SEM and AFM characterization of surface of two RMGICs for degradation before and after modification with bioactive glass ceramic.

*Estrella Osorio[1], Raquel Osorio[1], Edgar D. Zanotto[2], Oscar Peitl[2], Manuel Toledano-Osorio[1], Manuel Toledano[1]

[1]Department of Dental Materials, School of Dentistry, University of Granada, Granada, Spain

[2]Department of Materials Engineering (DEMa), Federal University of São Carlos, São Carlos, Brazil

*Corresponding author

Estrella Osorio

Address: Materiales Odontológicos, Facultad de Odontología

Colegio Máximo. Campus de Cartuja s/n

Universidad de Granada, 18071 Granada

Phone: 34 958 243793

Fax: 34 958 240908

E-mail: meosorio@ugr.es

Abstract

Objectives: The aim of this study was to evaluate the effect of bioactive glass-ceramic particles (Biosilicate®) addition on surface nanoroughness and topography of RMGICs. **Methods:** Experimental materials were made by incorporating 2 wt% of Biosilicate® into Fuji II LC® (FL) and Vitremer® (VT) powders. Disks of RMGICs (with and without Biosilicate®) measuring 0.5 cm (diameter) x 0.5 mm (thickness) were fabricated and polished. Samples were stored at 37ºC in dry or immersed in distilled water for 30 days. Digital images (20x20 microns) from the surfaces were obtained by means of an AFM microscope. Three images were acquired for each sample and four nanoroughness measurements were performed in each image. Nanoroughness (Ra, nm) was assessed by Nanoscope Software V7. Data were analyzed with ANOVA and Student–Newman–Keuls multiple comparisons ($p<0.05$). SEM images were obtained for surface topography analysis. **Results:** FL was significantly rougher than VT ($p<0.05$) in wet and dry conditions. The addition of Biosilicate® increased the surface roughness in VT and decreased in FL, regardless of the storage media ($p\leq0.05$). No differences existed between materials and storage conditions after Biosilicate® addition. **Significance:** The Biosilicate® particles addition produced changes on the surface nanoroughness of the RMGICs. These changes depended on the particles size of the original cements in dry conditions. In water storage, dissolution of the Biosilicate® particles, a silica-rich gel formation and a hydroxyl carbonate apatite precipitation on the surface of the materials changed the nanoroughness surface. FL was the roughest in both conditions.

1. Introduction

Resin-modified glass-ionomer cements (RMGICs) undergo an acid-base setting reaction and have been improved with monomers and initiators capable of generating photochemical polymerization. The set cement consists of interpenetrating networks of poly-(HEMA) and polyacrylate salts.[1] RMGICs are supposed to retain the advantages of glass-ionomer cements, such as anticariogenicity (due to release of fluoride), thermal compatibility with teeth [1] and show improved working characteristics and adhesion to dentin if compared with the conventional glass-ionomer cements (GICs). [2,3]

Bioactive glasses and glass-ceramics are synthetic materials generally made of calcium, phosphorus, silicon, and sodium oxides. Hench (2006) [4] concluded that these materials were surface-active which had the capacity to chemically bond to bone. The reaction sequence occurs in five stages resulting in hydroxycarbonapatite precipitation on the surface of the material. [5,6] Although most experience with bioactive glasses has been gained in bone medicine research, [7] they have also been used in dentistry, such as treatment of periodontal defects and dentin hypersensitivity. [8,9,10] A previous study reveals that bioactive glasses, especially nanometric particles, induces the odontogenic differentiation and dentin formation of dental pulp cells and may serve as a potential material for pulp repair and dentin regeneration. [11]

Modifications of GICs have been tried to obtain bioactivity. Previous studies, in which bioactive glasses have been incorporated into GIC and RMGIC, presented low mechanical properties [12, 13] although with similar setting ability, [12, 14] but yield high fluoride release [15] and positive bioactivity effects. [15, 16] A Ca-rich precipitation layer was observed on outer surfaces of the RMGIC *in vitro* [15] and also in the close vicinity of the restoration–dentin interface and in deeper parts of dentin tubules under *in vivo* conditions. [16, 17] BAG has been incorporated into GIC

composition to improve bioactivity and tooth regeneration and reconstruction capacity. [18, 19]

A fully crystalline glass–ceramic of the Na_2O–CaO–SiO_2–P_2O_5 system (Biosilicate®, PI 0300644-1) was developed. [20] In contrast to what might have been expected about the decrease of bioactivity with the increase of the material's crystallinity, under *in vitro* conditions, experiments demonstrated that Biosilicate® is highly bioactive and undergoes enhanced bone-like matrix formation compared to its parent glass and to Bioglass 45S5 in an osteogenic cell culture system. [21] It has been demonstrated that filling of sockets with Biosilicate® particles preserves alveolar bone ridge height and allows osseointegration of Ti implants. [22] Osteogenic activity was also been demonstrated on bone defects inflicted on the tibia of rats. [23]

However, there is a lack of studies evaluating the surface roughness and topography of RMGIC after the incorporation of bioactive glasses or glass-ceramics (Biosilicate®). The clinical significance of the increased roughness surface of the materials covers the increased plaque adhesion and its harmful effects on the tooth and periodontium, to surface discoloration and fatigue failure. [24] The nanoscale surface roughness has been considered as a factor positively influencing the adhesion of cells. [25]

The objectives of this study were to investigate the effects of a novel bioactive glass-ceramic (Biosilicate®) on nanoroughness and topography surface of RMGICs after wet and dry storage conditions. Null hypothesis tested was that there was no difference in nanoroughness and topography surface when incorporating Biosilicate® into RMGICs.

2. Experimental

2.1. Materials

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 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 [Table 2 near here]. 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 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 commercial mixtures were used as controls.

2.2. Specimen Preparation

Teflon molds were used for the preparation of disc specimens (0.5 cm in diameter and 0.5 mm in thickness). Eight discs were performed for control and experimental materials (VT, FL, VT-Biosilicate® and FL-Biosilicate®). The materials was directly injected into Teflon molds using a syringe injector (Centrix Incorporated, Shelton, USA) and covered with a polyester strip (Proben, Catanduva, Brazil). The specimens were light-cured, under a glass microscope slide, with an activated light source (Bluephase, Ivoclair/Vivadent, Schaan, Liechtenstein) polymerization unit. The light was tested for light output (600 mW/cm^2) by means of a Demetron radiometer

(Model 100, Demetron Research Corp., Danbury, CT, USA). The samples were irradiated in different positions for 40s until the entire area was exposed.

2.3. AFM imaging and nanoroughness

The specimens were stored in distilled water at 37 ºC for 7 days until to complete the setting reaction. They were metallographically polished to 1/4 μm diamond paste with water-proof. Specimens from each material were divided in two groups: 1) stored at room temperature at 37 ºC (dry) and 2) immersed in distilled water at 37 ºC. Both of them were store 30 days.

Surfaces were evaluated under an Atomic Force Microscopy (AFM, Multimode Nanoscope IIIa, Digital Instruments, Veeco Metrology group, Santa Barbara, CA, USA). Digital images were taken in air. The tapping mode was performed using a 1-10 Ohm-Cm phosphorus (n) dopes Si tip (at 50 μm).

Three 20 x 20 μm digital images were performed for each surface at a data scale of 1504 μm and recorded with a slow scan rate (0.1 Hz). In each image, five randomized boxes of 10x10 microns were created. Nano-roughness (Ra-nm) was automatically assessed with specific software (Nanoscope V530R35R). Each image was considered as a statistical unit, sample size per group was n= 24.

2.4. Scanning Electronic Microscopy (SEM) Analysis

Four discs from each group were selected, and prepared for SEM examination by desiccation in a Sampla Dry Keeper (Samplatec Co., Tokyo, Japan) for 48 h before sputter coating with gold by means of a coating Unit E-500 (Polaron Equipment Limited, Watford, England). Examination of all specimens under a scanning electron

microscopy (1430 VP, LEO Electron Microscopy Ltd., Cambridge, UK) at an accelerating voltage of 20 kV.

2.5. Statistical Analysis

Mean nanoroughness values were analyzed by two-way ANOVA and Student–Newman–Keuls multiple comparisons ($p < 0.05$).

3. Results

3.1. Nanoroughness surface

Mean nanoroughness (Ra-nm) and standard deviations are presented in Table 3. [Table 3 near here] Nanoroughness values were significantly affected by RMGIC type ($F = 57.70$; $p < 0.001$), by Biosilicate® particles addition ($F = 3.75$; $p = 0.07$) but storage conditions ($F = 3.09$; $p = 0.01$) affected it. Interactions were significant between factors. The power of ANOVA was 68%.

FL was rougher than VT ($p < 0.05$) in dry and wet conditions. Wet environment produced a nanoroughness increase in VT, while a nanoroughness decrease was produced in FL.

When Biosilicate® was added in FL, no differences were found between tested materials (FL-Biosilicate® and VT-Biosilicate®) in both storage conditions. Biosilicate® incorporation produced a nanoroughness decrease in FL, and an increase in VT ($p < 0.05$) in both storage conditions.

3.2. Topographical AFM study

AFM images are shown in Figures 1 and 2. Changes in surface textures were exhibited when 2% Biosilicate® was added in both materials as is shown in Figures: 1A, 1C, 2A and 2C. Wet storage induced changes in VT and FL as is shown in Figures: 1B, 1D and 2B, 2D. [Figures 1 and 2 near here] In the groups where Biosilicate® was used, changes on the surface topography were observed. These changes include matrix alterations and particles removal in wet conditions. No topographical differences can be observed between both materials when Biosilicate® was added in wet and dry conditions. The surface of the VT control groups reveals smoother surfaces in both conditions.

3.3. SEM Analysis

SEM micrographs (100x and 5000x) of the specimens are shown in Figures 3 and 4. Pores and microcracks were frequently found on the RMGICs surfaces with and without Biosilicate®.[Figure 3 and 4 near here] At a higher magnification, particles appeared to be adequately dispersed and no particle lost was evidenced.

SEM observations on VT surfaces revealed smooth surfaces. Filler particles are smaller than those of FL. The nanoroughness values of the Biosilicate® groups in wet environment were nearly the same in both cements. After the wet storage, some deposited particles could be observed on both materials with Biosilicate® surfaces.

4. Discussion

The RMGICs combine glass ionomer chemistry with resin composite technology. These materials can overcome the shortcomings of conventional glass ionomers, such as surface crazing during dehydration, and brittleness and flow fracture toughness. [26]

In previous studies, bioactive glass (BAG) has been incorporated into GICs. These experimental materials have been shown to be active in *vitro* condition, and they mineralized human dentin. [27] The BAG also possesses antimicrobial properties. [28]

The addition of bioactive ceramic or BAG to a biopolymer has been shown to alter the degradation rate of the material by changing parameters such as hydrophobicity, water absorption, weight loss and pH. [29] It has been shown that the addition of bioglass particles to the polymeric matrix significantly changes the surface morphology due to the exposure of the bioglass particles on the surfaces as is shown in Figures 3 and 4. [30] The changes on surface roughness play an important role in cell attachment. This fact in turn had a contributing effect on the bioactivity, water uptake and cytocompatibility of the materials. [31] Filler component in term of size, distribution, geometry and volume fraction have been investigated extensively. [32, 33]In the present study, a new BAG has been incorporated to two RMGICs to evaluate the surface roughness and topography after dry and wet storage.

Several methods exist for determining the surface characteristics of materials. Scanning electron microscopy (SEM) provides qualitative information about the variations on the surface with 2-D imaging. Quantitative methods can also be used to determine roughness parameters of a given surface, such as atomic force microcopy (AFM) that allows data acquisition at very high resolution. [27] In the present study both methods have been used to provide qualitative and quantitative information.

The results of this study do not support the acceptance of the null hypothesis. When surface nanoroughness values were compared in terms of the control materials, FL was rougher than VT. FL has bigger particle size (20 μm) than VT (10 μm). [26, 34] This can be observed in SEM images as is shown in Figures: 3A, 3B, 4A and 4B.The differences in the surface nanoroughness of these RMGICs might be ascribed to the

variation in their filler size. Smaller particles provide less vertical dimension and facilitate the adhesion to the resin matrix, thus providing a smoother surfaces [35, 36].

After wet storage, control specimens exhibited changes in surface. FL was rougher than VT but it showed a significant nanoroughness decrease. VT exhibited a nanoroughness increase after water storage.

In these cements the initial acid-base reaction is followed by a slower process, such us the hydration of reaction products and of dental cements. [37, 38] In an initial phase, the material absorbs water from environment and disintegration of a surface layer is the main problem. [39] In FL, water dissolves the components of the cement. It erodes the surface and causes hydrolysis and dissolution of some of their component. [40] This was in accordance with SEM observations (Fig. 3A, 3B). VT, in addition to being a simple mixture or HEMA with polyalkenoic acid, it is also modified by the attachment of pendant polymerizable methacrylate side groups on the polyalkenoic molecules; a higher number of inter-network links may be responsible for the lower values of water sorption and solubility.[38] On the other hand the higher adhesion of the particles to the resin matrix avoids water erosion as is shown un Figure 4B. But the absorbed water produced a volumetric expansion, which is also directly related to the amount of water taken up by the sample. [40] This can explain its minor nanoroughness surface change.

The incorporation of Biosilicate® into RMGICs produced a surface roughness increased on VT and decreased on FL after dry storage with respect to the control groups. Both materials plus Biosilicate® particles presented similar nanoroughness values. The Biosilicate® maximum particle size is 10 µm. This particle size is similar to the particles contained in VT, so the addition of these bioactive glass particles produced an increase of the particles percentage per volume and this fact augmented the surface

nanoroughness in this material. FL has a maximum particle size surrounding 20 μm. Incorporating Biosilicate® particles into FL, which exhibited a bigger particles size, decreased the surface nanoroughness of this material. Biosilicate® filler occupied the spaces between the particles of the FL. It has been shown that the introduction of finer particles among larger ones will result in a reduction of interparticle spacing. [35]

After storage in wet conditions, FL nanoroughness decreased while in VT samples the mean increased with respect to the control groups. This could be explained by the Biosilicate® particles presence. There were no differences between dry and wet conditions when Biosilicate® particles were incorporated in both materials. Beside the effect of the particle size of Biosilicate® on the surface roughness of both materials, the absorbed water in the polymeric matrix could allow surface reactions of bioactive glass [13, 41] and causes a partial dissolution of them. [42] The presence of water and the activity of the hidroxyl groups result in a breakdown of –Si-O-Si-O-Si-bonds. Disruption of silica networks results in the local release of [Si (OH)]. This leads the formation of a silica gel at the particle surfaces that can attenuate the peaks and valleys on the surface of the materials. [4, 18, 21] On the other hand, Calcium and phosphate ions released from the glass, forming a layer rich in calcium and phosphate on the surface. This layer crystallizes into hydroxyl carbonate apatite (HCA). These steps, which occur on the surface of the material do not require the presence of tissue and can happen in distilled water. [4, 18, 43] This could explain the changes produced in FL nanoroughenss which decreases in wet storage. The absence of extensive dissolution and erosion in VT, because its composition, can produce an increase of the nanoroughness surface because the gel layer production and apatite precipitation on the surface as is shown in Figures: 3D and 4D.

5. Conclusion

This study suggests that in laboratory conditions, mixing BAG particles into RMGICs powders produced changes on the nanoroughness surface of the RMGICs. These changes depended on the particles size of the original cements in dry conditions. In water storage, dissolution of the Biosilicate® particles, a silica-rich gel formation and a hydroxyl carbonate apatite precipitation on the surface of the materials changed the nanoroughness surface.

References

[1] Yelamanchili A, Darvell BW. Network competition in a resin-modified glass-ionomer cement. Dent. Mater. 2008;24:1065-9.

[2] Fagundes TC, Barata TJ, Bresciani E, Cefaly DF, Carvalho CA, Navarro MFL. Influence of ultrasonic setting on tensile bond strength of glass-ionomer cements to dentin. J. Adhes. Dent. 2006;8:401-7.

[3] Toledano M, Osorio E, Osorio R, Garcia-Godoy F. Microleakage of Class V resin-modified glass ionomer and compomer restorations. J. Prosthet. Dent. 1999;81:610-5.

[4] Hench LL. The story of Bioglass. J. Mater. Sci. Mater. Med. 2006;17:967-78.

[5] Mitchell JC, Musanje L, Ferracane JL. Biomimetic dentin desentizer based on nano-structured bioactive glass. Dent. Mater.2011;27:386-93.

[6] Tirapelli C, Panzeri H, Lara EH, Soares RG, Peitl O, Zanotto ED. The effect of a novel crystallised bioactive glass-ceramic powder on dentin hypersensitivity: A long-term clinical study. J. Oral. Rehabil. 2011;38:253-62.

[7] Thomas MV, Puleo DA, Al-Sabbagh M. Bioactive glass three decades on. J. Long. Term. Eff. Med. Implants. 2005;15(6):585-97.

[8] Jefferies SR, Appleby D, Boston D, Pameijer CH, Lööf J. Clinical performance of a bioactive dental luting cement--a prospective clinical pilot study. J. Clin. Dent. 2009;20:231-7.

[9]Lee BS, Kang SH, Wang YL, Lin FH, Lin CP. In vitro study of dentinal tubule occlusion with sol-gel DP-bioglass for treatment of dentin hypersensitivity. Dent. Mater. J. 2007;26:52-61.

[10] Sculean A, Windisch P, Keglevich T, Gera I. Clinical and histologic evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. Int. J. Periodontics. Restorative. Dent. 2005;25:139-47.

[11] Wang S, Gao X, Gong W, Zhang Z, Chen Z, Dong Y. Odontogenic differentiation and dentin formation of dental pulp cells under nanobiactive glass induction. Acta. Biomater. 2014;10:2792-803.

[12] Ana ID, Matsuya S, Ohta M, Ishikawa K. Effects of added bioactive glass on the setting and mechanical properties of resin-modified glass ionomer cement. Biomaterials 2003;24:3061-7.

[13] Yli-Urpo H, Lassila LV, Narhi T, Vallittu P. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass. Dent. Mater. 2005;21:201-9.

[14] Matsuya S, Matsuya Y, Ohta M. Structure of bioactive glass and its application to glass ionomer cement. Dent. Mater. J. 1999;18:155-66.

[15] Yli-Urpo H, Vallittu PK, Narhi TO, Forsback AP, Vakiparta M. Release of silica, calcium, phosphorus, and fluoride from glass ionomer cement containing bioactive glass. J. Biomater. Appl. 2004;19:5-20.

[16] Yli-Urpo H, Narhi M, Narhi T. Compound changes and tooth mineralization effects of glass ionomer cements containing bioactive glass (S53P4), an in vivo study. Biomaterials 2005;26:5934-41.

[17] Narayana SS, Deepa VK, Ahamed S, Sathish ES, Meyappan R, Satheesh Kumar K S. Remineralization efficiency of bioactive glass on artificially induced carious lesion an *in-vitro* study. J. Indian. Soc. Pedod. Prev. Dent. 2014;32:19-25.

[18] Khoroushi M, Keshani F. A review of glass-ionomers: From conventional glass-ionomer to bioactive glass-ionomer. Dent. Res. J. 2013;10(4):411-420.

[19] Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An *in-vitro* pH-cycling study. J. Conserv. Dent. 2014;17:3-7.

[20] Zanotto ED, Ravagnani C, Peitl O, Panzeri H, Lara EH. Process and compositions for preparing particulate, bioactive or resorbable Biosilicate® for use in the treatment of oral ailments, WO2004/074199, Fundação Universidade Federal de São Carlos, Universidade de São Paulo, 20 February, 2004, Int. C. C03C10/00.

[21] Moura J, Teixeira LN, Ravagnani C, Peitl O, Zanotto ED, Beloti MM et al. In vitro osteogenesis on a highly bioactive glass–ceramic (Biosilicate®). J. Biomed. Mater. Res. A2007;82:545-57.

[22] Roriz VM, Rosa AL, Peitl O, Zanotto ED, Panzeri H, de Oliveira PT. Efficacy of a bioactive glass-ceramic (Biosilicate®) in the maintenance of alveolar ridges and in osseointegration of titanium implants. Clin. Oral. Implants. Res. 2010; 21:148-55.

[23] Granito RN, Ribeiro DA, Rennó AC, Ravagnani C, Bossini PS, Peitl-Filho O, Zanotto ED, Parizotto NA, Oishi J. Effects of Biosilicate® and bioglass 45S5 on tibial bone consolidation on rats: a biomechanical and a histological study. J. Mater. Sci. Mater.Med.2009;20:2521-6.

[24] Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature.Dent.Mater.1997;13:258-69.

[25] Solar P, Kylián O, Marek A, Vandrovcová M, Bacáková L, Hanus J, Vyskocil J, Slavínská D, Biedrman H. Particles induced surface nanoroughness of titanium

surface and its influence on adhesion of osteoblast-like MG-63 cells. Appl. Surf. Sci. 2015;324:99-105.

[26] Mitsuhashi A, Hanaoka K, Teranaka T. Fracture toughness of resin-modified glass ionomer restorative materials: effect of powder/liquid ratio and powder particle size reduction on fracture toughness. Dent.Mater.2003, 19:747-757.

[27] Narayan P, Hancock BC. The relationship between the particle properties, mechanical behaviour, and surface roughness of some pharmaceutical excipient compatcts. Mater. Sci. and Engineering 2013;A355:24-36.

[28] Yli-Urpo H, Närhi T, Söderling E. Antimicrobial effects of glass ionomer cements containing bioactive glass (S53P4) on oral micro-organisms in vitro. Acta Odontol. Scand.2003;6:241-246.

[29] Li H & Chang J. pH compensation effect of bioactive inorganic fillers on the degradation of PLGA. Compos. Sci. Technol. 2005;65:2226-2232.

[30] Loher S, Reboul V, Brunner TJ, Simonet M, Dora C, Neuenschwander P & Stark WJ.. Improved degradation and bioactivity of amorphous aerosol derived tricalcium phosphate nanoparticles in poly(lactide-co-glycolide). Nanotechnology 2006;17:2054-2061.

[31] Misra SK, Ansarj T, Mohn D, Valappil SP, Brunner TJ, Stark WJ, Roy I, Knowles JC, Sibbons PD, Jones EV, Boccaccini AR and Salih V. Effect of nanoparticles bioactive glass particles on bioactivity and cytocompatibility of poly (3-hydroxybutyrate) composites. J. R. Soc. Interface 2009;20:1-13.

[32] Beun S, Glorieux T, Devaux J, Vreven J, Leloup G. Characterization of nanofilled compared to universal and microfilled composites. Dent. Mater. 2007;23(1):51-9.

[33] Cavalcante LM, Masouras K, Watts DC, Pimenta LA, Silickas N. Effect of nanofillers sizes on surface properties after toothbrush abrasion. Am. J. Dent. 2009;22(1):60-4.

[34] Misra SK, Ansari T, Mohn D, Valppil SP, Brunner TJ, et al., Effect of nanoparticle bioactive glass particles on bioactivity and cytocompatibility of poly(3-Hydroxybutyrate) composites. J. R. Soc. Interface 2009;20:1-3.

[35] Marghalani HY. Effect of filler particles on surface roughness of experimental composite series. J. Appl. Oral Sci.2010;18(1):59-67.

[36] Schibe KG, Almeida KB, Medeiros IS, Costa JF, Alves CM. Effect of different polishing systems on the surface roughness of microhybrid composites. J. Appl. Oral Sci.2009;17(1):21-6.

[37] Nicholson JW, Anstice HM, McLean JW. A preliminary report on the effect of storage in water in the properties of commercial ligh-cured glass-ionomer cements. Br. Dent. J. 1992;173:98-101.

[38] Toledano M, Osorio R, Osorio E, Fuentes V, Prati C, Garcia-Godoy F. Sorption and solubility of resin-based restorative dental materials. J. Dent.2003, 31:43-50.

[39] Um CM, Oilo G. The effect of early water contact on glass ionomer cements. Quintassence Int. 1992;23:209-14.

[40] Cattani-Lorente MA, Dupuis V, Payan J, Moya F, Meyer JM. Effect of water on the physical properties of resin modified glass ionomer cements. Dent. Mater. 1999;15:71-78.

[41] Andersson ÖH, Karisson KH, Kangasniemi K. Calcium phosphate formation at the surface of bioactive glass ion vivo. J. Non-Crys. Solids. 1990;119:290-6.

[42] Oréfice R, West J, La Torre G, Hench L and Brennan A. Effect of Long-Term in vitro testing on the properties of bioactive glass-polysulfone composites. Biomacromolecules 2010;11:657-665.

[43] Wiegand A, Buchalla W, Attin T. Review on fluoride-relasing restorative materials –Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent. Mater. 2007;23:343-62.

Legends for figures.

Figure 1- AFM images of FL (20_μm ×20_μm). (A) FL in dry conditions. (B) FL in wet conditions. (C) FL plus Biosilicate® in dry conditions. (D) FL plus Biosilicate® in wet conditions. In FL, wet conditions changed the surface roughness of resulting in an undulations decrease. When Biosilicate® is added, wet conditions became the surface flatter than in dry conditions.

Figure 2- AFM images of VT(20_μm ×20_μm). (A) VT in dry conditions. (B) VT in wet conditions. (C) VT plus Biosilicate® in dry conditions. (D) VT plus Biosilicate® in wet conditions. In control specimens, peaks and valleys increased when the material was storage in wet conditions. When Biosilicate® powder is added, the texture of the surface changed, producing higher undulations when the material was stored in wet conditions.

Figure 3- SEM images of polished surfaces of FLat 100xand 5000x. (A) FL in dry conditions. (B) FL in wet conditions. (C) FL plus Biosilicate® in dry conditions. (D) FL plus Biosilicate® in wet conditions. Some microcracks and pores can be observed on the surfaces of the specimens. Higher magnification showed the particles and the matrix of the material.

Figure 4- SEM images of polished surfaces of VT at 100xand 5000x. (A) VT in dry conditions. (B) VT in wet conditions. (C) VT Biosilicate® in dry conditions. (D) VT plus Biosilicate® in wet conditions. Some microcracks and pores can be observed on

the surfaces of the specimens. Higher magnification showed the particles sizes and the bond to the matrix of the material.
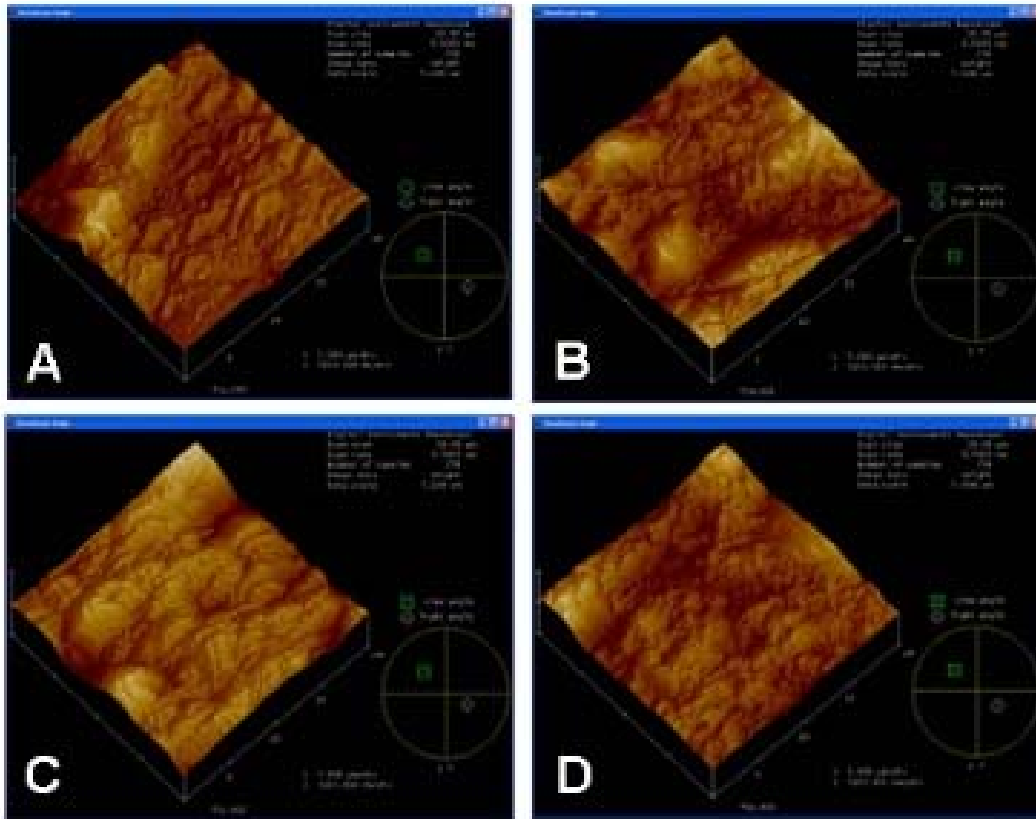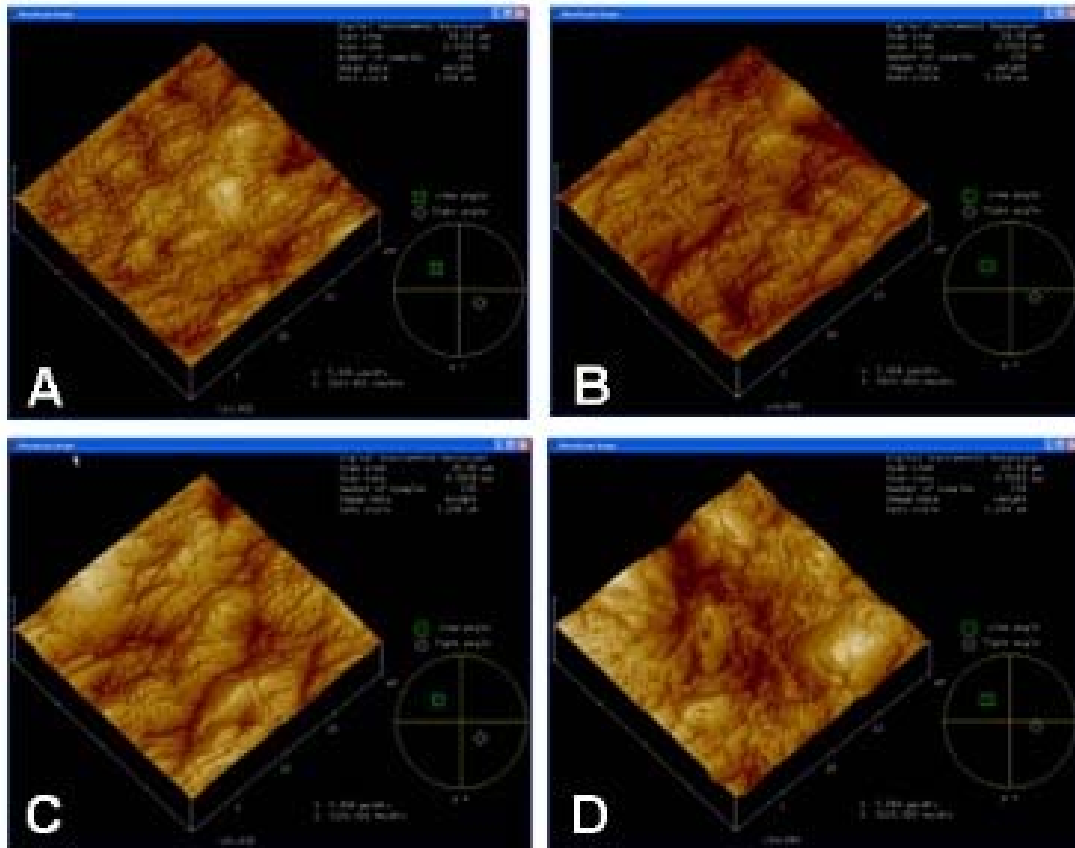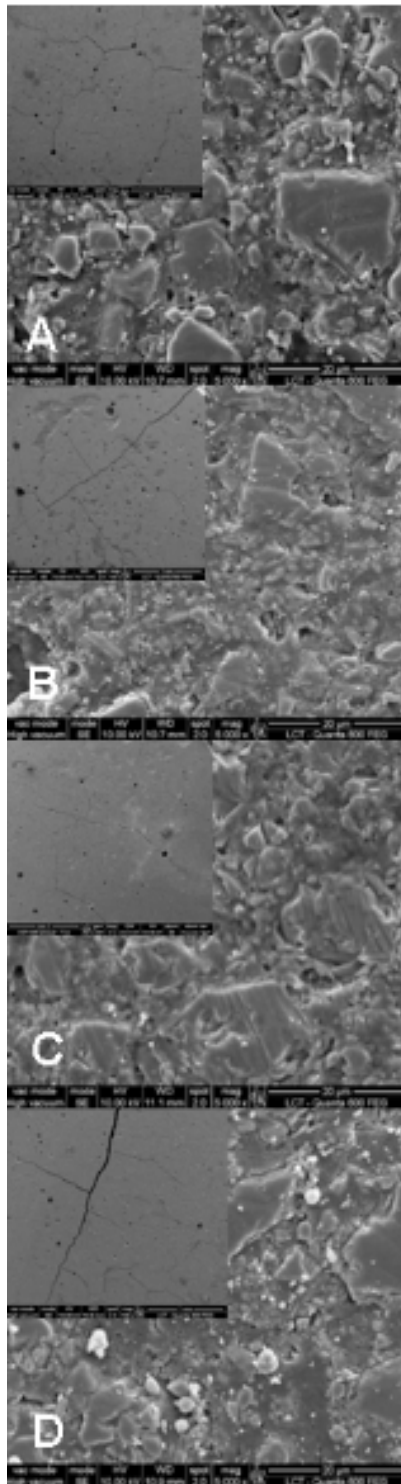
Figure 1
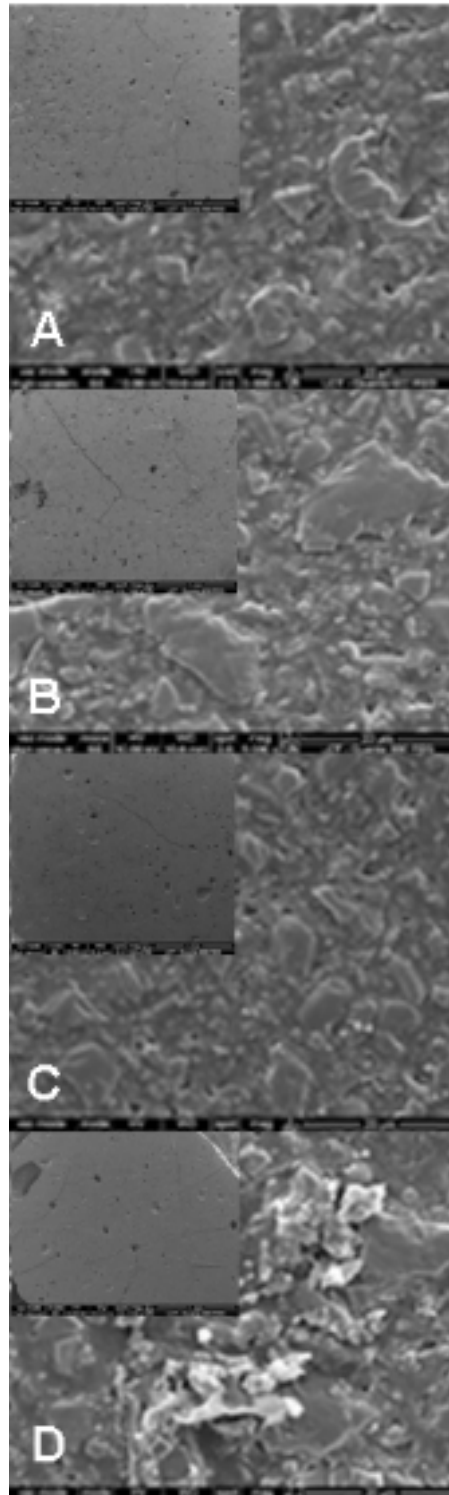
Figure 2

Figure 3

Figure 4

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 ($p > 0.05$).