



## Effect of Incorporation of Bioactive Glass Nano Particles in Adhesive Resin On Microtensile Bond Strength to Dentin in Self Etch and Etch and Rinse Mode

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### ABSTRACT

**Objectives:** This study aimed to evaluate the effect of addition of bioactive glass nano particles to an adhesive resin on the microtensile bond strength to dentin in etch and rinse and self-etch mode. **Materials and methods:** bioactive glass nano particles were prepared and added to a universal adhesive (Single Bond Universal, 3M ESPE) in concentrations of 10% and 20% by wt. 48 dentin specimens were prepared from human sound molars. The occlusal enamel was removed to expose dentin and the specimens were divided into six groups of 8, A1 and B1, received unmodified adhesive, A2 and B2 received adhesive with 10% bioactive glass and A3 and B3 received adhesive with 20% bioactive glass respectively. A groups were bonded using the etch and rinse approach while the B groups were bonded using the self-etch groups. Composite resin was applied to the bonded surfaces. Specimens were sectioned for microtensile bond strength testing. **Results:** the addition of bioactive glass fillers caused a significant reduction to the microtensile bond strength when compared to dentin with both adhesive techniques. The amount of filler had no significant effect on the self-etch mode, while with the etch and rinse there was a further significant reduction with added filler. **Conclusion:** addition of bioactive nano fillers caused a significant weakening of the bond strength.

### INTRODUCTION

Conservative dentistry is increasingly using bonded restorations due to their advantages in tooth conservative preparations as well as reliable function and esthetics. For the sake of tooth conservation, it is also commonly advocated to leave affected demineralized dentin under the restoration<sup>1</sup>. Remineralization of this affected dentin occurs over time aided by bioactive materials<sup>2</sup>. Among these materials are glass ionomer<sup>3</sup> as well as composite resins and adhesives containing bioactive material<sup>4</sup>. Hydroxyapatite, CaPO<sub>4</sub> and bio active glass have been incorporated in restorative materials to encourage remineralization<sup>5</sup>. The modification

### KEYWORDS

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of the adhesives to include material that encourage remineralization has also been documented<sup>6</sup>. Although the effect of these materials have been documented to aid in the remineralization of the affected dentin, their effect on the bond itself has been controversial<sup>7</sup>.

Bioactive glass in nano particle form has been used for remineralization successfully when used as a base material, in desensitizing agents, in tooth pastes and in composite and in adhesives<sup>8</sup>. The amount of filler in adhesives however has to be optimal to maintain proper adhesion<sup>9</sup>. The adhesive used in this paper has a filler loading of 5-15% approximately. Fillers in adhesives have been shown to improve the mechanical properties of the adhesive layer, however it is beneficial to bonding up to a limit to maintain workability of the adhesive resin<sup>10</sup>. In this paper bioactive glass (bioglass) nano particles have been added to a universal adhesive resin which can be used in both self-etch and etch and rinse modes. The presence of the bioglass itself as well as its concentration are studied to evaluate their effect of the microtensile bond strength to dentin. Evaluation was done in both etch and rinse mode and self-etch mode. The null hypothesis is that the incorporation of bioactive glass and its concentration in the adhesive have no effect on the microtensile bond strength of the adhesive resin to dentin.

## MATERIALS AND METHODS

### Preparation of the adhesive

Bioactive glass nanoparticles were prepared by synthesis using the alkoxide sol gel technique. The nano particles were then examined for characterization by transition electron microscope. The nanoparticles were of average size of less than 10 nm. The adhesive used in this study was Single Bond Universal Adhesive (3M ESPE) which can be used in either self-etch mode or etch and rinse mode. It is a light cure nano filled adhesive. The filler in the adhesive is silica based. The adhesive

was withdrawn from the bottle using a tape wrapped syringe and injected into an empty and clean amalgam capsule. The bioactive glass nanoparticles were weighed and were added to the adhesive with a ratio of 10% by weight. The capsules were closed and placed in an amalgamator for 30 seconds at 4400 rpm to homogenize the particles within the adhesive. The capsule was then opened and the adhesive with the incorporated particles were withdrawn into a wrapped syringe and sealed. The same procedure was done again but adding 20% by weight bioactive glass nanoparticles to the adhesive. A sample of each adhesive was subjected to TEM analysis for characterization of the adhesive with nano particles.

Forty-eight dentin specimens were prepared from extracted sound human molars. After initial cleaning and scaling of the molars, the occlusal enamel was removed from each tooth using a diamond disc at high speed with water cooling to expose a flat dentin surface. The dentin surface was inspected for remnants of enamel. The dentin specimens were then divided into their assigned groups (table 1). The specimens were divided into two different groups of twenty-four each. Group A was assigned for etch and rinse adhesive procedure, and was then randomly subdivided into three subgroups of eight specimens each according to the adhesive used. Group A1 was the control group and used the original adhesive as supplied by manufacturer unmodified by nano particles. Group A2 was assigned for the adhesive with 10% wt. bioglass nanoparticles, while group A3 was assigned for the adhesive with 20% wt. bioglass nanoparticles. Group B was assigned for the self-etch adhesive procedures and was similarly divided into three groups of eight specimens each; B1 for control, B2 for 10% wt. bioglass nanoparticles and B3 for 20% wt. bioglass nanoparticles.

The adhesive was applied to the dentin surface according to manufacturer instructions for each adhesive procedure. For group A (etch and rinse), 37% phosphoric acid gel was applied to the dentin surface for fifteen second. It was then thoroughly

**Table 1:** Assignment of the specimens to the experimental groups

	Control (unmodified adhesive)	10 % bioglass	20% bioglass	Total number of specimens
Group A Etch and rinse	A1	A2	A3	24
Group B Self-etch	B1	B2	B3	24

washed with an air water syringe and gently blotted by micro sponges to leave a visibly moist surface. The adhesives were the. Applied to the dentin specimens with A1 control, A2 10% bioglass and A3 20% bioglass. The adhesives were each applied using a brush and rubbed into the dentin surface for 20 seconds, after which it was gently air thinned. It was then cured for ten second with a light cure unit (woodpecker light cure unit, China). For group B the dentin surface was washed and gently dried, followed by the adhesive application as in group A; rubbing for 20 seconds, air thinning and light curing. Light cure resin composite (Filtek Z250, 3M) was then applied to the bonded dentin in 2 increment of 2 mm thickness each and light cured for 40 seconds for each increment. The samples were then stored in tap water for 24h at room temperatures after which they were sectioned into 1mm thickness slabs with a diamond saw under copious water coolant. They were then sectioned longitudinally into two beams of approximate cross section diameter of 1 mm. central beams were checked for size and used for measurement.

### Microtensile bond strength testing

For microtensile bond strength testing, each beam was attached to a special jig in a universal testing machine (Instron testing machine). The microtensile bond strength ( $\mu$ TBS) was determined as the samples were submitted to a tensile force at a crosshead speed of 1 mm/min and 500 N until failure. Load required to debond each specimen was recorder using computer software. After testing, samples were removed from the fixtures with a scalpel. Data was collected for statistical analysis.

### Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA). As data were parametric, significance of the difference between subgroups was evaluated using ANOVA test, followed by Tukey's post hoc test when ANOVA yielded a significant difference. The level of significance was set at  $P < 0.05$ .

## RESULTS

### Adhesive characterization:

TEM photomicrograph of adhesive with bioactive glass filler 20% shows fairly even dispersion of the fillers with some agglomeration of the fillers (fig 1)

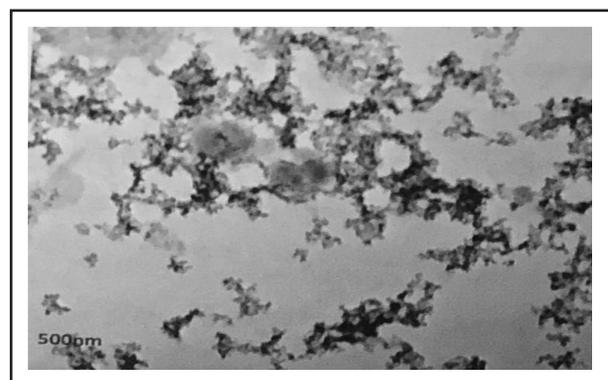


Fig. (1) TEM micrograph of filled adhesive Mag 50000x

**Microtensile bond strength results:**

Results of comparison of means and standard deviations of microtensile bond strength in MPa are shown in table 2 and fig 1. Group A compared A1 (control, A2 10% bioglass) and A3 (20% bioglass). Similarly, Group B compared B1 (control, B2 10% bioglass) and B3 (20% bioglass). In group A (etch and rinse group), the highest mean value was recorded in control, while the lowest mean value was noted in subgroup 20%. ANOVA test revealed that the difference was extremely significant ( $P < 0.0001$ ). Tukey HSD Post-hoc Test for group A (total etch) revealed a significant difference between control and 10% ( $p = 0.0013$ ), between control and 20% ( $p < 0.0001$ ) and between 10% and 20% ( $p = 0.0345$ ).

In group B (self tech), the highest mean value was recorded in control, while the lowest mean value was noted in subgroup 20%. ANOVA test revealed that the difference was extremely significant ( $P = 0.0044$ ). Tukey HSD Post-hoc Test for group B (self-etch) revealed a significant difference between control and 10% ( $p = 0.0064$ ), between control and 20% ( $p = 0.017$ ), while there was no statistically significant difference between 10% and 20% ( $p = 0.903$ )

**Table (2) Comparison between groups (ANOVA)**

	Group A (eth and rinse)			Group B (self etch)		
	Control E&R	10% E&R	20% E&R	Control se	10% se	20% se
Mean	42.30 <sup>a</sup>	25.46 <sup>b</sup>	14.47 <sup>c</sup>	18.49 <sup>a</sup>	11.11 <sup>b</sup>	12.03 <sup>b</sup>
SD	9.27	7.54	7.50	3.53	3.93	5.18
F	23.69			7.09		
P	<0.0001*			0.0044*		

Significance level  $p < 0.05$ , \*significant

Tukey's post hoc test: Within the same comparison, means sharing the same superscript letter are not significantly different

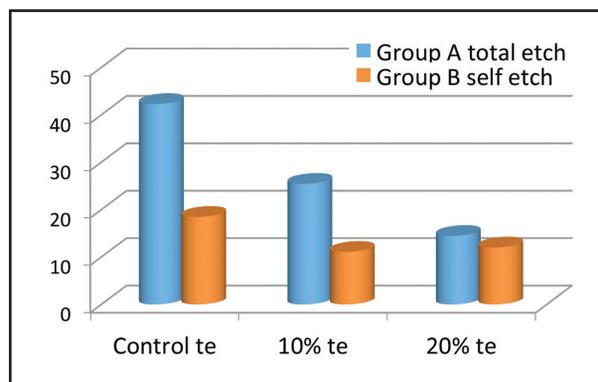


Fig. (2) Bar chart representing mean values of microtensile bond strengths of all groups

**DISCUSSION**

Bioglass has been used to aid in remineralization of incipient lesions, to help treat dentin hypersensitivity, to encourage the formation of a dentin bridge in deep cavities as well as to help remineralize affected dentin<sup>11</sup>. Bioactive glass is composed of calcium sodium phospho silicate. It is also used for polishing procedures and added to tooth pastes. The technique of remineralization comes from its ability to mimic and stimulate the body's natural mineralization mechanism<sup>12</sup>. Bioglass is hence useful for remineralization of affected dentin under restorations. While this is beneficial in enhancing pulp protection, it is also useful to strengthen the hybrid layer. Studies showing bond strength to affected dentin lacking when compared to sound dentin<sup>13</sup>. Transforming the affected dentin to sound dentin via mineralization could positively influence the dentin bond by improving the properties of the dentin itself<sup>14</sup>. There is another factor that is considered with the etch and rinse procedures, where it is a technique sensitive bonding mechanism. When micro pores are formed by strong etching, collagen fibers are stripped from their hydroxyapatite crystals and left bare. These bare collagen fibers are subject to hydrolysis and breakdown by enzymes over time causing bond degradation and nano leakage<sup>15</sup>. Bioglass by enhancing remineralization of dentin, can protect the bare collagen fibers from hydrolysis and thus serving to stabilize the bond. The addition of fillers

has also been seen to improve the mechanical properties of some adhesives <sup>6</sup>.

The results in this paper however have shown that the microtensile bond strength decreased by adding the fillers when compared to the control. For both etch and rinse, and self-etch systems, the addition of bioglass fillers has caused a significant drop in the microtensile bond strength. In the etch and rinse, the increase in concentration of bioglass caused a further decline in bond strength where the 20% group was significantly lower than the 10%. In the self etch, the amount of added filler was not the decisive factor, as both 10% and 20% showed similar results, but rather the addition of bioglass filler itself caused the decrease in strength. Hence the explanation for both technique results can be somewhat different.

For the etch and rinse technique, the etching procedure was not altered by the addition of the bioglass fillers, as the etching step is separate and precedes the adhesive application. Therefore we can assume the etching pattern is similar. However, the step that comes after that which is resin impregnation could have been affected. The etch and rinse adhesive depends on the resin bond to penetrate and fill the micro pores created from the etching procedure and polymerization in place. This process is essential for a successful bond<sup>16</sup>. In this case with the added filler, it could be that penetration into tubules has changed with change in composition of adhesive. Adhesive already contains 5-15% by wt. silica fillers. This adhesive is rubbed for twenty seconds to allow penetration. Studies have showed that 20 secs are sufficient for penetration into tubules <sup>17</sup>.

However, adding more fillers by adding the bioglass can have an effect on the adhesive viscosity, which may lead to incomplete penetration into the tubuli. This can be supported by the results where 10% added filler yielded higher strengths than 20%. This has also been supported in literature that added particles of both nano and micro sizes<sup>18</sup>. Another possible explanation is that the mixing method

could be better designed for more homogeneity and distribution of the fillers. Both mixing in a capsule or mixing by sonicator have been reported in literature<sup>19</sup>. Agglomeration is always a possibility with added fillers that can hinder the penetration process, as TEM characterization showed some agglomeration of fillers. Mechanical properties of the adhesive could also have been altered. Silica Fillers in adhesives have been shown to increase modulus of elasticity of adhesives in vitro, however how effective the filler itself is at strengthening the adhesive and aiding in stress transfer is not clear with this material and this method of incorporation.

With the self-etch technique, while it still applies that the increased viscosity may have hindered the function of the adhesive, it is also possible that the etching pattern has been affected as well. The adhesive in self etch mode depends on the demineralization of the dentin by the acidity of the adhesive itself. When trying to demineralize dentin with an adhesive that contains a remineralizing agent, it is possible that it had affected the etching process itself by altering the physiochemical properties of the adhesive. This is especially possible when we consider that the amount of added filler did not make a difference so the factor of viscosity is minimal. The adhesive is considered a mild self-etch, meaning its pH is more than 2. The pH of the adhesive might have changed with the addition of the bioglass to the bond. In a previous study,<sup>20</sup> the addition of powdered dentin to self etch adhesives pH to significantly increase from a range of 0.97-2.083 to a range of 6.3-7.11. This pH is neutral and can not produce a reliable etching process for bonding.

Ultimately the Idea of bioglass as an aid for remineralization is very beneficial, but should not adversely affect the bonding process. A balance has to be found between the need for remineralization and the need for high bond strengths. Several factors may be further investigated to find a better balance, either adjusting the amount of filler, substitution of silica fillers for bioglass, allowing more time for resin or more balance between silica and bio glass fillers.

The results of this study under these conditions have shown that the addition of bioglass fillers as well as the amount added had a detrimental effect on the micro tensile bond strength to dentin in both etching techniques and thus the null hypothesis is rejected.

## CONCLUSION

The addition of bioactive glass nano fillers to the adhesive resin have a negative effect on the microtensile bond strength to dentin.

## REFERENCES

1. Featherstone JD, Doméjean S. The role of remineralizing and anticaries agents in caries management. *Adv Dent Res* 2012;24:28-31.
2. Hala M, Heba M, HendM. Minimal Intervention Approaches in Remineralizing Early carious lesions. *J Am Sci* 2012; 8: 709- 17.
3. Ngo HC, Mount G, Mc Intyre J, Tuisuva J, Von Doussa RJ. Chemical exchange between glass-ionomer restorations and residual carious dentine in permanent molars: an in vivo study. *J dent.* 2004;34:608-13.
4. Samuel S & Marguerita F. Evaluation of NovaMin as an adjunct to fluoride for caries lesion remineralization. *NovaMin Res Rep* 2014; 32: 19-25
5. Cao CY, Mei ML, Li QL, Lo EC, Chu CH. Methods for biomimetic remineralization of human dentine: a systematic review. *Inter J molec Sci.* 2015;16:4615-27.
6. Suaro S, Osorio R, Watson TF, Toledano M. therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface *J Mater Sci.* 2012; 23: 1521-32
7. Tawfic HM, Niazy MA, Elsharkawy DA The Effect of Zn-doped Adhesive alone or Combined with Calcium Phosphate Nanoparticles on the Integrity of the Bonded ResinDentin Interface *ADJ girls.* 2017; 4: 289-96
8. Wang Z, Jiang T, Sauro S, Wang Y, Thompson I, Watson TF, Sa Y, Xing W, Shen Y, Haapasalo M. Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes. *J Dent.* 2011;39:746-56
9. Ikemura K, Tay FR, Yoshiyama M, Pashley DH Optimizing filler content in an adhesive system containing pre-reacted glass-ionomer fillers. *Dent. Mater.* 2003,19: 137-48
10. Belli R, Kreppel S, Petselt A, Homberger H, Boccaccini AR, Lohbauer U Strengthening of dental adhesives via particle reinforcement. *J Mech Beh. Biomed. Mater.* 2014, 37: 100-108
11. Vollenweider M, Brunner T, Knecht S. Remineralization of human dentin using ultrafine bioactive glass particles. *Acta Biomater.* 2007;3:936-943.
12. Sathya N, Vinoth K, Shafie A, Emmanuel S, Meyappan R, & Satheesh K. Remineralization efficiency of bioactive glass on artificially induced carious lesion an in-vitro study. 2014; 32: 19-25.
13. Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J Bonding to caries-affected dentin. *Jap Dent Sci Rev,* 2011; 47: 102-14
14. Bertassoni Le, Stefan Habelitz S, Pugach M, Soares Pc, Marshall Sj, And Marshall Jr Jw Evaluation of Surface Structural and Mechanical Changes Following Remineralization of Dentin. *SCANNING* 2010; 32: 312-9,
15. Anchieta RB, Machado LS, Sundfeld RH, Reis AF, Giannini M, Luersen MA, Janal M et al. Effect of partially demineralized dentin beneath the hybrid layer on dentin-adhesive interface micromechanics. *J Biomech.* 2015;48:701-7.
16. Manuja N, Nagpal R, Pandit K Dental Adhesion: Mechanism, Techniques and Durability *J clin. Ped. Dent.* 2012 36: 223-34
17. Leforestier E, Darque-Ceretti E, Bouchard PO, Bolla M determining the initial viscosity of 4 dentinal adhesives. Relationship with their penetration into tubuli. *Int J of Adh&Adh.* 2010; 30: 393-402
18. Martins GC, Reis A, Loguercio AD, Zander-Grande C, Meier M, Mazur RF et al. Does making an adhesive system radiopaque by filler addition affect its bonding properties? *J Adhes Dent* 2015; 17: 513-9
19. Corral Nunez C, Covarrubias C, Fernandez E, Oliviera Jr OB. Enhanced bioactive properties of biodentine TM modified with bioactive glass nanoparticles. *J Oral Sci.* 2017; 25:177-85
20. Iwasa M, Tsubota K, Ando S, Mmiyazaki M, Platt JA pH changes upon mixing of single step self-etching adhesives with powdered dentin. *J Adhes Dent* 2011, 13: 307-12