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Effect of Resin-Modified Glass Ionomer Containing Bioactive Glass on the Flexural Strength and Morphology of Demineralized Dentin

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Clinical Relevance

Flexural strength of the human dentin decreases after it is demineralized *in vitro*. This *in vitro* study demonstrates that resin-modified glass ionomer (RMGI) containing bioactive glass (BAG) can compensate for this loss of strength. RMGI without BAG does not restore the strength of such demineralized dentin.

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SUMMARY

Introduction: Recently, bioactive materials have been incorporated into glass ionomer cements to promote the precipitation of calcium phosphates in surrounding tooth structures. This *in vitro* study was undertaken to evaluate the effect of resin-modified glass ionomer (RMGI) containing bioactive glass (RMGI-BAG) on the flexural strength (FS) of demineralized dentin.

Materials and Methods: A total of 120 dentin bars (2×2×6 mm) were prepared from sound human third molars. Of these, 60 bars were immersed in a demineralizing solution for 96 hours. This produced dentin in two demineralization conditions (DC): untreated and demineralized. Each dentin bar was immersed for 14

days in simulated body fluid (SBF) at 37°C. Three immersion conditions (IC) were investigated: IC1–SBF only; IC2–SBF + an RMGI bar; IC3–SBF + an RMGI-BAG bar. The combination of the DCs and ICs produced six groups (n=20).

FS values of the specimens were measured using a three-point bending test. The microstructural changes and the elemental contents of dentin surfaces were evaluated by scanning electron microscopy. Data were analyzed using a two-way analysis of variance (ANOVA) for the effects of the two independent variables, ie, DC and IC, on mean flexural strength. Tukey multiple comparison tests and simple main effects models were used as needed. The significance level of all tests was set at α=0.05.

Results: Both DC (p=0.001) and IC (p=0.049) significantly influenced FS (two-way ANOVA). The interaction between DC and IC did not significantly affect FS (p=0.36). For undemineralized dentin, IC did not affect the mean FS (simple main effects model; p=0.4). However, for demineralized dentin, IC significantly affected FS (small main effects model; p=0.008). The Tukey test showed that for demineralized dentin, the mean FS produced by immersion in SBF + RMGI-BAG was significantly stronger than that produced by either immersion in SBF only (p=0.011) or in SBF + RMGI (p=0.034). Scanning electron microscope/energy-dispersive x-ray spectroscopy analyses revealed more calcium and phosphate ions on the surface of dentin immersed in SBF + RMGI-BAG than on dentin immersed in SBF + RMGI.

Conclusion: Immersion in SBF + RMGI-BAG increased the FS of demineralized dentin more than immersion in SBF + RMGI.

INTRODUCTION

Glass ionomer cements (GICs) were invented by Wilson and Kent in the late 1960s. These materials are water-based cements and are also known as polyalkenoate cements.[1] GICs are widely used in restorative dentistry.[2] The success of these restorative materials is attributed to their unique properties, such as direct adhesion to tooth structure,[3] anticariogenic properties as a result of fluoride release,[4,5] low coefficient of thermal expansion similar to that of tooth structure,[6] minimal microleakage at the interfaces, and low cytotoxicity.[5,6] Despite these advantages, lack of strength and toughness are major drawbacks.[2] In order to improve the mechanical properties of conventional GICs, resin-modified glass ionomers (RMGIs) were developed[6,7] that exhibit much higher mechanical strength.[8]

The first indication that an "active" rather than "passive" material could be more efficacious was the realization of the benefits of fluoride release from dental materials.[9] The potentially "smart" behavior of glass ionomer cements was first suggested by Davidson,[10] in light of its thermoresponsive behavior, fluoride release, and rechargeability. A previous study[11] has shown a chemical exchange between glass ionomer restorations and residual carious dentin in permanent teeth. Moreover, remineralization of hard dental tissues adjacent to RMGI restorations has been reported.[12] However, when demineralized dentin that had been in contact with conventional GIC was imaged using high-resolution transmission electron microscopy, no apatite deposition was detected.[13]

In 1969, Hench reported that some glass compositions form chemical bonds with bone. These glasses are referred to as bioactive glasses and have been used mostly as reconstructive materials for damaged hard tissues such as bone.

The first composition, 45S5 Bioglass, has been applied clinically since 1985.[14] Immersion of a bioactive glass in an aqueous solution, such as the body fluid or simulated body fluid (SBF), results in three main processes: leaching and formation of silanols, dissolution of the glass network, and precipitation of calcium and phosphate ions. These reactions rapidly release soluble ionic species and give rise to the formation of a large hydrated surface area of silica and polycrystalline hydroxycarbonate apatite on the glass surface.[15,16] An additional series of reactions is needed to achieve a bond with the tissue.[12] One of the indications of this material in dentistry is restoration of bony defects and augmentation of the alveolar ridge.[14]

Some previous studies have emphasized the remineralizing effect of bioactive glass materials on tooth structures.[16-18] In one study, the bioactive glass suspension, rather than the calcium hydroxide suspension, was recommended for treatment of apexification.[15] Sauro and others[19] recently used Raman spectroscopy and energy-dispersive x-ray spectroscopy (EDS) to show that a bioactive glass reacts with artificial saliva to deposit hydroxycarbonate apatite (HCA) within the demineralized collagen fibrils. These deposits appear to occlude dentinal tubules,[19] making this bioactive glass candidate material for both desensitizing and remineral-

alizing dentin. Mitchell and others[20] and Tirapelli and others[21] evaluated bioglass as a dentin-desensitizing agent. Another study[22] used attenuated total reflection Fourier transform spectroscopy, x-ray diffraction, and EDS to confirm that apatite forms when demineralized dentin disks are treated with either of two bioactive glasses and immersed in artificial saliva.

Recently, bioactive glass (BAG) has been added to GICs in some studies.[23-30] Some of these materials are already under patent.[23] These experimental GIC-BAG materials have been reported to exhibit bioactivity in simulated physiologic conditions, and they have been shown to remineralize human dentin[24-27] Some of the mechanical properties of this combination have been evaluated in recent studies.[23,28,29] Ana and others[30] reported that the compressive strength of RMGI containing BAG decreases but is still much higher than that of conventional GIC containing BAG. However, no studies have evaluated the effect of these new experimental materials on mechanical properties of tooth structures. Therefore, the aim of this *in vitro* study was to evaluate the effect of RMGI containing bioactive glass (RMGI-BAG) on the strength and microstructure of demineralized dentin compared with those of a RMGI.

METHODS AND MATERIALS

Dentin Bar Preparation

A total of 120 dentin bars were prepared from sound, extracted human third molars. Extractions were all in accordance with the ethical guidelines for medical research at the Isfahan University of Medical Sciences. Immediately after extraction, the teeth were cleansed of soft tissue debris and stored in 0.2% thymol solution at 4°C and used within three months after extraction.[31]

To fabricate dentin bars, the occlusal one-third of the crown was removed and rectangular bars (2×2×6 mm) were sectioned from the middle of the cut dentin surface (Figure 1) using a low-speed, water-cooled diamond saw (Servocut-M300, Kemet International Ltd, Kent, UK).

Preparation of RMGI-BAG and the Treatment Groups

The RMGI-BAG was prepared by adding a BAG (NovaBone) into the powder of a light-cured RMGI (Fuji II LC) and manually mixing with a mortar and pestle for 20 minutes.[32] BAG comprised 20 wt% of the mixture.[23] The brand names, manufacturers, and compositions of the BAG, the RMGI powder, and the RMGI liquid are given in Table 1. The RMGI liquid was used, without modification, as the liquid phase of RMGI-BAG.

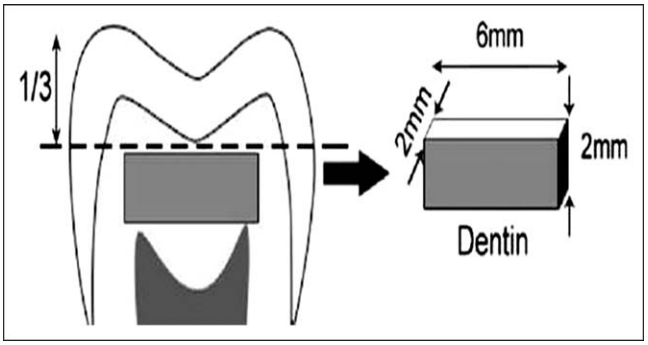

Figure 1. *Schematic view of a dentin bar, locations of cuttings, and resulting dentin bar configuration.*

Next, 40 rectangular blocks of RMGI and 40 rectangular blocks of RMGI-BAG (4×4×8 mm) were prepared with a powder-to-liquid ratio of 3:2 for RMGI as recommended by the manufacturer and 2:7 for RMGI-BAG based on ratios that have been used in the literature.[24,25,27,30] The mixtures were placed in stainless steel molds until the molds were slightly overfilled. The unset material was gently compressed with a transparent plastic matrix and a glass slab. The blocks were light cured (Dr's Light, Good Doctors Co Ltd, Seoul, Korea) for 40 seconds at a light intensity of 600 mW/cm^2. The blocks were gently retrieved from the molds and again polymerized from the other direction for 40 seconds. All the specimens were prepared at room temperature (22°C ± 1°C).

Half of the dentin bars (n=60) were randomly selected and each bar was demineralized by immersion in 10 mL of demineralizing solution (Table 1) for 96 hours at 37°C.[33,34] Note that the demineralized condition (DC) of each dentin bar was either DC1 (undemineralized) or DC2 (demineralized). Each dentin bar was then subjected to one of three immersion conditions (ICs): IC1 (immersion in SBF), IC2 (immersion in SBF containing a bar of RMGI), or IC3 (immersion in SBF containing a bar of RMGI-BAG).

The combination of DC and IC produced six different groups: 1) DC1 + IC1 (undemineralized control); 2) DC1 + IC2; 3) DC1 + IC3; 4) DC2 + IC1 (demineralized control); 5) DC2 + IC2; and 6) DC2 + IC3. The 60 undemineralized dentin bars were randomly assigned to groups 1–3 until 20 bars were assigned to each group. Similarly, the 60 demineralized dentin bars were randomly assigned to groups 4–6 until 20 bars were assigned to each group. Each

Table 1: *The Materials Used in the Study, Their Compositions (wt%), and Manufacturers*

Material	Product Name	Manufacturer	Composition
Resin-modified glass ionomer	Fuji II LC (Improved)	GC Corporation, Tokyo, Japan	Powder: fluoro-alumino-silicate glass Liquid: polyacrylic acid (20%-25%), 2-hydroxylethyl methacrylate (30%-35%), 2,2,4, trimethyl hexa methylene dicarbonate (1%-5%), proprietary ingredient (5%-15%)
Bioactive glass (45S5 Bioglass)	NovaBone	NovaBone Products LLC Alachua, Florida, USA	45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5
Demineralizing solution	–	–	2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, 0.05 M acetic acid; had the pH adjusted to 4.4 with 1 M KOH
Simulated body fluid (SBF)	–	–	NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, and Na_2SO_4 were dissolved in distilled and deionized water. The solution formed was buffered at physiological pH 7.4

$CaCl_2$: Calcium chloride; $CaCl_2{\cdot}2H_2O$: Calcium chloride dihydrate, CaO: Calcium oxide; $K_2HPO_4{\cdot}3H_2O$: Dipotassium hydrogen phosphate, KCl: potassium chloride; KH_2PO_4: Potassium hydrogen phosphate; KOH: Potassium hydroxide; $MgCl_2{\cdot}6H_2O$: Magnesium chloride hexahydrate, Na_2O: Sodium oxide; Na_2SO_4: Sodium sulfate; NaCl: Sodium chloride; $NaHCO_3$: Sodium bicarbonate; P_2O_5: Phosphorus pentoxide; and SiO_2: Silicon dioxide.

bar in each group was immersed in 10 mL of SBF and stored for 14 days at 37°C. The ion concentrations of the SBF are given in Table 2.

FS Measurement

The specimens were tested using a three-point bending test on a universal testing machine (DAR-TEC HC 10, Stourbridge, England). The crosshead speed was 0.5 mm/min. Flexural strength (ə) was calculated using the following equation:

$$\partial = \frac{3pl}{2bd^2}$$

in which p is the measured maximum load at the time of specimen fracture, L is the distance between the supports on the tension surface (L=6–(2×0.75)=4.5 mm), b is the mean specimen width, and d is the mean height of the specimen between the tension and compression surfaces.[16,17,31]

Scanning Electron Microscope Evaluation

To determine whether the dentin bars had undergone mineralization *in vitro*, the surfaces of one specimen from each group were examined using a scanning electron microscope (SEM) (model AIS2300C, Seron Technologies Inc, Gocheon-dong, Viwang-si, Gyeonggi-do, Korea) at magnifications of 200× and 1500×. Prior to examination, each dentin bar was dehydrated using a series of ethanol-water solutions where the ethanol increased[16] from 70% to 100%, and then desiccated.[24] The specimens were then sputter-coated in a vacuum using gold palladium with a thickness of 10–15 nm (BAL-TEC SCD 005, Germany). SEM

Table 2: *Compositions of Selected Simulated Body Fluid for the Study*

Ionic Concentration (mM) of the Prepared SBF							
SO_4^{2-}	HPO_4^{2-}	HCO_3^-	Cl^-	Ca^{2+}	Mg^{2+}	K^+	Na^+
0.5	1.0	4.2	147.8	2.5	1.5	5.0	142.0

Ca^{2+}: Calcium ion; Cl^-: chloride ion; HCO^-_3: Hydrogen carbonate ion; HPO^{2-}_4: Hydrogen phosphate ion; K^+: Potassium ion; Mg^{2+}: Magnesium ion; Na^+: Sodium ion; SO_4^{2-}: Sulphate ion.

Table 3: *Flexural Strength of the Specimens in Study Groups in MPa (n=20)*

Groups and Group Definitions	Mean ± SD	95% Confidence Interval		Min	Max
		Lower	Upper		
1. Undemineralized dentin +SBF	171.39 ± 44.97	152.6	190.2	87.7	275.6
2. Undemineralized dentin + RMGI + SBF	191.58 ± 64.80	172.7	210.4	97.8	371.2
3. Undemineralized dentin + RMGI-BAG + SBF	189.50 ± 41.95	170.7	208.3	110.2	275.6
4. Demineralized dentin + SBF	138.77 ± 35.61	119.9	157.6	97.8	222.7
5. Demineralized dentin + RMGI + SBF	142.87 ± 19.92	124.1	161.7	105.7	178.8
6. Demineralized dentin + RMGI-BAG + SBF	167.90 ± 34.44	149.1	186.7	108.0	211.5

Abbreviations: Min, minimum; Max, maximum; RMGI, resin-modified glass ionomer; RMGI-BAG, resin-modified glass ionomer containing bioactive glass; SBF, simulated body fluid.

micrographs were prepared using a standardized method with an accelerating voltage of 22 kV in a vacuum using a working distance of 20–25 mm.

Statistical Analysis

The experiment used a completely randomized design with two factors. A two-way analysis of variance (ANOVA) was use to determine whether the factors, the independent categorical variables DC (two levels: yes, no) and IC (three levels: SBF only, SBF + RMGI, SBF + RMGI-BAG), had a significant influence on strength, the continuous dependent variable. The ANOVA also tested for interactions between DC and IC. If IC was found to have a significant effect, a Tukey post hoc multiple comparison test was used to determine which IC levels were affecting the strength. For all these tests, the significance level was set at $\alpha=0.05$. All analyses were performed with the aid of statistical analysis software (SPSS, version 11.5, SPSS Inc, Chicago, IL, USA).

A simple main effects model was used to examine separately the effects of IC on demineralized and undemineralized dentin. To control for the type I error rate in the two simple main effects, the significance level for these tests was set at $\alpha=0.05$.

RESULTS

The descriptive statistics for the FS of the dentin bars for each group are given in Table 3 and are shown graphically in Figure 2. The null hypothesis that DC had no significant effect on mean FS was rejected (two-way ANOVA; $F_{2,114}=19.56$, $p=0.001$). Similarly, the null hypothesis that IC had no significant effect on mean FS was rejected (two-way ANOVA; $F=3.092$, $p=0.049$). The interaction between DC and IC was not statistically significant ($F=1.030$, $p=0.36$). However, for demineralized dentin, IC significantly affected FS (small main effects model; $p=0.008$). The Tukey test showed that for demineralized dentin, the mean FS produced by immersion in SBF + RMGI-BAG was significantly stronger than that produced by either immersion in SBF only ($p=0.011$) or in SBF + RMGI ($p=0.034$).

For demineralized dentin, Tukey multiple comparisons showed that mean FSs for ICs were not significant different except that the RMGI-BAG IC (group 6) was significantly stronger than either the SBF-only IC (group 4; $p=0.011$) or the SBF + RMGI IC (group 5; $p=0.034$). In addition, undemineralized dentin (group 1) and demineralized dentin exposed to SBF + RMGI-BAG (group 6) were not significantly different.

Figure 3 A-F shows SEM photomicrographs of the surface microstructures of dentin bars in each of the study groups.

DISCUSSION

The present study compared a mechanical property of undemineralized dentin with that of demineralized dentin after immersion in SBF containing either a RMGI or RMGI-BAG bar. Based on the results of the present study, FS values of sound dentin and demineralized dentin were 171.3 ± 44 and 138.7 ±

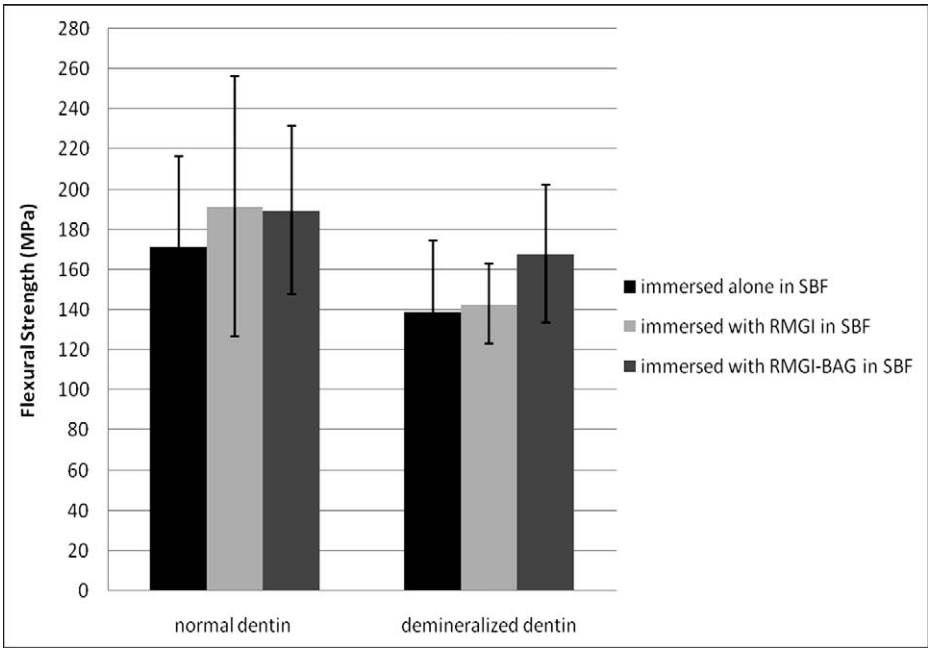

Figure 2. *Mean and standard deviation of flexural strength in the study groups. Abbreviations: RMGI, resin-modified glass ionomer; RMGI-BAG, resin-modified glass ionomer containing bioactive glass; SBF, simulated body fluid.*

35.6 MPa, respectively. Vollenweider and others[16] reported FS values of 180 ± 43 and 124 ± 19 MPa for sound dentin and demineralized dentin, respectively. The control specimens had been stored in water in that study.[16] Marending and others[17] reported a FS value of 203 ± 44 MPa for sound normal dentin after storage in normal saline solution for 30 days. Walker and others[31] reported a FS of 179 ± 26 MPa for dentin. Previous studies have shown that distilled water alters both the mechanical properties and spectroscopic views of human dentin.[32,33] Therefore, in the present study, SBF was used to store the samples. In addition, dentin bars were prepared from the coronal part of human third molars so that the placement of cervical restorations would be emulated as much as possible.[31]

In the present study, RMGI-BAG increased the FS values of undemineralized dentin and demineralized dentin around 10% and 20%, respectively, revealing statistically significant differences in the latter case. Xie and others[25] reported remineralization of dentin disks immersed in SBF along with BAG; however, they did not measure the strength of the dentin. Efflandt and others[18] evaluated the effect of BAG on dentin and chemically analyzed the dentin-material interface, reporting the presence of apatite. They attribute the bonding between the two materials to the affinity of collagen for the bioglass surface and to a chemical reaction between dentin and bioglass, resulting in the formation of apatite at the interface. They also suggested that the demineralization of the dentin produced by acid etching may produce ideal sites at which apatite can nucleate and grow.[18] Two other studies[20,21] have reported that BAG closes the entrances to the dentinal tubules by forming a layer of hydroxycarbonate apatite. The obstruction of the dentinal tubules is visible in SEM micrographs and is confirmed by a decrease in the hydrodynamic flow of dentin.[20] It is possible that the mineral deposits on the dentin bars in the present study are chemically bound to the underlying dentin. Further studies are necessary to investigate this possibility.

According to the results of the present study, the mean FS of demineralized dentin increased by almost 20% after immersion in SBF with RMGI-BAG. Its FS is significantly higher than that of SBF-treated demineralized dentin. It is important that its mean FS is not significantly different from that of undemineralized dentin immersed in SBF. Note that the SEM photomicrographs of dentin subjected to IC3 (Figure 3c,f: groups 3 and 6) reveal few open dentinal tubules, which might be the result of mineral deposition on the surface of demineralized dentin, and need future investigations. It appears remineralization of demineralized dentin by RMGI-BAG improves the (flexural) strength of demineralized dentin. Previously, Vollen-

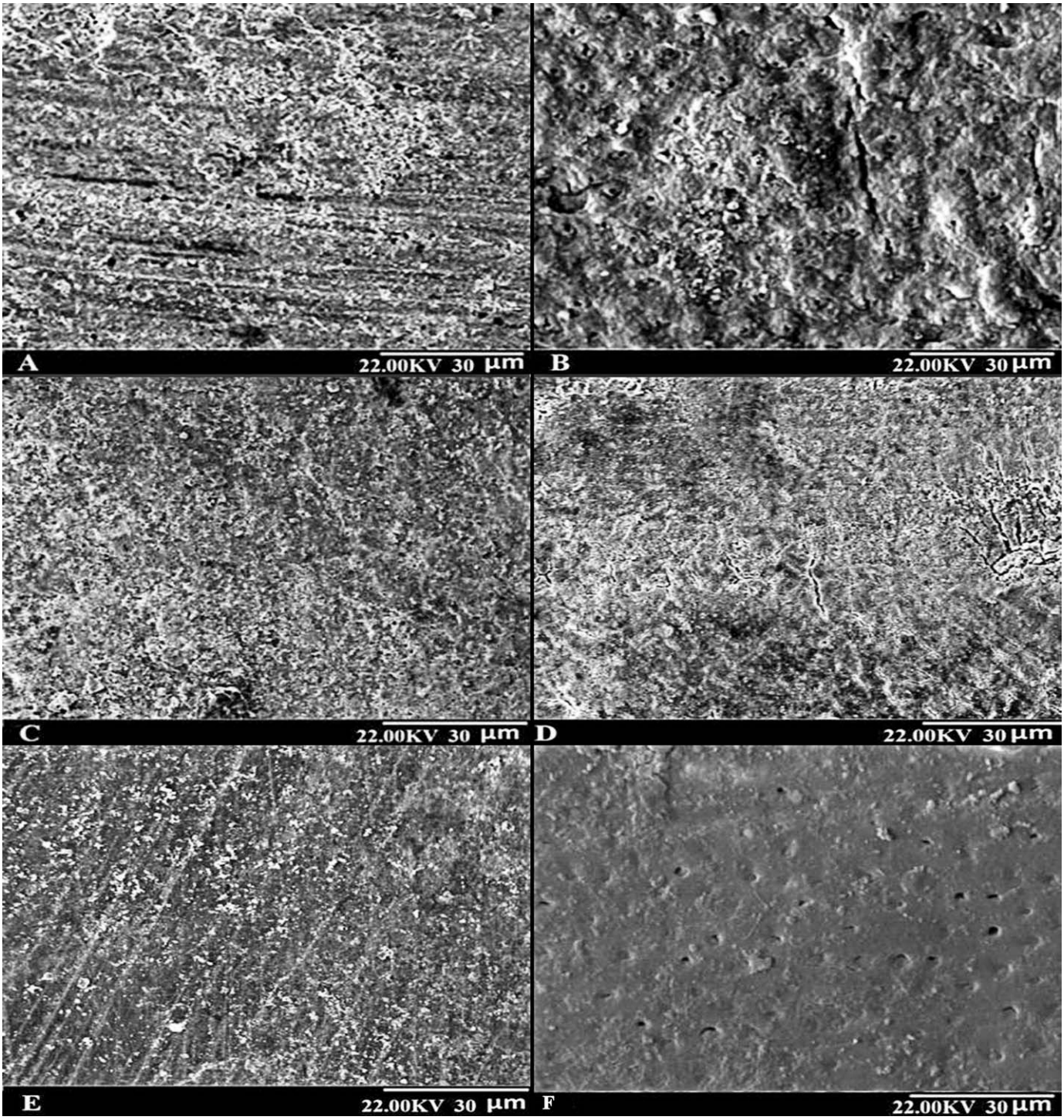

Figure 3. *(A–F): Scanning electron microscope photomicrographs of the dentin surfaces in the study groups 1–6, respectively (magnification: 500×)*

weider and others[16] reported a lower FS value (131 ± 47 MPa) for dentin remineralized by a kind of BAG (PerioGlass) compared with that of undemineralized dentin (180 ± 43 MPa); however, it was higher than FS values of demineralized dentin (124 ± 19 MPa). In a study by Marending and others[17] BAG decreased the FS values of dentin bars up to 20%. The decrease in the mechanical properties of dentin bars in that study was attributed to the basic pH of glass suspension.[17] The results of the present study cannot be compared with those because study conditions and parameters are different.

In the present study a synthetic demineralizing agent was used to demineralize the dentin bars. According to recent studies, this agent produced lesions that are similar to carious lesions that appear *in vivo*.[34-36] The lack of FS recovery in the study carried out by Vollenweider and others[16] might be attributed to the use of EDTA for the demineralization process. It has been reported that EDTA produces a worse condition in dentin compared with the natural process of cavity formation because it destroys the collagen network.[16] It is also possible that the lack of improvement in FS is due to the absence of a proper collagen scaffold for the deposition of minerals during remineralization and improper arrangement of new minerals in the matrix of demineralized tooth.[16] The formation of a bond between BAG apatite and tooth structure has been reported to be a chemical bond with dentin collagen.[14,18] If this is correct, an intact collagen scaffold may be necessary to promote remineralization by the BAG and consequently restore the strength of the demineralized dentin. The collagen network is largely unaffected in carious dentin.[36,37] To successfully interact with bioactive glasses *in vitro*, it may be essential that the demineralizing solution selectively dissolve the tooth mineral and leave the collagen relatively unaffected.

In the present study, no statistically significant differences were observed in the FSs of the three undemineralized dentin groups (1–3). It is possible that demineralization promotes remineralization. Pores in demineralized dentin may be low-energy sites for apatite deposition and therefore have a high mineralization potential. In studies that have evaluated the antibacterial properties of bioglass, investigators have hypothesized that undemineralized dentin powder functions as a receptor for ions in solution and therefore acts as a catalyst for dissolution of the BAG into aqueous solution.[38,39] Such ionic flow between the BAG and dentin powder apparently interferes with bacterial viability. Zehnder and others[38] demonstrated that solubility and release of ions from the BAG decreased in the presence of demineralized dentin. As a result, less hydroxyapatite forms than in the presence of undemineralized dentin powder.[38]

The present study provides no hint of demineralized dentin reducing the reactivity of the BAG. In the SEM micrographs of demineralized dentin after immersion in the SBF + RMGI-BAG solution (Figure 3f), the deposition of apatite on the dentin surface is pervasive. This result is inconsistent with the theory provided by antibacterial researchers and is more consistent with the explanations offered by Efflandt and others.[18] It could be that another process is accelerating the reaction. Perhaps the reduction in mineral content at the dentin's surface has increased the concentration gradient for the ions released from RMGI and RMGI-BAG to diffuse toward the dentin. The latter effect combined with the use of demineralizing solutions that leave behind collagen scaffolding may be all that is necessary to explain the observed kinetics. Additional experiments are needed before a definitive explanation of what is happening is possible.

The results of the present study clearly demonstrated the strengthening effect of RMGI-BAG on FS of demineralized dentin; however, the study had a number of limitations that are pointed out here. In the present study, SBF was produced based on a formula proposed by Kokubo and others,[40] and the SBF volume for each sample was calculated using this formula: $Vs = Sa / 10$, in which Vs is the SBF volume and Sa is the surface area of each disk.[41] Different SBF solutions are buffered solutions with a physiologic pH value of 7.4 and an ionic composition similar to that of plasma; they are predominantly produced by dissolving sodium chloride, sodium bicarbonate, potassium chloride, dipotassium phosphate, magnesium chloride, calcium chloride, and sodium sulfate in distilled water. [20,25-27] The ionic concentration of the prepared SBF for this study in terms of millimolars is presented in Table 2. As previously mentioned, in the majority of such studies, this solution has been used and good results have been reported.[20,25-27] A recent study showed that SBF solution might yield false positive and false negative results, necessitating more precise evaluations.[42]

Another important consideration is the fact that in the present study, NovaBone BAG (Table 1) was used, which is a synthetic bioactive material. This material is a 45S5 bioglass with a chemical composition of phosphorus pentoxide, 6%; calcium oxide, 24.5%; sodium oxide, 24.5%; and silicon oxide, 45%. Its particle sizes are 90-710 μm. A NovaBone package consists of 10 mL of solution equal to 13.2 g of the material. In different studies, various chemical compositions of the material with different particle sizes have been used; as a result, their comparison and selection of a more appropriate composition to incorporate into RMGI require further evaluations.

In addition, in the present study a dentin demineralizing agent (Table 1) was used for 96 hours to demineralize disks.[34] Further studies are

necessary to evaluate the materials used for this purpose; pH cycling conditions, too, can be used to better simulate oral cavity conditions. In addition, studies on caries-affected dentin will better represent conditions similar to clinical situations.

Future studies should focus on optimizing the RMGI + BAG combinations, evaluating other characteristics of the material such as bonding to tooth structures, and evaluation of the effects of RMGI-BAG restorations on dentin and enamel *in vivo*.

CONCLUSIONS

Dentin was tested in two conditions: undemineralized or demineralized by a method that produced demineralization similar to that which takes place *in vivo*.

- The flexural strength of demineralized dentin was less than that of undemineralized dentin, after the specimens were immersed in SBF.
- After the specimens were immersed in SBF, demineralized dentin immersed with RMGI-BAG was stronger than demineralized dentin immersed by itself.
- After the specimens were immersed in SBF, demineralized dentin immersed with RMGI was not stronger than demineralized dentin immersed by itself.

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Conflict of Interest Declaration

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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