Effect of bioactive glasses used as dentin desensitizers on the dentin-pulp complex in rats

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Bioactive glasses have been recommended for the occlusion of dentinal tubules in treating cervical dentin hypersensitivity. This study evaluates an *in vivo* model of dentin exposure, and tests the efficacy of bioglass treatments. Thirty male Wistar rats received gingival recession surgery on the upper left first molar. The treatments were applied over the surface of the exposed dentin every 4 days for 28 days. The groups were as follows: Naive; Gingival recession; Cavity varnish; Biosilicate®; Strontium bioglass; and Potassium bioglass. Changes in the dentin-pulp complex, and the presence of substance P, were evaluated through hematoxylin-eosin and immunohistochemical staining. The groups had similar results. Teeth with exposed dentinal tubules in rats showed a typical pattern in the dentin-pulp complex and immunotracing for substance P. The materials did not cause pulp damage. The effects of gingival recession and open dentinal tubules on pulp tissue require further clarification.

Keywords: Dentin, Biocompatible materials, Gingival recession, Inflammation

INTRODUCTION

Periodontal diseases are among the most prevalent diseases worldwide, with a 26% increase in the number of prevalent cases from 2006 to 2016[1,2]. This impacts on quality of life due to the consequences in relation to the oral cavity, such as tooth loss and masticatory dysfunction, and interactions with systemic diseases, such as diabetes and atherosclerosis, since oral biofilms release bacterial products that induce inflammatory mediators to reach hematogenous dissemination[3]. Periodontal treatment aims at a stable periodontium without the recurrence of disease, and this is achieved with subgingival biofilm removal through debridement. Subgingival debridement is used in initial, surgical, and supportive periodontal therapy. Debridement removes soft and hard deposits; however, it can also lead to the removal of cementum and root substances loss[2]. Furthermore, periodontal therapy can result in gingival recession, which leads to permanent exposure of the root portion[4,5].

Root sensitivity resulting from periodontal treatment can have adverse clinical effects, such as lower patient adherence to treatment, worse quality of life, postoperative pain, limitation or difficulty in eating, and even social limitations due to hypersensitivity[6,7]. Dental substance loss leads to the exposure of the inner dentin and a mild to moderate root sensitivity of short duration[8]. However, even dentin sensitivity can become long-lasting in cases where periodontal therapy results in gingival recession[4,5]. Teeth with exposed dentin can have inflammation in pulp tissues[9]. The pulp inflammation process can increase nerve-ending branches, thus expanding the innervation density in the pulp. This may be responsible for a more significant release of inflammatory mediators and neuropeptides, such as substance P, which can sensitize the nociceptor endings[10-13]. Nevertheless, *in vivo* evidence of such alterations is still scarce in the literature.

The treatments available for cervical dentin hypersensitivity are conducted to decrease the movement of dentinal fluid and/or block the nerve pulp response[9]. Desensitizing agents, such as products containing potassium salts and the application of low-intensity lasers, can interrupt neural activation and painful transmission[14]. The reduction of fluid movement by the induction of smear layer formation, or blocking the tubules, can be achieved with dentin adhesives, strontium chloride, oxalates, protein precipitants, and materials containing silica or calcium[8,15]. The treatment of cervical dentin hypersensitivity requires further studies to find a more effective solution[14,16) because the products currently available for clinical and home use provide immediate (up to 7 days), medium-term (up to 1 month), or long-term (from 3 to 6 months), but they do not offer a definitive solution[17].

Bioactive glasses were developed to be applied in bone tissue; their ability to release ions and promote hydroxyapatite formation allows excellent chemical adhesion to bone collagen fibrils and also promotes tissue

regeneration[18]. Bioactive glasses have already been used in dentifrices and dental polishing procedures[18-20]. However, the available bioglasses have limited effectiveness[19]. Studies have indicated that they can occlude dentinal tubules, and the current formulations are easily displaced[8]. The concentrations of bioglass in vehicles of more appropriate formulations require further investigation[20].

The consequences of exposed cervical dentin and open dentinal tubules are unknown, especially in relation to the effects on inflammation and the release of neurogenic substances in pulp tissue. Furthermore, there is no consensus in the literature regarding an ideal treatment for these conditions. The present study had as its first null hypothesis that cervical dentin exposure and the opening of tubules do not affect inflammation or the release of neurogenic substances in the pulp tissue. The second null hypothesis tested in this study was that the application of dentinal tubule obliterating substances does not affect inflammation and the release of neurogenic substances in the pulp tissue. Thus, this study evaluated the *in vivo* consequences of inflammation and substance P release in pulp tissue after exposure to cervical dentin and open dentinal tubules, after-treatment of the exposed dentin with different experimental bioactive glass formulations.

MATERIALS AND METHODS

Ethical statement
The Institutional Animal Use Ethics Committee approved the experimental animal protocols before the experiments started (#0392015). The use of rats in this study followed the guidelines of the Animal Research Reporting In Vivo Experiments (ARRIVE)[21].

Animals
The rats were maintained under the following controlled conditions: artificial lighting (12 h light/dark cycles); room temperature (22 ± 2°C), and free access to rodent food (Nuvilab CR-1, Quimtia, Colombo, Brazil) and tap water. Thirty male Wistar rats (358.8 ± 36.5 g) were randomly divided into the following six groups (n=5 per group): 1. Naive; 2. Gingival recession; 3. Cavity varnish; 4. Biosilicate® parent glass; 5. Potassium bioglass; and 6. Strontium bioglass. All the animals, except those in the Naive group, received a surgical procedure to induce gingival recession and the exposure of cervical dentinal tubules in the upper left first molar. The animals in the Naive group were anesthetized to simulate surgery. The animals in the Naive and Gingival recession groups only received inert treatment with saline solution (0.9% sodium chloride).

The sample size was based on a pilot study (unpublished) that used the same methodology previously described regarding the primary outcome (pulp inflammatory infiltrate). If the sample size in each experimental group was n=5, a two-sided test, we expected 86% (1-β) power at an effect size of 2.2, at a 0.05 significance level, to detect the minimum difference between the groups (G*Power, version 3.1.9.2; http://www.gpower.hhu.de).

Bioactive glasses
The experimental bioactive glasses were prepared using a melting route. The precursor substances ($CaCO_3$, Na_2CO_3, SiO_2, P_2O_5, K_2CO_3, and $SrCO_3$, depending on the composition of the bioactive glasses) were dried at 100°C for eight hours, melted at 1,400°C for three hours in platinum alloy crucibles (to avoid contamination), were quenched by splash cooling and annealing at 455°C for two hours to remove the internal residual stresses, followed by a slow cooling at a rate of 2°C/min. The bioactive glasses were ground in a high-speed planetary mill to obtain the desired powder distribution in the microparticles. The three bioglasses obtained were: potassium bioglass ($2Na_2O.1CaO.3SiO_2$-6%P_2O_5-5%K_2CO_3, particles between 1.5 and 20 µm (median: 6 µm); strontium bioglass ($2Na_2O.1CaO.3SiO_2$-6%P_2O_5-5%SrO, particles between 3 and 25 µm (median: 9 µm); and Biosilicate® parent glass, which had a quaternary formula containing $SiO_2.P_2O_5.Na_2O.CaO$, and particles between 1 to 25 µm (median: 7 µm)[22].

Experimental recession model and dentin treatments
Intraperitoneal injections of chloral hydrate were used to induce general anesthesia in all the animals (4%, 400 mg/kg, Vetec, Sigma-Aldrich, São Paulo, Brazil)[23]. After reflexes were lost, local anesthesia was injected in palatal soft tissues 2 mm from the gingival margin edge (2% mepivacaine with 1:100.000 epinephrine). A gingival collar, which extended from the mesial side of the upper left first molar to the distal side of the second molar, was removed after an incision 2 mm from the gingival margin edge. Approximately 1 mm height of palatal bone, and the cement that covered the exposed root of the first molar, were removed using a #36 Rhodes chisel. The contralateral tooth did not receive any surgical treatment to settle the within-animal control. A metallic device was attached around the cervical surface of the upper left first molar to maintain the recession during the experimental period (Fig. 1). The device was removed after 14 days, and then 24% ethylenediaminetetraacetic acid (EDTA) was applied for three minutes to remove the smear layer and open the dentinal tubules. The particles of the occlusion agents were suspended in the cavity varnish vehicle at a concentration of 5%. The treatments were performed by rubbing the occlusion agent (or inert substance) for 1 min and waiting 4 min before applying a 10% phosphoric acid solution for 20 s (acid challenge) to simulate an acid diet. The treatments and acid challenge were repeated every 4 days for 28 days.

Histological preparation
The animals were euthanized by cardiac exsanguination after sedation. The hemi-maxilla were dissected, fixed in 4% formaldehyde for 48 h, decalcified in 9% EDTA solution for 19 days, processed in an automated histological processor (TP 1020, Leica Biosystems,
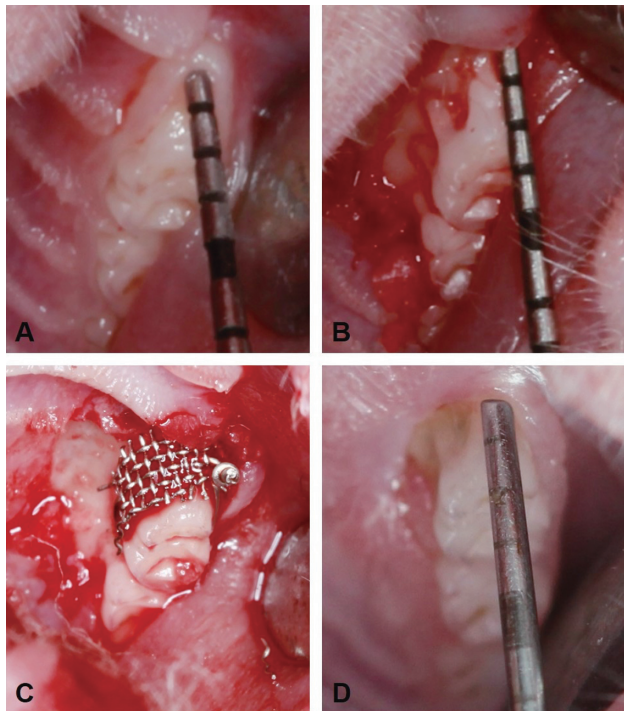
Fig. 1   The sequence of procedures for exposing cervical dentin of the upper left first molar.
(A) Pre-surgical probing, (B) Post-surgical probing, (C) A metallic device is placed around the tooth after the surgical procedure, (D) Gingival recession 24 days after removing the metallic device

Nussloch, Germany), and embedded in paraffin with the sagittal portion of the hemi-maxilla facing downwards. Slices of 5 μm thickness were obtained on a rotating microtome (RM 2235, Leica Biosystems) for the hematoxylin-eosin staining, and 3 μm thick slices were obtained for the immunohistochemical staining. The slices used in the immunohistochemical staining were dispensed on previously silanized slides.

Histological staining
The hematoxylin-eosin staining was performed using the conventional technique of deparaffinization in xylene for 20 min, followed by hydration in a sequence of alcohol concentrations of 100% (5 min), 80% (5 min), and 70% (5 min), rinsed in tap water (7 min), stained with hematoxylin (3 min), rinsed in tap water (3 min), stained with eosin (7 min), rinsed in tap water (2 min), and dehydrated in a sequence of alcohol concentrations of 70% (5 min), 80% (3 min) and 100% (3 min), followed by immersion in xylene for 15 min. The samples were mounted with Permount™ and examined by light microscopy (Olympus BX41 and Olympus DP72, Olympus, Tokyo, Japan).

In the immunohistochemical technique, the histological sections were deparaffinized in xylol, hydrated in ethyl alcohol in descending order, and rinsed in distilled water. Antigen retrieval was performed by immersion in sodium citrate buffer (pH 6.0), heating at 97.7°C for 20 min, rinsing in running tap water for 20 min, and immersion in 0.1M phosphate-buffered saline solution (pH 7.4). The sections were incubated in 6% H_2O_2 to inactivate endogenous peroxidase activity. To reduce non-specific binding, the slides were incubated in 2% bovine serum albumin for one hour in a humidified chamber at room temperature. Mouse anti-rat substance P primary antibody (mouse anti-SP, IgG1 monoclonal antibody, sc-58591, Santa Cruz Biotechnology, Dallas, TX, USA) was used at 1:500 dilution in phosphate-buffered saline 1% bovine serum albumin solution to incubate the slides for 20 h in a humidity chamber at 4°C. The slides were incubated with biotin-conjugated secondary antibody (sc-2017, ImmunoCruz mouse ABC Staining System, Santa Cruz Biotechnology) for 30 min in a humidity chamber at 37°C, and in avidin with biotin-conjugated horseradish peroxidase (sc-2017, ImmunoCruz mouse ABC Staining System, Santa Cruz Biotechnology) for 30 min in a humidity chamber at 37°C. Peroxidase substrate and 3,3′-diaminobenzidine tetrahydrochloride chromogen mixture (sc-2017, ImmunoCruz mouse ABC Staining System) were used for five minutes to reveal the antibody. Counterstaining (Mayer's hematoxylin) was applied for 90 s. The slides were dehydrated, immersed in xylene, mounted in Permount™, and examined by light microscopy (Olympus BX41 and Olympus DP72, Olympus). The immunohistochemical controls were performed without the primary antibody, and they showed no positive immunoreactivity.

Histological analysis
The hematoxylin-eosin and immunohistochemical slides were observed under light microscopy to identify the region of interest, *i.e.*, the coronary and cervical pulp of the upper first molar. The images were captured at a magnification of 400× and analyzed with ImageJ software. The immunohistochemical slides were qualitatively analyzed to identify positive immunoreactivity to substance P expression as brown pigmented structures. In the hematoxylin-eosin analysis, the evaluated criteria were: a) inflammatory infiltrate (qualitative analysis); b) characteristics of pulp tissue (qualitative analysis); c) characteristics of the odontoblastic layer (qualitative analysis); d) response of dentinal tissues (qualitative analysis) (Table 1); and e) vascular changes (quantitative analysis performed by measuring the area filled by blood vessels)[24,25].

Statistical analysis
The data analysis was performed by a single, trained, blinded investigator. The reliability and intra-examiner concordance tests were performed for all the criteria measured in this study. Analysis of the agreement percentage was performed for the ordinal qualitative data. The intraclass correlation coefficient was performed for the quantitative measurements.

The statistical analysis of the ordinal qualitative data was performed after considering the median of

Table 1 Scores attributed to the ordinal qualitative parameters used in the histological analysis

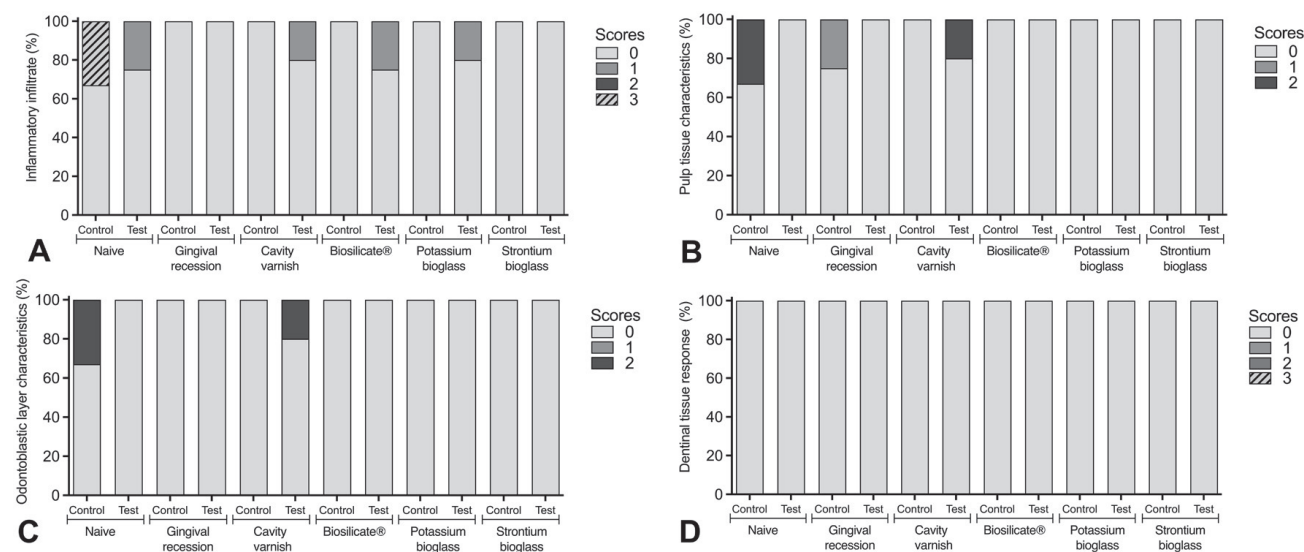| Score	Inflammatory infiltrate[24]	Pulp tissue characteristics[24]	Odontoblastic layer[25]	Dentinal tissue response[24]
0	None or few inflammatory cells	No detectable changes in the pulp tissue	Palisade pattern	Absence of reparative dentin
1	Slight inflammatory cell infiltrate	Pulp tissue fibrosis	Presence of odontoblast and odontoblast-like cells	Formation of reparative dentin
2	Moderate inflammatory cell infiltrate	Pulp tissue necrosis	Presence of odontoblast-like cells	Small nodules in the pulp tissue
3	Severe inflammatory cell infiltrate	—	Absent cell layer	Large nodules in the pulp tissue

Fig. 2   Within-subject comparisons of the percentage frequency distribution of the scores for (A) inflammatory infiltrate (0. no or few inflammatory cells; 1. mild inflammatory infiltrate; 2. moderate inflammatory infiltrate; 3. severe inflammatory infiltrate); (B) pulp tissue characteristics (0. no changes in pulp tissue; 1. fibrosis; 2. necrosis); (C) odontoblastic layer characteristics (0. palisade pattern of odontoblastic layer; 1. presence of odontoblast and odontoblast-like cells; 2. presence of odontoblast-like cells; and 3. absent cell layer); and (D) dentinal tissue response (0. absence of tertiary dentin; 1. tertiary dentin; 2. small nodules in the pulp tissue; 3. large nodules in the pulp tissue).
Non-significant differences (*p*>0.05, Wilcoxon test)

each animal's region of interest scores. The Wilcoxon test compared the teeth exposed to gingival recession and the contralateral (and healthy) teeth.The Kruskal-Wallis test compared data from the different groups (only considering teeth exposed to gingival recession). The tests were statistically significant when $p \leq 0.05$ (IBM SPSS 21.0 Statistics for Mac, Armonk, NY, USA; GraphPad Prism 7.00, for Mac, San Diego, CA, USA).

RESULTS

The animals were monitored to detect weight loss throughout the experimental period. There was a gradual increase in weight for all the groups. We excluded one animal from the Gingival recession group due to the loss of the metallic device.

Reproducibility
The intra-examiner calibration for ordinal qualitative measurements, which considered the absolute frequency analysis regarding difference to zero, had a 74% reproducibility rate for inflammatory infiltrate and 100% for pulp tissue characteristics, odontoblastic layer characteristics, and dentin tissue response. Regarding the continuous quantitative data (measurement of vascular area), the intraclass correlation coefficient indicated excellent reliability of 0.989.

Histopathological analysis
The within-subject comparisons (control tooth *vs.* test tooth) of the inflammatory infiltrate, pulp tissue characteristics, odontoblastic layer characteristics, dentinal tissue response, and the area filled by blood vessels were similar (control tooth and test tooth)[24,25]

(Figs. 2 and 3). In the between-subject comparison, in which only the teeth on the test side (upper left first molar) were considered, no statistically significant differences were observed between the groups (Figs. 3 and 4).

Immunohistochemical analysis
In the immunohistochemical technique, the photomicroscopy images of the pulp tissue of the upper left first molar sections detected, substance P as brown pigments in the pre-dentin layer and the periodontal ligament in all groups (Fig. 5). In the qualitative analysis, similar characteristics were observed between the groups regarding the staining intensity and staining patterns. No differences were observed between the groups. There was normal distribution of blood vessels and fibroblast
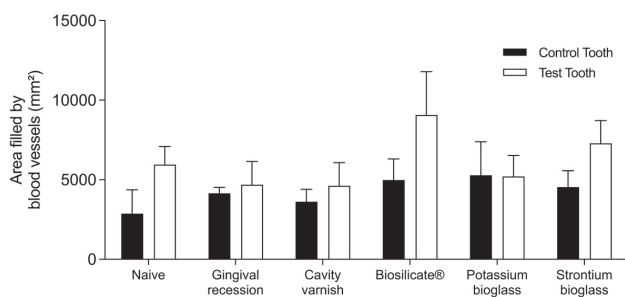
Fig. 3   Analysis of the area filled by blood vessels in pulp tissue.
Mean and standard error of the area filled by blood vessels in within-subject (control tooth vs. test tooth) and between-subject comparisons. Non-significant differences in within-subject (Wilcoxon test) and between-subject comparisons (Kruskal-Wallis test) ($p > 0.05$)
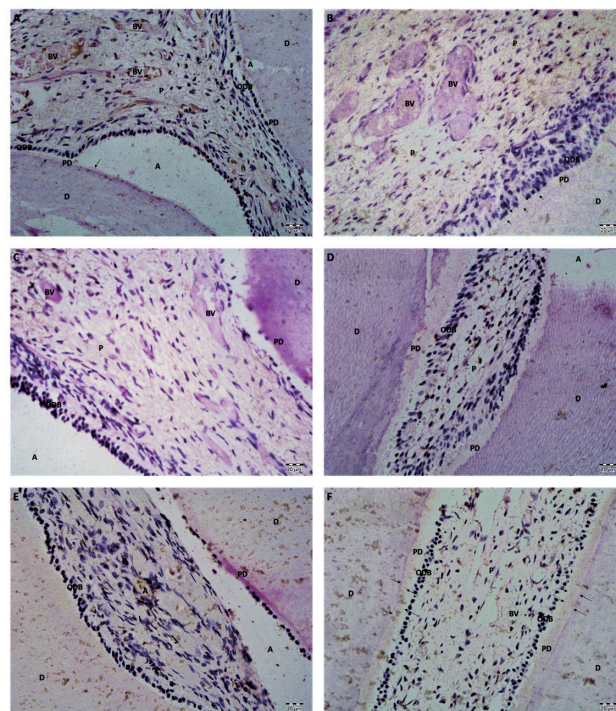
Fig. 5   Substance P (SP) immunohistochemical staining. Representative photomicroscopy images of the dental pulp (test teeth —upper left first molar). It was possible to observe markings (substance P) in the predentin layer (PD) in all groups. A. Naive group (N), B. Gingival recession group (S), C. Varnish group (V), D. Biosilicate® group (BV-BS), E. Bioglass group containing potassium (BV-K), F. Bioglass group containing strontium (BV-Sr). D: dentin; PD: pre-dentin; ODB: odontoblastic layer; P: pulp tissue; BV: blood vessels; A: artifact of the technique; Arrow: indicates antibody labeling
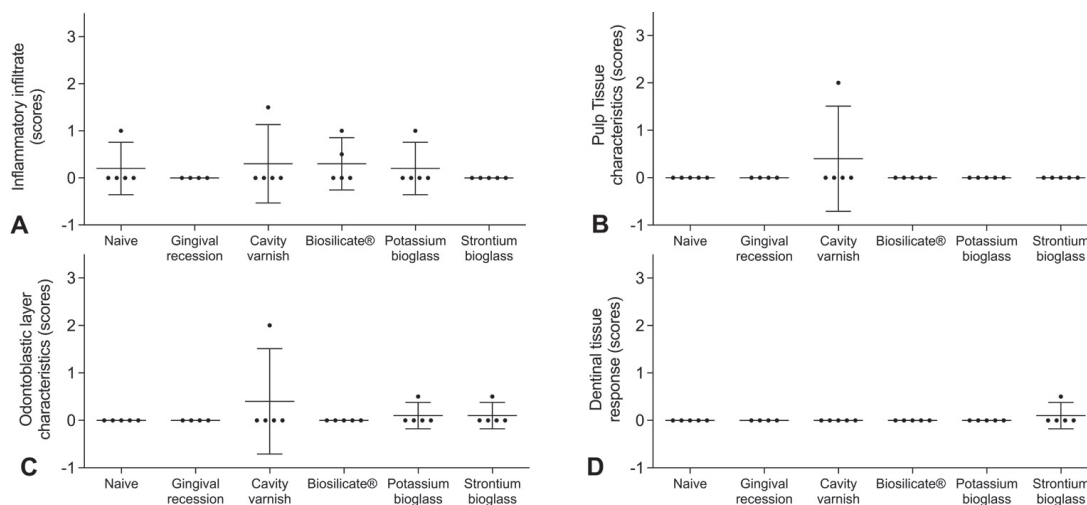
Fig. 4   Scatter dot plots of between-subject comparisons for (A) inflammatory infiltrate, (B) pulp tissue characteristics, (C) odontoblastic layer characteristics, and (D) dentinal tissue response.
Lines represent mean and 95% confidence intervals. Dots correspond to each animal (One animal from the Gingival recession group was excluded due to the loss of metallic device). Non-significant differences ($p > 0.05$, Kruskal-Wallis test)

cells in the loose connective tissue of the pulp. The arrows point (Fig. 5) to the pre-dentin layer, where the odontoblastic extensions were located. Nearby, there was the odontoblastic layer on the pulp side, and in the outer site, the intertubular dentin introduces the dentin layer. Immunolabeling for substance P were observed in the pre-dentin layer. Staining artifacts (brown spots) were observed on the dentin layer and across the lamina. In the sub-odontoblastic region, it was possible to observe the cell-rich zone, the cell-free zone, the odontoblastic layer, and the pre-dentin, which was immunostained by substance P (Fig. 5).

DISCUSSION

Our results indicated that gingival recession associated with dentinal tubules exposure seemed to be either slightly, or not at all, connected with pulpal alterations since no significant differences were observed between the groups for any of the considered histological and immunohistochemical parameters (inflammatory infiltrate, pulp tissue characteristics, odontoblastic layer condition, dentinal tissue response, area filled by blood vessels, and immunostaining for substance P). Changes in pulp tissues are relatively common. Many of these conditions have been studied in *in vivo* models (animal or human), such as cracked tooth syndrome, erosion, abrasion, carious processes, endodontic changes, adhesive treatments, and dentin hypersensitivity[9,26,27]. It is believed that neuroinflammatory pulpal reactions may result from non-harmful stimuli[9], such as those identified in our study design, *i.e.*, gingival surgery, dentinal tubules exposure, and acid diet simulation.

Exposure to open dentinal tubules is associated with symptoms of dentinal hypersensitivity[9,15,28,29]. Although the present study did not identify pulpal inflammatory response or neurogenic alterations, the literature provides some explanations for the acute pain associated with exposed tubules. Despite the lack of evidence, one theory defends the presence of synaptic structures that connect the odontoblasts to the pulpal nerves. Furthermore, the hydrodynamic theory supports nociceptive transduction in pulpal nerve fibers, which are induced through the movement of fluids present within the dentinal tubules[11,27,28]. There is emerging evidence that pain transduction, modulated or mediated by paracrine cell-cell communication through chemical mediators as chemo-, mechano-, and thermosensitive channels, has been identified in odontoblasts. There are also indications of autocrine/paracrine mechanisms for purinergic signaling involving adenosine triphosphate (ATP). Thus, nociceptive transduction in pulpal nerves appears to result from mechanosensitive responses from odontoblasts[30].

In the present study, we found similar results between the control groups (Naive, Gingival recession, and Cavity varnish) regarding pulp inflammation and the release of the neurogenic mediator. Such conditions probably did not occur in the pulp of the teeth with cervical dentinal tubule exposure or were not detectable due to the time of euthanasia and the evaluation methods used. No differences in terms of pulp inflammation and substance P release were observed compared to the bioactive glasses groups (Biosilicate® parent glass, Potassium bioglass, and Strontium bioglass). The topical application of these substances on exposed dentinal tubules cannot generate unwanted and inadvertent pulpal inflammation. Several *in vitro* and clinical studies have shown the efficacy of Biosilicate® parent glass in the occlusion of dentinal tubules[22,31,32]. An experimental bioglass containing strontium was used in the present study because *in vitro* studies using strontium salts, mainly strontium chloride or acetate in dentifrices, have demonstrated strontium ion affinity to dentin, with positive effects on the occlusion of dentinal tubules and dentin permeability. Potassium ions present in the commonly used formulations of bioglass have a higher release rate than Na^+ ions, resulting in higher levels of biological activity and a greater capacity to initiate the ionic exchanges necessary to form hydroxycarbonapatite on the aqueous surface. It is also known that the basic composition of the hydroxycarbonapatite $(Ca_{10}(PO_4,CO_3)_6(OH)_2)$ formed from bioglasses may undergo variability depending on its medium and the biomaterial[22]. Thus, the potassium and strontium present in the bioglasses may have remained as a constituent of the precipitate that formed after the reaction between bioglass and dentin (hydroxycarbonapatite and its variations), and may not have reached sufficient depth inside the dentinal tubules to promote an increase in the neuronal action potential and/or deep occlusion of the tubules. However, only specific compositional analyses, such as X-ray spectroscopy, could confirm such assumptions.

There is little solid evidence regarding histopathological changes in dental pulp concerning dentinal tubules exposure due to gingival recession[9,27,28]. Studies have indicated that modifications in the dentin-pulp complex, which occur in response to stimuli performed after periodontal procedures on teeth with exposed dentin, may lead to neurogenic changes in the pulp, which could increase the rate of fluid movement through the dentinal tubules, promoting nerve sprouting and changes in ion channel regulation in nerve membranes, thereby producing pain[9,12,27,33]. Neurogenic changes, and the increased release of neuropeptides, lower the pain threshold and excite nociceptors[27]. However, neurogenic inflammation and the release of neuropeptides seem to be associated with irreversible inflammation of the pulp, such as in pulpitis, where there is increased innervation and the release of substance P and calcitonin gene-related peptide (CGRP) in the early stages, as well as the release of neuropeptide Y in chronic cases[9,12,27,28,33].

Several studies have evaluated human teeth with irreversible pulp alterations[10-13,26,33-35], or have been conducted in animals, and they have generally used dental erosion models[36-39]. The study models used have not faithfully reproduced cervical dentinal exposure in clinical patients[29,40]. Our study design reproduced the

human oral environment, *i.e.*, the presence of saliva, biofilm, dental occlusion, mastication, and feeding. The stomatognathic system functions were preserved because no fractures and/or dental losses occurred in any animal. Furthermore, dentin exposure that occurs after a periodontal scaling session was reproduced because the surgical procedure for gingival recession removed gingival, bone, and cementum tissues in a single session ('acute' stimulus) and exposed the dentinal tubules, which were opened by EDTA. Root scaling increases the dental response to stimuli[15] in half of patients[41]. This acute condition is very particular to periodontal patients. It is different from gingival recession and/or the loss of cervical enamel due to trauma, bruxism, acid foods, and gastroesophageal reflux, which is a 'chronic' condition capable of causing pulp alterations[42]. After treatment, phosphoric acid simulates the oral conditions of acid foods and/or biofilm accumulation[14,16], challenging the biomaterials. The topical use of acid ensures the control of its application (contact, quantity, periodicity), which would not happen if animals ingested acid foods or beverages[36-39]. In addition, topical application does not cause stomach and/or systemic changes, which could cause bias in a study. Since there are no protocols for the duration and frequency of treatments to occlude dentinal tubules, the criterion for defining intervals between applications was to estimate a viable clinical reality. Thus a treatment model with one application every 4 days for 28 days was chosen.

However, the application of phosphoric acid immediately after the treatments may have removed or diluted the active ingredient, which was placed directly in contact with the dentin surface. Another possible limitation of our study was that the pulp alteration might have been more evident in the early periods of the acid challenge. These findings were not evident at the time of euthanasia of the animals.

The *in vivo* model was able to reproduce human buccal conditions despite the limitations of our study. The results suggest that the exposure of dentinal tubules, and treatment with bioactive glasses applied on the dentin surface, did not negatively affect the pulp tissues. Therefore, future studies should better elucidate pulp conditions and the mechanisms involved in the exposure of dentinal tubules.

CONCLUSION

In our study design, conventional histological and immunohistochemical techniques could not identify neuroinflammatory and neurogenic alterations in rat molars. Our results showed no significant changes in the pulp tissue of teeth with gingival recession and open dentinal tubules treated with different bioactive materials on the occlusion of dentinal tubules. Likewise, we found a similar neurogenic response in the samples.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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