Original Article

The Effect of a Fluoride-Bioactive Glass Paste on Demineralized Enamel Surface Characteristics and Bond Strength to the Orthodontic Bracket

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ABSTRACT

Background: Enamel white spot lesions (WSLs) are common in patients undergoing fixed orthodontic treatment. Remineralizing these lesions is necessary to prevent caries progression.

Purpose: This *in vitro* study aimed to assess the impact of fluoride bioactive glass (F-BAG) paste on the shear bond strength (SBS) of orthodontic brackets bonded to demineralized enamel.

Materials and Method: In this *in vitro* study, one hundred intact human premolars were obtained and randomly allocated into four groups (n= 25): intact (control), demineralized, remineralized using F-BAG paste, and remineralized with resin infiltration (Icon®). Surface characterization involved the scanning electron microscopy–energy dispersive X-ray spectroscopy (SEM-EDS) and the Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR). The brackets' SBS was evaluated using a universal testing machine, followed by recording the adhesive remnant index (ARI). Data analysis included one-way ANOVA, Tukey's post hoc, and chi-squared tests (α= 0.05).

Results: The control group exhibited the highest SBS values (p Value< 0.05). The F-BAG and resin infiltration groups had significantly higher bond strength than the demineralized enamel group (p< 0.05). SEM images revealed the presence of silica ions in the F-BAG group and resin tags in the resin infiltration group. The ARI scores were comparable across all groups.

Conclusion: The F-BAG paste used in this experiment was an effective remineralizing agent. Samples treated with F-BAG paste demonstrated clinically acceptable bond strength to brackets, with minimal enamel damage after orthodontic bracket debonding.

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Introduction

Orthodontic bracket debonding often occurs during treatment, either by the clinician or due to mechanical or thermal stress. Enamel white spot lesions (WSLs) are commonly found on the teeth's buccal surfaces during rebonding. Interestingly, WSLs are also observed in 15–40% of non-orthodontic individuals [1]. These lesions are caused by physicochemical reactions under bacterial acid attacks, leading to subsurface mineral loss and a porous structure, producing an opaque, chalky white appearance [2]. Early-stage WSLs are reversible until they reach surface cavitation [3-4].

Remineralizing these lesions can be challenging, prompting the suggestion of alternative treatments to halt lesion progression and mask their opaque color [3-6]. Several agents have been suggested for remineralizing early carious lesions [4]. A novel approach is the resin infiltration method, which involves acid etching to

increase enamel porosity and infiltrating a highly penetrating resin into the WSL [7-8]. An *in vitro* study showed that resin infiltration led to tooth mineral content restoration comparable to casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) [9].

On the other hand, remineralization offers a more conservative treatment by tipping the balance from demineralization to remineralization of primary carious lesions. Such treatments include topical fluorides, CPP-ACP, and bioactive glass (BAG) [3, 10-12]. BAG, traditionally used as a bone graft, is now utilized in dental remineralization. It is a rich source of calcium and phosphorus, quickly dissolving in aqueous solutions and forming a strong bond with tooth structures within two hours, promoting apatite formation [12-14]. According to a clinical trial, using a combination of fluoride and BAG (F-BAG) led to superior results in WSL remineralization compared to CPP-ACP [15]. However, the impact of F-BAG on the mineral content of WSLs and their bond strength to brackets is less studied. Moreover, no studies have compared F-BAG with resin infiltration in treating WSLs. Thus, this study was conducted under the hypothesis that different agents would not significantly affect orthodontic brackets' enamel surface ion composition and shear bond strength (SBS) values.

Materials and Method

The current *in vitro* study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.DENTAL.REC.1401.066). According to the findings of the study by El-Eesa *et al.* [10], and considering an effect size of 0.5, a power of 0.8, and a significance level of 0.05, the resulting sample size was 25 per group. One hundred intact premolars extracted for orthodontic purposes were obtained from the Oral and Maxillofacial Surgery Clinic of the Shiraz Faculty of Dentistry. In compliance with ethical standards, each patient provided signed consent. Teeth were cleaned and inspected under a light microscope to ensure they were free of cracks, demineralization, or defects. Then, they were stored in 0.1% thymol for 48 hours and preserved at 4°C in distilled water. Afterward, the samples' roots were removed at the cement–enamel junction using a high-speed disk with abundant water irrigation.

The preparation involved mixing measured quantities of sodium carbonate (22-24mol%), calcium carbonate (28-30mol%), phosphorus oxide (4-6mol%), silicon oxide (36-40 mol%), and calcium fluoride (1.5-3.0 mol%) in a glass mortar. This mixture was weighed using a digital scale with 0.001 g accuracy and melted at 1470°C for three hours. The resulting molten substance was rapidly cooled in water, forming a frit, which was subsequently milled in a mortar for 45 minutes. The F-BAG was ground using an agate grinder and passed through a 2-µm filter to ensure sample homogeneity. To produce the F-BAG paste (pH=2.5), 0.1g of F-BAG powder was mixed with two drops of 50 wt% phosphoric acid on a glass slab. Phosphoric acid was prepared by diluting 85 wt% phosphoric acid (3M Unitek; Morva Etch 37%, CA, USA).

Sample allocation

The specimens were allocated into four groups (n = 25 each) according to surface treatment. In the control group, the samples were incubated at 37°C for one day in artificial saliva (composition: 0.13 mM KCl, 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 5 mM NaN_3; pH= 7.0). The other samples were coated with dark, acid-resistant nail polish, exposing a 4-mm-diameter window. They were then immersed for one week in a demineralizing solution (2.2mM $CaCl_2$, 10mM NaH_2PO_4, 50mM acetic acid, 100 mM NaCl, 1 ppm NaF, 5 mM NaN_3; pH 4.5). The degree of saturation with respect to enamel was 4.44×10^9 [16-17]. In the demineralized group, no remineralizing agent was used.

For the third group, F-BAG paste was carefully applied to the demineralized enamel surfaces using a microbrush. This was then covered with a thin adhesive resin layer (3M Unitek, Monrovia, CA, USA) for 24 hours. Afterward, the adhesive and F-BAG paste were removed using an air–water jet, and a brush attached to a low-speed handpiece [18-20].

In the fourth group, WSLs underwent resin infiltration. The demineralized enamel was first cleaned with rubber cups and pumice, rinsed, and air-dried for 30 seconds. The 15% hydrochloric acid gel (Icon Etch, DMG Chemical-Pharmaceutical, Hamburg, Germany) was applied over the WSLs for two minutes. Then, the specimens were rinsed and dried. This process was repeated, after which the lesions were desiccated using ethanol (Icon Dry, DMG Chemical-Pharmaceutical, Hamburg, Germany) and air-dried for 30 seconds. The resin infiltrant (Icon Infiltrant, DMG Chemical-Pharma-

ceutical, Hamburg, Germany) was then applied to the tooth surfaces for three minutes; excess material was removed, and the teeth were light-cured (1,600 mW/cm²) for 40 seconds. This application and curing process was performed twice. Finally, the facial surfaces of the teeth were polished using a mildly abrasive polishing cup (Enhance, Dentsply, York, PA).

The enamel surfaces were cleaned using a mechanical brush attached to a low-speed contra-angle handpiece. Next, the specimens' buccal surfaces were uniformly etched using a 37% phosphoric acid etching gel (3M Unitek; Morva Etch 37%, CA, USA) for 30 seconds. Then, the surfaces were carefully rinsed and air-dried for 15 seconds. A layer of Transbond XT primer (3M Unitek, Monrovia, CA, USA) was then applied to the enamel. Moreover, adhesive resin (3M Unitek, Monrovia, CA, USA) was applied to the bases of stainless-steel standard edgewise premolar brackets (0.22 slot; American Orthodontics, Sheboygan, WI). The brackets were pressed firmly onto the enamel, and any excess resin was removed. The composite resin was cured for 10 seconds at each edge of the brackets, totaling 40 seconds of exposure. The samples were then incubated in artificial saliva at 37°C for one day.

The brackets from 10 randomly selected samples from each group were carefully removed using hand pliers. Any residual resin was meticulously eliminated using a 12-fluted tungsten carbide bur (Dentaurum no. 123-604; Ispringen, Germany) at low speed with air cooling. The surfaces were then polished using a PoGo polisher (Dentsply Caulk, Milford, DE, USA), a rubber cup, and a slurry of fine pumice for 10 seconds. Complete adhesive removal was confirmed under microscope with 10× magnification. The samples were subsequently analyzed using the following techniques. The size of five samples was reduced to 10×10mm. These samples were then examined using scanning electron microscopy- energy dispersive X-ray spectroscopy (SEM-EDS; JCM-6000 NeoScope; JEOL, Tokyo, Japan). Images of the top enamel surfaces were captured from the centers of each specimen to assess the tested groups, employing SEM-EDS for chemical analysis. Moreover, five samples from each group were subjected to analysis by a Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR) device (Nicolet iS5 FT-IR; Thermo Electron LLC, Madison, USA) [21].

The SBS values of the fifteen specimens of each group were calculated by a universal testing machine (Instron 900 Series; Norwood, MA 02062, USA) with the crosshead speed of 0.5mm/min.

Afterward, the adhesive remnant index (ARI) of the samples was examined using a light microscope (Best Scope 300, China) with 10× magnification. The ARI is categorized into the following scores: a score of 0 indicates no adhesive remnants, a score of 1 denotes remaining adhesive of less than half, a score of 2 signifies remaining adhesive of more than half, and a score of 3 represents the presence of all the adhesive [22].

Data analysis was performed using SPSS 22.0. One-way ANOVA and Tukey's post hoc tests were used to compare the SBS values of the groups. The chi-squared test was used to compare the ARI between the groups. The significance level was set at 0.05.

Results

SEM images revealed a layer of elements on the surface of healthy enamel in the control group (Figure 1a). However, these elements appeared noticeably diminished on the surface of the demineralized samples (Figure 1b). In the F-BAG group (Figure 1c) and the resin

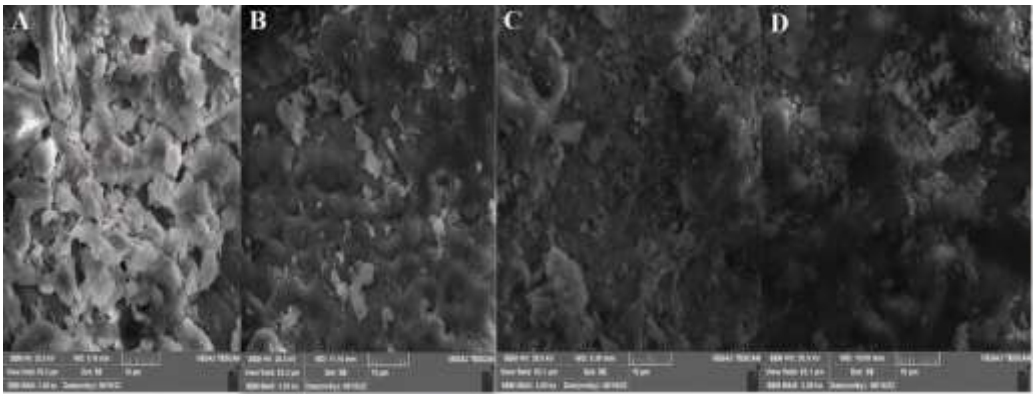

Figure 1: Scanning electron microscopy (SEM) surface examination of **a:** Intact enamel, **b:** Demineralized enamel, **c:** The fluoride bioactive glass (F-BAG) group, and **d:** The resin infiltration group

Table 1: Result of scanning electron microscopy- energy dispersive X-ray spectroscopy (SEM-EDS) analysis

Element	Study groups			
	Control	Demineralized	FBAG	Resin infiltration
Carbon	16.7	16.36	15.04	29.04
Oxygen	44.8	51.57	46.93	34.72
Fluoride	0.96	0.00	1.69	0.31
Phosphate	13.29	10.63	12.17	12.69
Calcium	24.33	21.44	24.17	23.39
Total	100	100	100	100

infiltration group (Figure 1d), silica ions and resin tags were observed, respectively.

The results of the SEM-EDS analysis, as presented in Table 1, indicate that calcium and phosphorus levels were lower in the demineralized group compared to the control group. Notably, the fluoride content was zero in the demineralized group. In contrast, the F-BAG group exhibited the highest fluoride levels among all groups. The F-BAG group also had higher percentages of fluoride and calcium than the resin infiltration group, although the latter group showed higher phosphorus content. The demineralized and resin infiltration groups had the highest amounts of oxygen and carbon.

The FTIR–ATR analysis of the control and demineralized groups identified typical bands, including a prominent band at 1086cm^{-1} attributed to the PO_4^{3-} group, as well as a distinct split band at 557 cm^{-1} linked with hydroxyapatite (Figure 2a-b). In the F-BAG group, four specific bands were noted at 875, 975, 1025, and 1094cm^{-1}, indicating HPO_4^{2-} stretching in brushite (Figure 2c). Another band at 975 cm^{-1} may represent the P-O(H) symmetric stretching vibration of the PO_4^{3-} group [23].

FTIR–ATR spectra from the resin infiltration group exhibited two distinct bands at 1061cm^{-1} and 1091cm^{-1}, associated with 2-hydroxyethyl methacrylate (HEMA) (Figure 2d). The band at 1061 cm^{-1} likely reflects the stretching mode of C-OH, while the band near 1091 cm^{-1} corresponds to the stretching mode of C-O-C.

The ANOVA test results revealed significant differences between the groups ($p< 0.05$). According to the post hoc test, the SBS of the control group (123.01± 22.48 MPa) was higher compared with the demineralized samples (78.64±31.29MPa; $p< 0.001$), F-BAG (93.94±32.64 MPa; $p= 0.032$), and resin infiltration groups (90.12±24.81 MPa; $p= 0.012$). The SBS values of the F-BAG and resin infiltration groups were higher than that of demineralized enamel ($p< 0.05$). According to the chi-squared test, the ARI was comparable among the groups (Table 2).

Table 2: Comparing the adhesive remnant index (ARI) scores among the groups

Groups	ARI scores					*p* Value
	0	1	2	3	Total	
Control	0	9	4	2	15	0.585
Demineralized	4	10	1	0	15	
FBAG	1	9	4	1	15	
Resin infiltration	2	10	3	0	15	

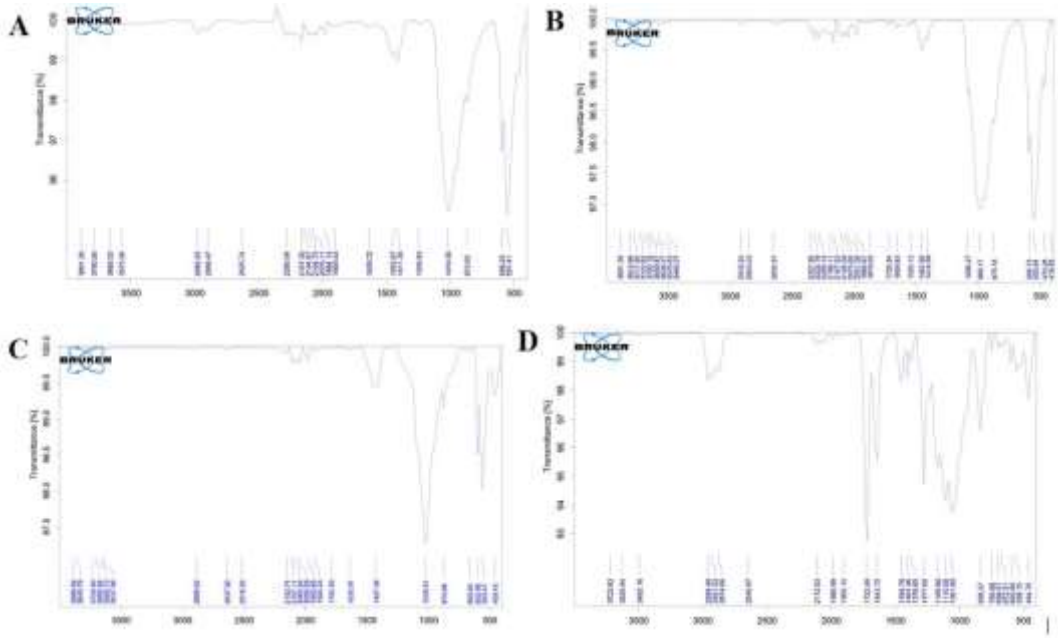

Figure 2: Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR) spectra of the, **a:** Intact enamel, **b:** Demineralized enamel, **c:** The fluoride bioactive glass (F-BAG) group, and **d:** The resin infiltration group

Discussion

In this study, applying F-BAG led to the establishment of fluoride and calcium ions on the demineralized enamel surface, potentially resulting in brushite formation. The control group displayed the highest SBS values among the groups. F-BAG and resin infiltration improved the SBS of demineralized enamel.

In the present study, SEM-EDX was utilized to quantitatively measure the mineral content in the samples. Rather than measuring the depth of mineral loss, this approach focuses on quantifying mineral loss, thereby providing a deeper insight into the biological processes of demineralization and remineralization [9]. Samples treated with F-BAG revealed the highest levels of calcium and fluoride compared to other groups.

F-BAG promotes remineralization primarily through the continuous release of calcium and phosphate ions; this process results in the development of a dense interaction layer that is approximately 10 μm thick and rich in calcium phosphate, with no observable gaps [24]. FTIR–ATR analysis of this layer in our study suggested that its composition is predominantly brushite, as indicated by commonly observed peaks. Typically, brushite crystals are formed in acidic conditions, which are conducive to the creation of calcium phosphate compounds [23]. After one week of storage in saliva, these brushite crystals gradually convert into stable hydroxyapatite crystals, supporting findings from other research on the remineralization properties of F-BAG [20]. Al-Eesa *et al.* [10] reported that F-BAG resin adhesive exhibits sustained release of fluoride, calcium, and phosphate ions, fostering apatite formation, in contrast to glass ionomer resins. Abbassy *et al.* [25] reported that fluoride bioactive pastes form an acid-resistant, hydroxyapatite-rich layer around orthodontic brackets. Similarly, Nam *et al.* [26] observed that nano-F-BAG orthodontic adhesive possesses antibacterial properties and prevents demineralization, potentially averting WSL formation. A randomized controlled trial highlighted that the application of BiominF slurry and toothpaste containing F-BAG over one month improved the esthetics of post-orthodontic WSLs and significantly reduced fluorescence intensity compared with Novamin and CPP-ACP [15]. Unlike Novamin, a 45S5 BAG product lacking fluoride, BiominF showed sustained and stable fluorapatite crystal formation, whereas lesion remineralization by Novamin relapsed during their study. Chen *et al.* [27] discovered that BAG toothpaste with 540 ppm fluoride released more calcium and phosphate ions and was more effective in rehardened artificial dentin carious lesions than toothpaste containing 1450 or 5,000 ppm fluoride.

According to the results of our experiment, the SBS values of F-BAG specimens were notably lower than that of intact enamel. The literature presents varied findings on the impact of BAG application on enamel bond strength, mainly due to differences in study design and materials tested. Some research indicates that applying BAG or F-BAG to the tooth surface or incorporating it into adhesive resin has no impact on the SBS of orthodontic brackets [24, 28-30]. For example, Abbassy *et al.* [24] discovered that BiominF® paste effectively remineralized the demineralized dental structures without impacting the bond strength to the adhesive. Their investigation compared the SBS of enamel and dentin surfaces treated with F-BAG to those treated with fluoride, whereas our study compared F-BAG with intact enamel. Al Shehab *et al.* [28] examined the impact of F-BAG and Alpha-Glaze® resin sealer on the SBS of orthodontic brackets bonded to enamel. Contrary to our findings, they observed no significant difference in SBS values between F-BAG and Alpha-Glaze compared with intact samples. However, our study applied F-BAG to demineralized enamel, while theirs used intact enamel, potentially explaining the differences in outcomes. Kohda *et al.* [31] reported that resin containing 4-methacryloxyethyl trimellitic anhydride/methyl methacrylate–tri-n-butyl borane and BAG could prevent enamel demineralization. They found that specimens bonded with resin containing 10–40% BAG had similar bond strengths, but resin with 50% BAG showed lower SBS than the control group. Shirazi *et al.* [29] also noted that adding BAG to resin-modified glass did not significantly affect SBS values. Similarly, Proença *et al.* [30] found that adding 5, 10, and 20 wt% 45S5 and NbG BAGs to adhesive resins did not significantly influence SBS. The differences observed could be attributed to the varied materials used in these studies, with our study focusing on F-BAG, while others examined BAG.

Conversely, consistent with the findings of the present study, Abbassy *et al.* [32] evaluated the effectiveness of 45S5 Bioglass in the remineralization of WSLs.

Their results were in agreement with ours, demonstrating that the SBS of samples treated with 45S5 Bioglass was higher than that of demineralized specimens but lower than that of control specimens. In contrast, the present study employed F-BAG; the incorporation of fluoride promotes fluorapatite formation, which exhibits greater acid resistance than hydroxyapatite [33].

In our study, the bond strength of the samples treated with F-BAG was comparable to that of the resin infiltration group, which is the most recent non-remineralizing enamel treatment used to camouflage the appearance of WSLs and arrest cavity formation. Notably, the resin infiltration group demonstrated lower bond strength than the control group. This could be due to the formation of pseudo-intact layers resulted from the sealing of microporosities on the demineralized enamel surface by low-viscosity resin, which likely blocked underlying pores in the carious lesions. Other studies have also reported these findings [9, 34], though there are contrasting results in the literature. Velİ *et al.* [35] and Baka *et al.* [36] found that the use of Icon® after demineralization notably enhanced bracket adhesion compared with treatments involving CPP-ACP, microabrasion, and fluoride varnish. While a previous study indicated that an SBS value of 4 MPa is acceptable for clinical bracket application [37], our results indicate the need for additional research in this field, especially concerning the optimal bracketing durations following the application of these agents.

The study also examined ARI scores, indicating differences in bond strength among various groups. Adhesive systems yield fewer remnants, facilitating safer and easier residual resin removal [38]. Our study groups showed comparable ARI scores, with a higher prevalence of scores 1 and 2 in the F-BAG, resin infiltration, and intact enamel groups. In contrast, the demineralized group predominantly exhibited ARI scores of 1. Consistent with these observations, Al Shehab *et al.* [28] reported no enamel cohesive failure when employing F-BAG as an orthodontic enamel sealer, with most samples (60%) achieving an ARI score of 1. The interaction layer formed by F-BAG might reduce the etching effect of phosphoric acid on enamel, leading to fewer resin tags and a decreased likelihood of enamel damage [39]. An ideal orthodontic biomaterial is expected to yield ARI scores of 1 or 2 [40]. Hence, the F-BAG group's frequent ARI scores of 1 and 2 place it well within these ideal ranges, suggesting its suitability as an effective system.

The present study has some limitations. Bracket failure may happen more often in a clinical setting due to factors specific to each patient such as individual chewing habits or trauma ; those which are difficult to replicate in a lab setting. Therefore, the findings of this study should be confirmed through clinical research to ensure their relevance.

Conclusion

The application of Icon® and F-BAG to treat WSLs before orthodontic treatment showed lower SBS values than intact enamel. However, applying both materials led to clinically acceptable bond strength and increased surface calcium, phosphate, and fluoride ions.

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Contributions

N.B. and HR.P. contributed substantially to the study's concept and design, supervised the study, and proofread the manuscript. H.K., S.M., and S.B. collected and analyzed the data and prepared the first draft of the manuscript. All authors have read and confirmed the final version of the manuscript and agreed to the submission.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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