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Effect of Bioactive Glass on Microshear Bond Strength of Composite Resin to Dentin Using a Universal Adhesive with Different Application Modes and Storage Times

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A B S T R A C T

Objectives: This study evaluated the effect of bioactive glass (BAG) in different forms on microshear bond strength (μSBS) of composite resin to dentin using a universal adhesive in self-etch (SE) and etch-and-rinse (E&R) modes after different storage times.

Materials and Methods: In this in vitro study, 120 extracted human third molars were sectioned 3mm occlusal to their cementoenamel junction to expose dentin. The teeth were then randomly assigned to three groups (n=40): (I) 20% BAG suspension for dentin pretreatment, (II) 1% BAG-modified adhesive (G2-Bond Universal), and (III) BAG-free control group. Each group was subdivided based on the adhesive application mode (SE or E&R) and storage time (immediate at 24 hours, or delayed at 3 months), with 10 teeth per each subgroup. The μSBS was measured by a universal testing machine. Data were analyzed by three-way and two-way ANOVA, followed by the Tukey's post-hoc test (α=0.05).

Results: Three-way ANOVA showed significant interaction effect of BAG incorporation and storage time on μSBS (P=0.017); while other interactions were not significant (P>0.05). The effects of BAG incorporation (P=0.951) and application mode (P=0.769) were not significant on immediate μSBS (P>0.05). After 3 months, the 1% BAG-modified adhesive showed a significantly higher μSBS than the 20% BAG suspension (P=0.001) and the control group (P<0.001), with no significant effect of application mode (P=0.417).

Conclusion: BAG incorporation did not affect the immediate μSBS of the adhesive but improved its long-term durability, such that the 1% BAG-modified adhesive showed the highest delayed μSBS, regardless of the application mode.

Keywords: Bioactive Glass 45S5; Dental Cements; Shear Strength; Dentin-Bonding Agents

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INTRODUCTION

The increasing demand for esthetic restorative treatments has led to the widespread use of adhesive restorations in

dentistry. Various adhesive systems, including the self-etch (SE), etch-and-rinse (E&R), and universal adhesives, have been developed to enhance the bond strength and durability [1]. Among these, universal adhesives have gained popularity due to their versatility, allowing application in both the SE and E&R modes based on clinical requirements. While the SE systems are favored for their simplified application, reduced procedural time, and lower technical sensitivity, the long-term durability of the resin-dentin bond remains a major challenge in adhesive restorations [1].

To improve bond longevity, several strategies have been explored, including the application of multiple adhesive layers in SE systems, addition of hydrophobic layers to prevent water penetration, and incorporation of bioactive agents such as matrix metalloproteinase (MMP) inhibitors and bioactive glass (BAG) [2, 3]. Among these, BAG has gained attention for its ability to promote remineralization and enhance the durability of the adhesive interface.

Remineralization plays a key role in maintaining the bond integrity by promoting recrystallization, replacing water in the adhesive layer, and inhibiting MMP activity, which is responsible for collagen degradation in dentin [4, 5].

BAG was first introduced by Larry Hench in 1969, and has since led to the development of various bioactive ceramic materials with applications in dentistry [6]. Studies suggest that incorporating BAG into dental adhesives can enhance the durability of bonding through its remineralization potential and antibacterial effects [7, 8]. Additionally, recent findings indicate that BAG facilitates dentin remineralization, and that the inclusion of polyhedral oligomeric silsesquioxane particles can further strengthen the bonds and reduce degradation over time [9-11]. However, the effect of BAG incorporation into universal adhesives with different application modes and under long-term storage conditions remains unclear.

Given the potential of BAG for use in adhesive dentistry, this study aimed to evaluate its effect on microshear bond strength (μSBS) of composite resin to dentin using a universal dental adhesive with different application modes (SE vs. E&R) and storage times (immediate vs. 3 months of aging in distilled water). The null hypothesis of the study was that addition of BAG to G2-Bond Universal adhesive, regardless of its application mode or storage duration, would have no significant effect on μSBS to dentin.

MATERIALS AND METHODS

Study design:

This in vitro experimental study was conducted at the Dental Materials Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, and was approved by the ethics committee of Isfahan University of Medical Sciences under the ethical code IR.MUI.REC.1400.64.

Sample selection and preparation:

Human extracted third molars were collected for this study. The inclusion criteria were intact caries-free teeth with no abnormality or cracks, stored in an appropriate solution (e.g., 0.1% thymol) to prevent dehydration and microbial growth. The exclusion criterion was teeth extracted earlier than 3 months before the study onset. A total of 120 third molars met the eligibility criteria and were included.

The selected teeth were thoroughly cleaned by removing the attached soft tissue, blood, and debris, and were then immersed in 0.5% chloramine-T solution at 4°C for one week. Next, the samples were stored in distilled water until use. Each tooth was cleaned using a low-speed handpiece with a rubber cup and prophylactic paste. The teeth were then mounted in auto-polymerizing acrylic resin blocks, ensuring that the cementoenamel junction remained at the level of the acrylic surface.

A horizontal section was made 3mm occlusal to the cementoenamel junction using a high-speed fine diamond bur (0.14; Tizkavan, Tehran, Iran), under continuous air and water cooling, to expose the underlying dentin. To simulate the smear layer typically formed in clinical conditions, the exposed dentin surfaces were polished with 400-, 600-, and 800-grit silicon carbide abrasive papers under water irrigation for 30 seconds.

Study groups and experimental design:
The samples were randomly divided into 3 main groups based on BAG incorporation:
Group 1: Pretreatment with 20% BAG suspension (n=40);
Group 2: 1% BAG-modified adhesive (n=40);
Group 3: BAG-free control (n=40).
Each main group was further subdivided into two subgroups based on the adhesive application mode: SE (n=20) and E&R (n=20). Each of these subgroups was then divided into two storage conditions: immediate testing (n=10) and delayed testing (n=10).

Preparation of BAG nanoparticles:
The BAG nanoparticles (Bioglass 45S5; Nik Seram Co., Isfahan, Iran) were synthesized using the sol-gel method [12, 13].
For group 1 (20% BAG suspension), the nanoparticles were dispersed in ethanol (20% v/v) and subjected to ultrasonication for 3 minutes in an ice bath using an ultrasonic probe (Sonoplus UW2200; Bandelin electronic GmbH, Berlin, Germany) [14]. The stability of the nanoparticle suspension was verified using a separation analysis device (LUMiReader® 416.1; LUM GmbH, Berlin, Germany) [15]. In this group, after primer application, the dentin surface was pretreated with the BAG suspension for 10 seconds, gently air-dried, and then bonded using the universal adhesive system (G2-Bond Universal; GC, Tokyo, Japan) per manufacturer's instructions.
For group 2 (1% BAG-modified adhesive), 1% BAG nanoparticles were incorporated into the bonding resin bottle of the universal adhesive system (G2-Bond Universal; GC, Tokyo, Japan) [12]. The mixture was stirred on a magnetic stirrer for 1 hour, followed by ultrasonic treatment for 2 hours to ensure homogeneous dispersion of nanoparticles. Stability was assessed using the LUMiReader® 416.1. In this group, bonding was performed using the modified resin instead of the original resin bottle.
For group 3 (BAG-free control), no BAG was applied in any form, and the bonding protocol followed the manufacturer's instructions without pretreatment.

Adhesive application protocols:
Each group was further categorized based on the adhesive application mode. In the SE application mode, the self-etch primer was applied actively on the dentin surface using a microbrush for 10 seconds, followed by strong air flow to evaporate the solvent. The resin layer was applied using an applicator, gently air-thinned, and light-cured for 20 seconds using a light-curing unit (Valo Grand LCU; Ultradent Products Inc., South Jordan, UT, USA) with an intensity of 650 mW/cm^2 at 1mm distance. The light intensity was confirmed using a LED radiometer (Demetron LED Radiometer; SDS/Kerr, Orange, CA, USA).
In the E&R application mode, 35% phosphoric acid was applied on the dentin surface for 15 seconds, and then rinsed for another 15 seconds. The self-etch primer and resin were subsequently applied following the same protocol as the SE technique.
After adhesive application, plastic tubes (with 0.5mm internal diameter and 1mm height) were positioned on the prepared dentin surface. The tubes were filled with the A2 shade of Gradia composite resin (GC, Tokyo, Japan,) and light-cured for 40 seconds using the same curing unit and parameters.

μSBS testing:
Following composite bonding, all samples were incubated in distilled water at 37°C and 100% humidity for 24 hours. The plastic tubes were carefully removed using a No. 11 blade (Fine Science Tools GmbH, Heidelberg, Germany).
The μSBS was measured using a universal testing machine (Bisco Inc., Schaumburg, IL, USA) equipped with a knife-edge loading fixture, with a crosshead speed of 0.5mm/min. In the immediate testing subgroup, the μSBS was measured 24 hours after bonding, and in the delayed testing subgroup, the measurements were made after 3 months of storage in distilled water at 37°C.
The μSBS values were calculated using the following formula:

$$\mu SBS\ (MPa) = \frac{\text{Force (N)}}{\text{cross} - \text{sectional area } (mm^2)}$$

Since the sample diameter was 0.5mm, and the radius was 0.25mm, the cross-sectional area was calculated to be $0.1963mm^2$.

Statistical analysis:

The required sample size was calculated based on an alpha level of 0.05, a beta of 0.80, and an estimated effect size of 1.25, resulting in a total of 120 samples (10 per subgroup).

Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Due to the relatively small sample size in each subgroup (n=10), data normality was assessed using the Shapiro-Wilk test, showing that the results had a normal distribution (P>0.05). Thus, three-way ANOVA was conducted to evaluate the effects of BAG incorporation, application mode, and storage time on micro-SBS. Since significant interactions were detected, and variance homogeneity was not confirmed, two-way ANOVA followed by the Tukey's post-hoc test were performed. P<0.05 was considered statistically significant.

RESULTS

Three-way ANOVA revealed the significant interaction effect of BAG incorporation and storage time on μSBS (P=0.017). However, the interaction effects of the application mode and storage time (P=0.718), and BAG incorporation and application mode (P=0.886) on μSBS were not significant. Additionally, the three-way interaction effect of BAG incorporation, application mode, and storage time on μSBS was not significant (P=0.715). Given the significant interaction effect of BAG incorporation and storage time on μSBS, two-way ANOVA was separately conducted for the immediate and delayed μSBS values.

In immediate μSBS testing, two-way ANOVA showed no significant effect of BAG incorporation (P=0.951) or application mode (P=0.769) on μSBS. The interaction effect of BAG incorporation and application mode on μSBS was not significant either (P=0.547; Table 1).

In delayed μSBS testing, a significant difference was found among the BAG incorporation groups (P=0.002); whereas, no significant difference was observed between the application modes (P=0.417). The Tukey's post-hoc test for pairwise comparisons in delayed μSBS testing showed no significant difference between group 1 (20% BAG suspension) and group 3 (BAG-free control) (P=0.066). However, significant differences were found between group 2 (1% BAG-modified adhesive) with both group 1 (20% BAG suspension) (P=0.001) and group 3 (BAG-free control) (P<0.001), with group 2 demonstrating the highest bond strength (35.4±6.332MPa). Similar to the immediate μSBS testing, the interaction effect of BAG incorporation and application mode on μSBS was not significant (P=0.820; Table 2).

DISCUSSION

This study evaluated the effect of incorporating BAG in different experimental groups on μSBS of composite resin using a universal adhesive with different application modes (SE and E&R) and different storage times (immediate testing at 24 hours and delayed testing at 3 months). A total of 120 extracted sound human third molars were used, with 10 teeth assigned to each subgroup.

Table 1. Immediate μSBS (MPa) of the study groups in different application mode subgroups

Groups	N	μSBS (Mean± SD)	P-value	Application mode	N	μSBS (Mean± SD)	P-value
Group 1; 20% BAG suspension	20	30.0±6.074	0.951	SE	10	29.5±5.886	0.769
				E&R	10	30.5±6.528	
Group 2; 1% BAG-modified adhesive	20	29.8.6±4.177		SE	10	29.7±4.317	
				E&R	10	29.9±4.261	
Group 3; BAG-free control	20	30.5±4.637		SE	10	31.6±2.013	
				E&R	10	29.3±6.186	

BAG: Bioactive glass; SD: Standard deviation; μSBS: Microshear bond strength; N: Number; SE: Self-etch; E&R: Etch and rinse.

Table 2. Delayed μSBS (MPa) of the study groups in different application mode subgroups

Groups	N	μSBS	P-value	Application mode	N	μSBS	P-value
Group 1; 20% BAG suspension	20	31.3±3.361[a]	0.002*	SE	10	30.2±2.964	0.417
				E&R	10	32.4±3.518	
Group 2; 1% BAG-modified adhesive	20	35.4±6.332[b]		SE	10	35.3±8.072	
				E&R	10	35.5±4.413	
Group 3; BAG-free control	20	29.5±5.073[a]		SE	10	29.0±3.301	
				E&R	10	29.9±6.556	

Significant differences are indicated by an asterisk (*). The post-hoc Tukey test results are presented using different uppercase letters to denote significant differences. BAG: Bioactive glass; SD: Standard deviation; μSBS: Microshear bond strength; N: Number; SE: Self-etch; E&R: Etch and rinse.

The three main experimental groups included dentin pre-treatment with a 20% BAG suspension, incorporation of 1% BAG into the adhesive, and a BAG-free control group.

One key observation in this study was absence of a significant three-way interaction effect among BAG incorporation, application mode, and storage time on μSBS. This finding suggests that the overall effect of BAG on μSBS is not significantly influenced by the type of adhesive application mode or the storage time when all factors are considered together. However, a significant interaction effect was observed between BAG incorporation and storage time on μSBS, indicating that the impact of BAG on μSBS becomes more pronounced over time. This finding suggests that while BAG may not provide immediate reinforcement, it plays a crucial role in enhancing the durability of the adhesive bond in the long term.

The present results showed no significant differences in immediate μSBS among the three BAG groups and between the two adhesive application modes. This finding suggests that the presence of BAG does not immediately enhance the μSBS of composite resin to dentin. Similar results were reported by Bauer et al, [16] and Carvalho et al, [15] where BAG particles, regardless of the application mode, did not influence the immediate bond strength. This consistency across studies reinforces the notion that the mechanisms of BAG require time to manifest their full effects.

Lack of an immediate improvement in μSBS can be attributed to the mechanism of action of BAG. BAG releases calcium and phosphate ions, which contribute to the remineralization of demineralized dentin and promote apatite formation [17,18]. However, these processes require a sufficient period of time to occur and do not significantly alter the bond strength within the first 24 hours. Additionally, BAG inhibits MMPs, which are responsible for collagen degradation [19]. The released calcium ions interact with MMP-2 and MMP-9, forming Ca-MMP complexes with reduced protease activity due to their high molecular weight and decreased mobility [20, 21]. BAG also increases the pH at the adhesive interface, further inhibiting the pH-dependent activity of MMPs [٢٢]. Despite these beneficial effects, the structural reinforcement provided by BAG is not immediate but instead contributes to the longevity of the adhesive bond.

Furthermore, BAG exhibits antimicrobial properties by releasing ions, increasing pH, and creating osmotic pressure through surface chemical reactions [23]. The formation of silanol (Si-OH) layers on the BAG surface regulates ion release and pH modulation, fostering an alkaline environment conducive to apatite nucleation and formation [24]. These attributes, while not directly affecting the short-term bond strength, may play a crucial role in preventing degradation over time.

In contrast to the immediate results, the delayed μSBS values significantly varied among the BAG groups in the current study. The adhesive modified with 1% BAG demonstrated a significantly higher μSBS after 3 months compared to both the 20% BAG dentin pre-treatment group and the BAG-free

control group. This finding suggests that BAG incorporation within the adhesive matrix provides a sustained benefit in bond stability over time. The enhanced long-term bond strength of BAG-modified adhesive can be attributed to continuous ion release, which promotes remineralization and preserves the integrity of the hybrid layer by inhibiting collagen degradation.

Interestingly, the 20% BAG suspension used for dentin pre-treatment also resulted in a slight and insignificant increase in μSBS. Previous studies have reported mixed findings regarding the effectiveness of BAG suspension. Some studies demonstrated that BAG suspension improved the bond strength, enhanced hardness, and increased the elastic modulus of the hybrid layer, regardless of the application technique [16, 25]. However, other reports suggested that BAG suspension was more effective only with E&R adhesives compared with SE adhesives [26-28]. These discrepancies may be due to variations in methodology, differences in BAG particle concentration and composition, or technical variability in dentin rehydration following BAG suspension application.

Similar to the immediate μSBS test results, the application mode did not significantly affect the delayed μSBS. This finding suggests that the positive influence of BAG on bond durability is independent of whether the adhesive is applied by the SE or E&R mode. This is a clinically relevant observation, as it suggests that BAG can enhance bond durability regardless of the adhesive protocol employed.

An important consideration is the potential interaction between BAG and adhesive due to the adhesive's acidic nature. Universal adhesives typically contain functional monomers, such as 10-MDP, which promote chemical bonding to dentin by interacting with hydroxyapatite. However, presence of BAG may interfere with this process. BAG's alkaline nature can neutralize the acidic components of the adhesive, altering the etching efficiency and the degree of demineralization at the dentin interface. This reaction could result in changes in hybrid layer formation and adhesive penetration. Additionally, BAG's ion release may influence polymerization kinetics, potentially affecting the mechanical properties of the adhesive layer. While the findings of this study did not show any significant impact of BAG on immediate μSBS, the long-term improvements observed in BAG-modified adhesive suggest that any initial interference might be outweighed by the long-term remineralization and stabilization effects of BAG. Future studies should further investigate the chemical interactions between BAG and adhesive monomers, particularly in different adhesive formulations.

From a clinical standpoint, BAG has the potential to improve bonding longevity by inhibiting MMP activity, promoting remineralization, and exerting antimicrobial effects through ion release and pH modulation. These mechanisms protect the exposed collagen fibers, reduce the risk of hydrolytic degradation, and maintain the integrity of the resin-dentin interface over time. Given the findings of this study, incorporating BAG into adhesives could be a promising strategy to enhance the durability of adhesive bonds, particularly in high-risk patients prone to secondary caries or adhesive failure.

One of the limitations of this study was the relatively short storage period of 3 months. While the delayed results provide preliminary evidence of BAG's long-term benefits, extended storage periods—such as 6 months, one year, or even longer—would provide more definitive conclusions regarding the durability of BAG-enhanced bonds. Additionally, incorporating thermocycling in future studies would better simulate the oral environment and assess the effects of thermal stresses on bond stability. Moreover, variations in BAG particle size, concentration, and composition could influence the bond performance. Future studies should investigate the optimal BAG formulation and its interaction with different adhesive systems. It would also be valuable to explore the efficacy of BAG in different clinical scenarios, such as bonding to carious or sclerotic dentin, to determine its efficacy in compromised bonding substrates. Finally, in vivo studies assessing BAG's effect on bonding

longevity in actual clinical conditions would provide more relevant data generalizable to clinical practice. The clinical significance of BAG in adhesive dentistry lies in its ability to inhibit enzymatic degradation, promote remineralization, and provide antimicrobial protection, all of which contribute to superior long-term adhesion. Future research should focus on optimizing BAG formulations, extending observation periods, and exploring its clinical applications to maximize its benefits in restorative dentistry.

CONCLUSION

This in vitro study demonstrated that while BAG incorporation did not significantly influence immediate μSBS, its long-term benefits became evident over time. The highest delayed μSBS value was observed in 1% BAG-modified adhesive, suggesting that BAG incorporation into adhesive systems enhances bond durability regardless of the adhesive application mode (SE vs. E&R).

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CONFLICT OF INTEREST STATEMENT

None declared.

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