Biocompatibility, induction of mineralization and antimicrobial activity of experimental intracanal pastes based on glass and glass-ceramic materials

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Abstract

de Araújo Lopes JM, Benetti F, Rezende GC, Souza MT, Conti LC, Ervolino E, Jacinto RC, Zanotto ED, Cintra LTA. Biocompatibility, induction of mineralization and antimicrobial activity of experimental intracanal pastes based on glass and glass-ceramic materials. *International Endodontic Journal*.

Aim To evaluate the biocompatibility, induction of mineralization and antimicrobial activity of experimental intracanal pastes based on two glass and glass-ceramic materials. Calcium hydroxide ($Ca(OH)_2$) paste was used as the positive control.

Methodology The glass-ceramic powder [two-phased Biosilicate (BS-2P)] and F18 bioactive glass were mixed with distilled water (ratio 2 : 1), inserted in polyethylene tubes and implanted in the subcutaneous tissues of 16 rats. Empty tubes were used as negative control. After 7 and 30 days ($n = 8$), the rats were euthanized for haematoxylin–eosin, von Kossa, polarized light and osteopontin (OPN) immunolabeling analysis. Direct contact tests using a suspension of each paste were performed with *Enterococcus faecalis* planktonic cells to evaluate antimicrobial activity (24 h of contact), in a pilot study. The number of CFU mL^{-1} was calculated for each group. The antimicrobial analysis data were submitted to one-way ANOVA and Tukey tests, whilst biocompatibility and immunohistochemical data were

submitted to the Kruskal–Wallis and Dunn tests ($P < 0.05$).

Results Most specimens of the control, BS-2P and $Ca(OH)_2$ groups were associated with moderate inflammation seven days following implantation, whilst F18 was associated with moderate to severe inflammation, without differences amongst the groups ($P > 0.05$). At 30 days, most specimens of control, F18 and BS-2P groups had mild inflammation, whilst $Ca(OH)_2$ had mild to moderate inflammation; however, no differences were determined amongst the groups ($P > 0.05$). The fibrous capsule was thick at 7 days, becoming thin at 30 days. All pastes induced von Kossa-positive structures and were birefringent to polarized light. At seven days, the BS-2P group had significantly more OPN immunolabeling compared to the control and $Ca(OH)_2$ groups ($P < 0.05$). At 30 days, the F18 group had significantly more OPN immunolabeling compared to the control and Ca$(OH)_2$ groups ($P < 0.05$). All pastes reduced the total number of *E. faecalis*; however, the reduction was only significant when comparing BS-2P and $Ca(OH)_2$ groups to the control ($P < 0.05$). Only calcium hydroxide eliminated *E. faecalis*.

Conclusions Experimental BS-2P and F18 pastes were biocompatible, stimulated biomineralization and induced significant OPN immunolabeling compared to $Ca(OH)_2$. Only the BS-2P paste demonstrated antimicrobial activity comparable to $Ca(OH)_2$.

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Introduction

The aim of root canal treatment is to eliminate microbial colonies and neutralize the toxic contents within the root canal system (Gomes-Filho *et al.* 2012). Mechanical preparation significantly reduces the number of bacteria within root canals (Siqueira & Rôças 2011, Neves *et al.* 2014, Nakamura *et al.* 2018); however, they are not eliminated completely (Herrera *et al.* 2015, 2016). Therefore, intracanal medication is used as an adjunct to canal cleaning and shaping (Zancan *et al.* 2016), resulting in reduced microbiological content and in the reduction of endotoxins (Vera *et al.* 2012, Xavier *et al.* 2013).

Calcium hydroxide $(Ca(OH)_2)$ has antimicrobial activity because of its ability to dissociate into hydroxyl and calcium ions, creating an unfavourable alkaline environment for microbial survival (Mohammadi & Dummer 2011, Zancan *et al.* 2016). Also, calcium hydroxide's bioactivity induces the formation of mineralized tissue (Holland *et al.* 1977, Chen *et al.* 2016, Cintra *et al.* 2017b). Calcium hydroxide pastes are used in procedures such as pulp capping, pulpotomy, intracanal medication, treatment of root resorption and apexification (Chen *et al.* 2016).

Other materials are known for their bioactivity, such as glass loosely denoted as bioglass (Hench 1991, Osorio *et al.* 2012, Wang *et al.* 2016), which have high osteoinductive and osteoconductive activity (Jones *et al.* 2007, Wang *et al.* 2016). Calcium silicate cements, often referred to as bioceramics, are also bioactive (Benetti *et al.* 2018a). The composition of bioglasses have been intensively studied for several decades (Hench 1991, Renno *et al.* 2013), primarily for applications in tissue engineering, by stimulating cell differentiation and inducing the formation of mineralized tissue, with promising results when compared to inert biomaterials (Khalid *et al.* 2018). Nevertheless, bioglass is relatively unexplored for endodontic applications.

The first bioglass was developed by Hench in 1970 for use in dentistry and is known as Bioglass 45S5® (Hench 1991). Hydroxycarbonate apatite (HCA), a compound chemically similar to the mineral phase of bone, is deposited on the surface of this material when this material is exposed to an aqueous medium (Jones 2015, Henao *et al.* 2019). From this first glass, several bioactive glasses and bioactive ceramics were developed, including a fully crystallized bioactive glass-ceramic, Biosilicate (BS) (Granito *et al.* 2009, Brandão *et al.* 2012, Renno *et al.* 2013, invention patent WO 97/41079). Biosilicate is not to be confused with the tricalcium silicate cement known as Biodentine (Septodont, Saint-Maur-des-fossés, France), which is marketed as a biosilicate (Primus *et al.* 2019).

Studies have demonstrated that BS allows rapid deposition of HCA (Moura *et al.* 2007, Granito *et al.* 2009, Azenha *et al.* 2010, Renno *et al.* 2010, Granito *et al.* 2011, Peitl *et al.* 2012). When Biosilicate was added to distilled water, it remineralized enamel and dentine (Tirapelli *et al.* 2010, 2011, Pintado-Palomino & Tirapelli 2015, Pintado-Palomino *et al.* 2015). In water, the Biosilicate releases calcium, sodium, silicon and phosphate ions (Bossini *et al.* 2011, Martins *et al.* 2011). The release of calcium and sodium ions increases the osmotic pressure and pH, creating an antimicrobial activity (Stoor *et al.* 1998, Martins *et al.* 2011).

Biosilicate is a fully crystalline glass-ceramic material that can be obtained with one (BS-1P) or two crystalline phases (BS-2P): sodium–calcium silicate $(Na_2CaSi_2O_6)$ or both $Na_2CaSi_2O_6$ and a sodium–calcium phosphate $(NaCaPO_4)$ phase (Alves 2011, Crovace *et al.* 2016). Both crystalline glasses have similar biocompatibility in bone tissue (Azenha *et al.* 2010); however, BS-2P has been reported to have greater osteogenic activity compared to BS-1P and Bioglass® 45S5 (Alves 2011).

F18 is a bioactive glass (Souza *et al.* 2015), which possesses a wider working range compared to other bioglasses – it has greater glass stability and crystallization does not occur easily during its processing, allowing the production of complex 3D shapes, fibres and meshes (Souza *et al.* 2015, 2016a, 2016b). F18 glass contributes to hard and soft tissue regeneration by induction of proliferation of osteoblast and fibroblasts (Gabbai-Armelin *et al.* 2015, 2017b). A previous study reported that F18 glass fibres were resorbed 60 days after implantation in the subcutaneous tissue of rats, with formation of HCA on the surface of the fibres (Gabbai-Armelin *et al.* 2017b). Thus, F18 has the potential of regenerating connective tissues to aid tissue repair.

These two glasses have been extensively evaluated in powder, fibre and block forms (Martins *et al.* 2011, Gabbai-Armelin *et al.* 2015, 2017a,b, Souza *et al.* 2017). Based on this literature, it was hypothesized that pastes from these materials could be used as intracanal medicaments after chemo-mechanical root canal preparation before canal filling. Their capacity of promoting osteogenesis and their antimicrobial action could stimulate biological sealing and reduce the residual microbial content. F18, in powder and fibres, has been shown to be antimicrobial against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Souza *et al.* 2017). Biosilicate has been shown to be antimicrobial against anaerobic bacteria (Martins *et al.* 2011). However, these materials have not been tested in paste form.

The performance of experimental pastes prepared from the BS-2P and F18 powders was evaluated, for biocompatibility and induction of mineralization (von Kossa technique, polarized light analysis, immunolabeling of mineralization protein). A pilot study was performed for the analysis of antimicrobial activity against *Enterococcus faecalis*. Ca(OH)$_2$ paste was used for comparison. The null hypothesis was that 'no difference exists in these parameters amongst the three pastes'.

Materials and Methods

Pastes preparation

The powders of BS-2P and F18 materials were obtained from the multidisciplinary research group of the Laboratory of Vitreous Materials, at the Federal University of São Carlos, SP, Brazil. Both materials had a mean diameter particle size of 10 μm. Calcium hydroxide powder was obtained commercially (Biodinâmica Química e Farmacêutica Ltda, Ibiporã, PR, Brasil). Pastes were prepared by spatulating each powder with distilled water, in the ratio 2 : 1 by weight.

Biocompatibility and biomineralization analysis

Sixteen 2-month-old male Wistar rats weighing from 250 to 280 g were used. The sample size was established based on previous studies involving the analysis of materials in the subcutaneous tissue of rats (Cintra *et al.* 2013, de Azevedo Queiroz *et al.* 2018, Benetti *et al.* 2018a). The animals were housed in a temperature-controlled environment (22 ± 1°C, 70% humidity) with a 12-h light–dark cycle and received water and food *ad libitum*. The study was approved and performed according to the guidelines of the ethical committee (CEUA 000692).

Sample preparation and surgical procedures

The surgical procedure was performed following previous protocols (Cintra *et al.* 2010, 2017a). The rats were anaesthetized, their dorsa were shaved, and a 2.0 cm incision was made in a head-to-tail orientation with a n° 15 Bard-Parker blade (BD, Franklin Lakes, NJ, USA). The skin was reflected to create two pockets on the right side and two pockets on the left side of the incision.

The pastes were mixed, inserted into polyethylene tubes (48 tubes with a 1.0 mm internal diameter, 1.6 mm external diameter and 10.0 mm long; Abbott Labs of Brazil, São Paulo, SP, Brazil). Empty tubes (16) were used as negative control. The tubes were filled with freshly mixed pastes and immediately implanted into the pockets. Each rat received randomly one tube with each paste group and one empty tube, and the skin was sutured with 4-0 silk sutures.

At 7 and 30 days after implantation, groups of 8 rats were euthanized with an overdose of anaesthetic solution. The polyethylene tubes, together with the surrounding tissues, were removed and fixed in a solution of 4% buffered formaldehyde for 24 h, at pH 7.0. The specimens were dehydrated, clarified and embedded in paraffin, and serially sectioned for staining with haematoxylin and eosin (5-μm sections), stained according to the von Kossa technique (10-μm sections), unstained for examination under polarized light (10-μm sections) or used for immunohistochemical reactions (5-μm sections).

Histological analysis was performed by a single, calibrated operator in a blinded manner, using light microscopy (DM 4000 B; Leica Microsystems, Wetzlar, Germany). The tissue inflammation was graded as follows: '0', no or minimal inflammatory cells and no reaction; '1', fewer than 25 cells and a mild reaction; '2', between 25 and 125 cells and a moderate reaction; and '3', 125 or more cells and a severe reaction (Cintra *et al.* 2017b). Fibrous capsules were considered thin when the thickness was less than 150 μm and thick when equal or greater than 150 μm (Cintra *et al.* 2017b). Positive structures for von Kossa and polarized light were recorded as absent or present (Cintra *et al.* 2013, 2017a, Benetti *et al.* 2018a).

Immunohistochemical analysis

The histological sections were deparaffinized in xylene and hydrated in a decreasing ethanol series. Antigen retrieval was achieved by immersing the histological slides in citrate buffer solution (Antigen Retrieval Buffer; Spring Bioscience, Pleasanton, CA, USA) in a pressurized chamber (Decloaking Chamber; Biocare Medical, Concord, CA, USA) at 95 °C for 10 min. The slides were rinsed with phosphate-buffered saline at the end of each stage of the immunohistochemical reaction. The histological sections were immersed in a 3% H_2O_2 solution for 1 h and 20 min and in 1% bovine serum albumin for 12 h to block the endogenous peroxidase activity and nonspecific sites. The histological slides were incubated with anti-osteopontin primary antibodies (primary antibody goat; SC-10593; Santa Cruz Biotechnology, Dallas, TX, USA), that was diluted (Antibody Diluent with Background Reducing Components; Dako Laboratories, Carpinteria, CA, USA). The slides were placed in a moist chamber for 24 h. The histological sections were incubated with a biotinylated secondary antibody for 1.5 h then treated with streptavidin–horseradish peroxidase conjugate for 1.5 h (Universal Dako Labelled Streptavidin-Biotin kit; Dako Laboratories). The slides were rinsed with phosphate-buffered saline, and a reaction was developed using chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB Chromogen kit; Dako Laboratories). The negative controls consisted of specimens submitted to the procedures previously mentioned without the primary antibodies (Benetti *et al.* 2018b, 2019, Dal-Fabbro *et al.* 2019). With the aid of image analysis software (×1000, Leica LAS 4.12; Leica Microsystems), the area corresponding to immunolabeling was delineated using the colour threshold tool to obtain the immunolabeling optical density, expressed as random unit percentage of optical density (mean ± SD) (Azuma *et al.* 2018, Statkievicz *et al.* 2018).

Antimicrobial activity – a pilot study

Direct contact with planktonic bacteria was performed. The pastes were prepared as previously described. The microbiological procedures were performed in a laminar flow chamber (Veco Bioseg 12 Ltda., Campinas, SP, Brazil).

A standard strain of *E. faecalis* (ATCC 51299) was used as a culture suspension. The microorganism was reactivated in 20 mL sterile Brain Heart Infusion (BHI) Agar (Difco Laboratories Inc., Detroit, MI, USA) and held at 37 °C for 24 h. The colonies were inoculated into 5.0 mL sterile BHI broth and held at 37 °C overnight, after which the medium optical density was measured with a spectrophotometer (BioTek Instruments, Winooski, VT, USA) set at 550 nm wavelength. The optical density was adjusted to 0.06 [approximately 9×10^7 colony-forming units per mL (CFU mL^{-1})].

The bacterial suspension was used within a 60 min period after the adjustment to colony-forming units per mL (CFU mL^{-1}). Sterilized physiological solution was used as the negative control, which served to confirm the initial number of CFU mL^{-1}. Two hundred fifty mg of each paste was weighed, manipulated in a falcon tube, and 10 mL of distilled water was added to obtain a suspension of intracanal medication with a concentration of 25 mg mL^{-1}. The suspension was agitated at room temperature in a vortex mixer (2800 rpm; Fisatom Equipamentos Científicos Ltda, São Paulo, SP, Brazil) for 1 min. Then, 700 µL of each paste suspension was placed in tubes (one tube for each paste; $n = 8$ per group) and received 25 µL of adjusted inoculum. The tubes were held at 37 °C for 24 h.

After the contact period, the suspensions of *E. faecalis* were serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) and 10 µL aliquots of each suspension were used for inoculation in Petri dishes containing sterile BHI Agar; triplicates of the petri dishes were then incubated at 37 °C for 24 h. The readings for each Petri dish were performed on areas of bacterial growth, where the dilutions generated were between three and 30 colonies. The number of CFU mL^{-1} (mean and standard deviation) was calculated for the eight specimens of each group.

Statistical analysis

Parametric data after the Shapiro–Wilk normality test were submitted to a one-way ANOVA and Tukey tests (data of antimicrobial analysis), and data not normal (immunohistochemical analysis) or nonparametric data (data of biocompatibility analysis) were submitted to the Kruskal–Wallis and Dunn tests ($P < 0.05$).

Results

Biocompatibility and biomineralization analysis

At seven days, the majority of the specimens of all groups had a moderate inflammatory process ($P > 0.05$), mainly represented by polymorphonuclear cells. Representative images of the histological

analysis are displayed in Fig. 1 and the scores attributed to each group in Table 1. The fibrous capsule was thick in all specimens at this time-point.

At 30 days, the numeric density of inflammatory cells was reduced, and mild inflammatory infiltrate was observed in the majority of the specimens of all groups ($P > 0.05$). The capsule was thin and organized throughout each group.

Representative images of the von Kossa analysis or under polarized light are presented in Fig. 2. All pastes induced the formation of von Kossa-positive structures and birefringent to polarized light.

Immunohistochemical analysis

Immunolabeling was observed in the cell cytoplasm and extracellular matrix. Representative images of the immunolabeling of the OPN are displayed in Fig. 2 and the results in Table 2. At seven days, a significant higher immunolabeling optical density was observed in the BS-2P group compared to the control and $Ca(OH)_2$ groups ($P < 0.05$). At 30 days, higher immunolabeling optical density was observed in the F18 group, similar to the BS-2P group ($P > 0.05$) and differing from both the control and the $Ca(OH)_2$ groups ($P < 0.05$).

Antimicrobial activity

The greatest number of CFU mL^{-1} was observed in the control group. The data are displayed in Table 3. All the evaluated pastes reduced the total CFU mL^{-1} number; however, this reduction was not significant for the F18 group compared to the control group
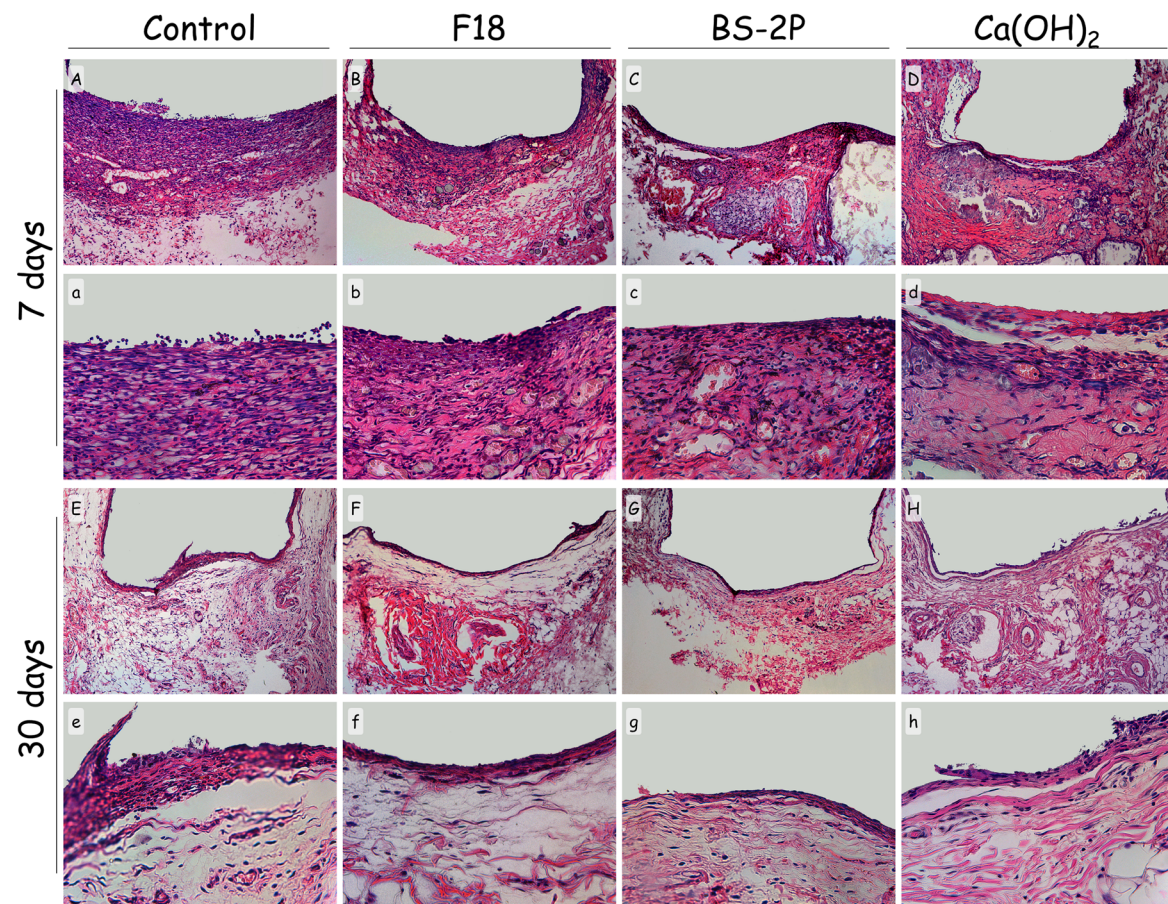
Figure 1 Representative images of inflammation reaction. (A,a–D,d) At 7 days: (A,a) Control, (B,b) F18, (C,c) BS-2P and (D,d) $Ca(OH)_2$ groups with moderate inflammatory cell infiltration and thick fibrous capsule. (E,f–H,h) At 30 days: (E,e) Control, (F,f) F18, (G,g) BS-2P and (H,h) $Ca(OH)_2$ groups with mild inflammatory cell infiltration and thin fibrous capsule in tube opening. [A–H: 100×; a–j: 400×; haematoxylin–eosin staining].

Table 1 Scores attributed to inflammatory infiltrate, fibrous capsule thickness, and percentage of biomineralization of each group

Analysis period/P	Material*	Inflammation scores				Inflammation median	Capsule	Biomineralization (%)	n
		0	1	2	3				
7 days	Control[a]	0	1	4	3	2	Thick	0	8
P = 0.439	F18[a]	0	0	4	4	2	Thick	100	
	BS-2P[a]	0	2	4	2	2	Thick	100	
	Ca(OH)$_2$[a]	0	2	4	2	2	Thick	100	
30 days	Control[a]	2	4	2	0	1	Thin	0	8
P = 0.403	F18[a]	2	4	2	0	1	Thin	100	
	BS-2P[a]	2	4	2	0	1	Thin	100	
	Ca(OH)$_2$[a]	0	4	4	0	1	Thin	100	

*Similar letters in same period indicate absence of significant difference between groups ($P > 0.05$).

($P > 0.05$). The BS-2P group was associated with a significant reduction of CFU mL^{-1} compared to the control group ($P < 0.05$), being similar to the Ca(OH)$_2$ group ($P > 0.05$), which indicated an absence of CFU mL^{-1} and differed from the control ($P < 0.05$). Only calcium hydroxide eliminated the *E. faecalis*.

Discussion

This study evaluated the performance of experimental pastes prepared from BS-2P and F18 for biocompatibility, induction of mineralization and antimicrobial activity to *E. faecalis*, compared to Ca(OH)$_2$ paste. The inflammatory tissue response decreased at 30 days in all groups, resulting in a thin fibrous capsule, indicating the biocompatibility of the pastes. All pastes induced the formation of positive structures to von Kossa and polarized light, which is the formation of crystals or biomineralization. However, the immuno-labeling of the OPN indicated greater expression in the BS-2P and F18 groups compared to the control and Ca(OH)$_2$ groups, at 7 and 30 days. Higher antimicrobial activity was observed in the BS-2P and Ca(OH)$_2$ groups. Thus, the null hypothesis was rejected for the induction of the OPN expression and for the antimicrobial activity.

The biocompatibility and bioactivity of F18 and BS-2P materials has been evaluated previously, however, not as an intracanal medicament (Brandão *et al.* 2012, Gabbai-Armelin *et al.* 2015, 2017b). F18 fibres used for bone regeneration demonstrated improvement in the immunolabeling of the osseous proteins in defects in the tibia of rats (Gabbai-Armelin *et al.* 2015), and in the biocompatibility and bioactivity both in rat subcutaneous tissue and in cell cultures

(Gabbai-Armelin *et al.* 2017b). BS-2P was biocompatible when used in the rabbit ophthalmic cavity (Brandão *et al.* 2012) and allowed bone formation *in vivo* (Azenha *et al.* 2010). Moreover, BS-2P and F18 induced the proliferation of osteoblast and fibroblast cells *in vitro* (Kido *et al.* 2013, Gabbai-Armelin *et al.* 2015, 2017b, Crovace *et al.* 2016). These data corroborate the results found in the present study.

Biomineralization is favourable for conventional root canal treatment to stimulate the closure of the apical foramen through the formation of mineralized tissue, potentially inducing a biological seal (Holland *et al.* 2007). Thus, the expression of proteins related to the mineralization process, such as OPN, would also be important. This protein was detected immuno-histochemically in the fibroblasts of the capsules present around the implanted tubes of all groups containing the pastes, but with lower expression in the Ca(OH)$_2$ group.

On the other hand, the bioactive glass and glass-ceramic-based pastes have the ability of inducing greater OPN expression than Ca(OH)$_2$. F18 is a highly bioactive material, capable of forming a layer of HCA in less than 4 h in solution of simulated body fluid, a phenomenon that leads to a rapid mechanism of bone binding (Souza *et al.* 2016a,b). The highly reactive surface of these materials in powder form provides the ability to increase the pH by leaching of Na$^+$ and Ca^{2+} (Stoor *et al.* 1998, Crovace *et al.* 2016). In addition, molecular analyses revealed that bioactive glass dissolution products activate seven gene families involved in the osteogenesis process (Xynos *et al.* 2001), which supports the results found in the present study.

Ca(OH)$_2$-based pastes are known to be biocompatible in rat subcutaneous tissue because its inflammation
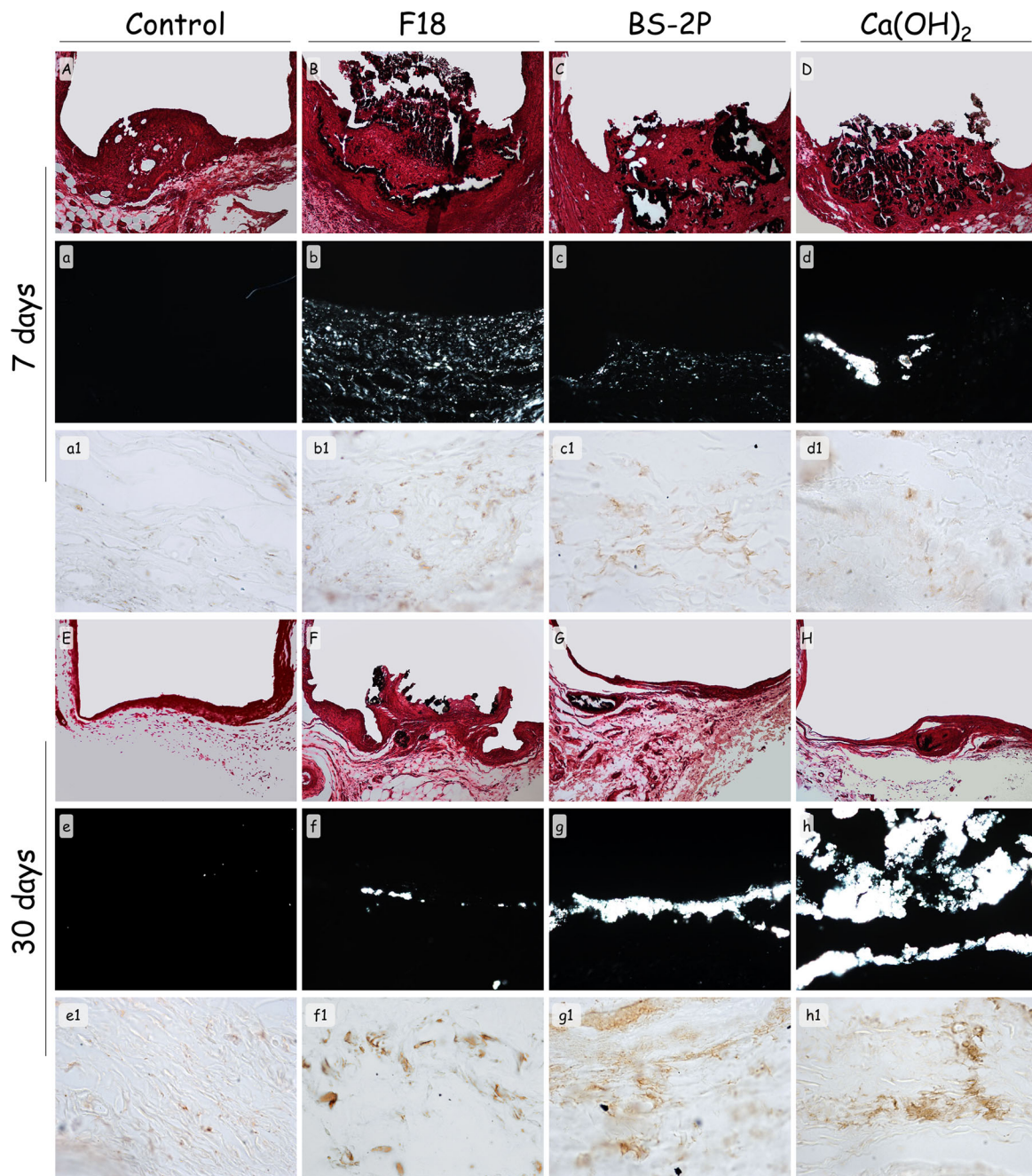
Figure 2 Representative images of biomineralization analysis. (A–H) Images of von Kossa and (a–h) polarized light analysis, of (A,a; E,e) Control group with absence of positive structures; (B,b; F,f) F18, (C,c; G,g) BS-2P and (D,d; H,h) Ca(OH)$_2$ groups with presence of positive structures for both analysis. Period of time: Day 7 (A,a–D,d), Day 30 (E,e–H,h). (a1–d1) Images of immunohistochemical analysis of OPN at seven days of (a1) Control and (d1) Ca(OH)$_2$ groups with lesser immunolabeling, (b1) F18 and, especially, (c1) BS-2P groups with higher immunolabeling; and at 30 days of (e1) Control group with lesser immunolabeling, and (g1) BS-2P, (h1) Ca(OH)$_2$ and especially (f1) F18 groups with higher immunolabeling of OPN. [A–H: 100×; von Kossa staining. a–h: 100×; polarized light visualization. a1–h1: 1000×; immunolabeling of OPN].

Table 2 Immunolabeled area percentage of osteopontin of each group

Analysis period/P	Material*	Mean (%)	±SD	n
7 days	Control[a]	0.24	0.16	8
$P < 0.001$	F18[bc]	2.82	1.62	
	BS-2P[c]	4.31	1.16	
	Ca(OH)$_2$[ab]	0.86	0.43	
30 days	Control[a]	1.08	0.32	8
$P < 0.001$	F18[b]	9.46	2.51	
	BS-2P[bc]	7.08	2.74	
	Ca(OH)$_2$[ac]	3.94	1.40	

*Similar letters in same period indicate absence of significant difference between groups ($P > 0.05$).

Table 3 Number of colony-forming units (CFU mL^{-1}) in each group after contact for 24 h

Material*	Mean	±SD	n	P
Control[a]	2.98×10^7	2.97×10^7	8	$P < 0.001$
F18[ab]	9.13×10^5	4.26×10^5		
BS-2P[bc]	2.10×10^4	0.88×10^4		
Ca(OH)$_2$[c]	0.00	0.00		

*Similar letters in same period indicate absence of significant difference between groups ($P > 0.05$).

reduces over time (Andolfatto *et al.* 2012, Mori *et al.* 2014). The action of biomineralization by Ca(OH)$_2$ occurs by the elution and diffusion of Ca^{2+} and OH^{3-} ions into dentine (Hauman & Love 2003, Camilleri 2007, Mohammadi & Dummer 2011). When exposed to carbon dioxide (CO$_2$) from the tissues, calcium carbonate (CaCO$_3$) is produced (Mohammadi & Dummer 2011). These calcium carbonate particles are positive in the von Kossa technique and birefringent to polarized light (Gomes-Filho *et al.* 2012, Viola *et al.* 2012, Cintra *et al.* 2017a). Thus, ions released by Ca(OH)$_2$ for biomineralization differ from the ions released by glass-ceramic materials, which may explain the observed difference in OPN expression in tissues.

Materials based on Ca(OH)$_2$ have the ability to form a thin necrotic layer on the initial contact with the tissue; the repair process follows afterwards. A thin necrotic layer may have delayed the expression of the OPN in the Ca(OH)$_2$ group, and OPN could be increased after a longer time. It is not known exactly if the bioactive glasses and glass-ceramics would behave the same way and induce necrotic layer for this application, such as Ca(OH)$_2$. However, in any other *in vivo* and clinical application such a tissue repair was induced by bioactive glasses (Jones 2015).

The ability to release ions, thus increasing pH, is key factor of an antimicrobial effect (Kido *et al.* 2013,

Crovace *et al.* 2016). In the present experiment, a pilot study was performed with direct contact with planktonic bacteria, indicating that the Ca(OH)$_2$-based paste completely eliminated *E. faecalis*. These results corroborate that of Aguiar *et al.* (2015), who reported that Ca(OH)$_2$ pastes completely eliminated *E. faecalis* after 30 s of direct contact. However, Ca(OH)$_2$ is not totally effective when there is no direct contact with the bacteria (Gomes *et al.* 2003, 2006), which highlights the limitation of this test. The medicament in direct contact with planktonic bacteria may have a different behaviour due to the absence of dentine as a substrate for bacterial growth. Consequently, disinfection strategies might appear to be more effective than they would be clinically in infected dentine (Hoedke *et al.* 2018). Therefore, future studies are necessary to evaluate the antimicrobial activity of these materials when challenged against dentine infected by bacterial biofilm.

Nevertheless, the direct contact test is a rapid and simple test to compare antimicrobial potential of experimental materials in an initial analysis (Zordan-Bronzel *et al.* 2019). As this was the first study evaluating these experimental pastes using F18 bioglass and BS-2P as possible intracanal medication, this pilot test was performed in order to identify whether these medications had antimicrobial properties. This is important because the vehicle could influence the activity of these materials in tissues or bacteria.

The antimicrobial activity of certain ceramic materials has been reported previously associated with the alkaline environment that these materials create (Stoor *et al.* 1998, Kido *et al.* 2013). The BS-2P group significantly decreased bacteria compared to the control group, although it did not completely eliminate the bacteria. In a previous study, BS completely eliminated *E. faecalis* after 60 min of direct contact (Martins *et al.* 2011). However, another experimental methodology was used (direct and indirect contact of the pure biomaterial) and a different particle size distribution was evaluated (<20 µm). In the present study, a 10 µm mean diameter of BS-2P was used, which had not been tested for antimicrobial activity. When comparing bioglass particle size fractions within 45–1000 µm, a previous study revealed that finer fraction allowed a higher pH value than a coarser fraction (Zhang *et al.* 2008), which could influence antimicrobial activity, but does not agree with the present results. On the other hand, another study reported that the difference in pH from particles of 16–90 µm was not significant (Cerruti *et al.* 2005).

Thus, further studies are needed to analyse the properties of different sizes of glass particles.

Fragments of vitreous particles, which are 'needle-like', may damage cell walls and inactivate bacteria (Zhang *et al.* 2007, Leppäranta *et al.* 2008, Begum *et al.* 2016). Previous data revealed that F18, in powder and fibre forms, was extremely efficient against gram-positive and gram-negative strains (*S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*), eliminating 100% of the bacterial cells after 24 h in direct contact (Souza *et al.* 2017). However, the present study indicates that F18 in paste form was not effective in eliminating *E. faecalis* in the direct contact test for the chosen experimental procedure. The antibacterial action of bioactive vitreous materials can be influenced by, in addition to the chemical composition, the glass concentration, particle size distribution and bacterial strain analysed (Zhang *et al.* 2007, Leppäranta *et al.* 2008, Gorriti *et al.* 2009). So, when different experimental settings, glass concentration, medium type, particle sizes, etc., are used, changes in response regarding bacterial elimination are normally expected.

It is known that the period for action of $Ca(OH)_2$ varies according to the vehicle that is used (Han *et al.* 2001). It should be noted that $Ca(OH)_2$ in aqueous vehicle must remain inside the root canal for a period of 7 days to achieve the full antimicrobial effect (Estrela *et al.* 2001, Han *et al.* 2001, Zancan *et al.* 2016). Similarly, the vehicle may influence the action of bioglasses. The optimum time for killing bacteria with the experimental pastes in infected dentine remains undefined.

Pastes from these two glass-based materials strongly induced mineralization, with more intense induction of OPN expression than $Ca(OH)_2$ paste. This study was the first to evaluate the antimicrobial activity of the pastes of these new materials. The effect of these pastes on bacterial colonies after a longer contact time is not known. Thus, these experimental pastes have properties that require further evaluation before being used as an intracanal medicament. In addition, this study may also indicate that the experimental pastes evaluated here may have potential use in direct contact with pulp tissue, such as pulpotomy or pulp capping, due to the induction of biomineralization. These are suggestions for future studies.

Conclusions

Experimental pastes of F18 and BS-2P bioactive materials were biocompatible, stimulated biomineralization and induced significant OPN expression compared to $Ca(OH)_2$. The BS-2P paste demonstrated antimicrobial activity comparable to $Ca(OH)_2$.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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