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Impact of silanization of different bioactive glasses in simplified adhesives on degree of conversion, dentin bonding and collagen remineralization

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ABSTRACT

Objective: To analyze simplified adhesive containing pure or silanized bioglass 45S5 (with calcium) or Sr-45S5 (strontium-substituted) fillers applied on dentin and to evaluate the microtensile bond strength (μTBS), interface nanoleakage, degree of conversion of adhesive, collagen degradation and remineralization.

Methods: Ambar Universal adhesive (FGM) was doped with 10 wt% bioactive glasses to form following groups: Control (no bioglass), 45S5 (conventional bioglass 45S5), Sr-45S5 (Sr-substituted bioglass 45S5), Sil-45S5 (silanized bioglass 45S5) and Sil-Sr-45S5 (silanized bioglass Sr-45S5). Adhesives were applied after dentin acid-etching using phosphoric acid at extracted human molars. Resin-dentin sticks were obtained and tested for μTBS, nanoleakage at 24 h or 6 months. Degree of conversion was measured using micro-Raman

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Strontium
Silanization

spectroscopy. Dentin remineralization was assessed by FTIR after 6-month storage in PBS. Hydroxyproline (HYP) release was surveyed by UV-Vis spectroscopy. Statistical analysis was performed using ANOVA and Tukey's test ($p < 0.05$).

Results: Regarding μTBS, Sr-45S5 and 45S5 presented higher and stable results ($p > 0.05$). Control ($p = 0.018$) and Sil-Sr-45S5 ($p < 0.001$) showed μTBS reduction after 6-month aging. Sil-Sr-45S5 showed higher HYP release than that obtained in the 45S5 group. Sil-45S5 showed mineral deposition and increase in μTBS ($p = 0.028$) after 6-months. All experimental adhesives exhibited higher degree of conversion compared to Control group, except for 45S5. All adhesives created gap-free interfaces, with very low silver impregnation, except for Sil-Sr-45S5.

Significance: The incorporation of silanized 45S5 bioglass into the universal adhesive was advantageous in terms of dentin remineralization, bonding performance and adhesive polymerization. Conversely, Sil-Sr-45S5 compromised the μTBS, interface nanoleakage and had a negative impact on HYP outcomes.

1. Introduction

Modern composite restorations fail mainly at the adhesive resin-dentine interface over the years [1,2], due to polymer hydrolysis [3], filler debonding [4] and unprotected collagen fibrils degradation caused by matrix metalloproteinases (MMPs) and other enzymes action [5,6]. It has been demonstrated that the weakest zone of the resin-dentine interface and prone to degradation is the bottom of hybrid layers that is typically characterized by the presence of unprotected collagen fibrils [8].

Several therapeutic strategies have been proposed to protect dentin collagen fibrils from degradation and to increase the longevity of the bonded interface. For instance, the use of biomodification agents (such as proanthocyanidins, curcumin and cardanol), which enhance the stability of dentin organic matrix by formation interfibrilar collagen crosslinking and reduction of proteases' activity, is a strategy that may overcome this problem [9]. A further alternative is based on the biomimetic remineralization to induce neoformation of apatite around collagen fibrils.

Several investigations have evaluated the addition of bioactive fillers into adhesives and composites, reporting a decrease in dentin collagen degradation [10–12]. Therefore, such tailored dental biomaterials may provide remineralization of dental hard tissues, as shown by laboratory and clinical studies [10,11]. Indeed, it was demonstrated the release of specific ions, which can subsequently precipitate in form of mineral complexes in presence of body fluids [12,13]. Furthermore, remineralization may interfere with collagenolytic activity of endogenous MMPs, as well as prevent bacterial growth by managing the micro-environment pH [13,15,18]. Besides the addition to resin composites [15] and dental bonding agents [18,19], bioactive fillers are also present in glass ionomer cements [17] and endodontic sealers [15].

One of the main bioactive glasses investigated in dentistry is bioglass 45S5, which presents high bioactivity, adequate biocompatibility and optimal ability to induce apatite precipitation in collagen fibrils [15,17–19]. Its common composition is based on 45 wt% SiO_2, 24.5 wt% Na_2O, 24.5 wt% CaO and 6%wt. P_2O_5. However, a feasible substitution could be undertaken by replacing calcium oxide by strontium oxide, thereby promoting the precipitation of strontium phosphate compounds [20]. To the best of our knowledge, the role of such modification and the silanization of the bioglass might create more reactive and therapeutic fillers; such scientific aspects have never been investigated so far.

Thus, the aim of this in vitro investigation was to evaluate the effect of the addition of silanized and non-silanized bioglass 45S5 or strontium substituted bioglass (Sr-45S5) in a two-steps etch-and-rinse adhesive on the dentin bond strength, dentin-adhesive interfacial nanoleakage, in situ degree of conversion of the experimental adhesives, collagen degradation, and dentin remineralization. The hypotheses of this study were that all the tested bioactive fillers would: 1- improve the dentin bond strength; 2- reduce the impregnation of silver ions at dentin-adhesive interface and collagen degradation; 3- not influence the degree of conversion of the experimental adhesives; 4- improve dentin remineralization.

2. Materials and methods

2.1. *Preparation of experimental adhesives and bioglass*

A dental adhesive (Ambar Universal adhesive, FGM Dental Group, Joinville, Brazil) was doped with bioactive glasses 45S5 (45S5) [20], and Sr-45S5 with or without pre-silanization, forming the following experimental groups: 45S5 (adhesive added with non-silanized conventional bioglass 45S5), Sr-45S5 (adhesive incorporated with non-silanized strontium-containing bioglass 45S5), Sil-45S5 (adhesive with silanized conventional bioglass 45S5) and Sil-Sr-45S5 (adhesive with silanized strontium-containing bioglass 45S5).

The bioglass fabrication was performed according to the protocol of Pintado-Palomino et al., 2015 [21]. Silanization was performed by stirring the fillers in 100 mL of 1 wt% γ-metacryoxi-propyl-silane for 48 h at 80 °C, double-filtering and washing of unbound silane with absolute ethanol. The bioglasses were added at 10% weight in Ambar Universal adhesive (FGM, Joinville, Brazil) and the homogenization was performed with slight stirring for 30 s. The pH of each

Table 1 – Control and experimental groups tested in this study.

Group/Adhesive	Code
Control (Ambar Universal)	Control (no bioglass)
Ambar Universal Adhesive + Bioglass 45S5	45S5
Ambar Universal Adhesive + Bioglass Sr-45S5	Sr-45S5
Ambar Universal Adhesive + Silanized Bioglass 45S5	Sil-45S5
Ambar Universal Adhesive + Silanized Bioglass Sr-45S5	Sil-Sr-45S5

adhesive was surveyed before and after bioglass addition and no modification was observed, with pH kept ~2.0 for all materials. The compositions of the experimental dentin bonding agents are shown in Table 1.

The bioactive glass 45S5 microfillers were produced by melting 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O and 2.5 mol% P_2O_5 in a platinum crucible at 10 °C/min up to 1,100 °C and kept for 1 h. Subsequently, the temperature was raised to 1450 °C (10 °C/min) and maintained for additional 30 min. The glass melt was rapidly quenched in water (~20 °C), dehydrated in absolute ethanol for 3 h, dried overnight in a furnace at 110 °C, milled and sieved (~5 μm). The Sr-45S5 was produced by melting 46.1 mol% SiO_2, 26.9 mol% SrO, 24.4 mol% Na_2O and 2.5 mol% P_2O_5 as aforementioned to produce similar fillers to those of calcium containing 45S5.

2.2. *Specimens preparation*

Thirty extracted human third molars were stored in distilled water and used within four months after extraction. This study was performed after the approval of the institutional ethical committee. Flat, deep dentin surfaces were prepared by cutting the crown of each tooth 2 mm below the cementum-enamel junction (CEJ) using a diamond saw (Isomet, Buehler; Lake Bluff, IL, USA), followed by a second parallel cut 3 mm above the CEJ. The exposed flat coronal dentin was then abraded using 320-grit SiC papers for 30 s to create standardized smear layers.

Dentin specimens were randomly divided into five groups (n = 6): Control (no bioglass addition), 45S5, Sr-45S5, Sil-45S5 and Sil-Sr-45S5 (Table 1). The bonding procedures were performed according to the manufacturer's instructions in the etch-and-rinse strategy. Afterwards, the restorative composite Opallis (FGM) was applied specimenusing three 2-mm-thick increments to obtain a 6-mm buildup light-cured composite.specimen. Adhesives and composite were light cured according to the manufacturers' instructions, using the poly-wave LED light-curing unit (Valo, Ultradent Product Inc., South Jordan, USA). The irradiance was kept at 2000 mW/cm^2, regularly monitored by a radiometer (Pujing, Beijing, China). The dentin specimens were stored in distilled water for 24 h at 37 °C.

2.3. *Microtensile bond strength (μTBS) and failure mode analysis*

The dentin specimens were serially sectioned to obtain resin-dentin sticks of approximately 1 mm^2 cross-sectional area suitable for μTBS testing. Sticks from same bonded tooth (approx.15 sticks per each tooth) were randomly divided into two storage period groups, as half was tested after 24 h and the further half was immersed in phosphate-buffered saline (PBS, Sigma Aldrich, St. Louis, USA) for 6 months prior to evaluation [5]. Before the bond strength assessment, the sticks were attached to jigs using cyanoacrylate glue (Superbonder gel, Loctite, Henkel, Rocky Hill, USA) and then tested until failure in a universal testing machine EMIC 23–2 S (EMIC-Instron, São José dos Pinhais, Brazil), using a 500-N load cell and 1 mm/min crosshead speed. The cross-sectional area of each stick was measured using a digital caliper. The bond strength was calculated and expressed in MPa. The bond strength mean values obtained from sticks originated from the same bonded tooth were used as statistical unit. The μTBS data were statistically analyzed by two-way ANOVA (adhesive and aging period) and Tukey's test ($p < 0.05$), after proving data normal distribution via Shapiro-Wilk normality test ($p > 0.05$). Subsequent to μTBS, the failure mode of each fractured stick was analyzed using a stereomicroscope at 100x magnification (Olympus SZ 40–50, Tokyo, Japan). Fractures were classified as adhesive, mixed, cohesive in composite or cohesive in dentin.

2.4. *Nanoleakage evaluation*

Two resin-dentin sticks per tooth (n = 12, six for 24 h period and six tested after 6-month aging) were processed for silver nanoleakage evaluation as described by Tay et al., 2002 [22]. The sticks were immersed in 50% ammoniacal silver nitrate solution in darkness for 24 h. To remove excess silver solution, they were subsequently rinsed with distilled water, immersed in photo-developing solution for 8 h under fluorescent light to reduce silver ions into metallic silver grains along the adhesive-dentin interface. The silver-impregnated sticks were embedded in epoxy resin, polished using SiC sandpapers (600-, 1200- and 2000-grit) and followed by polishing with disk-cloths and 6-, 3- and 1-μm diamond suspensions (Buehler, Lake Bluff, IL, USA). The specimens were cleaned in an ultra-sonic bath for 20 min between each polishing step and at the end of the procedure. They were then dehydrated and coated with carbon (MED 010, Balzers Union, Balzers, Liechtenstein). The nanoleakage evaluation was performed by SEM in backscattered electron mode at 20 kV voltage and with a 5 mm working distance (JSM-5600LV, JEOL, Tokyo, Japan).

2.5. *Remineralization assessment through ATR-FTIR*

Three adhesive bars (7 mm long, 2 mm wide and 1 mm thick) were created by light-curing the experimental adhesives in silicone molds. Dentin bars of similar size as the adhesive

resin specimens were cut perpendicularly to the long axis and demineralized in 10% phosphoric acid for 24 h, washed vigorously with distilled water and wired to the adhesive resin bars. They were immersed in PBS for 6 months (without solution exchange) and surveyed according to the method of Tezvergil-Mutluay et al. [23]. Dentin surfaces were analyzed prior to storage, and after 6 months, by Attenuated Total Reflectance Fourier-transform Infrared spectrophotometry (ATR-FTIR, Spectrum Frontier, Perkin-Elmer Corp, Norwalk, USA) in the spectral range of 2.000–400 cm $^{-1}$, with 4 cm^{-1} resolution and 16 scans per reading. Moderate pressure (0.034 MPa) was applied to ensure adequate contact between the tooth and the ATR device. Mineral analysis of dentin in ATR-FTIR was conducted similarly to the protocol used by Abuna et al. [25].

2.6. *In situ degree of conversion (DC)*

Micro-Raman spectroscopy analysis was used to assess the degree of conversion (DC) of experimental adhesives at bonded interface of several selected resin-dentin sticks (n = 3, three sticks per group). The micro-Raman spectrophotometer (Xplora, Horiba Jobin Yvon, Paris, France) was firstly calibrated using a silicon standard specimen supplied by the manufacturer. HeNe laser with 3.2 mW power and 532 nm wavelength was employed with 1.5 µm spatial resolution, 2.5 cm^{-1} spectral resolution associated with 10x magnification lens (Olympus, London, UK) to attain an approximate 60 × 70 µm field area and 5 µm laser spot. The degree of conversion of experimental adhesives and the Control was calculated based on a previous investigation method presented by Araujo-Neto et al. [25], through the following equation::

$$DC = \left(1 - \frac{R_{cured}}{R_{uncured}}\right) x100$$

Where R is the ratio between the heights of 1639 cm^{-1} and 1609 cm^{-1} peaks of uncured and light-cured adhesive at bonded interface, attained after baseline correction. Three readings were undertaken at adhesive layer of each specimen, which were averaged to obtain one statistical unit (n = 3). The data were statistically analyzed by one-way ANOVA (Factor under study: type of adhesive) and Tukey's test ($p < 0.05$), after verifying a normal data distribution via Shapiro-Wilk normality test ($p > 0.05$).

2.7. *Hydroxyproline assay*

Measurement of hydroxyproline (HYP) release was determined using a colorimetry assay kit (Sigma Aldrich), following the protocol described by Scheffel et al. [27]. Briefly, six (n = 6) supernatants of storage PBS solutions (after a six-month period), which contained 8 standardized sticks (with 0.9 ×0.9 mm cross-sectional area) from same bonded tooth (total 6 teeth per group) were resuspended in 10 mL deionized water and 4 mL of 2 N NaOH was added and were hydrolyzed (at 120 °C) for 90 min. Subsequently, the oxidation of the specimens was performed using a chloramine-T solution for 25 min and the chromophore was developed by incubation with Ehrlich's reagent for 40 min at 65 °C. Absorbance values were measured in the UV-Vis spectrophotometer (560 nm, Beckman Coulter DU-800, USA), standard curves of known concentrations of HYP (2, 5, 10, 15 and 25 mg/mL) were used as control and the solubilized collagen was expressed as micrograms of HYP per milliliter. The HYP data were statistically analyzed by one-way ANOVA (factor under study: type adhesive) and Tukey's test ($p < 0.05$), after proving normal data distribution in Shapiro-Wilk normality test ($p > 0.05$).

3. Results

3.1. *Microtensile bond strength (µTBS) and failure mode analysis*

The outcomes of microtensile bond strength test are depicted in Fig. 1. At 24 h, both experimental adhesives with Sr-45S5 and 45S5 fillers presented higher bond strength (p = 0.003 and p = 0.025 respectively) than that obtained with the Sil-45S5 adhesive. Control showed an initial bond strength of 34.1 MPa, without any significant difference compared to the other experimental adhesives ($p > 0.05$). After a 6-month storage in PBS, adhesives with 45S5 (p = 0.717) and Sr-45S5 (p = 0.816) fillers maintained stable bond strength, whilst the control adhesive (p = 0.018) and the adhesive with Sil-Sr-45S5 fillers ($p < 0.001$) presented significant reduction in bond strength. The adhesive containing Sil-45S5 fillers showed an increase in bond strength values after 6 months in comparison with the values obtained at 24 h (p = 0.028). Moreover, Sil-45S5 presented higher bond strength than the control group at 6 months, but it did not differ from both adhesives containing the non-silanized bioactive glasses.

Percentage results regarding the specimens' mode of failure are shown in Fig. 2. In general, most groups presented predominantly adhesive failures. Control and experimental groups showed high percentage of adhesive fractures

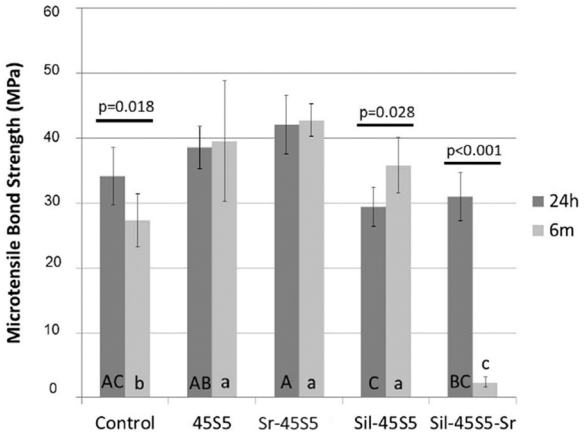

Fig. 1 – Microtensile bond strength outcomes (means and standard deviations) in MPa. Similar capital letters indicate no statistical difference among groups in 24 h evaluation time. Different lower case letters highlight significant difference among groups at 6 months. Bars above columns depict the p value for significant differences between 24 h and 6-month evaluation times.

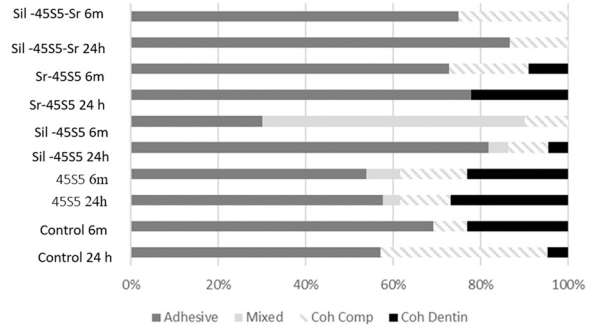

Fig. 2 – Results of the failure mode in percentage. "Coh dentin" means cohesive dentin fracture. "Coh Comp" means cohesive fracture in resin composite.

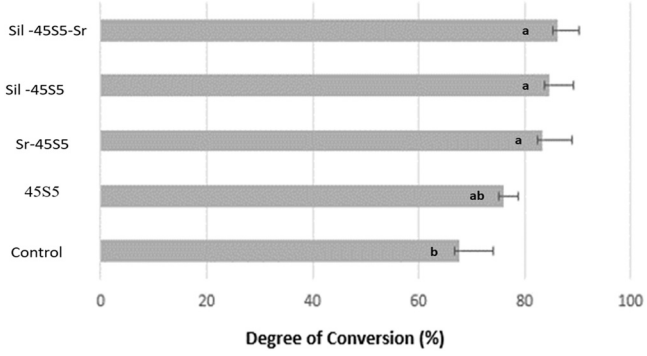

Fig. 3 – *In situ* degree of conversion outcomes obtained during Micro-Raman spectroscopy assessment. Different letters indicate statistically significant difference ($p < 0.05$).

regardless the evaluation time, except for Sil-45S5 at 6 months, which presented significant reduction when compared with 24 h.

3.2. *Degree of conversion*

The results of the degree of conversion of control and experimental adhesives are presented in Fig. 3. Both silanized bioglasses (Sil-45S5 and Sil-Sr-45S5) and non-silanized Sr-45S5 induced significant increase of monomeric conversion in comparison to the control adhesive ($p < 0.05$), which presented a mean degree of conversion of 67.7%. Conversely, the traditional non-silanized bioactive glass 45S5 showed 76.1% of monomeric conversion that did not differ significantly from the control ($p = 0.117$) and the other experimental groups ($p > 0.05$).

3.3. *Nanoleakage assessment*

Representative SEM micrographs of the nanoleakage patterns observed in the control and experimental groups are depicted in Fig. 4. Most groups showed very low silver impregnation within the dentin-adhesive interface, except for Sr-45S5 at 24 h. It was commonly observed that most dentin-adhesive interfaces were almost devoid of substantial defects at both periods of storage, except for Sil-Sr-45S5 that showed intense silver impregnation within the hybrid layer and large gaps at the adhesive interface at both evaluation times (Fig. 4H e 4I).

3.4. *Dentin Remineralization (FTIR)*

Representative FTIR spectra of dentin specimens before and after remineralization periods are depicted in Fig. 5. The experimental adhesives containing the silanized bioglasses (Sil-45S5 and Sil-Sr-45S5) were characterized by the precipitation of hydroxyapatite, which was identified by the presence of asymmetric vibration peaks of phosphate at 1019 cm^{-1} and 962 cm^{-1}, as well as carbonated apatite, which were assigned to peaks at 890 cm^{-1} and 870 cm^{-1}. Non-silanized bioglasses demonstrated very little mineral deposition; that was highlighted by the slight increase of aforementioned peaks. The control (without bioactive glasses) showed the no presence of mineral precipitation.

3.5. *HYP assay*

The outcomes of total collagen degradation represented by the release of HYP are shown in Fig. 6. The adhesive containing Sil-Sr-45S5 fillers (mean 0.648 μg/mL) presented the higher HYP release compared to the adhesive with non-silanized 45S5 fillers ($p = 0.002$). The Control, Sr-45S5 and Sil-45S5 groups achieved intermediate results, which did not differ from Sil-Sr-45S5 and 45S5 adhesives ($p > 0.05$).

4. Discussion

Bioactive glasses, once included within adhesive systems should be able to release ions and induce dentin remineralization, as well as to interfere with biofilm growth and enhancing the longevity of the bonding interface [14–16]. The 45S5 glass is an amorphous silicate-based mineral containing calcium, sodium and phosphate, and is vastly known to be able to induce the formation of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], also when incorporated in resin-based dental materials [24]. However, the role of silanization when such bioactive fillers are incorporated in modern universal adhesive is unknown. Furthermore, the effects of the substitution of calcium by strontium in the traditional Bioglass 45S5 composition regarding its bioactivity and its potential to remineralize and protect the collagen fibrils is also undetermined [15]. In the present study, we observed that the adhesive containing silanized calcium-containing bioactive glass exhibited an increase in bond strength after aging, thus, first hypothesis must be accepted. The experimental adhesive containing silanized strontium-containing bioactive glass applied on acid-etched dentin was characterized by evident collagen degradation, gaps and increase of silver impregnation within the interface; the second hypothesis must be also rejected. Most experimental bioglass-containing adhesives attained higher degree of conversion than control adhesive, so the third hypothesis must be rejected too. Finally, the fourth hypothesis was accepted, because all experimental adhesives improved dentin remineralization, although at different extent.

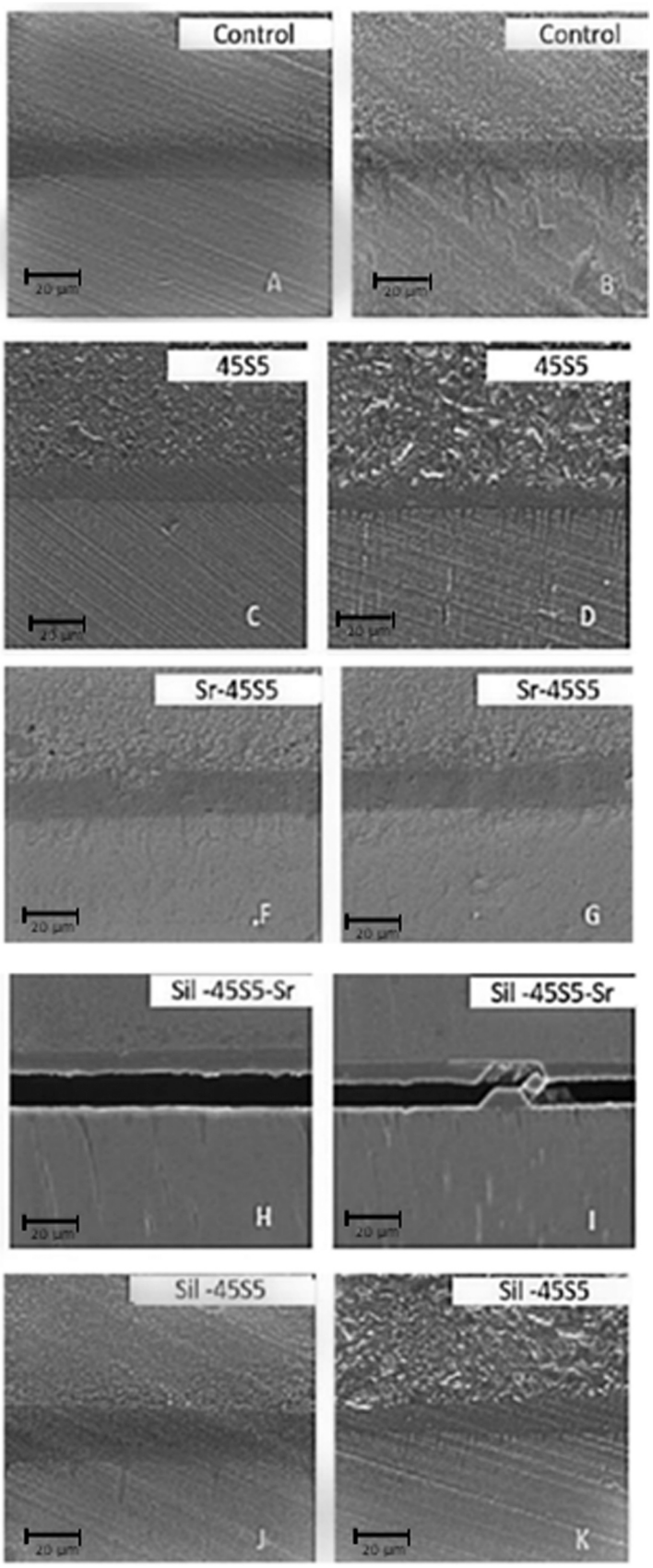

Fig. 4 – Representative SEM micrographs at 1000x magnification depicting dentin-adhesive-composite interface features and silver impregnation at resin-dentin interfaces. Adhesives with silanized strontium-containing 45S5 bioglass presented gaps along the bottom and top of hybrid layers at 24 h (G) and 6 months (H). The Control (Figs A and B), bioactive glass 45S5 (Figs. C and D) and its silanized version (Figs. I and J) showed lower silver impregnation, bonded and homogeneous interfaces.

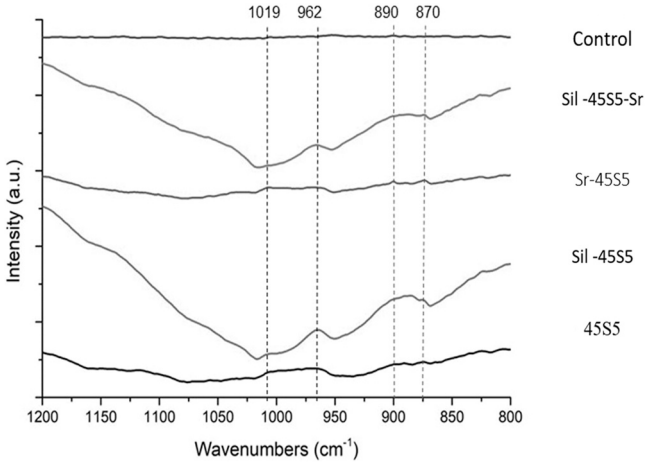

Fig. 5 – ATR-FTIR characterization of demineralized dentin after storage in contact with Control and bioactive glasses-containing experimental adhesives. Dotted lines highlight the presence of hydroxyapatite (peaks at 1019 cm^{-1} and 962 cm^{-1}) and carbonated apatite (peaks at 890 cm^{-1} and 870 cm^{-1}).

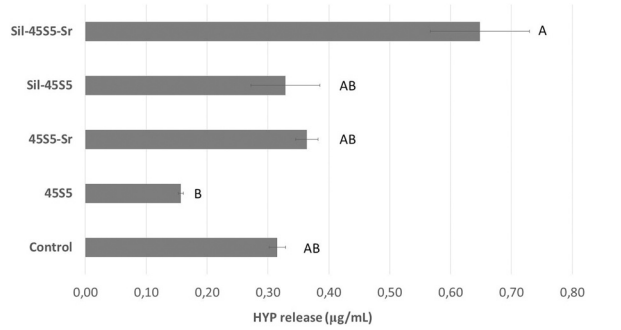

Fig. 6 – Hydroxyproline release (µg/mL) from demineralized dentin specimens that were in contact with the tested experimental adhesives.

Nowadays, universal dental adhesives represent the main clinical choice in composite restorations for many practitioners, as they simplify the bonding procedures and can be applied both in etch-and-rinse self-etching mode [29]. Nevertheless, some specific formulations, due to the mixture of different monomers, along with the presence of water, different solvents and filler particles may negatively influence the polymerization, which alters the degree of conversion and might yielded early degradation processes at the dentin-adhesive interface [30]. It is known that an environment with residual water and solvents can cause adhesive phase separation, compromising the polymerization process [31]. The low monomeric conversion can affect the mechanical properties of the adhesive resin and increase the rate of polymer degradation under continuous chewing forces and load cycling [32–35].

Recent investigations [17,18] reported that the use of bioglass fillers within the composition of simplified adhesives can improve their degree of conversion. This may be due to the fact that bioactive fillers, such as these inorganic glass particles, could diffract the light, activating more photoinitiator molecules (e.g. CQ), so enhancing the polymerization reaction among the monomers. Interestingly, silanized bioglass 45S5 favored a greater degree of conversion compared to the bioglass-free Control adhesive, as well as to the adhesive containing non-silanized bioglass 45S5. This outcome corroborated with the hypothesis that silane-coupling may further enhances the polymerization of resin-based materials [19–22].

However, the silanization of bioactive glass particles remains controversial. Although it may promote a crucial bonding between monomeric matrix and fillers, providing fillers dispersion, retention and mechanical reinforcement [35–39], the cured silane shell formed around the filler might reduce the ion-releasing ability, thereby reducing their bioactivity. Remarkably, the silanization of bioactive glasses was beneficial for calcium-containing 45S5 fillers, but it seemed to cause a reduction of the bonding outcomes in the adhesive containing the strontium-substituted bioglass. Regarding the ion-releasing and dentin remineralization (Fig. 5), the silanization of bioglasses did not interfere with their bioactivity, because they induced remarkable higher deposition of hydroxyapatite and carbonated apatite on the dentin. In particular for calcium-containing 45S5 fillers, this process had no impact on the dentin bonding and/or the physicochemical properties of the experimental adhesive.

The silane coupling agents present hydrophilic features, as they contain silanol and ester functionalities. This feature might enhance water infiltration [2] through resin matrix, promoting the bioactive glass dissolution and increasing the remineralization potential of the bioactive experimental adhesive containing bioglass 45S5, as previously reported [40]. Nevertheless, the silanization process seems to have jeopardized the initial bonding performance to dentin, in particular with the strontium-containing bioactive fillers. This may be explained by the chemical instability regarding the incorporation of Sr instead Ca.

According to an literature review paper by Kargozar et al. [41] the replacement of Sr with Ca in a bioglass composition was proved to slow down the formation of the apatite layer on the glass surface, specially at a nanoscale particle size. Several authors identified an inhibitory effect of Sr-doping on the crystallization of the deposited calcium phosphate layer mainly due to Sr incorporation into the hydroxyapatite layer. Rokidi and Koutsoukos's study [42] and Aina et al.,[43] reported that there was a decrease in crystallite size and degree of crystallinity after introducing Sr into hydroxyapatite due to the larger ionic radius of Sr^{2+} ion in comparison to Ca^{2+}, which leads to an increase in D-spacing and in the crystal cell unit parameters, showing that the presence of strontium in supersaturated solutions of calcium phosphate retarded the crystal growth of both octacalcium phosphate and hydroxyapatite [44]. From a bioactivity and remineralization point of view, a high Sr content may jeopardize the dentin bonding performance due to excess of ions and precipitation of minerals within the collagen fibrils, which can interfere with the infiltration of adhesive monomers and the micromechanical hybridization of dentin

[38,39]. The rapid degradation of such weak hybrid layer was expected, corroborating the striking reduction of bond strength of Sil-Sr-45S5 to dentin after six-months aging (Fig. 1).

The rationale behind replacing calcium for strontium in 45S5 bioactive glass is attributed to the chemical similarity between these two elements, but with the aim to have greater reactivity, with consequent increase of dissolution and bioactivity from the bioglass [45]. Moreover, the presence of strontium may stimulate osteoblast activity, impairing osteoclasts and enhancing mineral repair [45,46]. This caution may also offer a contribute in preventing the recurrence of caries or/and its progression via a similar mechanism as well as that of fluoride [46]. The higher atomic radius of strontium can also improve the radiopacity of bioglasses [47], which represents a significant clinical advance for resin-based materials doped with such materials. Although the incorporation of Sr into bioglasses have been shown to be positive, especially regarding to the hard tissue regeneration, the results presented herein showed that the strontium-containing bioactive glass did not yield noteworthy improvements in dentin bonding and remineralization when compared to silanized or non-silanized calcium-containing bioglass 45S5.

Silanization process might be the responsible for lower percentage of adhesive failures in both periods of storage for 45S5 bioglass (Fig. 2). Conversely, a decrease in adhesive failures and dentin remineralization (Fig. 5) may support the increase of bond strength after six-months aging with the experimental adhesive containing Sil-45S5. The silanization process was detrimental for Sr-45S5, because the reactivity of strontium with oxygen may cause the formation of a small pellicle of strontium salts during the reaction with silane coupling agent [44]. Observing 24-hours bond strength outcomes, the silanization process seemed to jeopardize dentin adhesion. Nevertheless, the optimal ion-releasing and dentin remineralization (Fig. 5) at six months demonstrated a clear bioactivity and an increase of bond strength with silanized calcium-containing 45S5 bioactive glass. Indeed, the remineralization of around the collagen fibrils and overall reduction of their degradation is feasible and favored by silanization of calcium-containing bioactive glass 45S5. Since the process of biomimetic remineralization demands some months to occur properly, unprotected collagen fibrils may undergo breakdown with HYP release.

Type I collagen from human dentin is composed by several amino acids, such as proline, lysine and majorly by HYP, which may be quantified by UV-Vis spectroscopy and using specific colorimetric kits [27]. Outcomes of HYP release (Fig. 6) supported the hypothesis that silanization of strontium-containing bioactive glass 45S5 had no preventive effect in terms of degradation of collagen fibrils at the dentin-adhesive interface (Fig. 1), even with high ion-releasing from the experimental bioactive adhesive. In general, the standard calcium 45S5 bioactive glass after silanization showed interesting outcomes in this study, but without significant difference from Control, Sil-45S5 and Sr-45S5 experimental adhesives in terms of HYP release.

Dentin collagen remineralization may be achieved when Sil-45S5 fillers were incorporated in universal adhesive as demonstrated by FTIR spectroscopy (Fig. 5). Silane coating can increase the hydrophilicity of bioglass particles, thus, providing more water infiltration, causing ion release [4,38]. Silane might also bind these minerals, stabilizing them to infiltrate into demineralized dentin [38,39]. Calcium phosphates precipitated in form of hydroxyapatite (peaks at 1019 cm^{-1} and 962 cm^{-1}) and carbonated apatite (peaks at 890 cm^{-1} and 870 cm^{-1}) within demineralized dentin specimens were observed. Such process might mimic a natural mechanism and inorganic compounds of genuine dentin collagen remineralization, thereby yielding dentin remineralization. This result requires further investigation to be corroborated, but, the present investigation highlighted the positive role of silanization of the calcium-containing 45S5 bioactive glass, with improvements on dentin bonding and remineralization promoted by an Universal Adhesives.

5. Conclusions

The substitution of calcium by strontium in the 45S5 bioactive glass may interfere with the ability of universal adhesives to create a durable dentin bond. However, silanization of the standard Ca-containing 45S5 bioglass fillers and subsequent incorporation into adhesive increases dentin remineralization and the bonding durability. Further experiments and clinical trials must be performed to confirm the potential improvement of the bond strength of composite restorations obtained by adding silanized 45S5 bioglass particles to universal adhesive.

Disclosure statement

The authors deny any conflicts of interest related to this study.

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