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

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Bioactive glasses are surface-active and able to induce remineralization of dentin. Two resin-modified glass-ionomer cements (RMGICs) doped with bioactive glass (Biosilicate[®]) were used as restorative materials in dentin. Experimental powders were made by incorporating 2, 5, and 10 wt% of Biosilicate[®] in Vitremer[®] (VT) and Fuji II LC[®] (FL) powders. Commercial FL and VT were used as control materials. Six cylinders of each material were tested for failure in compression (1.0 mm/min), after 24 h storage in distilled water at 37 °C. For microtensile bond strength (MTBS) test, cavity preparations were performed on 30 noncarious human molars and restored with the tested materials. Teeth were stored in water (37 °C) for 24 h or 7 days, sectioned into beams and tested for failure in tension (0.5 mm/min). Data were analyzed by analysis of variance and multiple-comparison tests ($p < 0.05$). Analysis of debonded specimens was performed by scanning electron microscopy. Only incorporation of 2 wt% of Biosilicate[®] did not decrease compressive strength of the RMGICs. Two weight percent of Biosilicate[®] into RMGICs produced an increase in MTBS after 24 h for FL and after 24 h and 7 days for VT. Two weight percent of Biosilicate[®] particles into RMGICs did not affect compressive strength and improved MTBS to dentin.

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

1. Introduction

Resin-modified glass-ionomer cements (RMGICs) were introduced in an attempt to improve the glass-ionomer cements properties.[1] In these materials, a resin component, which is set by a radical addition polymerization supplements the acid–base setting system of the conventional cements. These materials, whose setting is induced by visible light, exhibit some distinct advantages for clinician; e.g. the process of hardening of material is accelerated on command and the setting time is shortened.[2]

Previous studies [3,4] have suggested that bioactive glass could be used for remineralizing damaged dentin. As bioactive glass particles alone are easily displaced in a clinical environment, [5,6] a suitable carrier or matrix material is needed to facilitate its

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use in these settings. Previous studies showed that RMGICs with bioactive glasses yielded positive bioactivity effects, [7,8] a Ca-rich precipitation layer was observed on outer surfaces of the RMGICs *in vitro*, [7] in the close vicinity of the restoration–dentin interface and in deeper parts of dentin tubules *in vivo*. [8] However, these materials exhibited lower mechanical properties than RMGICs [8,9] with similar setting ability. [9,10]

Recently, a fully crystalline bioactive glass–ceramic of the Na_2O – CaO – SiO_2 – P_2O_5 system (Biosilicate[®], PI 0300644-1) was developed. [11] *In vitro* experiments demonstrated that Biosilicate[®] is highly bioactive and undergoes enhanced bone-like matrix formation compared with Bioglass 45S5[®] in an osteogenic cell culture system. [12]

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 strengths 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.

2. Experimental

2.1. Materials

Two different commercially available RMGICs were used: Fuji II LC[®] (FL) (GC Corporation, Tokyo, Japan) and Vitremer[®] (VT) (3 M ESPE, Seefeld, Germany). Table 1 displays manufacturers, powder/liquid ratios, components, and batch numbers. The

Table 1. Manufacturers, composition, and modes of application of tested materials.

Materials Manufacturer (batch number)	Mode/steps of application	Composition
Fuji II LC GC Corporation, Tokyo, Japan (0604191)	(1) Apply 10% of polyacrylic acid solution for 20 s, using a light scrubbing motion. (2) Rinse thoroughly and gently dry with absorbent paper. (3) Hand-mix manipulation. (4) Insert into a cavity using a syringe injector in a single increment. (5) Light cure for 40 s. (6) Apply finishing gloss and light-cure for 20 s.	Powder: Fluoro-aluminosilicate glass Liquid: Acrylic-maleic acid copolymer, HEMA, water, camphoroquinone
Vitremer 3 M ESPE, Seefeld, Germany (20061011)	(1) Apply primer for 30 s, using a light scrubbing motion. Apply mild air stream for 15 s. (2) Light-cure for 20 s. (3) Hand-mix manipulation. (4) Insert into a cavity using a syringe injector in a single increment. (5) Light-cure for 40 s. (6) Apply finishing gloss and light-cure for 20 s.	Primer: HEMA, ethyl alcohol, Vitrebond copolymer Powder: Fluoro-aluminosilicate glass, potassium persulfate, ascorbic acid Liquid: Polyacrylic acid copolymer, HEMA, water, carboxylic acid copolymer

Biosilicate[®] particles of the quaternary P₂O₅–Na₂O–CaO–SiO₂ system (Biosilicate[®], Vitrovita, São Carlos, SP, Brazil) were added to the RMGICs. The Biosilicate[®] percentages are shown in Table 2. 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 as is shown in Table 2. These powders were inserted into 0.5-ml Eppendorf 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 commercial mixtures were used as controls.

2.2. Compressive strength test

Compressive strength was tested following ISO 9917-1 standard. To FL and VT, 2, 5, and 10 wt% of Biosilicate[®] particles were added. Ten 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/cm² 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 ± 1 °C for 24 h. Six specimens were prepared for each group.

Compressive strength was tested in a universal testing machine (EFH/5/FR, Microtest SA; Madrid, Spain). A progressively increasing compressive load was applied along the long axis of the specimens at a cross-head of 1 mm/min. 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 (kg), r = radius of sample cylinder (cm)). Compressive strength values (kg/cm²) were converted into MPa by MPa = Kg/cm² × 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 ($p < 0.05$).

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
	5% wt BGC	2.1/1.0
	10% wt BGC	1.8/1.0
Vitremmer	Without BGC	2.5/1.0
	2% wt BGC	2.2/1.0
	5% wt BGC	2.0/1.0
	10% wt BGC	1.5/1.0

2.3. MTBS test

Twenty-four noncarious human molars were used. The permanent molars were obtained after the informed consent of 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 (7 mm × 5 mm × 2 mm 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.[13]

The teeth were randomly divided into eight groups according to the materials (VT and FL with or without 2 wt% of Biosilicate[®]) and times of storage (24 h and 7 days). Materials were applied following the manufacturers' instructions, using conditioner and primer when indicated as is shown in Table 1. For FL and VT application, the bonding procedures were performed in moist dentin following the manufacturer's instructions. 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 days at 37 °C 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 mm² for MTBS testing. Each beam was attached to a modified Bencor Multi-T testing apparatus (Danville Engineering Co., Danville, 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 crosshead speed of 1 mm/min. The fractured beams were carefully removed from the apparatus and the cross-sectional area at the site of failure was measured to the nearest ± 0.01 mm 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 40× magnification to determine the mode of failure. Failure modes were classified as adhesive and 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 tetroxide 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.

3. 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

Table 3. Mean compressive strength (MPa) and standard deviation obtained for each experimental group.

	Fuji II LC	Vitremer
Control	183 (16.3) A	142 (14.1) A*
2% BGC	151 (5.5) A	122 (9.6) A*
5% BGC	120 (7.7) B	87 (5.3) B*
10% BGC	22 (3.2) C	17 (7.2) C*

In rows, * indicates significant differences between subgroups ($p < 0.05$).

In columns, averages with the same letter are not significantly different ($p > 0.05$).

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 the compressive strength of the RMGICs tested.

The MTBS of 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. Pretesting failures (debonded before testing) were less than 2% and were not included in the analysis. When Biosilicate[®] was incorporated, higher bond strength of dentin was obtained, except for FL after 7 days of storage. When comparisons were made between RMGICs without Biosilicate[®], FL presented higher MTBS than VT after 7 days of storage. Regarding the different periods of storage, only FL without BCG and VT with Biosilicate[®] exhibited significantly higher MTBS values after 7 days, if it is compared to 24 h 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 (Tables 4 and 5). Some cohesive failures were observed when increased bond strength of dentin occurred as is shown in Tables 4 and 5.

SEM images of debonded specimens are shown in Figures 1 and 2. When FL was tested after 24 h of storage, most of the debonded specimens showed a dentin surface that was covered by cement remnants as is shown in Figure 1(a). After 24 h, when FL was doped with Biosilicate[®], similar features were encountered. A thin homogeneous layer of the cement was observed completely covering dentin surface as is shown in Figure 1(b). For specimens bonded with FL and stored during 7 days, mixed failures mostly occurred. Dentin was not exposed, even when tubule entrances

Table 4. Mean (SD) MTBS to dentin (MPa) and standard deviation obtained for each experimental group (number of beams from each subgroup = 30).

	Fuji II LC		Vitremer	
	24 h	7 days	24 h	7 days
Without BCG	6.7 ± 1.9 B	9.0 ± 1.8 A	7.9 ± 2.0 B	7.8 ± 1.5 B
With BCG	7.6 ± 1.4 C*	8.1 ± 1.6 C	9.0 ± 1.8 B*	10.8 ± 1.6 A*

In rows, averages with the same letter are not significantly different ($p > 0.05$).

In columns, * indicates significant differences between subgroups ($p < 0.05$).

Table 5. Percentage (%) distribution of failure modes of debonded specimens in each experimental group.

	Fuji II LC						Vitremer					
	24 h			7 days			24 h			7 days		
	A	M	C	A	M	C	A	M	C	A	M	C
Control	13.4	83.3	3.3	–	80.0	10.0	13.4	76.6	10.0	16.6	76.7	6.7
Experimental	10.0	90.0	–	6.7	86.7	6.6	–	86.7	13.3	–	80.0	20.0

A: adhesive; M: mixed; C: cohesive.

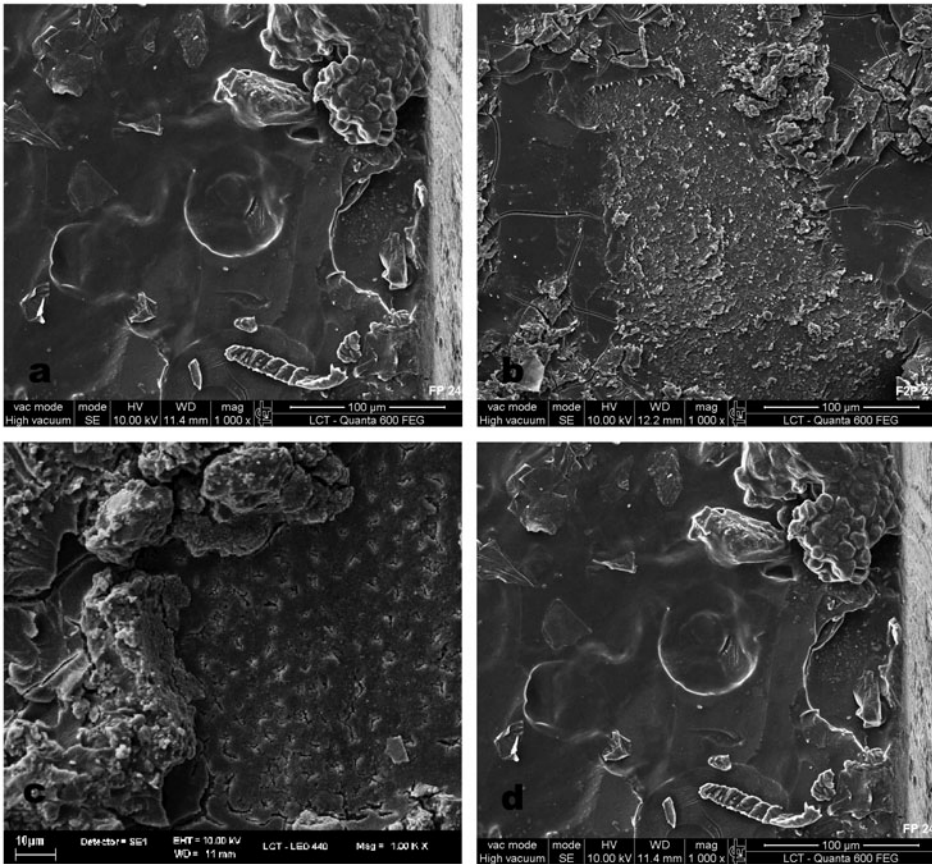


Figure 1. SEM image of debonded specimens restored with FL. (a) A cohesive failure within the cement, after 24 h of water immersion. Particles within the RMGIC were visible. No exposed dentin was observed. (b) A mixed failure is observed for a specimen restored with Biosilicate[®]-doped FL, after 24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

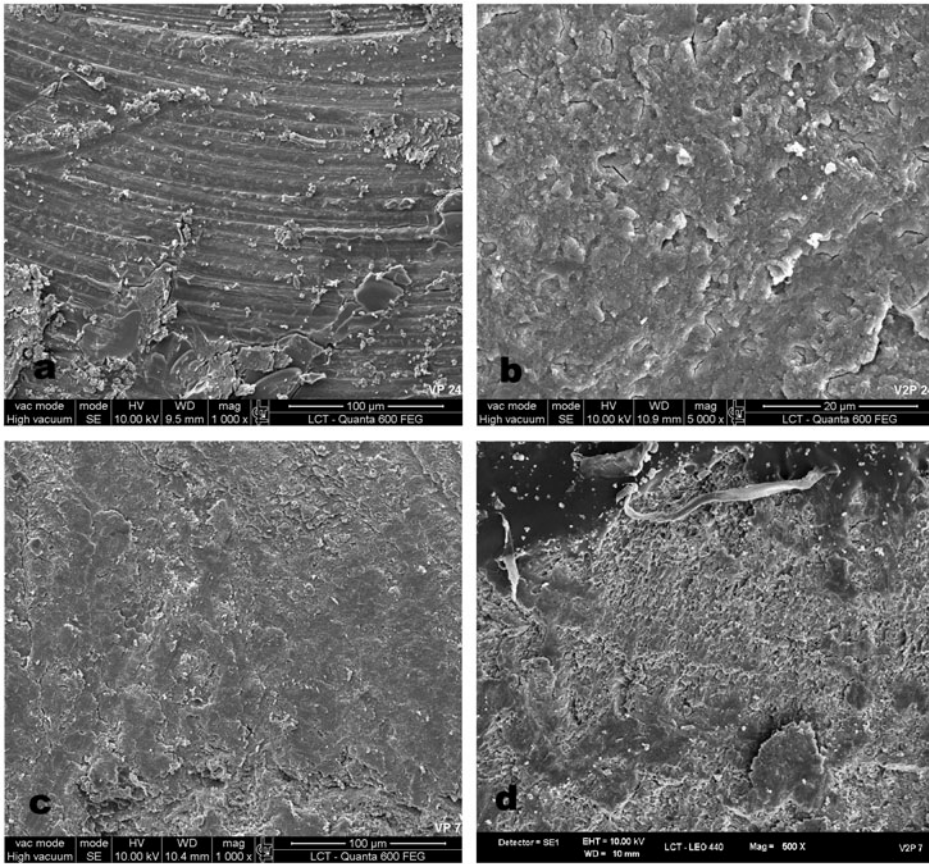


Figure 2. SEM observation of debonded specimens restored with VT along the dentin surface. (a) A VT bonded specimen showing an adhesive failure after 24 h of water immersion. Dentin as covered by the smear layer and adhesive remnants. (b) Biosilicate®-doped VT bonded specimen, after 24 h of storage.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

were discernible. Cements remnants are dispersed onto the dentin surface as is shown in Figure 1(c). Same observations were found when bonding with Biosilicate®-doped FL and testing after 7 days. Occluded dentinal tubules were exhibited in some areas of the surface, but cements remnants and rounded particles were visible onto the dentin surfaces as is shown in Figure 1(d). 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 as is shown in Figure 2(a) and (b). After 7 days 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 as is shown in Figure 2(c) and (d).

4. 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) as seen in Table 2. [7–9] If lower sized particles are employed, higher amount of liquid is needed, in order to obtain an adequate cement consistency, and the increased amount of liquid to obtain a homogeneous mixture promotes a weaker material. [8] It has also been reported that the released calcium ions from Biosilicate[®] may react with carboxylate groups, avoiding the adequate crosslink between carboxylate and aluminum ions.[9]

VT exhibited higher compressive strength than FL in all experimental groups. These results agree with another study.[15] Resin/glass-ionomer ratio in RMGICs also determines physical and mechanical properties.[14] VT attained improved integration of matrix and particles than FL, consequently, with less voids or defects.[16]

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 of dentin.

VT attained the same MTBS means as FL for the control groups after 24 h of storage as is shown in Table 4. Similar results have been found in previous studies.[17,18] However, after 7 days of water storage, FL showed significantly higher bond strength than VT.[19,20] When employing FL cement on dentin, a 10% polyacrylic acid is applied to remove the smear layer and to promote the micromechanical retention and chemical adhesion on dentin surface as is seen in Figure 1(c) and (d).[21] 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 (FL) exposed more dentinal structure than the VT primer. Therefore, when using VT cement, the bond is likely to be limited mostly to the smear layer as is shown in Figure 2(a).[22]

Dentin bond strength was increased with the addition of Biosilicate[®] after 24 h, in both cements. After 7 days, VT also exhibited these differences. Bioactive particles have been added to other resin-based materials in order to promote dentin remineralization [23,24] but it always produces a diminishing effect on MTBS of dentin, if tested after water storage,[25] probably due to increases in solubility and decreases in mechanical properties of new developed materials.[23,24]

The most important point is that these Biosilicate[®]-doped RMGICs are expected to induce dentin remineralization.[26] $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2-\text{P}_2\text{O}_5$ system suffers elution in the presence of water,[27] 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.[27] The $\text{Na}_3\text{P}_3\text{O}_4$ 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 polycarboxylic 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.

5. Conclusion

The addition of 2 wt% of Biosilicate[®] in the commercial RMGICs enhances the MTBS of the resulting cements to dentin. It also does not alter compressive strength of Biosilicate[®]-RMGICs cement and may favor dentin remineralization. Thus, Biosilicate[®] is promising additive for resin-modified glass-ionomer restorative dental materials with constructive properties.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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