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ORIGINAL ARTICLE

Obliteration of Dentinal Tubules by Desensitizing Agents Based on Silver Fluoride/Potassium Iodide or Pre-Reacted Glass Particles: An *in Vitro* Study

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ABSTRACT

Objective: To evaluate the efficacy of desensitizing agents for the obliteration of dentinal tubules subjected or not to a simulated oral environment. **Material and Methods:** Dentinal discs (n=8) treated with Riva-Star (RS) or PRG-Barrier-Coat (PRG) were submitted (cycled) or not submitted (control) to erosive-abrasive-thermal cycles and evaluated using scanning electron microscopy/energy dispersive spectroscopic analysis. The variables analyzed were tubule obliteration and dentin surface chemical composition. Data were analyzed by non-parametric tests ($p<0.05$). **Results:** The cycled and control groups did not differ significantly for the responses in each material. The PRG control and cycled groups had fewer visible tubules and a higher proportion of totally obliterated tubules than the RS groups. The percentages of silver coverage were higher in the RS-control than in the RS-cycled. There was a significant inverse correlation between the presence of silver and non-obliterated tubules ($R=-0.791$; $p<0.001$). The percentages of carbon, aluminum, strontium, and potassium were significantly higher in the PRG-control and PRG-cycled compared to the RS control. The percentages of calcium, phosphorus, and silver were significantly higher in the RS compared to the PRG groups. PRG-control showed a higher percentage of boron than RS-control. **Conclusion:** PRG promoted greater tubule obliteration than SR. Simulated stress did not affect the obliterating effect of each agent. Greater silver coverage corresponded to a lower proportion of non-obliterated tubules in RS. Carbon, aluminum, strontium, boron, and potassium predominated in the dentin surface treated with PRG, while calcium, phosphorus, and silver prevailed in RS groups.

Keywords: Dentin; Dentin Desensitizing Agents; Dentin Sensitivity; Microscopy, Electron, Scanning.

Introduction

Dentin hypersensitivity (DH) is a short, sharp pain arising from exposed dentin in response to thermal shock, tactile, osmotic, chemical, or evaporative stimulation not attributed to any other dental defect or pathology [1-4]. The average prevalence of DH is 33.5%, ranging from 4.8% to 62.3% [1]. DH affects more often canines and premolars in individuals aged 20-50 years old [3] with impacts on oral-health-related quality of life [2].

The most accepted among the theories used to explain the mechanisms of DH is the hydrodynamic theory, which states that the movement of fluid within the dentinal tubules stimulates mechanical receptors that are sensitive to pressure, resulting in transmission to the pulpal nerves, causing pain response [5].

The fundamentals of DH treatment are control of the etiologic factors, including occlusal adjustment, dietary advice, and tooth brushing instructions associated with desensitizing agents. According to their action mechanisms, these agents are classified as: physical obliterating agents containing particles or nanoparticles that promote physical occlusion of the dentinal tubules, reducing the dentin permeability; chemical obliterating agents that induce occlusion by mineral precipitation at the entrance of the dentinal tubules; neural desensitizing agents that block neural transmission by using potassium-based compounds to depolarize neural synapses; and photobiomodulation that increases the metabolism of odontoblasts by stimulating the formation of tertiary dentin [6].

Although many agents obliterate the dentinal tubules, their effects are often temporary, and HD recurs when the daily erosive challenges of the diet remove these substances [7,8]. Aiming to overcome this limitation, silver diamine fluoride with potassium iodide (SDF/KI), commercialized as Riva Star (RS) has emerged as a treatment option for DH, resulting in pain reduction through the obliteration of the dentinal tubules [9]. Ionic aggregates joined to proteins and precipitates of calcium fluoride and silver iodide inside the dentinal tubules obliterate them against the action of external agents [10,11]. Besides, coating materials have been developed to prevent and treat HD and protect the exposed dentin surface [12]. The remineralizing agent PRG Barrier Coat is a pre-reacted glass-based varnish whose particles are formed in an acid-base reaction between hot processed fluoraluminosilicate glass and polyacrylic acid, which results in the formation of a stable glass ionomer layer on the surface [13-16].

Thus, we designed this study to answer the following question: Are the effectiveness of dentinal tubule obliteration and chemical composition of the dentin surface influenced by the desensitizing agent used, whether the dentin has been submitted to simulated oral cavity conditions? This study aimed to evaluate, *in vitro*, the effectiveness of desensitizing agents based on SDF/KI or pre-reacted glass for dentinal tubule obliteration, comparing them when submitted or not submitted to simulated erosive, abrasive, and thermal stresses frequent in the oral environment. We also evaluated the coverage by silver particles on the dentin surface of specimens treated with RS. The tested hypothesis was: the desensitizing agents based on SDF/KI or pre-reacted glass do not differ for dentinal tubule obliteration and surface chemical composition when submitted or not submitted to simulated stress test.

Material and Methods

Study Design and Ethical Clearance

A randomized complete block *in vitro* study was designed. The factors evaluated were (1) the desensitizing agents based on SDF/KI silver diamine fluoride/potassium iodide (Riva-Star, SDI Brasil Indústria e Comércio Ltda., São Paulo, SP, Brazil) or pre-reacted glass particles (PRG Barrier Coat, Shofu

Dental, São Paulo, SP, Brazil) applied to the dentin surface; and (2) the dentin submitted or not submitted to the simulated stress. The response variables were (1) the effectiveness of dentinal tubule obliteration and (2) the chemical composition of the dentin surface. The local Ethics Committee approved this study (process number 934 16818.8.0000.5149).

The sample size was calculated by using percentages of tubule occlusion pre-and post-acid challenge demonstrated by a bioactive glass containing toothpaste [17]. The total sample size (n=8 per group) was computed for a two-sided test ($\alpha = 0.05$; $1-\beta = 0.95$) considering the difference between two dependent means (G*Power 3.1, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) and adding 10% for compensation specimen losses.

Specimen Preparation and Randomization of Groups

Eight freshly extracted caries-free human third molars were disinfected in 0.1% thymol solution at 5°C for 7 days and stored in distilled water for 7 days. Dentin discs of 2-mm thickness and a diameter proportional to the dental crown were obtained from the coronary middle third using a precision metallographic saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Each dentin disc was embedded in colorless polyester resin (Cristal 5061, Casa da Resina e do Silicone, Belo Horizonte, MG, Brazil). After 24 hours, each specimen was polished with #600, #1200, and #2500 silicon carbide abrasive paper (Norton Advance Water T223, Brazil) in a metallographic polisher (APL-4, Arotec Indústria e Comércio, Cotia, SP, Brazil). Specimens were ultrasonicated (model Cd4820l, Kondentech Indústria e Comércio Ltda., São Carlos, SP, Brazil) in distilled water for 15 minutes after each abrasive paper to remove residual particles. The specimens were then cut in two hemi sections with a diamond disc in a precision metallographic saw (Isomet 1000), resulting in 32 specimens that were manually polished with 1 µm, ½ µm and ¼ µm diamond polishing paste (Erios, São Paulo, SP, Brazil) on a felt disc for 30 seconds in each mesh. The specimens were ultrasonically cleaned for 15 minutes to remove the paste residues between each paste.

The specimens were randomly divided, using the =RANDOM () function of Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), into four groups (n = 8) as described below: RS-control: specimens treated with RS not submitted to the simulated stress test; RS-cycled: specimens treated with RS submitted to the simulated stress test; PRG-control: specimens treated with PRG not submitted to the simulated stress test; and PRG-cycled: specimens treated with PRG submitted to the simulated stress test.

Desensitizing Agent Application and Simulated Stress Test

Table 1 describes the desensitizing agents and their modes of use. For control groups, immediately after the application of the desensitizing agent, the specimens were stored in 2 mL of artificial saliva (0.96 g KCl, 0.67 g NaCl, 0.04 g $MgCl_2$, 0.27 g KH_2PO_4, 0.12 g $CaCl_2$, 0.1 nipagin, 8 g carboxymethyl cellulose, 24 g sorbitol, and 1000 mL double distilled water) (LenzaFarm, Belo Horizonte, MG, Brazil) in a biological incubator at 37°C for 15 days. For cycled groups, after the agents were applied, the specimens were submitted to simulated stress testing twice a day for 15 days.

Table 1. Description of the desensitizing agents.

Material (Manufacturer)	Composition	Mode of Application
Riva Star (SDI Brasil Indústria e Comércio Ltda)	Silver fluoride (35% to 40%), Potassium iodide (32%), Aqueous ammonia solution (15% to 20%)	1. Dry the tooth for 10 seconds; 2. Apply with a micro-applicator a generous drop of solution from the silver capsule onto the dry dentin surface; 3. Using a micro-applicator, apply a generous drop of the green

		capsule solution until the white precipitate becomes transparent; 4. Leave to dry for 10 minutes.
PRG Barrier Coat (Shofu Dental)	borofluoraluminosilicate, distilled water, methacrylic acid monomer, phosphonic acid monomer, Bis-MPEPP, carboxylic acid monomer, TEGDMA, polymerization initiator, and others	1. Wash and air dry for 10 seconds; 2. Add a drop of activator inside the cocoon with the Base; 3. Remove excess material from the brush at the edges of the cocoon. The working time of the material is about 2 minutes; 4. Apply an even layer on the surface from the cervical to the incisal edge. Wait 3 seconds; 5. Photoactivate for 10 seconds. Allow drying for 10 minutes.

Bis-MPEPP (bis-phenol A polyethoxymethacrylate); TEGDMA (triethylene glycol dimethacrylate).

The simulated stress test was adapted from Schmalz et al. [18] and consisted of 30 cycles of erosive challenge, thermal cycling, brushing, and remineralization in artificial saliva, performed twice a day for 15 days. In each cycle, the erosive challenge was performed by immersing the specimens in a soft drink with a pH of 2.95 (Sprite, The Coca-Cola Company, Rio de Janeiro, RJ, Brazil) for 5 minutes. Then, each specimen was individually placed in plastic bags containing 5 mL of distilled water, and thermocycling was performed in a cycling machine (Ethik Technology Equipamentos para Laboratórios, Vargem Grande Paulista, SP, Brazil) with 10 cycles of 1 minute each at 55°C and 5°C. The specimens were dried with an air jet for 5 seconds and brushed with an electric toothbrush (Professional Care 500, Oral B, Procter & Gamble, Rio de Janeiro, RJ, Brazil), whose head was positioned parallel to the surface of the specimen under a 175 g load, using an apparatus made with condensation silicone. Brushing was performed for 2 minutes, using 0.6g of sodium monofluorophosphate (1450 ppm F−) dentifrice (RDA = 70) (Colgate Triple Action, Colgate-Palmolive, São Paulo, SP, Brazil). After brushing, the specimens were rinsed for 5 seconds and dried for 5 seconds with an air jet.

After completing the simulated stress test, the specimens were stored in 2 mL of artificial saliva for 3 hours between the two same-day cycles and kept in a biological oven at 37°C. Between cycles on different days, the specimens were kept overnight for 12 hours until the next cycle.

Scanning Electron Microscopy (SEM) Analysis

All specimens were mounted on stubs with carbon tape and graphite paint, metalized by deposition of a thin carbon layer (20 nm), and kept in a vacuum desiccator until the time of SEM analysis (FEI Quanta 3D FEG Dual Beam Microscope, Hillsboro, OR, USA), equipped with an energy dispersive X-ray spectrometer – EDS (Quantax 400, Bruker Optics GmbH & Co. KG, Berlin, Germany). The surface of each specimen was analyzed with an operating voltage of 15 kV, resolution of 2048 × 1887 pixels, and magnification of 2500×. The images were analyzed by two trained and calibrated raters (agreement coefficients ≥ 0.99) using Image J 1.46 r software (National Institutes of Health, Bethesda, MD, USA) to determine: (1) the number of visible dentinal tubules in a total area of 2500 μm, and (2) the proportion of totally obliterated, partially obliterated, and non-obliterated tubules. In specimens treated with RS, the images were divided into quadrants, and the presence of silver particles was classified as follows: none - without silver particles in all the quadrants; low – the presence of silver particles in only one quadrant; medium - the presence of silver particles in two quadrants; high - the presence of silver particles in three or four quadrants.

Surface Chemical Analysis by EDS

The sample was illuminated at normal incidence by the 15 KV accelerated electron beam. The X-ray characteristic lines were detected at the Bruker Quantax 400 spectrometer equipped with a XFlash 5010 detector. The stoichiometric synthetic hydroxyapatite reagent (Sigma-Aldrich Quimica S.L., Sintra, Portugal), with a purity of 99.99%, was used as a calibration reference. The corresponding spectra of each sample were

processed and analyzed using the Bruker EDS ESPRIT Spectrum software. Carbon (C), oxygen (O), calcium (Ca), phosphorus (P), silver (Ag), fluorine (F), silicon (Si), boron (B), strontium (Sr), sodium (Na) magnesium (Mg), sulfur (S), nitrogen (N), chlorine (Cl), potassium (K), aluminum (Al), and iodine (I) were identified for each sample.

Statistical Analysis

The data obtained were organized in Microsoft Excel (Microsoft Corporation) spreadsheets and imported into the JAMOVI program (JAMOVI version 1.2.27, Sydney, Australia). The Shapiro–Wilk test verified that the data did not comply with the normality assumption ($p<0.05$). Comparisons between groups for the number of visible dentinal tubules, the proportion of obliterated dentinal tubules, and the chemical composition of the surface were performed by the non-parametric Kruskal-Wallis test, followed by the Dwass-Steel-Critchlow-Fligner post hoc test for paired comparisons. The presence of Ag particles in the RS specimens submitted or not submitted to simulated stress was compared by the χ^2 test followed by Bonferroni's post hoc test. Spearman's correlation coefficient verified the correlation between the presence of Ag particles and the obliteration of dentinal tubules. For all tests, the significance level adopted was 5%.

Results

Table 2 shows the medians and interquartile ranges of visible tubules number, the proportion of non-obliterated, totally obliterated, and partially obliterated dentin tubules in the surfaces treated with RS or PRG, submitted or not submitted to simulated stress cycles. PRG particles promoted high proportions of totally obliterated tubules (cycled=64.9; control=100) and a low proportion of non-obliterated tubules (cycled=12.9; control=0.0). Differently, SDF/KI agent presented a high number of visible tubules (cycled=75; control=61.5) and a low proportion of totally obliterated tubules (cycled = 3.96; control=14.4). For each desensitizing agent, cycled and control groups did not differ significantly for the measured responses ($p>0.05$). PRG-control showed a proportion of totally obliterated tubules higher than RS-control ($p=0.02$). PRG-cycled showed a proportion of totally obliterated tubules higher than RS-cycled ($p=0.017$). Moreover, PRG-cycled did not differ from RS-control ($p=0.188$), showing that PRG promoted more covering of the tubules (Figures 1 and 2).

Table 2. Comparison of medians (interquartile range) of the number of visible tubules number, as well as the proportion of non-obliterated, totally obliterated, and partially obliterated tubules, per field of dentin surface treated with Riva Star or PRG Barrier Coat, submitted (cycled) or not submitted (control) to simulated stress cycles.

Groups	Number of Visible Tubules	Proportion of Non-Obliterated Tubules	Proportion of Totally Obliterated Tubules	Proportion of Partially Obliterated Tubules
	Median (IR)	Median (IR)	Median (IR)	Median (IR)
Riva Star (cycled)	75.0 (45.0)a	54.4 (39.9)a	3.96 (11.24)a	31.0 (27.0)a
Riva Star (control)	61.5 (27.5)a	8.59 (9.08)a	14.4 (20.12)ac	66.3 (36.5)ac
PRG Barrier Coat (cycled)	48.0 (65.9)ac	12.9 (35.0)ac	64.9 (55.2)bc	17.7 (24.0)ab
PRG Barrier Coat (control)	0.00 (0.00)bc	0.00 (0.00)bc	100 (0.00)b	0.00 (0.00)b

Different lower-case letters in the same column indicate differences in the comparison between groups. Kruskal-Wallis test and Dwass-Steel-Critchlow-Fligner post-hoc test ($p<0.05$).

Table 3 shows the coverage of the dentin surface by Ag particles in RS-cycled and RS-control. The percentages of high and medium Ag coverage were higher in RS-control. The percentages of low or no coverage were higher in RS-cycled. PRG-cycled and PRG-control did not present Ag particle coverage.

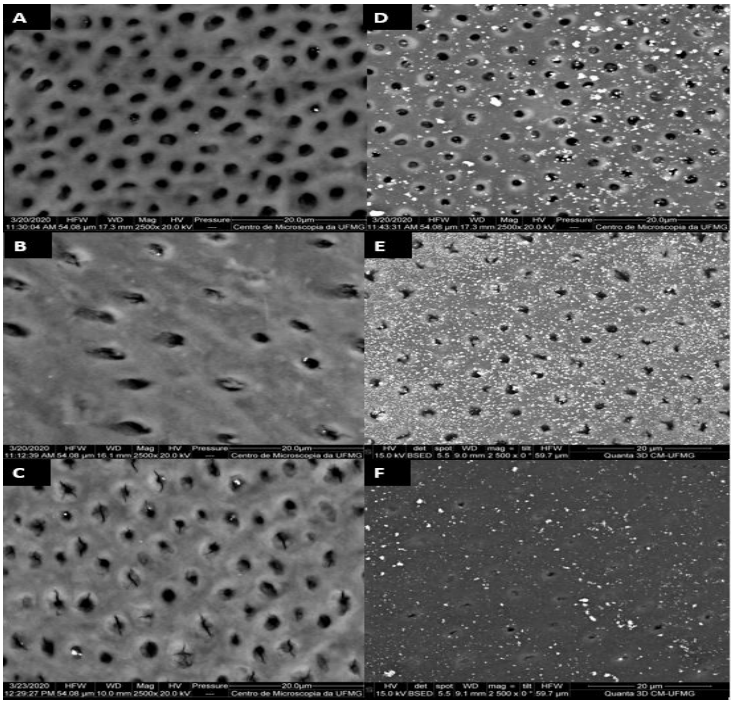

Figure 1. Photomicrographs obtained by scanning electron microscopy (SEM) of the dentin surface in the Riva-Star-cycled (A–C) and Riva-Star-control (D–F) groups. (A) Dentinal tubules non-obliterated, low silver coverage; (B) dentinal tubules partially obliterated and low silver coverage; (C) few dentinal tubules totally obliterated and low silver coverage; (D) dentinal tubules non-obliterated, high silver coverage; (E) dentinal tubules partially obliterated and high silver coverage; (F) dentinal tubules totally obliterated and high silver coverage.

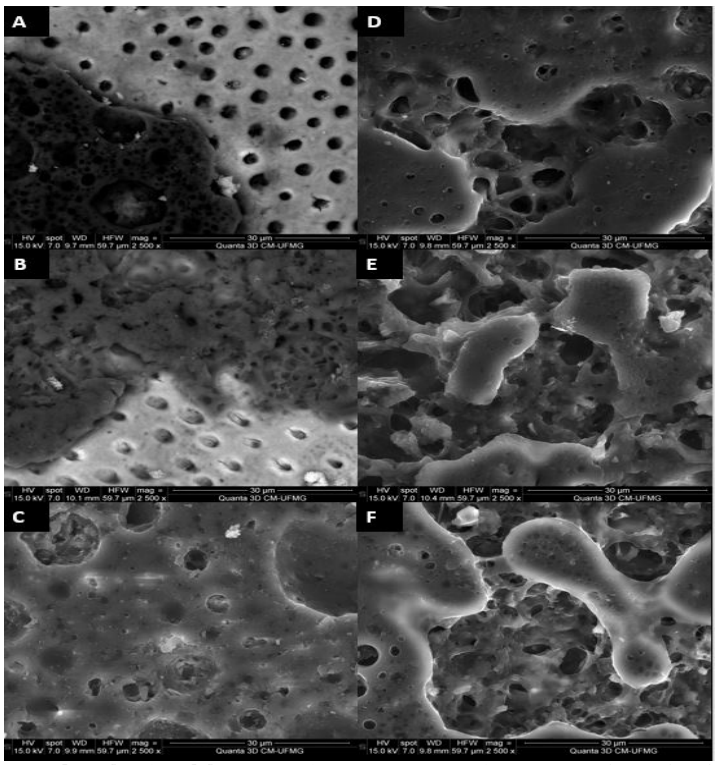

Figure 2. Photomicrographs obtained by scanning electron microscopy (SEM) of the dentin surface in the PRG-cycled (A–C) and PRG-control (D–F) groups. (A) Dentinal tubules non-obliterated, protective layer remaining; (B) dentinal tubules partially obliterated, protective layer remaining; (C) dentinal tubules totally obliterated by the protective layer; (D) dentinal tubules totally obliterated by the protective layer; (E) dentinal tubules totally obliterated by the protective layer; (F) dentinal tubules totally obliterated by the protective layer.

Table 3. Absolute and relative frequencies of specimens with silver particles covering the dentin surface in Riva Star and PRG Barrier Coat groups, submitted (cycled) or not (control) to simulated stress.

Groups	Silver Coating			
	No Coverage N (%)	Low Coverage N (%)	Medium Coverage N (%)	High Coverage N (%)
Riva-Star-cycled	2 (25.0)a	5 (62.5)a	0 (0.0)a	1 (12.5)a
Riva-Star-control	0 (0.0)b	0 (0.0)b	2 (25.0)b	6 (75.0)b
PRG-Barrier-Coat-cycled	8 (100.0)c	0 (0.0)c	0 (0.0)c	0 (0.0)c
PRG-Barrier-Coat-control	8 (100.0)c	0 (0.0)c	0 (0.0)c	0 (0.0)c

Different lower case letters in the same column indicate differences in the comparison between groups; Chi-square and Bonferroni post hoc test ($p<0.05$).

There was a significant inverse correlation between the presence of Ag and non-obliterated tubules ($R=-0.791$, $p<0.001$). There was a positive correlation between the number of partially obliterated tubules and the presence of Ag ($R=0.591$, $p=0.016$). There was no significant correlation between the presence of Ag and totally obliterated tubules ($R=0.143$, $p=0.60$) (Figure 1).

Table 4 shows the medians and interquartile ranges of the percentages of the elements C, O, Ca, P, B, F, Na, Mg, S, Ag, Si, N, I, Al, Cl, Sr, and K obtained by EDS in each experimental group. The percentages of O, F, Na, Mg, N, I, Cl did not differ among the groups ($p>0.05$). The control and cycled groups of both materials did not differ for C, P, B, Ag, Al, Sr, and K. The percentages of C, Al, Sr, and K were significantly higher in PRG-control and PRG-cycled compared with RS-control.

Table 4. Medians (interquartile ranges) of the percentages of chemical elements identified in energy dispersive X-ray spectroscopy (EDS).

Elements	Groups			
	Riva-Star-cycled	Riva-Star-control	PRG-Barrier-Coat-cycled	PRG-Barrier-Coat-control
Carbon	0.00 (8.08)ab	0.00 (8.89)a	32.7 (9.10)b	36.2 (10.30)b
Oxygen	37.7 (2.50)a	37.4 (4.00)a	45.9 (2.30)a	44.7 (9.40)a
Calcium	30.6 (4.90)a	37.2 (3.20)a	11.8 (11.18)ab	2.07 (0.73)c
Phosphorus	12.9 (1.40)a	13.9 (2.00)a	4.13 (3.93)b	3.41 (2.28)b
Boron	4.96 (4.33)a	4.14 (5.70)ab	15.5 (19.2)a	23.3 (4.0)ac
Fluorine	0.98 (0.46)a	1.49 (0.98)a	0.98 (0.55)a	1.44 (2.57)a
Sodium	0.61 (0.37)a	0.62 (0.26)a	0.54 (0.33)a	0.36 (0.31)a
Magnesium	0.21 (0.20)a	0.60 (0.45)a	0.19 (0.14)a	0.10 (0.14)a
Sulfur	0.11 (0.12)a	0.04 (0.06)a	0.16 (0.14)ab	0.02 (0.05)ac
Silver	0.07 (0.78)a	0.39 (0.27)a	0.00 (0.00)b	0.00 (0.00)b
Silicon	0.04 (0.06)a	0.01 (0.02)a	0.22 (0.54)ab	2.37 (0.61)ac
Nitrogen	2.78 (6.44)a	0.00 (1.53)a	0.00 (0.00)a	0.00 (0.00)a
Iodine	0.00 (1.84)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a
Aluminum	0.00 (0.02)a	0.00 (0.00)ab	1.46 (0.99)ac	2.71 (3.04)ac
Chlorine	0.00 (0.00)a	0.00 (0.11)a	0.00 (0.00)a	0.00 (0.00)a
Strontium	0.00 (0.00)a	0.00 (0.00)ac	0.85 (0.26)b	1.25 (3.63)ab
Potassium	0.00 (0.00)a	0.00 (0.00)a	0.37 (0.07)ab	0.71 (0.35)ab

Different lower case letters in the same row indicate differences in the comparison between groups; Kruskal-Wallis test and Dwass-Steel-Critchlow-Fligner post-hoc test ($p<0.05$).

Discussion

PRG is a resinous, bioactive material composed of resin matrix and glass ionomer particles containing F-, Sr2+, BO33-, Al3+, SiO32-, and Na+ ions [12]. It is formed by the acid-base reaction between a fluoride-containing glass and polyacrylic acid in the presence of water, forming a hydrogel with Si particles. After freeze-drying, PRG is desiccated to form a xerogel, which is subsequently ground and silanized to form PRG filler particles in a specific size range [14].

PRG acts as a surface barrier, besides interacting with the hydroxyapatite of the dental structure. This barrier occurs by forming a thicker layer of PRG and high resistance of the material against erosive, abrasive, and thermal challenges. The high release of fluoride among other ions and a tight seal of the dentinal tubules promotes the material's resistance to acid, forming an effective physical and chemical barrier [12,13,19,20]. Due to both physical and chemical adhesion potential forming hydroxyapatite, strontium apatite, and fluorapatite, this material has been used as a desensitizing agent to cover dentin structures [13,15,21,22].

In the present study, PRG demonstrates improved tubule coverage as even after cycling, it did not differ from RS-control. This result agrees with the report of a thicker covering layer formed by PRG application (200 µm) compared with that of RS (20 µm) [9,20]. On the other hand, RS promotes a protein precipitate and the formation of Ca deposits in dentinal tubules [10,23], with a low resistance to acid, abrasion, and thermal cycling [15,20]. Willershausen et al. [9] demonstrated the formation of a thin RS layer (20 µm) with penetration into the tubules. However, unlike the present study, all dentin samples were treated for 60 seconds with 36% phosphoric acid (36%) and rinsed thoroughly to remove the smear layer before RS application. These findings can explain how RS provides occlusion of the tubules through an insoluble precipitate of calcium fluoride, silver phosphate, potassium iodide, and silver protein formed after the application of diamine silver fluoride (38%)/potassium iodide (32%) [9,23,24].

We observed the highest percentages of high and medium Ag coverage in RS-control, low or no Ag coverage in RS-cycled, and no Ag particle coverage in PRG-cycled and PRG-control. After the simulated stress test, Ag particles that have a diameter size of 2 to 15 µm can diffuse through the dentinal tubules, obliterating them inside [9,10,23,24]. There was a significant inverse correlation between Ag presence and non-obliterated tubules. The number of partially obliterated tubules was directly proportional to the presence of Ag, confirming its participation in the obliteration mechanism. The greater the amount of Ag on the dentin surface, the greater the obliteration of dentinal tubules. However, a pilot study showed a short duration of the desensitizing effect of silver diamine fluoride [10], which may result from the low obliteration of dentinal tubule surface *in vivo*.

The identification of the chemical composition of the dentin surface, after the application of PRG and RS, submitted or not submitted to simulated stress, sought to elucidate, together with the images obtained, the probable mechanisms of action of the desensitizing agents. There was no difference for the elements O, F, Na, Mg, N, I, and Cl among the groups studied. The control and cycled groups of both materials did not differ regarding the percentages of C, P, B, Ag, Al, Sr, K, showing that the simulated stress did not affect the detection of these elements. The percentages of C, Al, Sr, and K were significantly higher in PRG-control and PRG-cycled compared with RS-control. B was significantly higher in PRG-control than in RS-control. Al, Sr, and B are PRG active ions dispersed at the points where the analyzer microprobe penetrated. The higher concentration of C in PRG may be related to its resin component (methacrylate polymers), while Al ions have an obliterating action when released in small amounts, forming fluoraluminosilicate [13,14]. Sr has a modulatory effect by forming strontium apatite, contributing to remineralization and obliteration of tubules [13]. B may affect remineralizing, in addition to antibacterial action. K was identified in our study and can contribute to the obliterating effect, besides having a neural effect, although the PRG manufacturer does not indicate K in its formula. These elements in a more significant proportion in PRG seem to promote a more resistant surface against the action of acid, abrasion by brushing, and thermal cycling [14].

P was significantly higher in the RS groups compared to PRG. The manufacturer does not mention P in the material composition, but it may be in greater quantity in the RS groups because it forms a thin layer

allowing the penetration of the microprobe partly into the dentin hydroxyapatite. Ca was significantly higher in the RS groups than in the PRG groups, with a lower concentration in PRG-control. This result may also be due to the thin film formed by RS, allowing the microprobe to penetrate demineralized dentin. The presence of a tick material layer covering the dentin surface can explain the lower Ca concentration in PRG-control.

Si only differed between PRG-control and PRG-cycled, with no difference in the other comparisons. Si is one of the structural elements of PRG [13]; it helps form a smooth and regular layer on the dentin surface. In addition, Si helps form hydroxyapatite through nucleation triggered by the presence of silica gel on the dentin surface [16]. While PRG forms ions aggregated resinous matrix that interacts chemically and physically with the tooth structure, becoming an effective barrier against chemical, abrasive and thermal stresses [12,13,16], RS promotes silver fluoride, silver iodide, and silver diamine fluoride precipitates from the ammonia solution to dentin, favoring the synthesis of fluor-hydroxyapatite [23,24].

In agreement with other studies, we did not perform surface conditioning before applying the obliterating agents to simulate the presence of open dentinal tubules, a common situation in HD [12,19]. However, conditioning with ethylenediaminetetraacetic acid [8,24] or smoothing the surface with a curette [22] before applying the desensitizing agent has been reported. Pre-conditioning the dentin surface promotes greater opening, increasing the patency of the tubules, which could facilitate the penetration of desensitizing agents. On the other hand, artificially opened tubules could be more difficult to obliterate.

To simulate the stress coming from the oral environment, we submitted the specimens to erosive, abrasive, and thermal cycles, according to a protocol adapted from Schmalz et al. [18]. The standardization of the number and duration of cycles, the erosive pH, the load applied and the volume of dentifrice during brushing, the temperature of the baths, and the storage medium were sought. There was no consensus in defining cycling protocols, with significant variation among the studies reviewed [8,12,18,24]. In the present study, a matched pairs experimental design was chosen to evaluate the groups submitted or not submitted to simulated stresses allowing to control of the individual variation of the dentin substrate. However, this design is suitable for only two treatments due to the restriction of the specimen's area and precluded the inclusion of a negative control group.

We have considered the obliteration of dentinal tubules a clinical assumption for reducing DH and the consequent impact on quality of life [2,6]. The desensitizing agent modulates the pain effect by obliterating dentinal tubules, which decreases the intratubular interstitial flow, resulting in the reduction of pulpal excitation. Besides these effects, the release of ions that promote remineralization may be a mechanism of action attributed to RS and PRG. Long-term clinical studies are needed to evaluate the resistance of these desensitizing agents in the oral environment.

Conclusion

The PRG desensitizing agent application produced a dentinal surface with fewer visible tubules and more partially obliterated and totally obliterated tubules than the RS application. The submission to simulated stress did not affect the obliterating effect of either agent. The percentage of high Ag coverage on dentin surfaces treated with RS was reduced by simulated stress. The higher the Ag coverage, the lower the proportion of non-obliterated tubules. The elements C, Al, Sr, B, and K predominated in PRG submitted or not submitted to simulated stress, while the elements Ca, P, and Ag prevailed in both RS groups. The distribution of elements was consistent with the chemical composition and the mechanisms of action attributed to the desensitizing agents.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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References

[1] Zeola LF, Soares PV, Cunha-Cruz J. Prevalence of dentin hypersensitivity: systematic review and meta-analysis. J Dent 2019; 81:1-6. https://doi.org/10.1016/j.jdent.2018.12.015

[2] Douglas-De-Oliveira DW, Vitor GP, Silveira JO, Martins CC, Costa FO, Cota LOM. Effect of dentin hypersensitivity treatment on oral health related quality of life - a systematic review and meta-analysis. J Dent 2018; 71:1-8. https://doi.org/10.1016/j.jdent.2017.12.007

[3] Jena A, Shashiekha G. Comparison of efficacy of three different desensitizing agents for in-office relief of dentin hypersensitivity: a 4 weeks clinical study. J Conserv Dent 2015; 18(5):389-95. https://doi.org/10.4103/0972-0707.164052

[4] Majji P, Murthy KRV. Clinical efficacy of four interventions in the reduction of dentinal hypersensitivity: a 2-month study. J Dent Res 2016; 27(5):477-82. https://doi.org/10.4103/0970-9290.195618

[5] Brännström M, Astrom A. A study on the mechanism of pain elicited by dentin. J Dent Res 1964; 43:619-25. https://doi.org/10.1177/00220345640430041601

[6] Moraschini V, Da Costa LS, Dos Santos GO. Effectiveness for dentin hypersensitivity treatment of non-carious cervical lesions: a meta-analysis. Clin Oral Investig 2018; 22(2):617-31. https://doi.org/10.1007/s00784-017-2330-9

[7] Chen CL, Parolia A, Pau A, Porto ICCM. Comparative evaluation of the effectiveness of desensitizing agentes in dentine tubule occlusion using scanning electron microscopy. Aust Dent J 2015; 60(1):65-72. https://doi.org/10.1111/adj.12275

[8] Wang Z, Jiang T, Sauro S, Pashley DH, Toledano M, Osorio R, et al. The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an in vitro study. Aust Dent J 2011; 56(4):372-81. https://doi.org/10.1111/j.1834-7819.2011.01361.x

[9] Willershausen I, Schulte D, Azaripour A, Weyer V, Briseño B, Willershausen B. Penetration potential of a silver diamine fluoride solution on dentin surfaces. An ex vivo study. Clin Lab 2015; 61(11):1695-1701. https://doi.org/10.7754/clin.lab.2015.150401

[10] Craig GG, Knight GM, Mcintyre JM. Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. Aust Dent J 2012; 57(3):308-11. https://doi.org/10.1111/j.1834-7819.2012.01700.x

[11] Greenhill JD, Pashley DH. The effects of desensitizing agents on the hydraulic conductance of human dentin in vitro. J Dent Res 1981; 60(3):686-98. https://doi.org/10.1177/00220345810600030401

[12] Arita S, Suzuki M, Kazama-Koide M, Shinkai K. Shear bond strengths of tooth coating materials including the experimental materials contained various amounts of multi-ion releasing fillers and their effects for preventing dentin demineralization. Odontology 2017; 105(4):426-36. https://doi.org/10.1007/s10266-016-0290-1

[13] Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. Dent Mater J 2010; 29(4):92-7. https://doi.org/10.4012/dmj.2010-015

[14] Ikemura K, Tay FR, Endo T, Pashley DH. A review of chemical-approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers. Dent Mater J 2008; 27(3):315-39. https://doi.org/10.4012/dmj.27.315

[15] Ito S, Iijima M, Hashimoto M, Tsukamoto N, Mizoguchi I, Saito T. Effects of surface pre-reacted glass-ionomer fillers on mineral induction by phosphoprotein. J Dent 2011; 39(1):72-9. https://doi.org/10.1016/j.jdent.2010.10.011

[16] Murayama R, Furuichi T, Yokokawa M, Takahashi F, Kawamoto R, Takamizawa T, et al. Ultrasonic investigation of the effect of S-PRG filler-containing coating material on bovine tooth demineralization. Dent Mater J 2012; 31(6):954-9. https://doi.org/10.4012/dmj.2012-153

[17] Ma S, Imazato S, Chen JH, Mayanagi G, Takahashi N, Ishimoto T, et al. Effects of a coating resin containing S-PRG filler to prevent demineralization of root surfaces. Dent Mater J 2012; 31(6):909-915. https://doi.org/10.4012/dmj.2012-061

[18] Schmalz G, Hellwig F, Mausberg RF, Schneider H, Krause F, Haak R, et al. Dentin protection of different desensitizing varnishes during stress simulation: an in vitro study. Oper Dent 2017; 42(1):E35-E43. https://doi.org/10.2341/16-068-L

[19] Han L, Okamoto A, Fukushima M, Okiji T. Evaluation of a new fluoride-releasing one-step adhesive. Dent Mater J 2006; 25(3):509-15. https://doi.org/10.4012/dmj.25.509

[20] Saori M, Saori T, Matsuda Y, Naoki H, Sano H, Masamitsu K. Caries prevention after surface reaction-type prereacted glass ionomer filler-containing coating resin removal from root surfaces. J Nanosci Nanotechnol 2016; 16(12):12996-13000. https://doi.org/10.1166/jnn.2016.13653

[21] Selvaraj K, Sampath V, Sujatha V, Mahalaxmi S. Evaluation of microshear bond strength and nanoleakage of etch-and-rinse and self-etch adhesives to dentin pretreated with silver diamine fluoride/potassium iodide: An in vitro study. Indian J Dent Res 2016; 27(4):421-5. https://doi.org/10.4103/0970-9290.191893

[22] Mei ML, Ito L, Cao Y, Li QL, Lo EC, Chu CH. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. J Dent 2013; 41(9):809-17. https://doi.org/10.1016/j.jdent.2013.06.009

[23] Cai J, Burrow MF, Manton DJ, Tsuda Y, Sobh EG, Palamara JEA. Effects of silver diamine fluoride/potassium iodide on artificial root caries lesions with adjunctive application of proanthocyanidin. Acta Biomater 2019; 88:491-502. https://doi.org/10.1016/j.actbio.2019.02.020

[24] Machado AC, Rabelo FEM, Maximiano V, Lopes RM, Aranha ACC, Scaramucci T. Effect of in-office desensitizers containing calcium and phosphate on dentin permeability and tubule occlusion. J Dent 2019; 86:53-9. https://doi.org/10.1016/j.jdent.2019.05.025