Received: 13 January 2021 | Revised: 13 August 2021 | Accepted: 22 August 2021

DOI: 10.1002/jbm.b.34931

RESEARCH ARTICLE

Society For Biomaterials WILEY

Effect of bioactive glasses containing strontium and potassium on dentin permeability

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Funding information
São Paulo Research Foundation—FAPESP, Grant/Award Number: 2013/07793-6; Fundação Araucária, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant/Award Number: 461774/2014-3

Abstract

Dentin hypersensitivity (DH) is characterized by pain caused by an external stimulus on exposed dentin. Different therapeutic approaches have been proposed to mitigate this problem; however, none of them provide permanent pain relief. In this study, we synthesized and characterized experimental bioactive glasses containing 3.07 mol% SrO or 3.36 mol% K_2O (both equivalent to 5 wt% in the glass), and evaluated their effect on dentin permeability to verify their potential to treat DH. The experimental materials were characterized by field-emission scanning electron microscopy, Fourier transform infrared spectroscopy, micro-Raman spectroscopy, and X-ray diffraction to confirm the respective structures and chemical compositions. The reduction in the hydraulic conductance of dentin was evaluated at the three stages: minimum permeability; maximum permeability (24% ethylenediaminetetraacetic acid [EDTA] treatment); and final dentin permeability after treatment with the bioactive glasses. They all promoted a reduction in dentin permeability, with a significant difference for each sample and posttreatment group. Also, a significant reduction in dentin permeability was observed even after a simulated toothbrushing test, demonstrating effective action of these materials against DH. Besides, incorporating 3.07 mol% SrO was a positive factor. Therefore, strontium's desensitizing and re-mineralizing properties can be further exploited in bioactive glasses to promote a synergistic effect to treat DH.

KEYWORDS
dentin sensitivity, glass, oxides, potassium, strontium

1 | INTRODUCTION

Dentin hypersensitivity (DH) is a clinically relevant and population-wide problem that affects patients' quality of life and requires appropriate action in terms of research, dental education, and dental treatment. This clinical condition affects 35% of the worldwide adult population.[1,2] DH is described as acute, localized pain of variable intensity; it is generally associated with external stimuli. Different theories have been proposed to explain the origin of DH-related pain, and the hydrodynamic theory is one of the most accepted. This theory states that fluid movement within dentine tubules can cause pain after physical or osmotic stimulation. According to this theory, the occlusion of dentinal tubules may be a solution for DH.[3,4]

Different treatments have been proposed for DH; some of these focus on the occlusion of dentin tubules, and others center on a reduction in the conduction of painful nervous stimuli. Some examples of these treatments include toothpaste, gels containing Sr^{2+} or K^+, and fluoride varnish, which are widely used as a home or dental office

treatment.[4] Another method to treat DH is laser therapy. Different types of lasers are used to treat DH by employing variable energy settings and wavelengths. This form of treatment has shown a desensitizing effects, which can be either physical in nature (resulting in the reduction or occlusion of dentin tubules) or related to laser activity at the level of the nervous system.[2] However, none of these treatments provide permanent pain relief. Hence, the development of dental materials which are bioactive and interact with tissues without producing adverse effects, such as bioactive ceramics, have been the subject of several studies, either through creating new materials or by improving the characteristics of those that already exist.[5-8]

Bioactive ceramics have been intensively studied since Bioglass® was first used as a material for bone regeneration in the early 1970s.[9] These materials can quickly (in a few hours or days) bond with hard tissues and some soft tissues, stimulating bone growth away from the bone-implant interface. In dentistry, bioactive ceramics can be used for the treatment of DH because they can promote the formation of a hydroxyapatite layer on the dentin surface (sealing the dentinal tubules).[10-12] Since the hydroxyapatite crystals formed are similar to the mineral phase present in dentin, both can be chemically bonded to promote a stronger adhesion of the glass particles to the dentin surface. For example, there are commercially available products containing bioactive glass (Novamin®) as a re-mineralizing agent.[13-15] Such toothpaste can decrease dentin permeability by inducing hydroxyapatite microcrystals inside dentinal tubules.[14-16] It is relevant to note that this property of bioactive glasses and other bioactive ceramics depends primarily on their chemical composition, surface area, and crystalline phases (in case of glass–ceramics and ceramics).[5-12] Thus, different modifications can be tested, aiming to improve their efficiency according to the desired application.

Since bone and dentine have similar composition, and based on the reported characteristics of bioactive glasses (i.e., the ability to form in vivo a hydroxyapatite layer on their surfaces, promoting an interface and strong bonds to bone and teeth)[9] and the desensitizing property of Sr^{2+} and K^{+},[4,7] it is possible to design new glass compositions seeking to maximize its positive interaction with those tissues. For instance, controlling the rate of glass degradation by changing the composition, the material may properly release specific ions to provide stimuli for several biological activities.[17-21] Therefore, considering the possible improvements in bioactive glass properties to be gained by incorporating Sr^{2+} and K^{+}, we developed two experimental bioactive glasses derived from 48.68%SiO_2–16.23%CaO–32.46%Na_2O–2.63%P_2O_5 composition (mol%); one containing 3.07 mol% SrO and the other containing 3.36 mol% K_2O. Our aim was to evaluate new experimental formulations of bioactive glasses with SrO or K_2O in the occlusion of dentinal tubules and verify the effect of these biomaterials on dentin permeability. All glass compositions tested were based on the 50.00%SiO_2–16.67%CaO–33.33%Na_2O system, one of the few glass compositions that crystallizes in the volume without nucleating agents. Therefore, this study opens the possibility of finding new glass compositions for dental applications with better mechanical properties by crystallization. The capacity of the materials to occlude the dentin tubules was assessed in vitro, and they were characterized by field emission scanning electron microscopy (FESEM), Fourier transform infrared (FTIR) spectroscopy, micro-Raman spectroscopy, and X-ray diffraction (XRD) to confirm the respective structures and chemical compositions.

2 | MATERIALS AND METHODS

2.1 | Synthesis of experimental bioactive glasses

We synthesized bioactive glass samples (Bv1, Bv2, and Bv3) using the melting/quenching method (Table 1).[9] For 100 g of Bv1, 47.07 g silicon oxide (SiO_2 99.9%, Zetasil 3® Santa Rosa Ltda, Paqueri, MG, Brazil), 26.38 g calcium carbonate ($CaCO_3$ 99%, Labsynth, Diadema, SP, Brazil), 55.59 g sodium carbonate (Na_2CO_3 99.5%, Labsynth, Diadema, SP, Brazil), and 6.01 g phosphorus pentoxide (P_2O_5 99.9%, Labsynth, Diadema, SP, Brazil) were mixed for homogenization and subsequently dried in an oven at 100°C for 8 h. The dried mixture was then melted in a platinum crucible, using an electric furnace and a heating rate of 10°C/min up to 1400°C, kept at this temperature for 3 h and finally poured and remelted ×3 during for homogenization. The melt was subsequently poured and cooled by splat cooling and then annealed for 2 h at 455°C. This procedure was followed by cooling at a rate of 2°C/min for residual stress relief. Finally, the material was wet milled with isopropanol in a high-energy mill for 3 h at 550 rpm (reversing the cycles every 30 min), using agate spheres and a jar to obtain powders with particle sizes smaller than 4 μm, which were selected by filtration. We employed the same synthesis procedure for the samples Bv2 and Bv3, mixing for each batch 44.57 g SiO_2, 24.98 g $CaCO_3$, 52.62 g Na_2CO_3, 6.01 g P_2O_5, and 7.35 g strontium carbonate ($SrCO_3$ 97%, Vetec/Sigma-Aldrich, Duque de Caxias, RJ, Brazil) or 7.40 g potassium carbonate (K_2CO_3 99% Vetec/Sigma-Aldrich, Duque de Caxias, RJ, Brazil), respectively. As reference materials, we used Bioglass® and Biosilicate® samples with the same particle sizes, which were prepared and selected using the same procedure described for the experimental samples.

Biosilicate® is an almost fully crystalline glass–ceramic, and like Bioglass®, has also been successfully tested in various medical and dental applications.[4,8,11,12] Both reference materials were provided by

TABLE 1 Group identification and nominal composition of the tested materials

Group	Composition (mol%)
Bv1: experimental	48.68%SiO_2–16.23%CaO–32.46%Na_2O–2.63%P_2O_5
Bv2: experimental	47.12%SiO_2–15.71%CaO–3.07%SrO–31.41%Na_2O–2.69%P_2O_5
Bv3: experimental	46.98%SiO_2–15.66%CaO–3.36%K_2O–31.32%Na_2O–2.68%P_2O_5
Bv4: bioglass®	46.14%SiO_2–26.91%CaO–24.35%Na_2O–2.60%P_2O_5
Bv5: biosilicate®	49.16%SiO_2–25.79%CaO–23.33%Na_2O–1.72%P_2O_5

researchers of the Vitreous Materials Laboratory (LaMaV) of the Materials Engineering Department of the Federal University of São Carlos (DEMa/UFSCar), Brazil.

2.2 | Field-emission scanning electron microscopy and energy-dispersive X-ray spectroscopy analyses

Field-emission scanning electron microscopy (FESEM) was used to analyze all the bioactive glass powders for their morphological characterization. A scanning electron microscope (Mira 3, TESCAN, Czech Republic) coupled to an energy-dispersive X-ray (EDX) spectrometer was used, which allowed qualitative chemical analysis.[6,8] About 3 mg of each sample were added in isopropanol, and then the system was submitted to a vortex shaker (Biomixer QL-901, Taboão da Serra, SP, Brazil) for dispersion of the particles. After this procedure, 5 μl of the solution was removed and placed on a metallic sample holder and taken to the oven at 50°C for 12 h before the analysis. The photomicrographs were obtained using accelerating voltages from 10 to 15 kV collecting secondary electrons (SEs) originated from surface regions of the analyzed particles. This signal is originating from the atoms of the sample as a result of inelastic interactions between the electron beam and the sample, and is especially useful for the inspection of the sample's surface. Before the analysis, the dried samples were coated with a thin evaporated gold/palladium (Au/Pd) layer using a SC7620 Mini Sputter Coater (Quorum Technologies, East Sussex, UK), that makes the surface electro-conductive and improving SEs emission. The mean of the largest particle sizes in the SEM images was estimated using the software ImageJ,[22] selecting at least 10 particles per image.

2.3 | Raman spectroscopy

The Raman spectra of each sample were collected at room temperature using a Senterra micro-Raman spectrometer (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany), with a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser line at 532 nm and 20 mW as a source of excitation, attached to an optical microscope. A magnification of ×50 with an opening of 50/1000 was used to visualize the samples and to determine the exact location of the scans.[23,24] The detector (CCD) was cooled by thermoelectric effect at −65°C, being the power over the sample of 2 mW, scanning range from 2000 to 400 cm^{-1} with a spectral resolution of 3–5 cm^{-1}, accumulation time of 30 s, and number of scans of 4. Finally, a postprocessing of the collected spectra was performed using the dedicated Opus Spectroscopy software, version 6.5 (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). The data were subjected to background correction and smoothing.

2.4 | Fourier transform infrared spectroscopy

The characteristic functional groups of the materials were analyzed using an IRPrestige-21 spectrometer (Shimadzu, Tokyo, Japan). The equipment operated in absorption mode using the potassium bromide (KBr) pellet technique, with a spectral resolution of 4 cm^{-1} from 2000 to 400 cm^{-1}.[6,8,17,25] We collected the mean spectra of 32 scans. We used a mixture with 4 mg of each sample and 196 mg KBr spectroscopic grade (2% m/m) to prepare the pellets.

2.5 | X-ray diffraction

The diffraction patterns of the particulate materials were obtained using an Ultima IV, X-ray diffractometer (Rigaku, Japan). The scanning parameters were 2° min^{-1} from 2θ at 5–80°, with CuK_α radiation (λ = 1.5418 Å), a current of 30 mA, and voltage of 40 kV.[6,8,17,25] We performed the XRD analysis to check the possible crystalline phases formed in the materials.

2.6 | In vitro evaluation of dentin permeability

We used 84 bovine incisors for the in vitro evaluation of dentin permeability. The teeth were disinfected in 0.5% chloramine and stored in distilled water at 4°C until use. For each tooth, two grooves were made on the vestibular surface at the cervical region using a round diamond bur (1012F KG Sorensen, Barueri, SP, Brazil). The first groove was made in the enamel-cement junction, and the second groove was 4 mm apical from the first. The area between the two grooves was worn 0.3 mm to simulate a class V noncarious lesion using a cylindrical diamond bur (3099F KG Sorensen, Barueri, SP, Brazil). The crown was removed at the first groove level using a double-sided flexible diamond disc (7020 KG Sorensen, Barueri, SP, Brazil) at low speed, and then we removed the root by cutting in the second groove. We separated the vestibular surface from the palatine by cutting with the same flexible diamond disc. Only the vestibular fragment was used, the palatine was discarded. Finally, each external dentine surface of each specimen was wet-grinded with 1000, 1200, 1500, 2000, 2500, and 4000-grit SiC papers to leave a uniform surface. Subsequently, the internal surface was adjusted with 600-grit paper to standardize the thickness of the specimens to 1 mm (Figure 1).

2.6.1 | Dentin permeability (hydraulic conductance)

The dentin hydraulic conductance was evaluated using a test involving the passage of distilled water through the dentin tubules, a process measured by the movement of air bubble trapped within a microcapillary tube. We used a measuring device (THD03 Permeabilidade dentinária Analógico, Odeme Biotechnology, Luzerna, SC, Brazil) working with a pressure of 10 Psi for 1 min. The measurement was repeated three times at each time of assessment. The values obtained were converted into dentin permeability (Lp) according to the relationship $Lp = Q/P(A)$, where Q is the fluid flow (μl/min), P is the hydrostatic pressure across the dentin in cm H_2O, and A is the

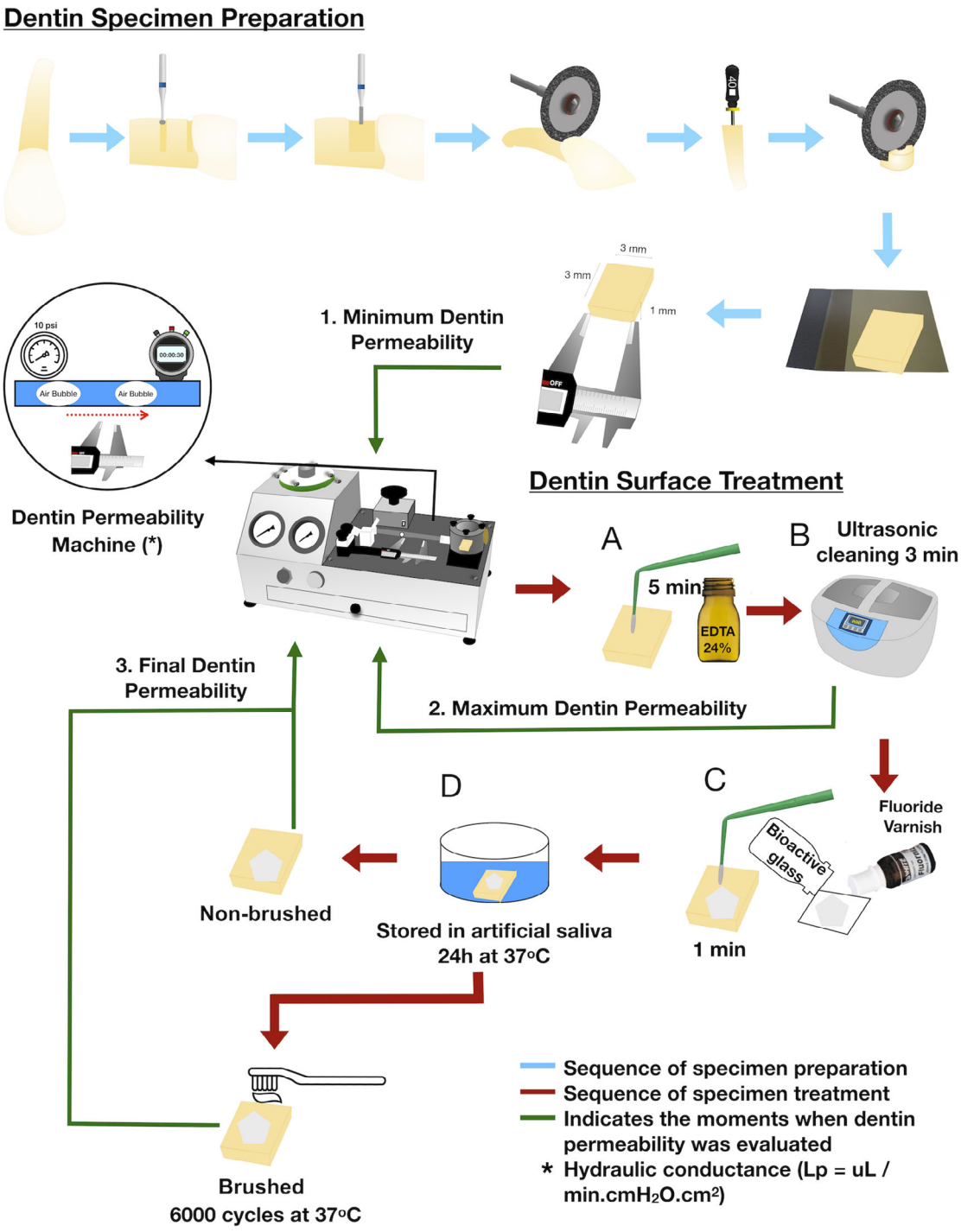

FIGURE 1 Flowchart of the steps involved in preparing dentin specimens from bovine incisors. Dentin surface treatment sequence, posttreatment, nonbrushed or brushed, with simulated toothbrush machine, and evaluating different moments of the measurement of dentin permeability (hydraulic conductance)

exposed dentin surface area (cm^2). Thus, the minimum hydraulic conductance value obtained from the dentin specimens was considered before any treatment.[26] After this initial measurement we applied 24% ethylenediaminetetraacetic acid solution (EDTA) on the vestibular surface of all the samples for 5 min to open the dentinal tubules and to simulate more permeable dentin. The specimens were subsequently washed and soaked in an ultrasonic cleaner (Cristófoli, Campo Mourão, PR, Brazil) for 3 min to remove all EDTA from the dentine surface. The permeability of the specimens was measured again and the new values were considered to be the maximum permeability.

The dentin specimens were randomly distributed and then treated using fluoride varnish as a vehicle for the glass powders in order to evaluate the effectiveness of the bioactive glasses on dentinal tubule occlusion. The treatments were as follows: FV: 5% sodium

fluoride varnish; Bv1: FV + bioactive glass; Bv2: FV + bioactive glass with 3.07 mol%SrO; Bv3: FV + bioactive glass with 3.36 mol%K_2O; Bv4: FV + Bioglass®; and Bv5: FV + Biosilicate®. The treatments were applied once over the dentin surface for 1 min using a micro brush (Brush Regular, KG Sorensen, Barueri, SP, Brazil). We submitted the samples to two different treatments (simulated toothbrushing): nonbrushed and brushed. This procedure was performed using a simulated toothbrush machine (ElQuip, São Carlos, SP, Brazil). The specimens were submitted to 6000 cycles in linear motion strokes under a load of 400 g with toothpaste slurry (Figure 1).[16] Each dentin specimen was then stored in 5 ml of artificial saliva (Table 2) at 37°C for 24 h before measuring the hydraulic conductance.

TABLE 2 Composition of the artificial saliva

Chemical	Manufacturer/ batch number	Concentration (g L^{-1})
NaF, P.A.	Synth, Diadema, SP, Brazil/42953	0.0044
$MgCl_2$, P.A.	Synth, Diadema, SP, Brazil/112987	0.06
$CaCl_2$, P.A.	Synth, Diadema, SP, Brazil/10257	0.17
KCl, P.A.	Synth, Diadema, SP, Brazil/25564	0.62
$NaH_2PO_4{\cdot}H_2O$, P.A.	Synth, Diadema, SP, Brazil/105427	4.82
$Na_2HPO_4{\cdot}H_2O$, P.A.	Synth, Diadema, SP, Brazil/93438	4.04
Distilled water	q.s 1 liter	–

2.7 | Statistical analysis

In order to confirm the minimum and maximum dentin permeability (before and after exposure to 24% EDTA) for the hydraulic conductance measurements, statistical differences between the groups were established using the paired Student's *t*-test. We compared the percentage reduction of dentin permeability after the treatment involving the different bioactive glasses (nonbrushed and brushed) using two-way ANOVA and Bonferroni post-hoc tests. The tests were considered statistically significant when $p < .05$ (GraphPad Prism version 7.00 for Windows, GraphPad software, La Jolla, California, and IBM® SPSS® 21.0 Statistics, IBM Corp., Armonk, New York).

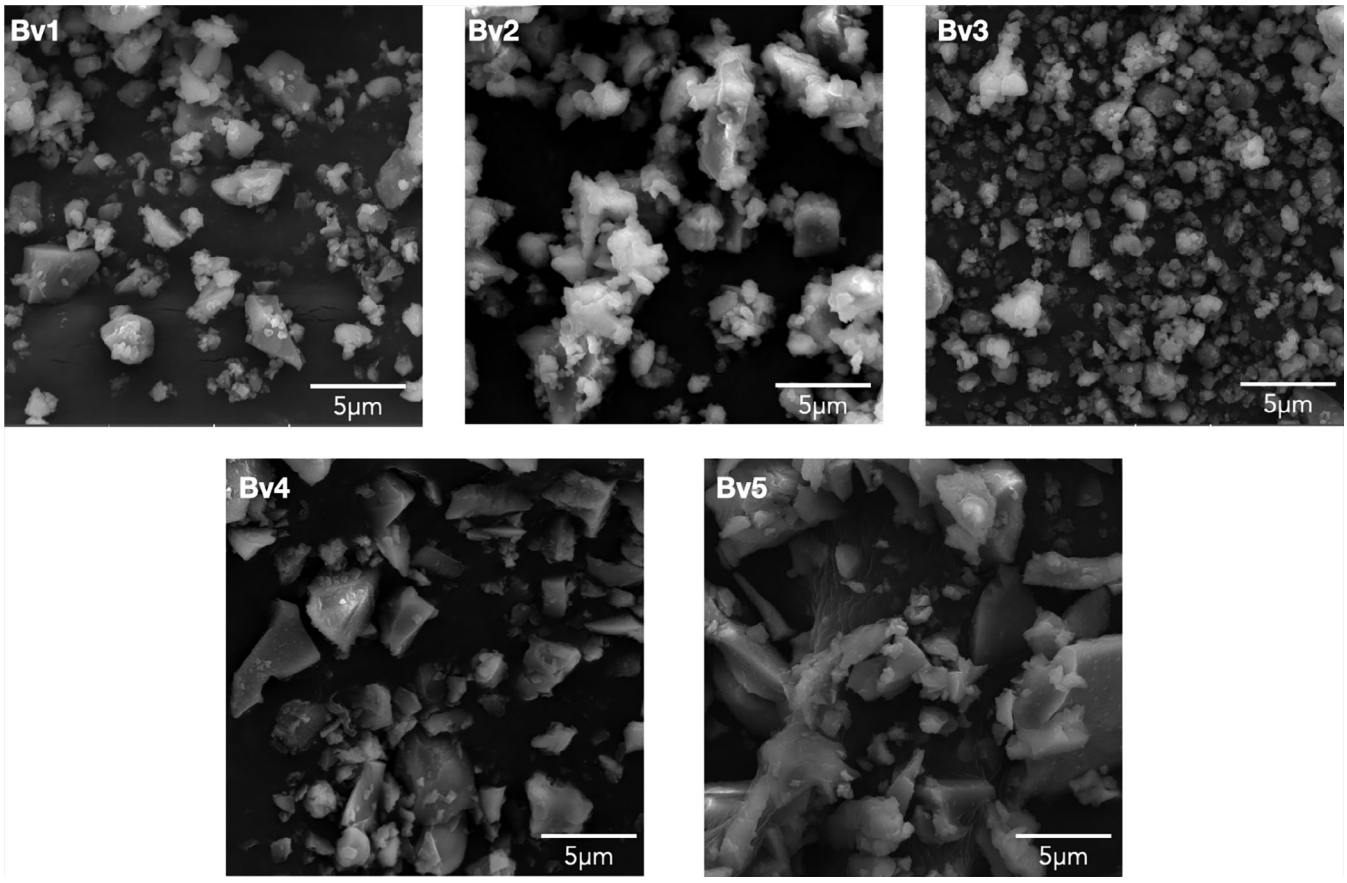

FIGURE 2 Field-emission scanning electron microscopy (FESEM) micrographs of the evaluated materials (Bv1: bioactive glass; Bv2: bioactive glass with 3.07 mol%SrO; Bv3: bioactive glass with 3.36 mol%K_2O; Bv4: Bioglass®; and Bv5: Biosilicate®). All the bioactive glasses had irregular morphology

3 | RESULTS

3.1 | Characterization of materials

The FESEM micrographs (Figure 2) showed that all the bioactive glasses had irregular morphology, and the mean of the largest particle sizes was 5.9 ± 1.2 μm. Although the planned particle size was around 4 μm, these larger particle sizes still serve the purpose of DH treatment.[11,12] The EDX spectra demonstrated the different chemical composition of the materials, indicated the incorporation of Sr and K in the experimental bioactive glasses by a qualitative analysis (Table 3).

The Raman spectra shown in Figure 3A are characterized by the bands situated at approximately 1070 cm^{-1} (which are associated with the asymmetric stretching vibration of Si–O–Si bonds); at 950 cm^{-1} (which relate to the stretching of nonbridging oxygen [NBO] bonds); and at 600 cm^{-1}, which are due to the Si–O–Si bending vibration in depolymerized structural units. In addition to the silica features, the intensity of these bands also contributed to the signals related to the PO_4^{3-} groups observed in the same region.[23,24] The FTIR spectra shown in Figure 3B were mainly characterized by intense bands in the regions of 1100 and 500 cm^{-1}. The bands at ~1100 and 1030 cm^{-1} were related to the stretching vibrations of Si–O bonds. The bands at ~930 and 500 cm^{-1} were attributed to the stretching vibrations of the Si–O(NBO) bonds, which are formed by the presence of cations such as Na^+ and Ca^{2+} Si–O bond bending, respectively.[25,27,28] Similarly to the Raman spectra, the intense bands assigned to the silicate groups overlapped with some phosphate bands that are usually observed at around 1200 and 800 cm^{-1} for P–O bond stretching, and about 650 to 400 cm^{-1} for O–P–O bond bending, which were related to PO_4^{3-} vibrations.[27] Asymmetric C–O stretching vibration of carbonate groups (CO_3^{2-}) was also visible in the region of ~1460 cm^{-1}.

Finally, the XRD patterns shown in Figure 4 confirmed the crystalline character of the Bv5 sample (Biosilicate®), which was formed by the sodium-calcium silicate $Na_2CaSi_2O_6$ phase (PDF #77-2189).[8] Although the other samples had an essentially glassy nature, which was confirmed by the broad halo centered at ~32° (2θ), low intensity peaks appeared in Bv2 and Bv3. The peaks at approximately 26.50 and 33.25° (2θ) corresponded to the traces of $Na_2CaSi_2O_6$ (PDF #77-2189), while the peak at approximately 32.30 (2θ) corresponded to the $NaCaPO_4$ phase (PDF #29-1193).

TABLE 3 Chemical compositions under investigation (mol%)

Sample	Na	Ca	Si	P	Sr	K
Bv1	52.5	10.8	33.3	3.4	–	–
Bv2	51.3	9.6	32.8	4.0	2.3	–
Bv3	54.1	8.7	31.9	3.6	–	1.7
Bv4	41.9	19.4	35.0	3.7	–	–
Bv5	40.4	20.2	37.1	2.4	–	–

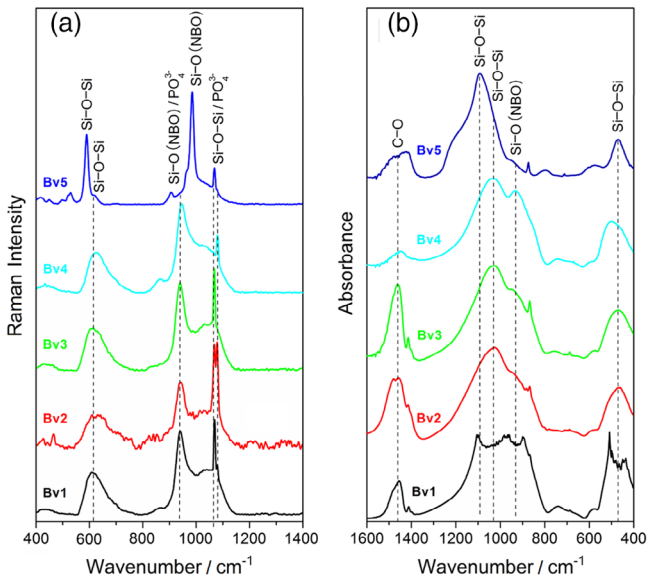

FIGURE 3 Raman (A) and FTIR (B) spectra of the evaluated materials (Bv1: bioactive glass; Bv2: bioactive glass with 3.07 mol% SrO; Bv3: bioactive glass with 3.36 mol%K_2O; Bv4: Bioglass®; and Bv5: Biosilicate®)

3.2 | Dentin permeability (hydraulic conductance)

The hydraulic conductance values of the dentin specimens, before and after the application of 24% EDTA to open the dentinal tubules, were statistically different ($p < .0001$), showing that this treatment was successfully performed and resulted in more permeable dentin (Figure 5A).

The reduction in dentin permeability was significantly different for the type of bioactive glass and treatment (nonbrushed and brushed), with $p < .0001$ (Figure 5B). Nonsignificant difference was verified for the interaction between these factors (material and

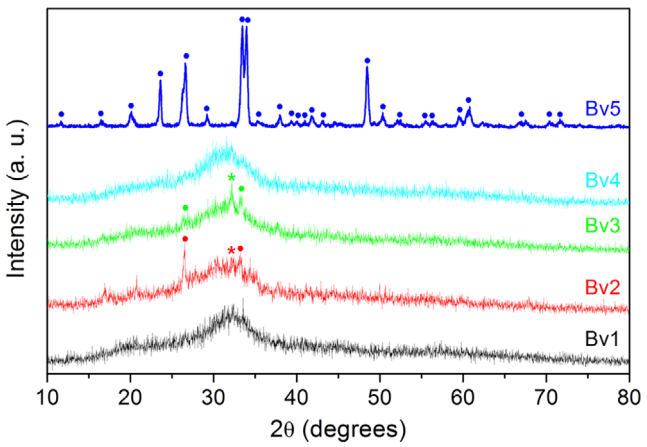

FIGURE 4 XDR patterns of the evaluated materials (Bv1: bioactive glass; Bv2: bioactive glass with 3.07 mol%SrO; Bv3: bioactive glass with 3.36 mol%K_2O; Bv4: Bioglass®; and Bv5: Biosilicate®). ● = $Na_2CaSi_2O_6$ (PDF #77-2189); ✱ = $NaCaPO_4$ (PDF #29-1193)

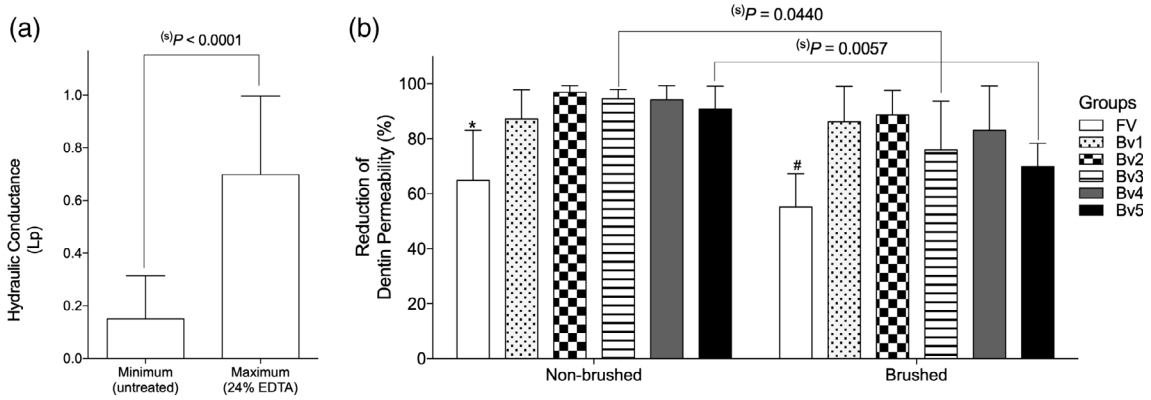

FIGURE 5 Dentin permeability. (A) Mean values and standard deviation of hydraulic conductance (Lp = μl/min cmH_2O cm^2) before (minimum permeability) and after treatment with 24% EDTA (maximum permeability). Significant difference (*p < .0001, paired Student's t-test). (B). Mean values and standard deviation of reduction in dentin permeability (hydraulic conductance) after 24 h of treatment with bioactive glasses (nonbrushed and brushed). Nonsignificant difference (p = .2395) verified for the interaction between these factors (material and treatment). There was a significant difference (nonbrushed) between *FV and Bv1 (p = .0014), Bv2 (p < .0001), Bv3 (p < .0001), Bv4 (p = .0002) and Bv5 (p < .0001). In the brushed samples, a significant difference was observed comparing #FV with Bv1 (p = .0011), Bv2 (p = .0004) and Bv4 (p = .0039). A (s)significant difference between the nonbrushed and brushed specimens for Bv3 (p = .0440) and Bv5 (p = .0057). Two-way ANOVA and Bonferroni post-hoc tests. FV: 5% sodium fluoride varnish; Bv1: FV + bioactive glass; Bv2: FV + bioactive glass with 3.07 mol%SrO; Bv3: FV + bioactive glass with 3.36 mol%K_2O; Bv4: FV + Bioglass®; and Bv5: FV + Biosilicate®

treatment), with p = .2395. There was a significant difference (nonbrushed) between FV and Bv1 (p = .0014), Bv2 (p < .0001), Bv3 (p < .0001), Bv4 (p = .0002) and Bv5 (p < .0001). In the brushed samples there was significant difference comparing FV with Bv1 (p = .0011), Bv2 (p = .0004) and Bv4 (p = .0039). Finally, there was a significant difference between the nonbrushed and brushed groups for Bv3 (p = .0440) and Bv5 (p = .0057).

4 | DISCUSSION

We tested the capacity of different experimental bioactive glasses (one containing SrO and the other K_2O) to reduce dentin permeability to evaluate their potential to treat DH. As references, we used samples of Bioglass® and Biosilicate®, materials that have been successfully tested in various medical and dental applications.[5,8,9] SEM micrographs confirmed the particle sizes (<4 μm) of the selected powders, as well as the presence of Sr and K (EDX) in the Bv2 and Bv3 samples, respectively. In addition to their ability to act as desensitizing agents, thereby assisting in the treatment of DH, the addition of SrO and K_2O may also alter the glass solubility and crystallization tendency.[17,18,20] These are important features because allow us to control the glass degradation rate, by changing its composition. Consequently, ionic products including Sr^{2+} and K^+ were released in physiological conditions that provided stimuli to several biological properties.[17-21]

Incorporating 3.07 mol% SrO (Bv2) or 3.36 mol% K_2O (Bv3) into the base 48.68%SiO_2–16.23%CaO–32.46%Na_2O–2.63%P_2O_5 glass did not provide significant structural changes in the system. This is because the amounts of SrO and K_2O were small; and both compounds behave as glass network modifiers, similar to CaO and Na_2O, which were already existent in the base glass.[17,20,24] A very small crystalline fraction was verified in the Bv2 and Bv3 samples; however the phases that were formed did not necessarily represent a problem in relation to the characteristics of the biomaterial. First, the Bv5 sample was solely composed of the $Na_2CaSi_2O_6$ phase, which can increase the mechanical properties of glass without significantly affecting its bioactivity.[8,25] The $NaCaPO_4$ phase, as a component in the silica-based composites, can improve bioactivity and overall resorption compared to 45S5 bioglass.[29] Furthermore, a bioactive glass–ceramic based on the SiO_2–P_2O_5–CaO–Na_2O–SrO system, with hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and $NaCaPO_4$ as its crystalline phases, proved to be effective in reducing dentin permeability under a simulated oral environment similarly to commercial Sensodyne repair toothpaste containing NovaMin®.[10,15,16]

In the present study, powders with a granulometry of <4 μm were selected because they are suitable for DH treatment.[11] Different materials, either in micro or nanoscale, can also be used for this purpose.[30-32] It has been shown that microparticles can deposit over the dentin surface. Furthermore, their capacity to penetrate the opened dentinal tubules is reduced compared to nanoscale materials.[30] However, it is worth noting that some material compositions in microparticles can partially dissolve in a humid medium like dentin surface, releasing small fragments and ions that could penetrate in dentin tubules with excellent retention.[10,12,14] Moreover, the production of nanometric powder on a large scale is expensive in practice, as well as being a difficult process.[32] Thus, to evaluate the occlusion capacity of our micrometric glass powders, a hydraulic conductance test was performed using bovine dentin specimens. This was due to the probability of obtaining good reproducibility by using bovine dentin specimens because of their histological and morphological similarity to the dentin of human teeth.[33-35]

The application of 24% EDTA to open the dentinal tubules and to simulate a characteristic dentin surface of teeth with DH was an efficient strategy. EDTA is an organic compound ($C_{10}H_{16}N_2O_8$) with a remarkable ability to chelate different metal ions, clean dentinal tubules, and increase dentin permeability.[36] The analysis of the reduction in permeability showed that all the treatments were significant. However, a significant difference was observed between FV and all the bioactive glasses in the nonbrushed samples. The use of FV as a vehicle for glass powders made the application quick and easy, and varnishes are less likely to be diluted by the presence of saliva than gels.[37] Thus, we consider that this feature may help maintain bioactive glass in contact with the dentin surface for as much time as possible.

These results are relevant for future studies of our experimental materials in relation to DH treatment. They showed a similar reduction in dentin permeability compared to Bioglass® and Biosilicate®. Bioactive glass–ceramic containing Sr could also be successfully tested to reduce dentin permeability in vitro.[10] Regarding DH treatment by promoting dentin tubule occlusion, strontium acetate ($C_4H_8O_5Sr$) proved to have this ability and also to promote small changes in the mechanical properties of dentin.[7] It seems that a combination of Sr and fluorine (F) has a positive effect on dentin and tooth enamel re-mineralization. Sr and F improve hydroxyapatite crystallization and reduce its dissolution in an acidic medium.[38,39] Sr could enhance enamel remineralization, having a synergistic effect combined with F.[40] Furthermore, Sr could potentially differentiate dental pulp stem cells to induce dentine-like matrix formation.[41] Therefore, the incorporation of Sr and the controlled release of different materials appear to be beneficial for several dental applications.

When we compared the hydraulic conductance values before and after simulated toothbrushing we observed that all the applied treatments were suitable to promote a reduction in dentin permeability. However, the percentage reduction was lower for the brushed samples, with a significant difference for the treatments Bv3 (3.36 mol% K_2O) and Biosilicate®. A reduction in dentin permeability is associated with lower stability of the materials on the dentin surface, which is caused by the application vehicle, abrasion, or a certain solubility when bioactive glasses come into contact the toothpaste during brushing.[14,16] Consequently, further studies are needed to understand this result better and to explain it adequately. An important fact is that the occlusion of dentinal tubules using bioactive materials can occur through a chemical reaction with the dentin surface and not only through mechanical contact.[10,30] These materials exhibit the ability to form a hydroxyapatite layer on their surfaces, creating an interface and strong bonds to bone and teeth.[8,9,23,25] Therefore, the formation of a continuous, tightly adherent, phosphate layer and rod-shaped crystals, which occlude the exposed dentinal tubules, can occur when this type of material is applied for DH treatment.[6,10,14,15,30,31]

Finally, as the reduction in dentin permeability was similar for some of our experimental bioactive glasses both before and after the simulated toothbrushing, we consider that these materials could be an efficient alternative for DH treatment. However, an in vivo study is essential to ascertain whether the concentration of Sr and K added to these materials affects the nervous system, thereby leading to reducing the painful sensations associated with DH. Furthermore, specific assessments to determine the minimum contact time required to reduce dentin permeability, as well as the depth of penetration of the material into the dentinal tubules (which would provide an idea of the material's stability when applied, as well as the possible longevity of its effect), are also relevant parameters that are worth investigating to identify potential clinical application.

5 | CONCLUSION

This study demonstrated that our experimental bioactive glasses, with SrO or K_2O and associated with fluoride varnish as a vehicle, significantly reduced dentin permeability before and after brushing. This result indicates that the tested glasses promoted the occlusion of dentinal tubules. The SrO glass presented a certain prominence, maintaining a reduction of hydraulic conductance around 90%, even after brushing. Therefore, these experimental bioactive glasses are promising for DH treatment, however, further clinical studies are required to validate their potential in reducing DH under real conditions, and also to verify treatment duration over time.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the following Brazilian agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [Finance code 001]; Fundação Araucária, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—Project No. 461774/2014-3; and the São Paulo Research Foundation—FAPESP—Project No. 2013/07793-6, for generous funding. The authors are also grateful to CLABMU/UEPG for the use of their experimental facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Luisa Alegria Acevedo was responsible for conceptualization, research, methodology, validation and writing the original draft. Letícia Antonelo Campos, Iolanda Cristina Dechandt, Gustavo Alegria, and Renato Luiz Siqueira were responsible for research, validation, and writing the original draft. Edgar Dutra Zanotto and Francisco Carlos Serbena were responsible for research, supervision, and drafting the original draft. Fabio André Santos was responsible for formal analysis, methodology, supervision, writing the review, and editing. All the authors gave their final approval and agreed to be accountable for all aspects of the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Acevedo, L. A., Campos, L. A., Dechandt, I. C., Alegria, G., Siqueira, R. L., Zanotto, E. D., Carlos Serbena, F., & Santos, F. A. (2021). Effect of bioactive glasses containing strontium and potassium on dentin permeability. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 1–10. https://doi.org/10.1002/jbm.b.34931