associada a um processo inflamatório subsequente. A dentina é exposta após destruição da camada de pré-cemento e torna-se suscetível à atuação de osteoclastos (GOLD e HASSELGREN, 1992). As RRE são frequentemente associadas a infecções de origem microbiana e geralmente ocorrem sem nenhuma sintomatologia clínica (ASGARY e AHMADYAR, 2011).

O princípio fundamental relacionado ao tratamento de qualquer reabsorção é a paralisação da atividade das células clásticas, que pode ser conseguida pela remoção da fonte de estímulo, sendo necessária posterior indução do reparo (VAANANEN, 2005). Nos casos de RRE a remoção da fonte de estímulo está intimamente relacionada à realização do tratamento endodôntico. Assim, se a reabsorção provém de um processo inflamatório infeccioso, o estímulo deve ser interrompido por meio do tratamento endodôntico.

1.2 Hidróxido de cálcio no tratamento das RRE

O tratamento das RRE geralmente consiste na desinfecção do sistema de canais radiculares (SCR). Essa desinfecção é feita por meio da modelagem e desinfecção do SCR , e manutenção de pasta à base de hidróxido de cálcio (HC) como medicação intracanal (HC) por um período que varia entre 6 e 24 meses (CONSOLARO e FURQUIM, 2014; RAFTER, 2005). Essa manutenção de HC não é permanente, portanto necessita de trocas periódicas para manter seu efeito antibacteriano. Esse efeito ocorre devido ao aumento do pH da dentina pela difusão de íons hidroxila (OH) através dos túbulos dentinários (FUSS e colab., 2003).

A atividade antimicrobiana do HC está relacionada à liberação de OH em ambiente aquoso elevando o pH local. Ocorre devido aos danos a membrana citoplasmática bacteriana, desnaturação protéica e lesão do DNA bacteriano (RAHIMI e colab., 2014). Entretanto, alguns microrganismos associados a infecções endodônticas são resistentes ao HC, como o *Enterococcus faecalis* e a *Candida albicans* (RAHIMI e colab., 2014). O *E. faecalis* é um microorganismo anaeróbio facultativo e um dos mais resistentes a desinfecção do SCR. Geralmente está presente nas periodontites apicais resistentes (MEHRVARZFAR e colab., 2011).

O aumento do pH local causado pelo HC paralisa a ação das células clásticas e ativa a função das células odontoblásticas. Durante esse processo ocorre a formação cementoblástica que recoloniza a superfície radicular. Em seguida, forma-se novo cemento reinserindo as fibras colágenas entre a nova camada cementoblástica (CONSOLARO, 2011; FUSS e colab., 2003).

A pasta de hidróxido de cálcio tem efeito limitado devido a sua dissolução, dessa forma com o tempo ela perde a capacidade de manter o pH alcalino. Na dependência do veículo utilizado, o tempo de dissolução pode ser maior ou menor, sendo necessária a troca da medicação (SIMON e colab., 1995). No entanto, além de ter efeito com duração

limitada causada pela sua dissolução rápida com posterior necessidade de troca, a manutenção intracanal da pasta de HC por longo período de tempo altera o módulo de elasticidade, resistência flexural e resistência à fratura da dentina radicular (MARENDING e colab., 2009; RIBEIRO e colab., 2017; VALERA e colab., 2015).

1.3 Agregado Trióxido Mineral (MTA) no tratamento das RRE

Além da pasta de HC, a utilização de cimentos de reparo endodôntico tem sido indicada no tratamento das RRE. O mais utilizado são os cimentos de silicato tricálcico, entre eles o Agregado Trióxido Mineral (MTA). Atualmente, o MTA é usado com segurança para tratamentos conservadores da polpa, apicificações e no tratamento de reabsorções radiculares (PARIROKH e TORABINEJAD, 2010b). A utilização do MTA é importante principalmente quando o dente se encontra fragilizado pela extensão da reabsorção. Esse material interage com os fluidos teciduais, age sobre os tecidos periodontais, induz o reparo dos tecidos periapicais, apresenta boa capacidade seladora e baixas propriedades mecânicas, principalmente resistência à compressão e módulo de elasticidade (NATALE e colab., 2015; PARIROKH e TORABINEJAD, 2010a). A hidratação dos componentes dicálcio e tricálcio silicato induz a liberação de hidróxido de cálcio, responsável por sua alta alcalinidade (pH entre 10,5 e 12,5), o que interrompe o processo odontoclástico (PARIROKH e TORABINEJAD, 2010a; SCHWARTZ e colab., 1999).

A liberação de OH com consequente aumento da alcalinidade pelo MTA, pode variar de acordo com o meio onde ele é empregado ou solução onde é testado. Natale et al. (2015) (NATALE e colab., 2015) comparando a liberação de cálcio entre diferentes formulações de MTA verificaram que em meios ácidos (como em condições de inflamação periapical) esses cimentos diminuem em até 24% a capacidade de liberação iônica de cálcio. Meios ácidos têm mostrado capacidade em inibir a cristalização do MTA com consequente redução das propriedades mecânicas e aumento da solubilidade desse material (ROY e colab., 2001). Nesse contexto, é importante ressaltar que o MTA diminui a resistência à flexão e módulo de elasticidade da dentina radicular pela degradação do colágeno dentinário (WHITE e colab., 2002).

1.4 Biovidros no tratamento das RRE

Desde a introdução do ProRoot MTA em 1998 (SCHMITT e colab., 2001), várias associações de compostos ao MTA tem sido feitas com objetivo de diminuir as desvantagens desses cimentos (ASGARY e colab., 2008). Para ser utilizado no tratamento das RRE o cimento reparador ideal deve ser antibacteriano, radiopaco, biocompatível, ter fácil manipulação, manter pH alcalino e promover o reparo dos tecidos perirradiculares (CHANIOTIS, 2016; PARIROKH e TORABINEJAD, 2010b).

Além dessas características básicas, a bioatividade e capacidade de paralisação dos processos reabsortivos são propriedades que novos cimentos reparadores devem alcançar. Biovidros que liberam compostos de silicato solúveis apresentam capacidade em acelerar a formação de tecido ósseo pela promoção da atividade gênica em células ósseas reguladoras (XYNOS e colab., 2001).

Tem sido mostrado que vidros alcalinos bioativos do sistema SiO_2-Na_2O-CaO-P_2O_5 atuam como antibactericidas em canais radiculares infectados (MEHRVARZFAR e colab., 2011; ZHANG e colab., 2010). Esses biovidros já demonstraram atividade antibacteriana contra *E. faecalis* (MEHRVARZFAR e colab., 2011). Além desse efeito bactericida que também é apresentado pelo MTA e hidróxido de cálcio, os vidros bioativos possuem bioatividade e potencial remineralizador (JONES, 2015). Diferente do hidróxido de cálcio e MTA, a hidratação das partículas do biovidro leva a liberação de cálcio, sílica, fosfato e sódio. Essa liberação iônica promove um efeito antibacteriano adicional indiretamente relacionado ao pH (GUBLER e colab., 2008; STOOR e colab., 1998). Ademais, os efeitos negativos dos biovidros nas propriedades mecânicas dentinárias parecem ser menores que as causadas pelo hidróxido de cálcio e MTA (MARENDING e colab., 2009; NATALE e colab., 2015).

Dentre os tipos de biovidro existentes, o Bioglass 45S5® (BG) se destaca pelas concentrações de SiO, Ca, NaO, H e P, assim como as proporções moleculares de óxidos de cálcio e fósforo similares as encontradas nos ossos humanos (KRISHNAN e LAKSHMI, 2013; WANG, Xiaodu e colab., 2001). Estudos prévios demonstraram que a instabilidade química superficial de vidros bioativos leva a alta liberação iônica em curto espaço de tempo (HU e colab., 2009; ZHANG e colab., 2010). Em ambiente aquoso, uma série de reações ocorre na superfície das partículas do BG, incluindo liberação iônica de Ca, Si e $NaPO_4$, com aumento na pressão osmótica do meio (BELLANTONE e colab., 2002; HENCH e JONES, 2015; STOOR e colab., 1998). Hu et al. (2009) apontam que o BG alcança pH em 9,8 numa concentração de 50 mg/mL em uma hora (HU e colab., 2009).

O BG apresenta capacidade em regenerar tecidos, formar hidroxiapatita (HA) e ligação com tecidos duros (KRISHNAN e LAKSHMI, 2013; XYNOS e colab., 2001). A HA é química e estruturalmente similar ao tecido ósseo humano. Desse modo, osteoblastos podem proliferar-se na camada de HA formada sobre o biovidro estabelecendo uma forte ligação com o tecido ósseo (HENCH e JONES, 2015). Quando utilizado em modelos simulados de reabsorções esse material inibiu significativamente a formação dos osteoclastos e expressões genéticas de reabsorção óssea *in vitro* (MLADENOVIĆ e colab., 2014).

Considerando essa bioatividade e biomodulação celular vários estudos tem sido conduzidos associando o BG a diferentes materiais dentários, mostrando resultados positivos referentes ao seu potencial em remineralizar estruturas dentais desmineralizadas (BAKRY e colab., 2014; JONES, 2015; WANG, Zhejun e colab., 2011; YANG e colab., 2016). Associações de BG a cimentos endodônticos e guta-percha foram conduzidas demonstrando melhoras no selamento apical, precipitação de Ca/P e indução de alto pH (HEID e colab., 2016; MOHN e colab., 2010). Apesar disso, ainda não existem estudos que considerem a associação de BG a cimentos de reparo endodônticos.

Diante da necessidade de um cimento de reparo endodôntico bioativo para o tratamento das RRE, que tenha propriedades mecânicas necessárias para sua utilização num ambiente intracanal, promover um ambiente com pH alcalino e liberar íons necessários para estimular a mineralização dos tecidos, ser radiopaco, além de possuir atividade antimicrobiana (CARVALHO e colab., 2016), este estudo avaliou a resistência máxima compressiva (RMC), o módulo de elasticidade (E), variação de pH, liberação iônica, radiopacidade (Rd) e resposta histológica de um cimento de reparo endodôntico experimental à base de BG.

2. CAPÍTULO 1

(Artigo submetido ao periódico *International Endodontic Journal*)

Physicochemical and histological analysis of an experimental endodontic repair cement containing 45S5 bioactive glass

Michael Ranniery Garcia Ribeiro[1,2], Vandilson Pinheiro Rodrigues[2], Hudson Guterres Guilherme[3], Alina Neres Braga[3], Afonso Gomes Abreu Júnior[4], Érika Martins Pereira[3], José Bauer[2], Soraia de Fátima Carvalho Souza[2]

[1]School of Dentistry, CEUMA University, Imperatriz, Brazil

[2] Postgraduate Dentistry Program, Federal University of Maranhão, São Luís, Brazil

[3]School of Dentistry, Federal University of Maranhão, São Luís, Brazil

[4]School of Dentistry, CEUMA University, São Luís, Brazil

Running Title: **45S5 experimental endodontic repair cement**

Keywords: Bioglass. Endodontics. Histological. MTA. Root resorption

Corresponding author:

Soraia de Fátima Carvalho Souza

Universidade Federal do Maranhão, Programa de Pós-Graduação em Odontologia, Avenida dos Portugueses, 1966, Bacanga, 65.085-580, São Luís, MA, Brazil.

Tel. +5598991901911

E-mail:

endosoraia@gmail.com

Abstract

An in vitro and in vivo study with an experimental endodontic repair cement containing 45S5 bioactive glass was conducted. There were three endodontic repair cement groups: 45S5 bioactive glass-based (BioG), zinc oxide-based (ZnO), and Mineral Trioxide Aggregate (MTA). In vitro tests were used to evaluate their physicochemical properties: compressive strength, modulus of elasticity, radiopacity, pH variation, and the ionic release of Ca^{+} and PO_4. An animal model was used to evaluate the bone tissue response to endodontic repair cement. Comparisons employed an unpaired t-test or ANOVA and Tukey's test (α = 5%). BioG showed the lowest compressive strength and ZnO showed the highest radiopacity among the groups, respectively ($p < 0.05$). There were no significant differences in the modulus of elasticity among the groups. BioG and MTA maintained an alkaline pH during the 7 days of evaluation, both at pH 4 and in a pH 7 buffered solutions. PO_4 was elevated in BioG, peaking at 7 days ($p < 0.05$). Histological analysis showed less intense inflammatory reactions and new bone formation in MTA. BioG showed inflammatory reactions that decreased over time. The BioG experimental cement had good physicochemical characteristics and biocompatibility required for bioactive endodontic repair cement.

Keywords: Bioglass. Endodontics. Histological. MTA. Root resorption

Introduction

Different approaches have been proposed for the treatment of external inflammatory root resorption (EIRR), and most of them have focused on procedures using intracanal materials (Yoshpe *et al.* 2020). Calcium hydroxide pastes have been used as intracanal medication in cases of EIRR. However, the need for periodic changes and long-term use can alter the mechanical properties of the root dentin (Ribeiro *et al.* 2017).

An alternative to calcium hydroxide pastes are endodontic repair cements. The ideal repair cement for EIRR must be antibacterial, radiopaque, biocompatible, easy to handle, maintain an alkaline pH and promote the repair of periradicular tissues (Parirokh & Torabinejad 2010b). Repair cements for endodontic use are not subjected to direct occlusal loads, but resistance to compression is important for their handling and insertion into resorptive defects. Although there is still no material with all of these characteristics, the Mineral Trioxide Aggregate (MTA) is currently considered the gold standard among the repair cements available on the market (Parirokh & Torabinejad 2010b). However, the difficulty of its manipulation and insertion into the appropriate sites, prolonged setting time, and dentin discoloration, are disadvantages of this kind of cement (Parirokh & Torabinejad 2010a).

Previous research has suggested that alkaline bioactive glasses of the SiO_2-Na_2O-CaO-P_2O_5 system act as bactericides in infected root canals (Mehrvarzfar *et al.* 2011, Zhang *et al.* 2010). In addition to the bactericidal effect, bioactive glasses have bioactivity and remineralizing potential (Jones 2015). Hydration of the bioglass particles causes the release of calcium, silica, phosphate and sodium. This ionic release indirectly promotes an additional pH-related antibacterial effect (Mehrvarzfar et al., 2011). The adverse effects of bioglass on dentin mechanical properties appear to be less than those caused by calcium hydroxide and MTA (Marending *et al.* 2009, Natale *et al.* 2015, Ribeiro *et al.* 2017).

Among the existing types of bioglass, Bioglass 45S5® stands out for being composed of molecular proportions of calcium oxides and phosphorus similar to those found in human bones (Krishnan & Lakshmi 2013). This bioglass has the ability to regenerate tissues, form hydroxyapatite and bind to hard tissues (Xynos *et al.* 2001). Hydroxyapatite is chemically and

structurally similar to human bone tissue. In this way, osteoblasts can proliferate in the hydroxyapatite layer formed on the bioglass, establishing a strong connection with the bone tissue (Hench & Jones 2015). In vitro resorption models have shown that bioglass significantly inhibited the formation of osteoclasts and the expression of genes involved in bone resorption (Mladenović *et al.* 2014).

Associations of 45S5 with endodontic sealers and gutta-percha demonstrated effectiveness in apical sealing, greater Ca/P precipitation and high pH induction (Heid *et al.* 2016). Despite this, there are no studies evaluating 45S5 as an endodontic repair cement. In view of the need for an effective bioactive material for the treatment of EIRR, this study evaluated the maximum compressive strength, the modulus of elasticity (E), pH variation, ionic release, radiopacity (Rd) and biological response of an experimental endodontic repair cement based on Bioglass 45S5®.

Materials and methods

Study design and endodontic repair materials

This study was conducted to evaluate the in vitro physicochemical properties and in vivo bone tissue response using an animal model. The experimental protocol was approved by the Ethics Committee on Animal Use of CEUMA University (no. 06/2017). The tests were performed on three endodontic repair material groups: 45S5 bioactive glass-based (BioG), zinc oxide-based (ZnO), and MTA.

The BioG experimental cement was prepared with 40% 45S5 bioactive glass (NovaBone Products, Alachua, FL, USA) and 60% zinc oxide (Sigma Aldrich, St Louis, MI, USA). The ZnO and MTA (Angelus, Londrina, SC, Brazil) cements were used at a 100% concentration as control groups. The BioG and ZnO cements were mixed with distilled and deionized water (DDW) at the proportion of 1:3, weighed using a high precision electronic balance (AD200 model, Marte, SP, Brazil). The MTA cement was mixed according to the manufacturer's instructions. The materials were placed in polypropylene tubes (1.5 mL) and homogenized using a vortex for 1 minute.

Specimens' preparation

Specimens were made in a plastic matrix (12 mm × 6 mm Ø) according to the ANSI / ADA specification No. 66 (Mallmann *et al.* 2007), and distributed among the tests of maximum compressive strength, pH variation and ionic release. A polyester strip was inserted below and above the matrix for packaging the materials and promoting surface smoothness. The cements were inserted in three increments interspersed with 1 minute of vibration to avoid the formation of air bubbles. After 24 hours in an oven at 37ºC ± 1ºC and 100% humidity, they were removed from the dies and their edges were smoothed with sandpaper No. 1200 (Skill-Tec, São Paulo, SP, Brazil).

Compressive strength and modulus of elasticity

Thirty specimens were submitted to the compressive test (n = 10, per group) using a universal mechanical testing machine (Instron 3342, Canton, MA, USA) with a 50 N load and a speed of 0.5 mm/min. The compressive strength (in MPa) and modulus of elasticity (in GPa) values were calculated directly by the testing machine software.

Radiopacity (Rd)

To determine the radiopacity, 3 acrylic matrices with 5 holes (1 mm × 10 mm Ø) were used to make the specimens (n = 5 per group). After conditioning, the cements were flattened with a glass plate overlay. The set was kept in an oven at 37ºC ± 1ºC and 100% humidity for 48 hours. The thickness of the specimens was checked with a digital caliper (Mitutoyo, Tokyo, Japan) and the excess was removed with sandpaper No. 1200. An aluminum 12-step penetrometer (1 mm per step) was placed over the semi-direct digital plate sensor (Orion Corporation Soredex, Helsinki, Finland) to allow for analysis of the radiographic density. The radiographs were taken by an X-ray machine (Focus, Kavo Brasil, Joinville, SC) at 70 KVP and 7 mA, for 0.25 seconds with a 40 cm focus-film distance, according to specification No. 57 ANSI / ADA (Institute, 1984). After each exposure, the sensor was read on a Digora Optime® scanner (Orion Corporation

Soredex, Helsinki, Finland). The images were exported to Kodak® Dental Imaging software to read the Rd in pixel values, ranging from 0 to 255. These values were converted to units in mm Al (Húngaro Duarte *et al.* 2009).

pH measurements

Thirty specimens were used for this analysis (n = 10, per group). Buffering was carried out at pH 4 with hydrochloric acid and pH 7 with sodium hydroxide (n = 5, for each solution). Each specimen was immersed in 5 mL of solution and kept in a refrigerator at 4°C ± 1ºC until reading. The pH values were measured using a digital pHmeter (Quimis Q400A, São Paulo, SP, Brazil) in triplicate at 15 min, 30 min, 1 hour, 24 hours, and 7 days after submersion (Xie *et al.* 2017).

Ion release of Ca^+ and PO_4

Fifteen specimens were used to determine the ion release of Ca^+ and PO_4 (n = 5, per group). After 24 hours, 7 and 30 days submerged in 1 mL of DDW, 10 µL and 100 µL aliquots were collected to measure Ca^+ and PO_4, respectively. Colorimetric analysis was performed in triplicate with Arsenazo III and Phosphomobilidate (Doles, Goiânia, GO, Brazil) by using a multiplate spectrophotometer (630 nm; Elx800UV, Biotek Instruments, Winooski, VT, USA) (Carvalho *et al.* 2016, Vogel *et al.* 1983).

Bone tissue response

The in vivo experiment included 45 male rats (*Rattus norvergius, albinus, Wistar*), aged approximately 8 weeks and weighing 300–400 g. The animals were conditioned in plastic boxes (dimensions, 37.5 × 32 × 16 cm^3) in a controlled-environment room (temperature, 23°C ± 3°C; humidity, 25%; light/dark cycle, 12/12 h). The animals received balanced feed and water ad libitum. The animals were divided into three experimental groups (BioG, ZnO and MTA) and each group in three subgroups according to the experimental times (15, 30, and 45 days).

Anesthesia was performed with xylazine hydrochloride solution (Xilazin 2%, Syntec do Brasil Ltd.) and ketamine (Cetamin 10%, Syntec do Brasil Ltd.) Intramuscularly at a dose of 1.5 mL/kg. Then, trichotomy and asepsis were performed, followed by 1.0 cm incisions made parallel to the long axis of the right and left tibias. After syndesmotomy, a cavity 2 mm in diameter was made in the anterior wall of the right and left tibiae with a low rotation carbide bur, under saline irrigation. The cavity was filled with endodontic repair material (BioG, ZnO or MTA) according to its group, and then the surgical wounds were sutured. A solution of iodinated polyvinylpyrrolidone was applied after suturing to the surgical wounds. In the first 48 hours after surgery, the animals received single daily intraperitoneal doses of 0.05 ml of injectable sodium dipyrone 500 mg/mL (Novalgina, Aventis Laboratory, Brazil).

At the end of each experimental period, the rats were euthanized in a gas chamber according to humanitarian techniques (SISBIO: 16716-1-14 / 07/2008). The BioG, ZnO and MTA animals were euthanized at 15, 30 and 45 days after the surgical intervention (n = 5 per each subgroup).

A sample of each subgroup 1 cm equidistant from the endodontic material area was collected to serve as a control group. The tibial areas, where the material was filled during the surgical intervention, were removed and fixed by immersion in a 10% formaldehyde solution buffered in 0.1 M sodium phosphate buffer, pH 7.4 at 4ºC.

The samples were demineralized in a 10% EDTA solution for 30 days followed by routine histological processing. After this step, the specimens were embedded in paraffin and subjected to serial sectioning of 5 μm, followed by hematoxylin and eosin staining. Histological analysis was performed using a light microscope and Axio Vision Rel. 4.8 software (Carl Zeiss, Germany).

The area corresponding to the lower interface between the implanted material and bone was evaluated considering: the presence or absence of necrosis; the frequency and intensity of acute and chronic inflammatory cells (neutrophils, lymphocytes, macrophages, mast cells, plasmocytes and giant foreign body cells); the formation and characteristics of a fibrous capsule

around the implanted material; the possible resorption and replacement of the material by tissue; degeneration and disintegration along with inflammatory cells and blood vessels aspects.

Statistical analysis

The data analysis was conducted using GraphPad Prism version 8 (GraphPad Software Inc., San Diego, USA). Data are presented as mean values ± standard deviation. Normality assessments were performed using the Shapiro-Wilk test. Comparison analysis was made using an unpaired t-test or one-way ANOVA and Tukey's test. The significance level adopted was 5%.

Results

Compressive strength, modulus of elasticity, and radiopacity

Figure 1a shows the significant differences among the three groups ($p < 0.05$). MTA presented the highest compression force while BioG was the lowest. There were no statistically significant differences in the elasticity module among the groups (Fig. 1b). In Figure 1c, the radiopacity was higher in the ZnO than in the other groups ($p < 0.05$).

pH evaluation

Figure 2 shows the variations of the pH values in the tested solutions. At pH 4 (Fig. 2a) and pH 7 (Fig. 2b), both BioG and MTA alkalized the medium, showing a more significant increase after the first 15 minutes ($p < 0.01$), and the pH peaked after 7 days.

Ion release of Ca^+ and PO_4

Figure 3a illustrates the analysis of Ca concentration. There was a significant gradual increase in Ca^+ release in the BioG group during the analyzed period ($p < 0.05$). The peak of Ca concentration in the MTA group occurred at 7 days ($p < 0.05$). In the comparison among groups, MTA showed higher concentrations than BioG at both 24 hours and 7 days ($p < 0.05$).

The PO_4 data are shown in Figure 3b. In the BioG group, the 7 days and 30 days timepoints showed PO_4 concentrations statistically higher than at 24 hours ($p < 0.05$). While in

the MTA group, 30 days showed a statistically lower concentration than at previous times ($p < 0.05$). BioG compared to MTA showed a lower concentration at 24 hours but it was higher at 7 days and 30 days ($p < 0.05$).

Histological analysis

Figure 4 illustrates representative regions of the histological sections in the study groups. The Control Group showed characteristics of integrity with osteocytes and matrix, and normality of the periosteum and medullary region. The BioG group presented with connective tissue and a larger number of blood vessels surrounding the material, the presence of an intense inflammatory infiltrate close to the bone surface region, without osteoclasts, bone marrow rich in connective tissue, inflammatory cells and fibroblasts. In BioG over time, the material showed closer contact with the bone surface and at 45 days there was a reduction in the proportion of inflammatory cells. Internal erosion of the bioactive glass particles was not observed in any of the specimens. The ZnO group showed an intense inflammatory reaction during the entire period evaluated, with subtle resolution in the last period, congested blood vessels and a large number of inflammatory cells close to the material. In the MTA group, connective tissue was observed, rich in fibroblasts and collagen, inflammatory cells in small amounts covering the material and with few blood vessels, the material in close contact with the bone tissue, and signs of bone neoformation.

Discussion

The main findings of this study, which compared maximum compressive strength, pH variation and ionic release tests, among the BioG, ZnO and MTA groups, suggest that the experimental endodontic repair cement at a 40% concentration of 45S5 has the required physicochemical requirements for a bioactive material.

The compressive strength of the endodontic repair cements varies according to the type of material and vehicle used, their proportion, the applied pressure, the mixing technique and the storage conditions. These factors were considered in the standardization of the experimental

groups. According to this study, the highest compressive strength values were recorded for MTA. This characteristic of MTA represents a disadvantage when it comes to the handling of this material and insertion into the location where it should act (Parirokh & Torabinejad 2010a). Although endodontic repair cements are not subjected to direct forces, they do experience compressive forces from intracanal insertion and from masticatory mechanics. High compressive strength values similar to that of MTA can hinder the handling and maintenance of the cement in the resorptive cavity. On the other hand, it is believed that the formation of a superficial porous layer in Bioglass-based cements can reduce their compressive strength values, as noted in this study (Bellucci *et al*. 2011).

DDW was selected as a vehicle for BioG and ZnO cements because it provides faster ionic release of these materials. Eugenol is known to be a known vehicle for ZnO. However, despite the formation of zinc eugenolate being a factor that increases the strength of this cement, this vehicle was not used in this study because it is a genotoxic and mutagenic agent (Hunag *et al.* 2001). In this study, the physical evaluation of the cements, in all groups, presented low values of modulus of elasticity, which is expected for dental cements.

Evaluations of digital radiographic images using photodensiometry and aluminum scales have been previously performed (Carneiro *et al.* 2018, Húngaro Duarte *et al.* 2009). Radiopacity is an important property for endodontic filling materials to confirm the quality of the filling of the root canal system (Carneiro *et al.* 2018). Even without the inclusion of added opacifying agents, BioG obtained radiopacity values greater than 3 mm of Al, complying with specification No. 57 ANSI / ADA (Institute, 1984).

The radiopacity of the MTA is guaranteed by the addition of bismuth oxide. Camilleri *et al*. (2004) suggest that this radiopacifier affects the precipitation of calcium hydroxide released from the MTA and that it does not stimulate cell proliferation (Camilleri *et al.* 2004). In addition, bismuth oxide undergoes greater release when MTA is used under conditions of tissue inflammation (Parirokh & Torabinejad 2010b). Thus, the addition of bismuth oxide can reduce the biocompatibility of MTA.

BioG alkalized both the acidic and neutral medium, as well as releasing Ca and PO_4 over time. These results are consistent with that proposed by Cunha *et al.* (2011), who considered it desirable to increase the pH in the resorptive sites to neutralize the action of clastic cells (Cunha *et al.* 2011). Thus, the ideal bioactive material should favor an increase in pH in the reabsorption area and remineralization of the reabsorbed area. Researchers have pointed out that the induction capacity of dentin remineralization gradually improves when ionic release occurs (Rabiee *et al.* 2015). In this in vitro evaluation, the BioG group reached its peak of Ca^+ and PO_4 release at 30 days, thus being slow and gradual. A rapid ionic release was observed in the MTA at 7 days, when it reached the peak of Ca^+ release and in the first 24 hours for the release of PO4. The saturation of these compounds, mainly Ca^+, as observed in the MTA, induces the formation of crystalline hydroxyapatite that does not participate effectively in tissue binding (Bingel *et al.* 2015).

Alkaline ions such as Na^+ and Ca^{2+} on the surface of the bioglass are exchanged for H^+, causing hydrolysis of silica and changes in pH (Rabiee *et al.* 2015). The formation of hydroxyapatite is significantly faster in acidic challenges than in neutral ones due to the rapid ion exchange and the use of Ca^+ and PO_4 released from the bioglass (Bingel *et al.* 2015). These authors reiterate that such results are of interest for clinical applications of 45S5, since they suggest that low pH conditions are unable to inhibit the formation of hydroxyapatite (Bingel *et al.* 2015).

With respect to the release of Ca^+, the MTA showed high ionic release of this compound associated with an increase in pH, being the highest among all groups ($p < 0.001$). High pH values may not be as clinically desirable, as they induce negative changes in the mechanical properties of root dentin, which can lead to dental fractures (Ribeiro *et al.* 2017). Considering the strong increase in local pH promoted by MTA, we could assume that when indicated for the treatment of EIRR, it could cause negative changes in the dentinal structure (White *et al.* 2002). It is believed that an increase in pH just enough to paralyze the osteoclastic process is more advisable.

Histological analysis showed characteristics related to the formation of fibrous tissue involving the material in the BioG and ZnO groups. In the connective tissues adjacent to the

bioglass particles, a moderate number of fibroblasts, macrophages, and lymphocytes are present. These results are similar to those found by Melo *et al.* in 2005.

The formation of blood vessels related to the three experimental groups at 30 and 45 days, as well as the quantitative decrease in inflammatory cells over time, are in accordance with the evidence available in the literature (Azenha *et al.* 2010). The excellent biocompatibility of 45S5 has already been demonstrated by Azenha *et al.* (2010) in Bioglass 45S5 implants in rabbit femurs. These researchers observed in the implanted sites bone formation and a layer of soft tissue in close contact with the implant surfaces in the spinal canal (Azenha *et al.* 2010).

In both the BioG and MTA groups, the material had intimate contact with the bone tissue, with the formation of blood vessels in this connection. The areas in contact with BioG and MTA showed bone tissue closely related to the material particles, probably due to the osteoinductive property of these materials (Xynos *et al.* 2001). However, MTA was the only group that presented bone neoformation.

The MTA has a fast-setting , even in a humid environment. In addition, the lack of membrane covering the filling materials could justify the lack of bone formation by BioG. This material has a considerable ionic dissociation rate, which can facilitate its dilution in the overlying connective tissue (Jones 2015).

No new bone formation was observed in the Bioglass 45S5 group. We attribute this to the short evaluation period (up to 45 days), which can be considered a limitation of our study. However, it must be considered that the repair of bone defects is achieved not only by the osteoconductive properties of bioactive glass granules, but also by their osteostimulatory effect (Melo *et al.* 2005).

Moreover, BioG has already shown its ability to activate genes that control osteogenic activity and growth factors, inhibition of osteoclast formation and gene expression and bone resorption (Mladenović *et al.* 2014), in addition to stimulating the formation of hydroxyapatite (Jones 2015) and remineralization of dental hard tissues (Xynos *et al.* 2001). Thus, it is believed that BioG could also induce repair of the defect caused by the EIRR (Mladenović *et al.* 2014).

Conclusion

We concluded that the experimental endodontic repair cement with a concentration of 40% of Bioglass 45S5 seems to be a promising material for the treatment of EIRR, as it presents the biocompatibility and resistance to be inserted in the root canal or applied in resorptive areas, can alkalize acid and neutral environments with the gradual release of PO_4 ions, which are involved in its bioactivity.

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References

Azenha MR, Peitl O, Barros VMR (2010) Bone response to biosilicates® with different crystal phases. *Brazilian Dental Journal* **21**, 383–389.

Bellucci D, Cannillo V, Sola A (2011) A new potassium-based bioactive glass: Sintering behaviour and possible applications for bioceramic scaffolds. *Ceramics International*, **37**, 145–157.

Bingel L, Groh D, Karpukhina N *et al.* (2015) Influence of dissolution medium pH on ion release and apatite formation of Bioglass® 45S5. *Materials Letters* **143**, 279–282.

Camilleri J, Montesin FE, Papaioannou S *et al.* (2004) Biocompatibility of two commercial forms of mineral trioxide aggregate. *International Endodontic Journal* **37**, 699–704.

Carneiro KK, Araujo TP, Carvalho EM *et al.* (2018). Bioactivity and properties of an adhesive system functionalized with an experimental niobium-based glass. *Journal of the Mechanical Behavior of Biomedical Materials* **78**, 188–195.

Carvalho CN, Freire LG, de Carvalho APL *et al.* (2016). Ions release and pH of calcium hydroxide–, chlorhexidine– and bioactive glass–based endodontic medicaments. *Brazilian Dental Journal* **27**, 325–331.

Cunha RS, Abe FC, Araujo RA *et al.* (2011). Treatment of inflammatory external root resorption resulting from dental avulsion and pulp necrosis: clinical case report. *General Dentistry*, **59**, e101–e104.

Heid S, Stoessel PR, Tauböck TT *et al.* (2016). Incorporation of particulate bioactive glasses into a dental root canal sealer. *Biomedical Glasses* **2**, 29–37.

Hench LL, Jones JR (2015). Bioactive glasses: Frontiers and challenges. *Frontiers in Bioengineering and Biotechnology* **3**, 194.

Hunag TH, Lii CK, Kao CT (2001). Root canal sealers cause cytotoxicity and oxidative damage in hepatocytes. *Journal of Biomedical Materials Research* **54**, 390–395.

Húngaro Duarte MA, de Oliveira El Kadre GD, Vivan RR *et al.* (2009). Radiopacity of Portland Cement Associated With Different Radiopacifying Agents. *Journal of Endodontics* **35**, 737–740.

Institute ANS (1984). American National Standards Institute. Specification no 57 for endodontic filling materials. *J Am Dent Assoc*, 108:88.

Jones JR (2015). Review of bioactive glass: From Hench to hybrids. In *Acta Biomaterialia* **23**, s53-s82.

Krishnan V, Lakshmi T (2013). Bioglass: A novel biocompatible innovation. *Journal of Advanced Pharmaceutical Technology & Research*, **4**, 78–83.

Mallmann A, Ataíde JCO, Amoedo R *et al.* (2007). Compressive strength of glass ionomer cements using different specimen dimensions. *Brazilian Oral Research* **21**, 204–208.

Marending M, Stark WJ, Brunner TJ *et al.* (2009). Comparative assessment of time-related bioactive glass and calcium hydroxide effects on mechanical properties of human root dentin. *Dental Traumatology* **25**, 126–129.

Mehrvarzfar P, Akhavan H, Rastgarian H *et al.* (2011). An in vitro comparative study on the antimicrobial effects of bioglass 45S5 vs. calcium hydroxide on Enterococcus faecalis. *Iranian Endodontic Journal* **6**, 29–33.

Melo LGN, Nagata MJH, Bosco AF *et al.* (2005). Bone healing in surgically created defects treated with either bioactive glass particles, a calcium sulfate barrier, or a combination of both materials: A histological and histometric study in rat tibias. *Clinical Oral Implants Research* **16**, 683–691.

Mladenović Ž, Johansson A, Willman B *et al.* (2014). Soluble silica inhibits osteoclast formation and bone resorption in vitro. *Acta Biomaterialia* **10**, 406–418.

Natale LC, Rodrigues MC, Xavier TA *et al.* (2015). Ion release and mechanical properties of calcium silicate and calcium hydroxide materials used for pulp capping. *International Endodontic Journal* **48**, 89–94.

Parirokh M, Torabinejad M (2010a). Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties. *Journal of Endodontics* **36**, 16–27.

Parirokh M, Torabinejad M (2010b). Mineral Trioxide Aggregate: A comprehensive literature review-part III: Clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics* **36**, 400–413.

Rabiee SM, Nazparvar N, Azizian M *et al.* (2015). Effect of ion substitution on properties of bioactive glasses: A review. *Ceramics International* **41**, 7241–7251.

Ribeiro MRG, Thomaz ÉBAF, Lima DM *et al.* (2017). Chlorhexidine Prevents Root Dentine

Mineral Loss and Fracture Caused by Calcium Hydroxide over Time. *International Journal of Dentistry*.

Vogel GL, Chow LC, Brown WE (1983). A Microanalytical Procedure for the Determination of Calcium, Phosphate and Fluoride in Enamel Biopsy Samples. *Caries Research* **7**, 23–31.

White JD, Lacefield WR, Chavers LS *et al* (2002). The effect of three commonly used endodontic materials on the strength and hardness of root dentin. *Journal of Endodontics* **28**, 828–830.

Xie X, Wang L, Xing D *et al.* (2017). Novel dental adhesive with triple benefits of calcium phosphate recharge, protein-repellent and antibacterial functions. *Dental Materials* **33**, 553–563.

Xynos ID, Edgar AJ, Buttery LDK *et al.* (2001). Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution. *Journal of Biomedical Materials Research* **55**, 151–157.

Yoshpe M, Einy S, Ruparel N *et al.* (2020). Regenerative Endodontics: A Potential Solution for External Root Resorption (Case Series). *Journal of Endodontics* **46**, 192–199.

Zhang D, Leppäranta O, Munukka E *et al.* (2010). Antibacterial effects and dissolution behavior of six bioactive glasses. *Journal of Biomedical Materials Research - Part A*, **93**, 475–483.

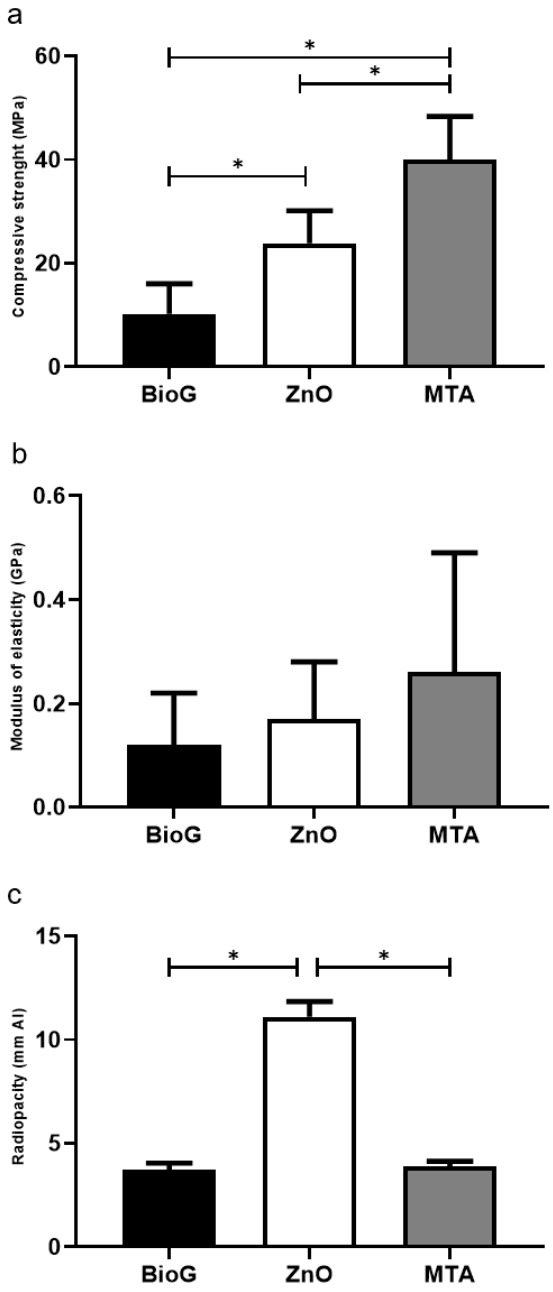

Figure 1. Mean and standard deviation of the mechanical and radiographic evaluation of the groups. Maximum compressive strength (a), modulus of elasticity (b) and radiopacity of the groups after hydration and setting time (c). (*) Indicates statistically significant differences between the material groups ($P < 0.05$).

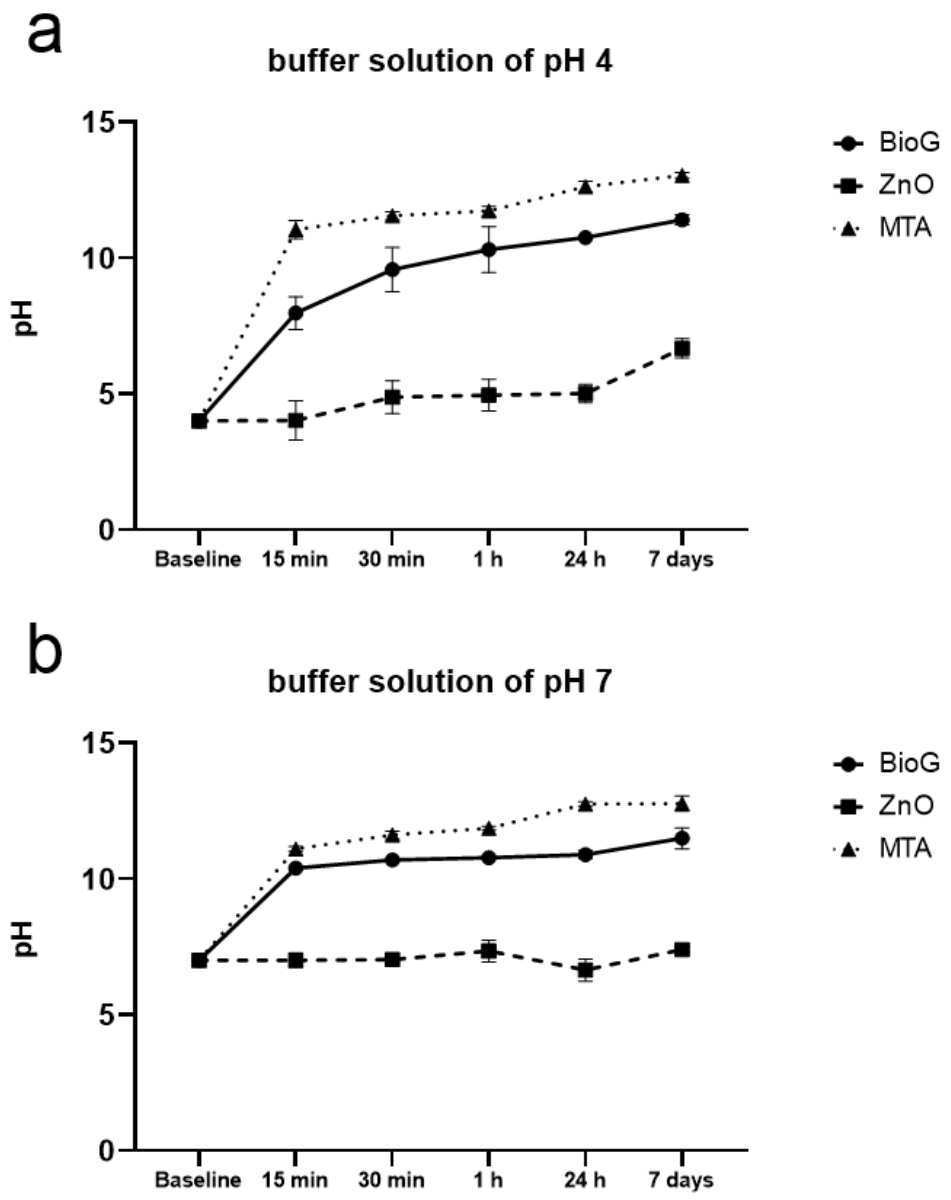

Figure 2. Mean and standard deviation of the pH variation of the groups tested in the buffered solutions (pH 4 and pH 7) at the experimental times evaluated.

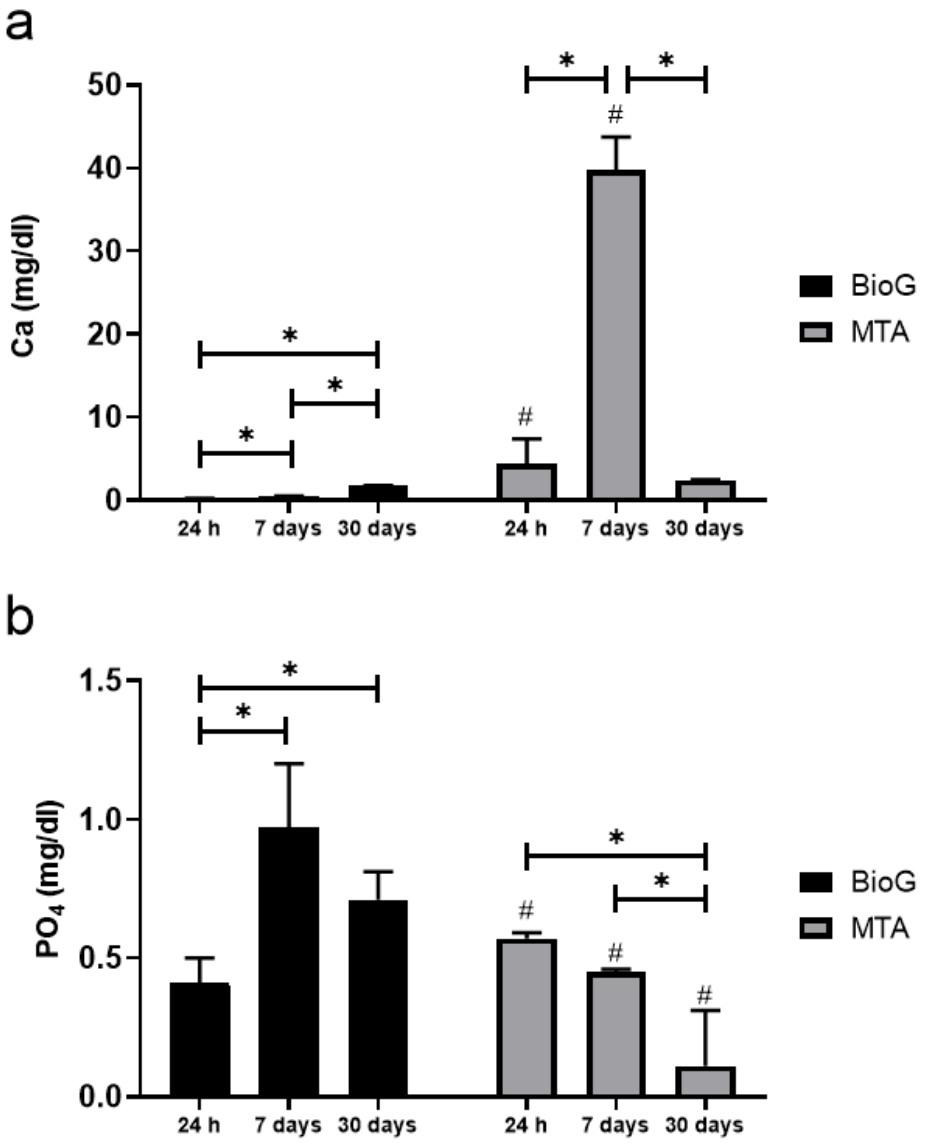

Figure 3. Mean and standard deviation of calcium and phosphate concentration between the BioG and MTA groups and the evaluation period. (*) Indicates statistically significant differences between times in the same material group ($P < 0.05$). (#) Indicates statistically significant differences between materials at the same experimental time ($P < 0.05$).

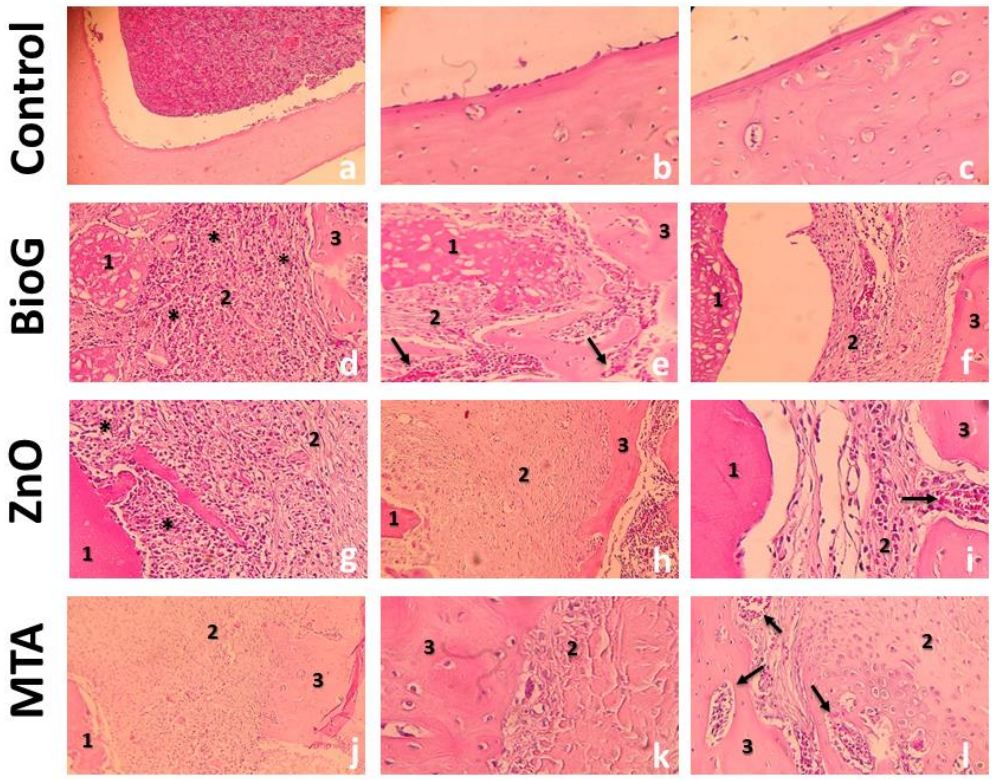

Figure 4. Photomicrographs of histological sections of the bone regions and connective tissue surrounding the tested materials. Control group: (a, b, c; 50×, 100×, and 200× magnification). BioG: (d, e, f; 200× increase at 15, 30, and 45 days). ZnO: (g, h, i; 200× increase at 15, 30, and 45 days). MTA: (j, k, l; 200× increase at 15, 30, and 45 days). (1) Type of material, (2) Dense connective tissue, (3) Bone trabeculae, (*) Diffuse mononuclear inflammatory infiltrate, (→) Congested blood vessels.

3. CONSIDERAÇÕES FINAIS

Este estudo foi conduzido com o objetivo de testar um material à base de Bioglass 45S5® comparando-o ao MTA. A utilização de um cimento endodôntico definitivo no tratamento das Reabsorções Radiculares Inflamatórias Externas é importante para evitar a reintervenção num elemento dental fragilizado pelo processo patológico da reabsorção.

O cimento testado na concentração de 40% de 45S5 demonstrou liberação iônica de Ca e PO_4 que garantem sua bioatividade e formação de HA. Do mesmo modo, esse cimento elevou os valores de pH a níveis suficientes que paralisam a atividade osteoclástica. A radiopacidade desse cimento mostrou que o preenchimento intracanal pode ser verificado com segurança. O cimento experimental pareceu ser um material

promissor para o tratamento das Reabsorções Radiculares Inflamatórias Externas a ser inserido no canal radicular ou em áreas de reabsorções.

4. REFERÊNCIAS

ANBINDER, Ana Lia e CARVALHO, Yasmin Rodarte e DA ROCHA, Rosilene Fernandes. **Os bifosfonatos e a Odontologia / The biphosphonates and the Dentistry**. Rev. ABO nac, v. 14, n. 6, p. 373–379, 2007.

ASGARY, Saeed e colab. **The properties of a new endodontic material**. Journal of Endodontics, v. 34, n. 8, p. 990–993, 2008.

ASGARY, Saeed e AHMADYAR, Maryam. **One-visit RCT of maxillary incisors with extensive inflammatory root resorption and periradicular lesions: a case report**. Iranian Endodontic Journal, v. 6, n. 2, p. 95–98, 2011.

BAKLAND, LK. **Root resorption**. Dent Clin North Am, v. 36, n. 2, p. 491–507, 1992.

BAKRY, Ahmed Samir e colab. **The effect of a bioglass paste on enamel exposed to erosive challenge**. Journal of Dentistry, v. 42, n. 11, p. 1458–1463, Nov 2014.

BELLANTONE, Maria e WILLIAMS, Huw D. e HENCH, Larry L. **Broad-spectrum bactericidal activity of Ag2O-doped bioactive glass**. Antimicrobial Agents and Chemotherapy, v. 46, n. 6, p. 1940–1945, 2002.

CARVALHO, C. N. e colab. **Comparative analyses of ion release, pH and multispecies biofilm formation between conventional and bioactive gutta-percha**. International Endodontic Journal, v. 49, n. 11, p. 1048–1056, Maio 2016.

CHANIOTIS, A. **The use of a single-step regenerative approach for the treatment of a replanted mandibular central incisor with severe resorption**. International Endodontic Journal, v. 49, n. 8, p. 802–812, 2016.

CHAVES NETTO, HDM e colab. **Association of several degrees of root resorption with etiological-predisposing factors**. Revista da Associação Paulista de Cirurgiões-Dentistas, v. 63, n. 1, p. 49–56, 2009.

CONSOLARO, Alberto. **O conceito de reabsorções dentárias**. Dental Press Journal of Orthodontics, v. 16, n. 4, p. 19–24, 2011.

CONSOLARO, Alberto e FURQUIM, Laurindo Zanco. **Extreme root resorption**

associated with induced tooth movement: A protocol for clinical management. Dental Press Journal of Orthodontics, v. 19, n. 5, p. 19–26, 2014.

DARCEY, J e QUALTROUGH, A. **Resorption: part 2. Diagnosis and management.** British dental journal, v. 214, n. 10, p. 493–509, 2013.

FERNANDES, Marina e DE ATAIDE, Ida e WAGLE, Rahul. **Tooth resorption part II - external resorption: case series**. Journal of Conservative Dentistry, v. 16, n. 2, p. 180–185, 2013.

FILIPSSON, R e LINDSTEN, J e ALMQVIST, S. **Time of eruption of the permanent teeth, cephalometric and tooth measurement and sulphation factor activity in 45 patients with turner's syndrome with different types of x chromosome aberrations**. Acta Endocrinol (Copenh), v. 48, p. 91–113, 1965.

FUSS, Zvi e TSESIS, Igor e LIN, Shaul. **Root resorption--diagnosis, classification and treatment choices based on stimulation factors.** Dental traumatology : official publication of International Association for Dental Traumatology, v. 19, n. 4, p. 175–182, 2003.

GOLD, Steven I. e HASSELGREN, Gunnar. **Peripheral inflammatory root resorption. A review of the literature with case reports**. Journal of Clinical Periodontology, v. 19, n. 8, p. 523–534, 1992.

GUBLER, M. e colab. **Do bioactive glasses convey a disinfecting mechanism beyond a mere increase in pH?** International Endodontic Journal, v. 41, n. 8, p. 670–678, 2008.

HEGDE, Nanditha e HEGDE, Mithra N. **Internal and External Root Resorption Management: A Report of Two Cases**. International Journal of Clinical Pediatric Dentistry, v. 6, n. 1, p. 44–47, 2013.

HEID, Susanne e colab. **Incorporation of particulate bioactive glasses into a dental root canal sealer**. Biomedical glasses, v. 2, n. 1, p. 29–37, 2016.

HENCH, Larry L. e JONES, Julian R. **Bioactive glasses: Frontiers and challenges**. Frontiers in Bioengineering and Biotechnology, v. 3, p. 194, 30 Nov 2015.

HU, Sheng e colab. **Study on antibacterial effect of 45S5 Bioglass®**. Journal of

Materials Science: Materials in Medicine, v. 20, n. 1, p. 281–286, 2009.

IGLESIAS-LINARES, A. e HARTSFIELD, J.K. **Cellular and Molecular Pathways Leading to External Root Resorption**. Journal of Dental Research, v. 96, n. 2, p. 145–152, 5 Fev 2017.

JONES, Julian R. **Review of bioactive glass: From Hench to hybrids**. Acta Biomaterialia. [S.l: s.n.]. , 2015

KRISHNAN, Vidya e LAKSHMI, T. **Bioglass: A novel biocompatible innovation**. Journal of advanced pharmaceutical technology & research, v. 4, n. 2, p. 78–83, 2013.

MARENDING, Monika e colab. **Comparative assessment of time-related bioactive glass and calcium hydroxide effects on mechanical properties of human root dentin**. Dental Traumatology, v. 25, n. 1, p. 126–129, 2009.

MEHRVARZFAR, Payman e colab. **An in vitro comparative study on the antimicrobial effects of bioglass 45S5 vs. calcium hydroxide on Enterococcus faecalis**. Iranian Endodontic Journal, v. 6, n. 1, p. 29–33, 2011.

MLADENOVIĆ, Živko e colab. **Soluble silica inhibits osteoclast formation and bone resorption in vitro**. Acta Biomaterialia, v. 10, n. 1, p. 406–418, Jan 2014.

MOHN, D e colab. **Composites made of flame-sprayed bioactive glass 45S5 and polymers: bioactivity and immediate sealing properties.** International Endodontic Journal, v. 43, n. 11, p. 1037–1046, Nov 2010.

NATALE, L. C. e colab. **Ion release and mechanical properties of calcium silicate and calcium hydroxide materials used for pulp capping**. International Endodontic Journal, v. 48, n. 1, p. 89–94, 2015.

PARIROKH, Masoud e TORABINEJAD, Mahmoud. **Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties**. Journal of Endodontics, v. 36, n. 1, p. 16–27, 2010a.

PARIROKH, Masoud e TORABINEJAD, Mahmoud. **Mineral Trioxide Aggregate: A comprehensive literature review-part III: Clinical applications, drawbacks, and mechanism of action**. Journal of Endodontics, v. 36, n. 3, p. 400–413, 2010b.

RAFTER, Mary. **Apexification: A review**. Dental Traumatology, v. 21, n. 1, p. 1–8, 2005.

RAHIMI, Saeed e colab. **A Review of Antibacterial Agents in Endodontic Treatment**. Iranian Endodontic Journal, v. 9, n. 3, p. 161–168, 2014.

RIBEIRO, Michael Ranniery Garcia e colab. **Chlorhexidine Prevents Root Dentine Mineral Loss and Fracture Caused by Calcium Hydroxide over Time**. International Journal of Dentistry, 2017.

ROBINSON, PB e HARVEY, W. **Tooth root resorption induced in rats by diphenylhydantoin and parathyroidectomy**. British journal of experimental pathology, v. 70, n. 1, p. 65–72, 1989.

ROY, Charles O. e JEANSONNE, Billie G. e GERRETS, Thomas F. **Effect of an acid environment on leakage of root-end filling materials**. Journal of Endodontics, v. 27, n. 1, p. 7–8, 2001.

SCHMITT, D e LEE, J e BOGEN, G. **Multifaceted use of ProRoot MTA root canal repair material.** Pediatric dentistry, v. 23, n. 4, p. 326–30, 2001.

SCHWARTZ, R S e colab. **Mineral trioxide aggregate: a new material for endodontics.** Journal of the American Dental Association (1939), v. 130, n. 7, p. 967–75, Jul 1999.

SIMON, ST e BHAT, KS e FRANCIS, R. **Effect of four vehicles on the pH of calcium hydroxide and the release of calcium ion**. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, v. 80, n. 4, p. 459–464, Out 1995.

STOOR, Patricia e SODERLING, Eva e SALONEN, Jukka I. **Antibacterial effects of a bioactive glass paste on oral microorganisms**. Acta Odontologica Scandinavica, v. 56, n. 3, p. 161–165, 1998.

TRONSTAD, Leif. **Root resorption--etiology, terminology and clinical manifestations.** Endodontics & dental traumatology, v. 4, n. 6, p. 241–252, 1988.

VAANANEN, K. **Mechanism of osteoclast mediated bone resorption—rationale for the design of new therapeutics**. Advanced Drug Delivery Reviews, v. 57, n. 7, p. 959–

971, 25 Maio 2005.

VALERA, Marcia Carneiro e colab. **Fracture resistance of weakened bovine teeth after long-term use of calcium hydroxide**. Dental Traumatology, v. 31, p. 385–389, 2015.

WANG, Xiaodu e colab. **The role of collagen in determining bone mechanical properties**. Journal of Orthopaedic Research, v. 19, p. 1021–1026, 2001.

WANG, Zhejun e colab. **Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes**. Journal of Dentistry, v. 39, n. 11, p. 746–756, 2011.

WHITE, J. Derek e colab. **The effect of three commonly used endodontic materials on the strength and hardness of root dentin**. Journal of Endodontics, v. 28, n. 12, p. 828–830, 2002.

XYNOS, Ioannis D. e colab. **Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution**. Journal of Biomedical Materials Research, v. 55, n. 2, p. 151–157, Maio 2001.

YANG, SY e colab. **Enamel Surface with Pit and Fissure Sealant Containing 45S5 Bioactive Glass**. Journal of Dental Research, v. 95, n. 5, p. 550–570, 2016.

ZHANG, Di e colab. **Antibacterial effects and dissolution behavior of six bioactive glasses**. Journal of Biomedical Materials Research - Part A, v. 93, n. 2, p. 475–483, 2010.

ANEXO 1

Normas de publicação do periódico "*International Endodontic Journal*"

Author Guidelines

Content of Author Guidelines:

1. General
2. Ethical Guidelines
3. Manuscript Submission Procedure
4. Manuscript Types Accepted
5. Manuscript Format and Structure
6. Graphical Abstracts
7. After Acceptance

Useful Websites: Submission Site, Articles published in International Endodontic Journal, Author Services, Wiley's Ethical Guidelines, Guidelines for Figures

The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

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Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in International Endodontic Journal. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

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Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2 Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

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2.3.1 Randomised control clinical trials

Randomised clinical trials should be reported using the Preferred Reporting Items for RAndomized Trials in Endodontics (PRIRATE) 2020 guidelines. A PRIRATE checklist and flowchart (as a Figure) should also be completed and included in the submission material. The PRIRATE 2020 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/prirate/

It is recommended that authors consult the following papers, which explains the rationale for the PRIRATE 2020 guidelines and their importance when writing manuscripts:

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Pigg M, Rechenberg DK, Vaeth M, Dummer P. PRIRATE 2020 guidelines for reporting randomized trials in Endodontics: a consensus-based development. Int Endod J. 2020 Mar 20. doi: 10.1111/iej.13294. (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13294)

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Dummer P. PRIRATE 2020 guidelines for reporting randomized trials in Endodontics:

Explanation and elaboration. Int Endod J. 2020 April 8. doi: 10.1111/iej.13304 (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13304)

2.3.2 Epidemiological observational trials

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Nagendrababu V, Duncan HF, Tsesis I, Sathorn C, Pulikkotil SJ, Dharmarajan L, Dummer PMH. PRISMA for abstracts: best practice for reporting abstracts of systematic reviews in Endodontology. Int Endod J. 2019 Mar 19:1096-07. doi: 10.1111/iej.13118.
Nagendrababu V, Dilokthornsakul P, Jinatongthai P, Veettil SK, Pulikkotil SJ, Duncan HF, Dummer PMH. Glossary for systematic reviews and meta-analyses. Int Endod J. 2020 Feb;53(2):232-249. doi: 10.1111/iej.13217. Epub 2019 Nov 25.

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Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.

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Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and

Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to International Endodontic Journal should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 350 words giving details of what was done using the following structure:

• Aim: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.
• Methodology: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
• Results: Give the main results of the study, including the outcome of any statistical analysis.
• Conclusions: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Systematic Review Articles should be no more than 350 words using the following structure where applicable:

• Title: Identify the report as a systematic review, meta-analysis, or both.
• Background: Provide a brief introduction of the subject and why it is important.
• Objectives: The research question including components such as participants, interventions, comparators, and outcomes. Use PICO format.
• Methods: Briefly describe i) the inclusion criteria, ii) provide databases searched and dates, iii) mention the method used to assess study quality (risk of bias) iv) meta-analysis methodology (if appropriate).
• Results: i) Number and type of included studies and participants ii) results for main outcomes (benefits and harms). If a meta-analysis was undertaken, include summary measures and confidence intervals. iii) direction of the effect in terms that are meaningful to clinicians and patients.
• Discussion: i) Strengths and ii) limitations of evidence.
• Conclusions: General interpretation of the results and important implications.
• Funding: Primary source of funding for the review (if no funding: say 'none').
• Registration: Registration number and name.

Abstract for Review Articles (narrative)
The Abstract should be unstructured and no more than 350 words.

Abstract for Case Reports should be no more than 350 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.
• Summary: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• Key learning points: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 350 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.
• Methodology: Describe the methods adopted.
• Results: Give the main results of the study.
• Conclusions: State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion:

• Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.
• Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the PRIRATE 2020 guidelines. A PRIRATE 2020 checklist must be completed and included along with a flow diagram (as a Figure) in the submission material. These are available at http://pride-endodonticguidelines.org/prirate/.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out.

Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

• Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.
• Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.
• Conclusion: should contain a summary of the findings.

Main Text of systematic review articles should be divided into Introduction, Methods, Results and Conclusions:

• Introduction: Should be focused to place the subject matter in context and to justify the need for the review.
• Method: Divide into logical sub-sections in order to improve readability and enhance understanding (e.g. details of protocol registration, literature search process, inclusion/exclusion criteria, data extraction, quality assessment, outcome(s) of interest, data synthesis and statistical analysis, quality of evidence).
• Results: Present in structured fashion (e.g. results of the search process, characteristics of the included studies, results of primary meta-analysis, additional analysis, publication bias, quality of evidence).
• Discussion: Should summarize the results, highlighting completeness and applicability of evidence, quality of evidence, agreements and disagreements with other studies or reviews, strength and limitations, implications for practice and research.
• Conclusion(s): Section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Case Reports should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality.

Case reports should be written using the Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines. A PRICE checklist and flowchart (as a Figure) should also be completed and included in the submission material. The PRICE 2020 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/price/.

It is recommended that authors consult the following papers, which explains the rationale for the PRICE 2020 guidelines and their importance when writing manuscripts:

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH. PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development. Int Endod J. 2020 Feb 23. doi: 10.1111/iej.13285. (https://www.ncbi.nlm.nih.gov/pubmed/32090342)

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH. PRICE 2020 guidelines for reporting case reports in Endodontics: Explanation and elaboration. Int Endod J. (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13300)

Acknowledgements: International Endodontic Journal requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by et al. is sufficient, e.g. (Tobias et al. 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias et al. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add et al.
(ii)Year of publication in parentheses
(iii) Full title of paper followed by a full stop (.)
(iv) Title of journal in full (in italics)
(v) Volume number (bold) followed by a comma (,)
(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article
Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. International Endodontic Journal 24, 8-14.

Corporate author
British Endodontic Society (1983) Guidelines for root canal treatment. International Endodontic Journal 16, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html
[accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms

should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

Permissions: If all or part of previously published illustrations are to be used, permission must be obtained from the copyright holder concerned. This is the responsibilty of the authors before submission.

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(including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley Blackwell's guidelines for figures: http:/authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp

5.6. Guidelines for reporting of DNA microarray data

The International Endodontic Journal gives authors notice that, with effect from 1st January 2011, submission to the International Endodontic Journal requires the reporting of microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available at http://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.

5.7. Supporting Information

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only. Authors need to work closely with the editors in developing or using such new publication formats.

Supporting information, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted. It should be clearly stated at the time of submission that the supporting information is intended to be made available through the online edition. If the size or format of the supporting information is such that it cannot be accommodated on the journal's website, the author agrees to make the supporting information available free of charge on a permanent Web site, to which links will be set up from the journal's website. The author must advise Wiley Blackwell if the URL of the website where the supporting information is located changes. The content of the supporting information must not be altered after the paper has been accepted for publication.

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6. GRAPHICAL ABSTRACT

Authors are invited to submit a graphical abstract along with their manuscript to be published in the International Endodontic Journal's:

Online table of contents.
Content alert emails.
Within the article.
The graphical abstract should visually convey the key findings of the report and present a clear message to the reader. It should be used as a means of attracting the readers' attention and promoting further engagement with the article.

To create an effective graphical abstract, authors should focus on presenting to the reader what they can learn from the report, communicating only the key message.

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Creating a graphical abstract does not mean just copying and pasting a figure from the manuscript.
Use text sparingly, so the graphical abstract does not become cluttered, but ensure that you have clearly stated the purpose of the report, research design, clinical case and the

outcome of the study or case. Use language consistent with terms and definitions in the article that are free of editorialization (personal opinion) or bias.

Use only images that you have a legal right to use. Authors are responsible for obtaining permission to use any images that they include from outside sources, including articles, web pages, stock photo sites or Google image searches. Any needed permissions must be submitted along with your graphical abstract or identified in the Acknowledgements section of your manuscript.

Exclude imagery that can be viewed as advertisement, such as trade names, logos, or images of trademarked items.

The Graphical Abstract should be submitted along with the manuscript though our ScholarOne platform and uploaded with the file designation “Graphical Abstract”.

Required file properties:

Resolution: 700 pixels (width) x 600 pixels (height).

Font size: at least 12pt.

Font: Calibri.

File size should not exceed 1MB.

A good example of how a graphical abstract should look can be seen here: https://onlinelibrary.wiley.com/doi/10.1111/joim.13141

Please contact the editorial office at IEJeditor@cardiff.ac.uk if you have any questions.

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Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

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Hard copies of all figures and tables are required when the manuscript is ready for publication. These will be requested by the Editor when required. Each Figure copy should be marked on the reverse with the figure number and the corresponding author’s name.

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The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html. This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt. As changes to proofs are costly, we ask that you only correct typesetting errors. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

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