Title: A novel bioactive agent improves adhesion of resin-modified glass-ionomer to dentin.

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Keywords: resin-modified glass-ionomer cement; dentin; adhesion; compression; bioactive materials.

Abstract

Bioactive glasses are surface-active materials able induce remineralization of dental structure. **Objectives**: to evaluate the effect of doping two resin-modified glass ionomer cements (RMGICs) with a bioactive glass (Biosilicate) in compressive and microtensile bond strength (MTBS) to dentin. **Methods**: Experimental powders were made by incorporating 2, 5, and 10 wt% of biosilicate in Vitremer (VT) and Fuji II LC (FL) powder. FL and VT were used as control materials. Six cylinders of each experimental material were tested for failure in compression at 1.0mm/min, after 24h storage in distilled water at 37ºC. For the bond strength test, thirty non-carious human molars underwent cavity preparation previous to restoration. Restorative materials were VT or FL with and without 2 wt% biosilicate. The teeth were stored in water at 37ºC for 24h or 7d, sectioned into beams, and tested for failure in tension at 0.5 mm/min. Compressive and bond strength data were analyzed by analysis of variance and multiple-comparison tests ($p < 0.05$). Analysis of de-bonded specimens was performed by scanning electron microscopy (SEM). **Results**: Only incorporation of 2 wt% of biosilicate did not decrease the compressive strength of the tested RMGICs. The incorporation of 2 wt% biosilicate into RMGICs produced and increase in bond strength after 24h for FL, and after 24h and 7d for VT. **Significance:** Incorporation of 2 wt% of biosilicate particles into RMGICs did not affect the compressive strength and improved bond strength to dentin.

Introduction

Resin-modified glass ionomer cements (RMGICs) were introduced in an attempt to improve the glass ionomer cements properties [13]. In these materials a resin component which set by a radical addition polymerization, supplement the acid-base setting system of the conventional cements. These materials of which the setting is induced by visible light exhibit some distinct advantages for clinician: the process of hardening of material starts on command, the working time is lengthened, and the setting time is shortened [11].

Previous studies [6] have suggested that bioactive glass could be used for remineralizing damaged dentin. As bioactive glass particles alone are easily displaced in a clinical environment [9], a suitable carrier or matrix material is needed to facilitate its use in these settings. Previous studies showed that RMGICs with bioactive glasses yielded positive bioactivity effects [24, 23], a Ca-rich precipitation layer was observed on outer surfaces of the RMGICs, *in vitro* [24], in the close vicinity of the restoration–dentin interface and in deeper parts of dentin tubules *in vivo* [23]. However, these materials exhibited lower mechanical properties than RMGICs [2, 23] with similar setting ability [2, 12].

Recently, a fully crystalline bioactive glass–ceramic of the Na_2O–CaO–SiO_2–P_2O_5 system (Biosilicate, PI 0300644-1) was developed [25]. *In vitro* experiments demonstrated that Biosilicate is highly bioactive and undergoes enhanced bone-like matrix formation compared to Bioglass 45S5 in an osteogenic cell culture system [15].

The adhesion to dentin of RMGICs after incorporation of bioactive glasses or glass-ceramics has not been evaluated extensively. The objectives of this study were to investigate the effects of the novel bioactive glass (Biosilicate) incorporation into RMGICs on compressive and bond strength to dentin.

The null hypothesis to be tested is that no difference in compressive strength and in dentin bond strength exists when incorporating Biosilicate particles into RMGICs formulations.

Materials and Methods

Two different commercially available RMGICs were used: Fuji II LC (FL) (GC Corporation, Tokyo, Japan) and Vitremer (VT) (3M ESPE, Seefeld, Germany). Table 1 displays manufacturers, powder/liquid ratios, components, and batch numbers. The biosilicate particles of the quaternary P_2O_5-Na_2O-CaO-SiO_2 system (Biosilicate, Vitrovita, São Carlos, SP, Brazil) were added to the RMGICs. The size of these particles ranged from 0.2 to 10 μm, and the average size was about 2 μm.

Experimental powders were made by incorporating 2, 5 and 10 wt% of Biosilicate particles with FL and VT powders for compressive strength, and 2 wt% for microtensile bond strength (MTBS). Different powder/liquid ratios were used according to the percentage of Biosilicate particles incorporated (Table 2). These powders were inserted into 0.5 ml Ependorf plastic test tubes, and were agitated in an automatic mixer (Ghimas 92, Imperial, Casaluchio, Italy) for 30 s to obtain a uniform distribution of filler particles. FL and VT powder were used as controls.

Compressive strength test

Compressive strength was tested following ISO 9917-1 standard. 2, 5 and 10 wt% of Biosilicate particles were added to FL and VT. Cylindrical specimens were made by placing 2 mm thick layers of the mixed material into teflon molds (height 6 mm, diameter 4 mm), the last layer was compressed with a glass plate. The layers were polymerized with a Translux EC halogen light-curing unit (Kulzer GmbH, Bereich Dental, Wehrheim, Germany) for 40 s. The output intensity was monitored with a Demetron Curing

Radiometer (Model 100, Demetron Research Corporation, Danbury, CT, USA). A minimal output intensity of 600 mW/cm^2 was required for the experiments. The specimens were kept in the mold for 15 min under a load of 150 g to avoid dimensional and mechanical changes. After this period, the cement was removed from the mold and stored individually in 6 mL of deionized water at 37±1ºC for 24 h. Six specimens were prepared for each group.

Compressive strength was tested in a universal testing machine (EFH/5/FR, Microtest S.A.; Madrid, Spain) at a cross-head speed of 1 mm/min. A progressively increasing compressive load was applied along the long axis of the specimens. The maximum load applied to fracture the specimens was recorded. Compressive strength was calculated using the following formula: $P/\pi r^2$ (P= load at fracture (kgf), r= radius of sample cylinder (cm). Compressive strength values [kg/cm^2] were converted into MPa by MPa=Kg/cm^2 x 0.09807. Compressive strength data were tested for Normal distribution by Kolmogorov-Smirnov test ($p<0.05$) and after analysis of variance was performed. Post hoc multiple comparisons were performed using the Student-Newman-Keuls test ($p<0.05$).

Microtensile bond strength (MTBS) test

Twenty non-carious human molars were used. The permanent molars were obtained after the informed consent from donors. The research was approved by the Institutional Research Ethics Commission. The teeth were cleaned with pumice/water slurry, rinsed, and stored in distilled water in a refrigerator (4°C) until use. The root orifices were sealed with composite resin and their cusps flattened with 220-grit abrasive paper. Occlusal Class I cavities (7mm x 5mm x 2mm deep) were prepared using a high-speed handpiece with a cylindrical medium-grit (100 µm) diamond bur (#842, Komet,

Lemgo, Germany) under water irrigation. Each diamond point was replaced for every five preparations [5].

The teeth were randomly divided into 8 groups according to the materials (VT and FL with or without 2 wt% of Biosilicate) and times of storage (24 h and 7 d). Materials were applied following the manufacturers' instructions, using conditioner and primer when indicated (Table 1). For FL application dentin was gently dried with absorbent paper and for the VT group, dentin was carefully air dried in order to maintain a moist dentinal surface. Polymerization was performed. Occlusal surfaces of restorations were ground to assure that the bonded dentin-restorative material interfaces were exposed and specimens were stored for 24 h or 7 d at 37 ºC in distilled water containing 0.02% sodium azide (Sigma-Aldrich, S.A., Madrid, Spain).

After each storage period, the bonded teeth were vertically sectioned into serial slabs and further into beams with cross-sectional square areas of approximately 1 mm^2 for microtensile bond strength testing. Each beam was attached to a modified Bencor Multi-T testing apparatus (Danville Engineering Co., Danveile, CA, USA) with cyanoarylate adhesive (Zapit, Dental Venture of America Inc., Corona, CA, USA) and stressed to failure in tension using an universal testing machine (Instron 4411, Instron Corp., Canton, MA, USA) at a cross-head speed of 1 mm/min. The fractured beams were carefully removed from the apparatus, and the cross-sectional area at the site of failure measured to the nearest 0.01mm with a pair of digital callipers (Sylvac Ultra-Call III, Fowler Co. Inc., Newton, MA, USA). Bond strength values were expressed in MPa. Bond strength values were analyzed by ANOVA. Post hoc multiple comparisons were performed using the Student–Newman–Keuls test ($p < 0.05$).

The fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV, Olympus, Tokyo, Japan) at 40x magnification to determine the mode of failure.

Failure modes were classified as adhesive, cohesive within the cement or mixed. Four representative debonded samples from each group were fixed in a solution of 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer for 24 h, rinsed three times in 0.1 mol/L sodium cacodylate buffer, and postfixed in 1% osmium tetraoxide solution for 2 h. They were then rinsed in distilled water and dehydrated in an ascending ethanol series (30%, 50%, 70%, 80%, 95%, and 100%) for 15 min each. Samples were placed in an apparatus for critical point drying. Specimens were gold-coated and observed with a scanning electron microscope (SEM) (Zeiss DSM-950, Karl-Zeiss, Germany) at an accelerating voltage of 20 kV to examine the morphology of the debonded interfaces.

Results

RMGIC commercial types (F=99.614; p<0.0001) and Biosilicate incorporation (F=521.148; p<0.0001) significantly affected compressive strength. Interactions among these factors were also significant (p<0.0001). Mean compressive strength values obtained for the different groups are shown in Table 3. The incorporation of Biosilicate particles into the commercial cements decreased compressive strength in all groups, except when 2 wt% of Biosilicate was incorporated, this percentage did not decrease compressive strength of the RMGICs tested.

The MTBS to dentin was affected by RMGIC commercial types (F=31.0; p<0.0001), storage period (F=77.1; p<0.0001) and Biosilicate incorporation (F=4.8; p<0.0001). Interactions among factors were also significant (p<0.0001). The mean bond strength values obtained for the different groups are shown in Table 4. Pre-testing failures (debonded before testing) were less than 2%, and were not included in the analysis. When Biosilicate was incorporated, higher bond strength to dentin was obtained, except for FL after 7d of storage. When comparisons were made between RMGICs without Biosilicate,

FL presented higher MTBS than VT after 7d of storage. Regarding the different periods of storage, only FL without BCG and VT with Biosilicate exhibited significantly higher MTBS values after 7d, if it is compared to 24h of storage.

Table 5 summarizes the percentage of failure modes of the debonded specimens in the tested groups. Mixed fracture modes were frequently identified in all groups. More adhesive failures were observed in groups in which lower values of bond strength were found (Table 4 and 5). Some cohesive failures were observed when increased bond strength to dentin occurred (Table 4 and 5).

SEM images of debonded specimens are shown in Figures 1 and 2. When FL was tested after 24h of storage, most of the debonded specimens showed a dentin surface that was covered by cement remnants (Fig. 1a). After 24h, when FL was doped with Biosilicate, similar features were encountered. A thin homogeneous layer of the cement was observed completely covering dentin surface (Fig. 1b). For specimens bonded with FL and stored during 7d, mixed failures mostly occurred. Dentin was not exposed, even when tubule entrances were discernible. Cements remnants are dispersed onto the dentin surface (Fig. 1c). Same observations were found when bonding with Biosilicate-doped FL and testing after 7d. Occluded dentinal tubules were exhibited in some areas of the surface, but cements remnants and rounded particles were visible onto the dentin surfaces (Fig. 1d). When using VT and testing after 24 h, regardless of Biosilicate doping, dentin was covered by smear layer showing a bur-cut pattern with few remnants of the RMGICs onto the surfaces. Dentin tubules were not directly exposed. Main fractures occurred within the modified smear layer or on the top of the formed gel-phase (Fig. 2a and 2b). After 7 d of storage, it was possible to observe a layer of the cement remaining on top of dentin for specimens bonded with VT. Cement attained a porous and rough surface (Fig. 2c and 2d).

Discussion

The null hypothesis is rejected as Biosilicate particles addition modifies mechanical properties and dentin bond strength of tested RMGICs.

The compressive strength decreased when 5 and 10 %wt of biosilicate particles were incorporated. Alteration in viscosity can interfere severely with the strength of these cements [14]. Bioactive glasses used in the present study have a powder average particle size of 2 μm, which is a lower size than that used in other studies (20 μm) [2, 23, 24]. If lower sized particles are employed, higher amount of liquid is needed, in order to obtain an adequate cement consistency (Table 2), and the increased amount of liquid to obtain a homogeneous mixture promotes a weaker material [23]. It has also been reported that the released calcium ions from Biosilicate may react with carboxylate groups, avoiding the adequate crosslink between carboxylate and aluminium ions [2].

Vitremer exhibited higher compressive strength than FL in all experimental groups. These results agree with other study [3]. Resin/glass ionomer ratio in RMGICs also determines physical and mechanical properties [14]. Vitremer attained improved integration of matrix and particles than Fuji II LC, consequently, with less voids or defects [22].

When 2 %wt Biosilicate was incorporated powder was diminished just in 0.5 for FL and in 0.3 for VT, and no differences in compressive strength were encountered. This percentage of particles was selected to test MTBS to dentin.

VT attained the same MTBS means as FL for the control groups after 24 h of storage (Table 4). Similar results have been found in previous studies [4, 10]. However, after 7 d of water storage, FL showed significantly higher bond strength than VT [16, 19]. When employing FL cement on dentin, a 10% polyacrylic acid is applied to remove the smear layer and to promote the micromechanical retention on dentin surface [20] (Fig.1c and 1d). When using VT, a primer is applied previous to the insertion of the material. This primer

is an acidic light-polymerizing liquid composed by HEMA, ethyl alcohol, polycarboxylic acid and photoinitiators. The active conditioning with 10% polyacrylic acid (Fuji II LC) exposed more dentinal structure than the Vitremer primer. Therefore, when using Vitremer cement, the bond is likely to be limited mostly to the smear layer [7] (Fig. 2a).

Dentin bond strength was increased with the addition of Biosilicate after 24 h, in both cements. After 7 d, VT also exhibited these differences. Bioactive particles have been added to other resin-based materials in order to promote dentin remineralization [8, 17] but it always produced a diminishing in microtensile bond strength to dentin, if tested after water storage [18], probably due to increases in solubility and decreases in mechanical properties of new developed materials [8, 17]. Being this new proposed material advantageous over those previously tested.

The most important point is that these Biosilicate-doped RMGICs are expected to induce dentin remineralization. $Na_2O–CaO–SiO_2–P_2O_5$ system suffers elution in presence of water [1], leading to cations and anions release. One of the anionic species released is P_3O_9 which exhibited the highest liberation rate, and it is also correlated with the trend seen in the cation Na^+ release profile. It was suggested that P_3O_9 is unbranched, with Na^+ ions taking up the residual charge on this anion, due to the correlation seen between sodium and P_3O_9 ion release [1]. The $Na_3P_3O_4$ formation may be possible and this polyphosphate is a recognized matrix phosphoprotein analog for extrafibrillar mineralization. The other factor necessary to produce the interfibrillar and durable mineralization is the polycarboxilic acid. VT contains carboxylic acid copolymer in its formulation which could help to produce this effect. If this remineralization may also be a factor increasing MTBS should be further researched.

Within the limitations of this study, it can be concluded that 2 wt% of Biosilicate addition into tested RMGICs does not alter compressive strength, increased MTBS to dentin and may favor dentin remineralization.

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Legends for figures

Figure 1– SEM image of debonded specimens restored with Fuji II LC. **(a)** A cohesive failure within the cement, after 24 h of water immersion. Particles within the RMGIC were visible. No exposed dentin was observed. **(b)** A mixed failure is observed for a specimen restored with Biosilicate-doped Fuji II LC, after 24 h of storage. A thin homogeneous layer of the cement was observed. **(c)** A mixed failure is presented, it occurred in a Fuji II LC specimen, after 7 d of storage. Dentin was not exposed even when tubule entrances may be discernible, the complete surface was covered by cements remnants. **(d)** Biosilicate-doped Fuji II LC, after 7 d of storage. Occluded dentinal tubules were exhibited in some areas of the surface. Cements remnants and rounded particles were visible onto the dentin surface.

Figure 2– SEM observation of debonded specimens restored with Vitremer along the dentin slide. **(a)** A Vitremer bonded specimen showing an adhesive failure after 24 h of water immersion. Dentin as covered by the smear layer and adhesive remnants. **(b)** Biosilicate-doped Vitremer bonded specimen, after 24 h of storage. Dentin was completely covered by adhesive and cement remnants, polishing scratches on dentin were not visible. **(c)** Surface bonded with Vitremer after 7 d storing. RMGIC covered the complete dentin surface. **(d)** Sample bonded with Biosilicate-doped Vitremer, dentin surface was not visible as remained covered by the cement. It showed a porous morphology.
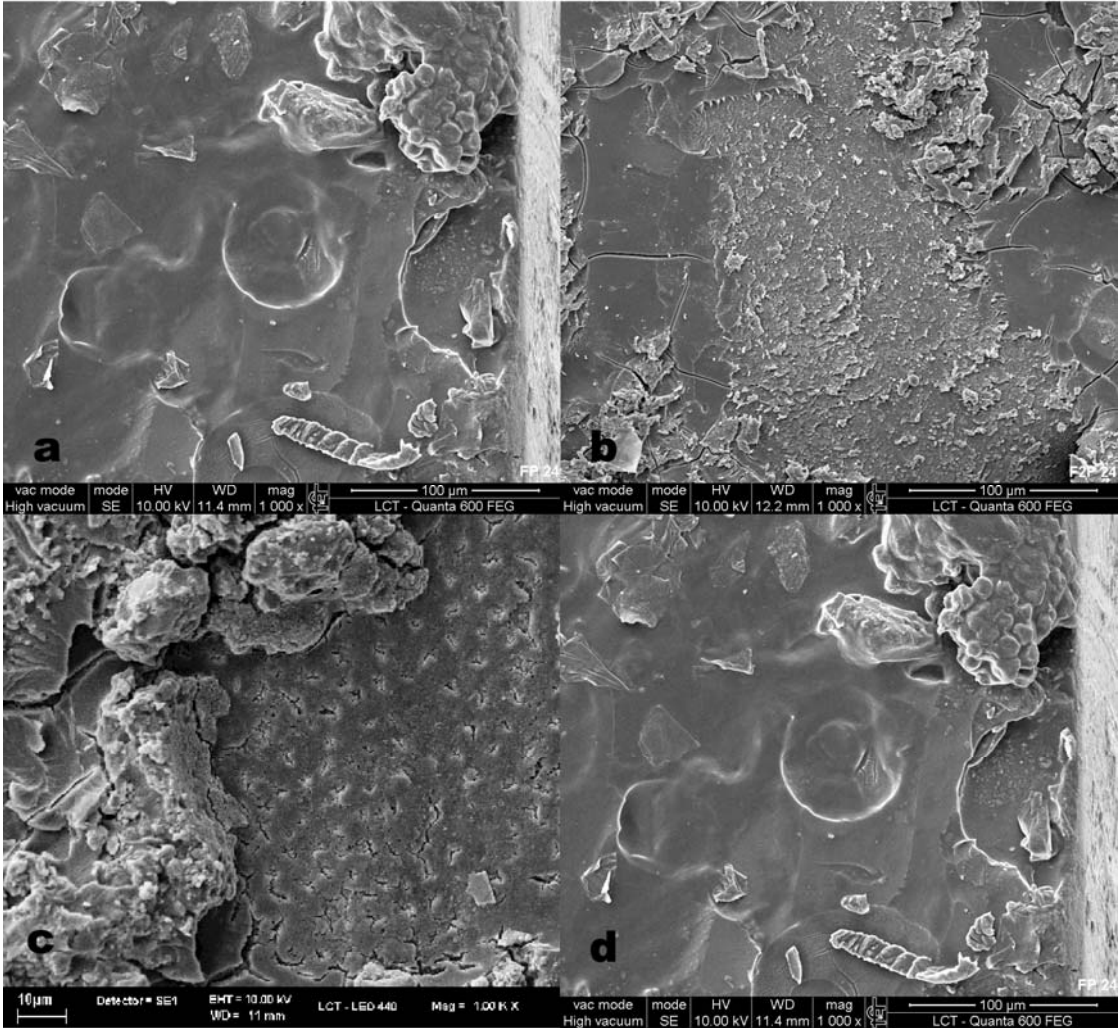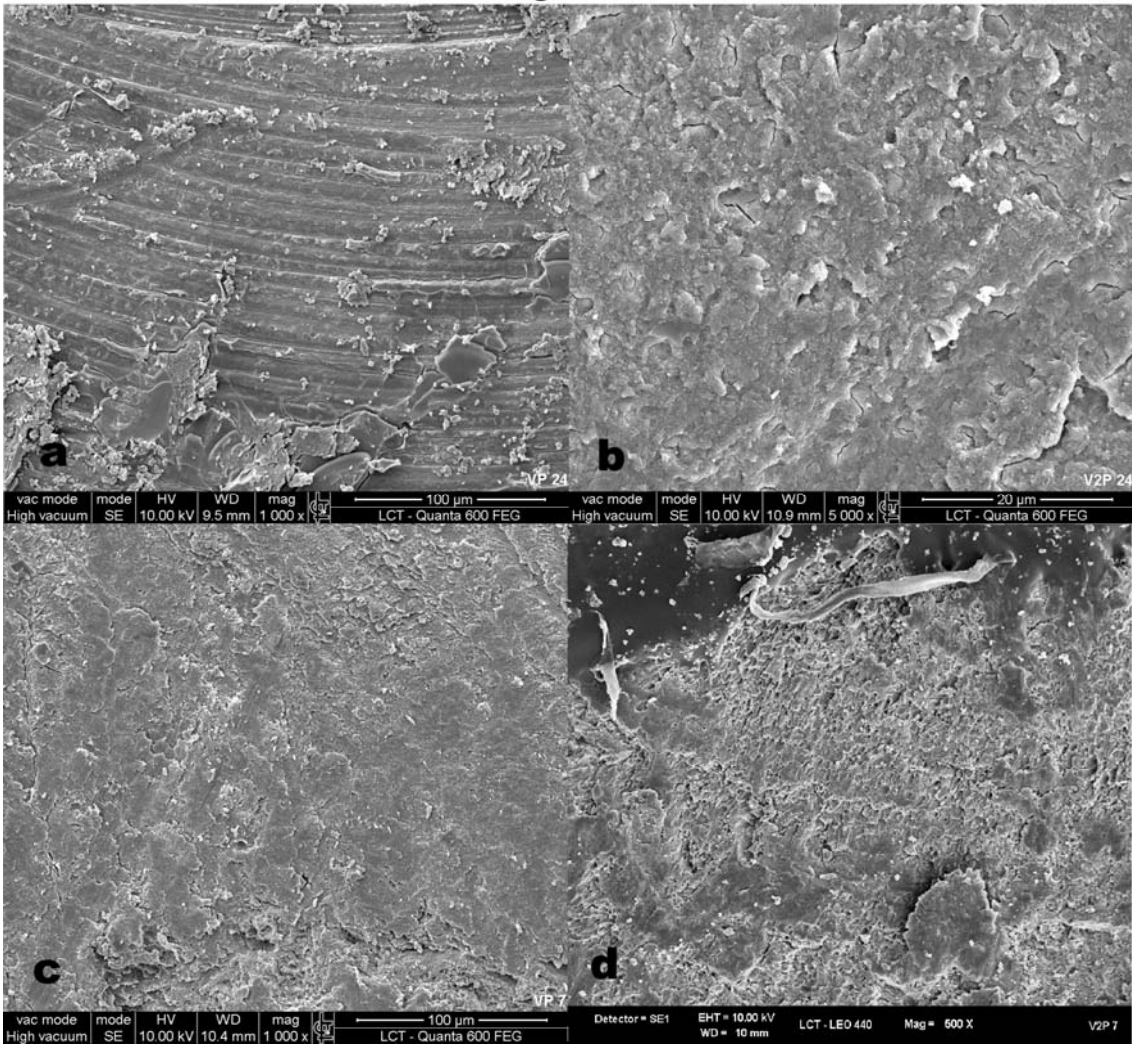
Figure 1

Figure 2

Table 1 – Descriptions of manufacturers, powder/liquid ratios, compositions, and batches number of materials.

Materials Manufacturer	P:L ratio Control	P:L ratio Experimental	Composition	Batch #
Fuji II LC GC Corporation, Tokyo, Japan	3.0:1.0	2.5:1.0	P: Fluoro-aluminosilicate glass L: Acrylic-maleic acid copolymer, Hydroxyethyl methacylate (HEMA), water, camphoroquinone	0604191
Vitremer 3M ESPE, Seefeld, Germany	2.5:1.0	2.2:1.0	P: Fluori-aluminosilicate glass, potassium persulfate, ascorbic acid L: 50% Polyacrylic acid copolymer, 20% HEMA, water, 13% carboxylic acid copolymer	20061011
Biosilicate™ Vitrovita, São Carlos, Brazil	----------		P_2O_5-Na_2O-CaO-SiO_2	----------

Table 2. Power/Liquid ratio of tested materials

Materials		Power/Liquid
Fuji II LC	Without BGC	3.0/1.0
	2%wt BGC	2.5/1.0
Vitremer	Without BGC	2.5/1.0
	2%wt BGC	2.2/1.0

Table 3- Mean (standard deviation) average roughness values (Ra- nm) of the glass ionomer surfaces, with and without BGC addition, under two different storage conditions.

	2% Biosilicato		Control	
	Dry storage	Wet storage	Dry storage	Wet storage
FUJI II LC	295.65 (19.61) A1	271.20 (37.64) A1	616.91 (119.43) B2	359.05 (37.79) C2
VITREMER	226.80 (42.14) a1	273.50 (15.12) a1	112.23 (27.41) b1	172.38 (7.35) c1

Same alphabetical letters in rows and numbers in columns indicate groups that are statistically similar ($p > 0.05$).