

# Effect of 0.12% chlorhexidine and zinc nanoparticles on the microshear bond strength of dentin with a fifth-generation adhesive

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## ABSTRACT

**Objective:** In this study, we compared the effects of 0.12% chlorhexidine (CHX) and nano zinc oxide (NZO) on the microshear bond strength of dentin with a fifth-generation adhesive after acid etching. **Materials and Methods:** Forty molar teeth were randomly divided into four main groups based on dentin surface treatment technique (a) control (single bond 2); (b) NZO; (c) CHX; and (d) NZO + CHX. In each group, half of the samples underwent thermocycling, with no thermocycling in the other half. Then, failure mode was evaluated under a stereomicroscope. Statistical analysis was performed using *t*-test, two-way ANOVA, and Chi-squared test. **Results:** The mean microshear bond strength of the groups without thermocycling was more than that of the groups with thermocycling, but there were no statistically significant differences between the groups with and without thermocycling in pair-wise comparisons. **Conclusion:** Pretreatment with NZO and CHX separately and simultaneously had no effect on the microshear bond strength of a fifth-generation adhesive.

**Key words:** Acid etching, bond strength, chlorhexidine, nano zinc oxide

## INTRODUCTION

Lack of durability of the bond to tooth structure is one of the most important problems in the application of dental adhesive systems, especially in the etch-and-rinse adhesives.<sup>[1]</sup> The bond to dentin is more

complex than that to enamel, due to its heterogeneous nature, with a larger organic content and water.<sup>[2]</sup> One problem in restorative dentistry is complete removal

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of carious dentin. If the bacteria remain in the cavity preparation, recurrent caries might occur.<sup>[3,4]</sup> Even, despite good seal, bacteria might be able to replicate. Bacterial growth under the restoration leads to an increase in tooth sensitivity, a decrease in its strength, recurrent caries,<sup>[3,4]</sup> and inflammation and necrosis of the pulp.<sup>[5]</sup> The use of disinfectant solutions such as chlorhexidine (CHX) is an alternative method to reduce or eliminate bacteria from cavity preparations.<sup>[6]</sup>

Recent studies have described the relationship between dentin collagen hybrid bond layer degradation and matrix metalloproteinases (MMPs) after their release by etch-and-rinse ( $\text{pH} \leq 4.5$ )<sup>[7]</sup> and self-etch adhesives<sup>[8,9]</sup> that can reduce the bond strength over time.<sup>[10]</sup> It results in the creation of secondary caries and marginal discoloration, and ultimately in restoration failure.<sup>[8,9]</sup>

MMP activation is mediated by binding of Zn ion to N-terminal branches. However, in two cases and by some inhibitors of the activation, function has been impaired, which might be attributed to the following reasons: (1) other enzymes and ions compete with Zn ion and hence deactivation occurs and (2) by increasing the amount of Zn ion, the other binding sites that are less likely to be activated are occupied by zinc, and by deforming the space, MMP activity is inhibited.<sup>[11]</sup> Hitherto, the inhibitory effects of CHX, zinc, galardin, and others on MMPs activity have been studied. In addition, CHX can inhibit the activation of dentin MMPs even at low concentrations.

Osorio *et al.*<sup>[12]</sup> investigated the effect of zinc on MMPs and deduced that zinc oxide nanoparticle, in combination with the etch-and-rinse adhesives such as single bond (SB), has the best inhibitory effect on the MMP activity, with no deleterious effect on the microtensile bond strength. Leitune *et al.*<sup>[13]</sup> indicated that short applications of CHX at low concentrations prevent hybrid layer degradation and positively affect bond strength over time.

Alaghemand *et al.*<sup>[11]</sup> evaluated the inhibitory effect of CHX and zinc nanoparticles on the degradation of collagen scaffolds, and in this study, we investigated the effect of CHX and zinc nanoparticles on the microshear bond strength of a fifth-generation adhesive after etching. Anti-MMP benefits of ethanol-wet-bonding were also assessed and confirmed by Sadek *et al.* in comparison with the use of CHX that is a nonspecific anti-MMP agent.<sup>[14]</sup> The null hypotheses examined were (1) the effect of CHX and zinc nanoparticles separately and simultaneously on the microshear

bond strength to dentin after acid etching with a fifth-generation adhesive is different between the test groups and is different from the control group as well. (2) adhesive failure after application of CHX and zinc nanoparticles individually and simultaneously to etched dentin is different between the test groups and is different from the control group as well.

## MATERIALS AND METHODS

### Tooth preparation

Forty extracted human molar teeth with no caries and no visible anatomical defects were selected. The teeth were extracted for the periodontal disease under a protocol reviewed and approved by the Ethics Committee in Babol University of Medical Sciences, Babol, Iran. The teeth were cleaned and polished by water and pumice with a brush using a low-speed handpiece. To disinfect the teeth, they were placed in 0.2% thymol solution for 24 h, followed by storage in a saline solution until used for the purpose of the study.

The occlusal enamel was removed with a diamond bur under water and air spray perpendicular to the tooth long axis. The flat dentin surfaces were polished with 400 and 600 grit silicon carbide abrasive paper to provide a standardized smear layer.<sup>[15]</sup>

### Bonding procedures

The dentin surface was etched with 37% phosphoric acid for 15 s, rinsed for 30 s with tap water, and then dried with oil/water free air. The materials used are listed in Table 1.

The samples were randomly divided into four main groups ( $n = 10$ ), based on the dentin surface pretreatment as follows:

- Group A: The control group without pretreatment. SB 2 was applied as a bonding agent to the cavity walls with a microbrush; after 10 s of gentle air-drying, 20 s of light-curing (LED VALO, Ultradent, USA) was performed at a light intensity of  $600 \text{ mW/cm}^2$
- Group B: Pretreatment with nano zinc oxide (NZO). NZO powder was added to SB 2 and mixed for 1 min in a tube agitator (SB 2 + 10 wt% of NZO) in the dark area<sup>[12]</sup> and applied to the cavity walls and then light-cured for 20 s
- Group C: Pretreatment with 0.12% CHX. CHX was used for dentine rehydrate for 30 s using a new microbrush until the emergence of a sleek appearance. After 60 s, excess solution was removed with absorbent paper,<sup>[11]</sup> and the

**Table 1: List of the materials used in this study**

Product	Company	Ingredients	Descriptions
Composite filtek Z 250	3M, USA	Silanated ceramic, Bis-EMA 6, UDMA, Bis-GMA, TEGDMA, benzotriazole, EDMAB	Shade A2
Adper single bond 2	3M, USA	Ethyl alcohol, Bis-GMA, silane-treated silica (Nanofiller), HEMA, copolymer of acrylic and itaconic acids, glycerol 1,3-dimethacrylate, water, UDMA, diphenyliodonium hexafluorophosphate, EDMAB	10 s gentle air drying 20 s curing
Condac 37%	37 Condac, FGM, Joinville, Brazil	Aqueous-based gel containing 37% phosphoric acid	15 s etching, 10 s rinsing and drying
Nano zinc oxide powder	Penta, Czech		
Chlorhexidine 0.12%	3M, USA		
TEGDMA: Triethylene glycol dimethacrylate, Bis-GMA: Bisphenol A diglycidyl ether dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, UDMA: Diurethane dimethacrylate, EDMAB: Ethyl 4-dimethylaminobenzoate, Bis-EMA 6: Bisphenol A polyethylene glycol diether dimethacrylate			

adhesive without NZO was applied similar to the first group

- Group D: pretreatment with 0.12% CHX plus adhesive containing NZO. After using of CHX similar to Group C, adhesive (SB 2 + 10 wt% of NZO) was applied similar to Group B.

In all the groups, Filtek Z250 composite resin (shade A2) was used incrementally (1.5 mm) on all the exposed dentin surfaces at a thickness of 4.5 mm, and each layer was light-cured for 20 s.

In each group, the specimens were divided into two subgroups for 2500 cycles of aging in the NEMO thermocycling machine (Mashhad, Iran) at 5°C-55°C, with a dwell time of 30 s and an interval of 30 s. Half of the samples were thermocycled and the other half was not.

### Microshear bond testing

The specimens were mounted in epoxy resin. Then, the teeth were placed in a cutting machine, and each tooth was sectioned at levels of approximately 1 mm × 1 mm. The microshear test was performed using a universal testing machine (Zwick, Roell, Germany). A knife-edge shearing rod was used to load the specimens until fracture at a crosshead speed of 1 mm/min. Microshear bond strength was calculated in MPa from the peak load at failure divided by the specimen's surface area; the cross-sectional surface area was measured 3 times using a digital caliper (Shinwa Rules Co., Niigata, Japan) and then the mean was calculated.

After testing, the failure modes were evaluated under a stereomicroscope (Carl Zeiss Inc., Oberkochen, Germany) at ×10 and classified according to the predominant mode of fracture including (1) adhesive, (2) cohesive in dentin, (3) cohesive in composite resin, and (4) mixed, i.e., a combination of adhesive and cohesive.

### Energy dispersive X-ray analysis

Adhesive with 10 wt% of NZO was placed in 1 mm × 1 mm × 1 mm stainless steel quadrangular molds and light-cured using a light-curing unit for 20 s. The samples were coated with gold, and Map-energy dispersive X-ray (EDX) analysis was performed.

### Statistical analysis

Statistical analysis was performed using *t*-test to determine the level of significance, and two-way ANOVA was used for comparison between the groups. Chi-squared test was used for comparison of bond failure at the 0.05 level of significance. All statistical calculations were performed with SPSS 17.0 software (SPSS Inc., Chicago, IL).

## RESULTS

The mean of microshear bond strength of the subgroups without thermocycling was higher than that of the subgroups with thermocycling, but there were no statistically significant differences between the subgroups with and without thermocycling ( $P > 0.05$ ) [Table 2 and Figure 1].

Pair-wise comparisons did not reveal any statistically significant differences in the mean microshear bond strength values between the subgroups with and without thermocycling.

The results of failure modes are presented in Table 3. The most frequently observed failure mode was cohesive failure in all the experimental groups.

## DISCUSSION

In this study, we evaluated the effect of CHX and NZO on the microshear bond strength of a fifth-generation adhesive after etching in two subgroups with and without thermocycling.

The results showed that separate and simultaneous application of CHX and NZO had a similar effect on the microshear bond strength and failure mode. Therefore, the first and second null hypotheses were rejected.

The comparison of groups in two subgroups with and without thermocycling showed neither significant differences between the experimental groups themselves nor between these groups and the control group, which can be explained by the effect of CHX as a broad-spectrum disinfecting agent.<sup>[3]</sup> When CHX is applied on the prepared tooth surface after acid etching, it is not washed off, and the adhesives are applied, and then, the procedure is continued. It has the potential to bind to both organic and inorganic components of the dentin.<sup>[16]</sup> CHX binds to hydroxyapatite (inorganic component) of dentin, which is believed to be mediated by the formation of a phosphate salt that leads to an increase in the surface energy of dentin, thus enhancing the dentin wetting ability of primers. The interaction of CHX with the organic component of dentin is believed to be mediated by binding to Type I collagen.<sup>[16]</sup> The binding of CHX to the dentin matrix component is probably the best way for CHX to inhibit collagen bond proteases<sup>[17]</sup> such as MMPs 2, 8, and 9.<sup>[8,18]</sup> CHX is an effective synthetic MMP inhibitor even in low concentrations (0.02%–0.0001%).<sup>[18]</sup> At low concentrations, the inhibitory effect of CHX on MMPs is thought to be related to a cation-chelating mechanism, wherein

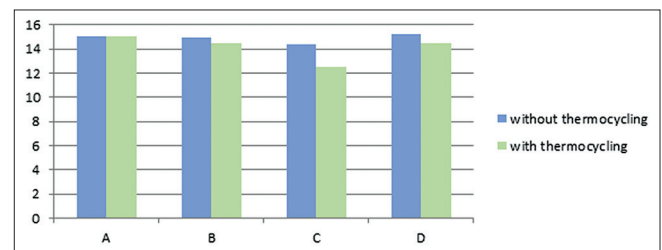
the sequestration of metal ion, such as zinc, would hamper the activation of the catalytic domains within MMPs.<sup>[19,20]</sup> Notwithstanding the MMP inhibitory effect of CHX seems to be dose-dependent,<sup>[18]</sup> and at high concentrations, it may likely inactivate MMPs by enzyme denaturation rather than by chelation of cation.<sup>[10]</sup> *In vitro*<sup>[19]</sup> and *in vivo*<sup>[20]</sup> studies have shown that CHX has a beneficial effect on the preservation of dentin-resin bonds. The use of CHX (0.12%) mouthwash to rub on the preparation might seem to be the most immediate way to treat self-etch dentin bonds. Since CHX (0.12%) (Peridex, 3M, USA) has a routine use in dental offices and for dentistry patients, we decided to use this CHX concentration applying it before bonding in etch-and-rinse adhesives.<sup>[11]</sup>

According to this study, amounts of bond strength in CHX group (Group C) (with and without thermocycling) were less than control group (Group A) but did not have statistically significant differences. This is showing that using of CHX (0.12%) after acid etching in SB 2 adhesive has no effect on bond strength. This is hit off Soaresc's study.<sup>[21]</sup>

The zinc ion has also an inhibitory effect on the MMP activation, in a manner similar to CHX. By increasing the amount of Zn ion, binding sites are

**Table 2: Microshear bond strength mean of groups with and without thermocycling**

	A	B	C	D
Without thermocycling	15.02±4.76	14.91±2.75	14.4±4.06	15.2±3.02
With thermocycling	13.45±4.71	14.48±3.55	12.52±4.14	14.45±3.1



**Figure 1:** Comparison between microshear bond strength of groups with and without thermocycling

**Table 3: Mode of failure**

Group	Cohesive dentin		Cohesive composite		Adhesive		Mix		Significant level	
	With thermocycling (%)	Without thermocycling (%)	With thermocycling (%)	Without thermocycling (%)	With thermocycling (%)	Without thermocycling (%)	With thermocycling (%)	Without thermocycling	With thermocycling	Without thermocycling
A	15 (75)	11 (55)	5 (25)	5 (25)	0	2 (10)	0	2 (10)	0.062	0.181
B	8 (40)	6 (30)	9 (45)	12 (60)	1 (5)	0	2 (10)	2 (10)		
C	6 (30)	12 (60)	7 (35)	6 (30)	4 (20)	0	3 (15)	2 (10)		
D	8 (40)	9 (45)	5 (25)	10 (50)	5 (25)	0	2 (10)	1 (5)		

A: Control group, B: Nano zinc oxide group, C: Chlorhexidine group, D: Nano zinc oxide+chlorhexidine



occupied by zinc, resulting in the spatial deformation and finally inhibiting MMP activity. A determining factor in the efficacy of nanoparticles in improving microshear bond strength is their dispersion quality in the matrices. A proper distribution of ZnO element is observed in the Map-EDX [Figure 2], which is an indicator of relatively homogeneous distribution of the NZO in the adhesive.

Osorio *et al.*<sup>[12]</sup> evaluated the collagen degradation and bond strengths using SB adhesive containing NZO (etch-and-rinse) versus Clearfil self-etching primer (self-etch adhesive) for 24 h and 1 and 4 weeks, concluding that Zn-doping of SB resin inhibited and stabilized collagen degradation at the hybrid layer (up to 4 weeks) and did not affect 24-h bond strength, but increased bond stability of SB after 3 months. In the Osorio's study, dentin beam was immersed for 8 h in a zinc-containing resin bonding agent. In this study, we applied NZO-containing bonding agent according to the manufacturer's instructions to simulate the clinical steps.

ZnO is an amphoteric oxide although it normally exhibits basic properties. It is nearly insoluble in water and alcohol but is soluble in and degraded by acids. Small size particles of ZnO were selected to induce the highest interfibrillar infiltration. The high solubility of ZnO when combined with acid could also account for the effective release of zinc ions at the resin-dentin interface.<sup>[12]</sup>

In this study, bond strength in group with ZnO especially with thermocycling was a little better than group with CHX that was showing using of ZnO had no effect on bond strength. Nevertheless because ZnO

was added to SB 2 adhesive, Group B (with ZnO) had clinically working steps less than CHX group that this is better.

Thermocycling is one broadly used artificial aging methodology, and in this study, we evaluated the effect of aging on the long-term performance of the bond; half of the samples were thermocycled and the other half was not. The results indicated no significant differences between the above circumstances.

Some studies have shown that during thermocycling, the specimens are subjected to thermal changes and also additional exposure to water.<sup>[22,23]</sup> Thermal stresses generate mechanical stresses by differences in the coefficient of thermal expansion<sup>[23]</sup> and can result in bond failure at the tooth-restoration interface. The main cause for the reduction in bond strength is believed to be the possible effect of hydrolysis at bonding interfaces.<sup>[23]</sup> Consistent with a study by Titley *et al.*,<sup>[24]</sup> our study indicated that the shear bond strength of SB was not significantly affected by thermocycling. The positive effect of CHX and NZO separately and simultaneously on the microshear bond strength did not result in a decrease in bond strength after thermocycling. In the evaluation of failure, cohesive failure was dominant in all the groups. The cohesive failures of bonding agents are often associated with high bond strength values, indicating effective bonding.<sup>[25]</sup> These findings are in line with the fact that thermocycling has no deleterious effect on the bond strength.

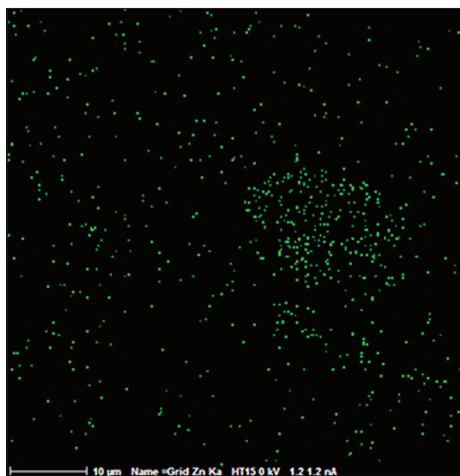
Various studies<sup>[26,27]</sup> have suggested that acid etching is one way of activation of MMPs. Mazzoni *et al.*<sup>[28]</sup> in a study with conflicting results stated that high acidity of phosphoric acid might lead to denaturation of the MMPs. Perhaps, that is why the use of CHX and NZO before and after thermocycling had no effect on the microshear bond strength. It is advised to investigate CHX with more concentration and NZO simultaneously in a fifth-generation adhesive for more evaluation.

## CONCLUSION

Pretreatment with NZO and CHX separately and simultaneously did not affect microshear bond strength of SB 2.

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**Figure 2:** The Map-energy dispersive X-ray of cured adhesive containing 10 wt% of nano zinc oxide, which indicates a relatively homogeneous distribution of nano zinc oxide in the adhesive

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## Conflicts of interest

There are no conflicts of interest.

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