Original Article

# The Effect of CuO Nanoparticles on Antimicrobial Effects and Shear Bond Strength of Orthodontic Adhesives

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**KEY WORDS**
Nanoparticles;
Orthodontic Adhesive;
Shear Bond Strength;
Antibacterial Effect ;
Streptococcus Mutans;

**ABSTRACT**

**Statement of the Problem:** Orthodontic appliances facilitate microbial plaque accumulation and increase the chance of white spot lesions. There is a need for new plaque control methods independent of patient's cooperation.

**Purpose:** The aim of this study was to determine the effects of incorporating copper oxide (CuO) nanoparticles on antimicrobial properties and bond strength of orthodontic adhesive.

**Materials and Method:** CuO nanoparticles were added to the composite transbond XT at concentrations of 0.01, 0.5 and 1 wt.%. To evaluate the antimicrobial properties of composites containing nanoparticles, the disk agar diffusion test was used. For this purpose, 10 discs from each concentration of nano-composites (totally 30 discs) and 10 discs from conventional composite (as the control group) were prepared. Then the diameter of *streptococcus mutans* growth inhibition around each disc was determined in blood agar medium. To evaluate the shear bond strength, with each concentration of nano-composites as well as the control group (conventional composite), 10 metal brackets were bonded to the human premolars and shear bond strength was determined using a universal testing machine.

**Results:** Nano-composites in all three concentrations showed significant antimicrobial effect compared to the control group ($p< 0.001$). With increasing concentration of nanoparticles, antimicrobial effect showed an upward trend, although statistically was not significant. There was no significant difference between the shear bond strength of nano-composites compared to control group ($p= 0.695$).

**Conclusion:** Incorporating CuO nanoparticles into adhesive in all three studied concentrations added antimicrobial effects to the adhesive with no adverse effects on shear bond strength.

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## Introduction

Conventional methods to deal with tooth decay and white spot lesion (WSL), focused on control of oral biofilm through oral hygiene care and mechanical removing of microbial plaque using fluoride and antimicrobial agents, all depend on patient's cooperation. [1-2] Another possible method to prevent enamel demineralization, independent of patient's cooperation, is the use of adhesives resistant to bacterial adhesion and biofilm formation.

Consequently, various antimicrobial agents have been tried to be add to orthodontic adhesives. [3] One of the antimicrobial agents that have recently received a lot of attention is metal nanoparticles. [4] The unique antimicrobial properties of metal nanoparticles are attributed to their small size and related high surface area to volume ratio. [5] These physiochemical properties of nanoparticle provide a large interaction with bacterial membranes causing a wide range of antimicrobial effects, other than releasing metal ions. [6]

The use of adhesives containing nanoparticles could play an important role to inhibit development of WSL. It is reported that restorative composite containing 1 wt% quaternary ammonium polyethylene imine can completely inhibit the growth of *streptococcus mutants* (S.mutans). [7] Brackets which were coated with copper oxide(CuO) and zinc oxide (ZnO) nanoparticles showed better antimicrobial effects on S.mutans compared to control group. [8] It is also reported that CuO and ZnO nanoparticles have strong antimicrobial effects on inhibition of bacterial colonization and plaque development. [9] CuO has showed antimicrobial effects against a wide range of pathogenic bacteria. [10] Since CuO is more low-priced and more stable, both chemically and physically, [11] this study was designed to evaluate the antimicrobial properties of CuO nanoparticles and its shear bond strength on orthodontic adhesives.

**Materials and Method**

In order to have nano-composites with concentration of 0.1, 0.5, and 1.0 wt. %, 0.003, 0.15, and 0.03 g CuO nanoparticles (Avijeh nano structure company, Tehran, Iran) were weighed by a digital scale respectively. Each were mixed with 3 grams orthodontic composite (Transbond XT, 3M Unitek, USA) with a mixer spatula and glass slab in a semi-dark environment to reach to a uniform concentration.

40 maxillary premolar teeth, extracted for orthodontic purposes within 6 months, were recruited in this study. Teeth were free from caries, filling, and endodontic treatment. Teeth with enamel cracks, detected under the direct light of dental unit, were omitted from study. Teeth preserved in 0.1% thymol and then divided randomly in 4 groups of 10 teeth, mounted on acrylic blocks. Buccal surfaces of all teeth were cleaned with prophylaxis brush, washed with water and then dried. Teeth were etched for 30 seconds with phosphoric acid 37% (Fineetch, Korea); then washed for 15 seconds, and dried with an oil-free air spray. A thin layer of primer (Transbond XT primer, 3M Unitek, USA) was applied to buccal surface of teeth and brackets were bonded using adhesives assigned to each group.

Metal brackets (Dentaurum, Discovery, Germany) were placed on buccal surface of teeth, 4 millimeter from cusp tip, and fitted completely on the tooth surface using a scaler pressure. Then excessive adhesive was removed with a scaler before curing. Nano-composites of 0.1%, 0.5%, and 1% (wt.%) were used for first, second, and third groups respectively. Transbond XT (3M) adhesive was also used in control group. Then brackets were light-cured using a LED curing unit (MORITA, Japan) at 450 nm wavelength, and 500 mv/cm$^2$, for 20 seconds (5 seconds from each aspect). All samples were thermo-cycled (Vafaee, Iran) for 1500 times between 5 to 55$^0$C, 15 seconds in each temperature with 10 seconds intervals. Then shear forces were applied to the brackets of all 4 groups, including three study groups and one control group, using a universal testing machine (Walter+bai, Switzerland) at 0.5 mm/min crosshead speed, until the bond failure occurred. Debonding force was recorded in Newton, changed to mega-Pascal by dividing to surface area of bracket base.

Disc agar diffusion test was used in this study to investigate the antibacterial effects of adhesive containing CuO nanoparticles against S.mutans. A suspension equivalent to 0.5 McFarland turbidity ($1.5\times10^8$. CFU/ml) in the brain-heart-infusion (BHI) medium was produced from new cultured (24 hours) standard S.mutans. Plastic molds with a diameter of 6 mm and a thickness of 1 mm were used to make composite discs.

The molds were placed on a glass slab, and were covered with another slab after putting adhesive in the mold; then the adhesives were cured for 20 seconds (10 seconds from side) with light curing device. In this way, 10 discs of 6mm diameter and 1 mm thickness were produced from each adhesive group. In order to complete curing, discs were cured for 10 seconds again. Then, using swaps, some prepared bacterial suspension was meadow cultured on the Mueller Hinton medium (Merck, Germany) enriched with 5% sheep blood. Then a disc from each group was placed on the plates containing medium. All culture media plates were kept in a $CO_2$ incubator at 37$^o$C for 24 hours. Now the diameter of bacterial growth inhibitory zone around the discs was measured in millimeters.

One-Way ANOVA test was used in this study to compare the shear bond strength. Kruskall-Wallis and Mann-Whitney tests were used to compare and investigate the diameter of inhibition zone. in all statistical tests, the significance level was considered 0.05

## Results

Based on the obtained results, incorporating CuO nanoparticles into the adhesives in all three concentrations of 0.01, 0.5, and 1 wt.% not only had no negative effect on shear bond strength; but even increased it. As summarized in Table 1, all three groups of adhesives containing nanoparticles showed a mean shear bond strength more than that of control group; yet there were no significant differences according to One- Way ANOVA test and $p= 0.695$ for all four studied groups. As illustrated in Figure 1, increasing the concentration of CuO nanoparticles up to 0.5 wt.% increased the shear bond strength, but in 1 wt.% concentration the shear bond strength decreased compared to other two groups. Antimicrobial examination showed that the greatest diameter for S. mutans growth inhibition zone (the mean of 9.20mm) was belonged to the third group consisting of 1 wt.% CuO nanoparticles (Table 2).

**Table 1:** Mean of shear bond strengths of four groups

| Groups | N | Bond | | | |
|---|---|---|---|---|---|
| | | Mean (MPa) | SD | Min | Max |
| Control | 10 | 15.90 | 1.46 | 12.57 | 19.22 |
| Nanocomposite0.01% | 10 | 16.69 | 1.16 | 15.05 | 19.33 |
| Nanocomposite 0.5% | 10 | 17.81 | 1.18 | 15.12 | 20.50 |
| Nanocomposite 1% | 10 | 16.22 | 0.91 | 14.14 | 18.30 |

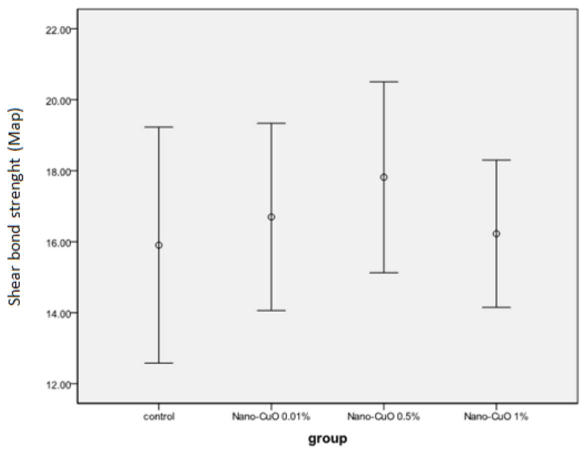


**Figure 1:** Comparison of shear bond strengths of four groups

**Table 2:** Comparison of S.mutans growth inhibition zone (millimeter)

| Control | N | Mean±SE | Median | Min | Max |
|---|---|---|---|---|---|
| | 10 | 6±0.00 | 6 | 6 | 6 |
| Nanocomposite 0.01% | 10 | 8±0.36 | 8 | 6 | 9 |
| Nanocomposite 0.5% | 10 | 9±0.29 | 9 | 8 | 10 |
| Nanocomposite 1% | 10 | 9.20±0.38 | 9 | 8 | 11 |

The results of Kruskal-Wallis test showed that there was a significant difference in the inhibition of S. mutans growth among four groups ($p< 0.001$). Pairwise comparison of antimicrobial effects, using Mann-Whitney test, showed that there is a significant difference between each of the three groups containing nanoparticles and the control group (Table 3). Among the three groups containing CuO nanoparticles, in pairwise comparison, there was no significant difference; however, the findings showed that the increase in the concentration of nanoparticles resulted in a greater inhibition zone of S. mutans growth (Figure 2).

## Discussion

Due to unique antimicrobial properties of metal nano particles, antibacterial and mechanical effects of CuO nanoparticles on orthodontic adhesives were investigated in this study. According to the results obtained by this study, although incorporating CuO nanoparticl-

**Table 3:** Pairwise comparison of shear bond strengths of three groups

| Group | Control | 0.01% | 0.5% | 1% |
|---|---|---|---|---|
| Control | — | 0.002* | 0.000* | 0.000* |
| 0.01% | 0.002* | — | 0.105 | 0.105 |
| 0.5% | 0.000* | 0.105 | — | 0.796 |
| 1% | 0.000* | 0.105 | 0.796 | — |

*Significant $p< 0.005$

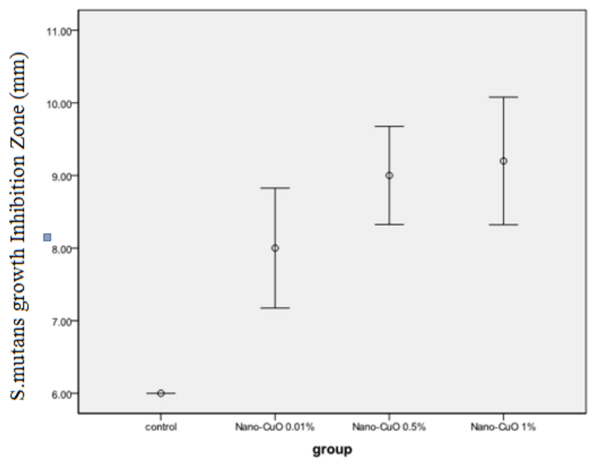


**Figure 2:** Comparison of S.mutans growth inhibition zoon in all four groups

es into adhesive in all three studied concentrations increased shear bond strength but it was not significant compared to control group. In all three concentrations of 0.01, 0.5 and 1.0 wt.%; adhesives containing CuO nanoparticles showed significant antimicrobial effects against S.mutans compared to control group. Increasing in the concentration of CuO nanoparticles did not result in increase of S.mutans inhibition zone statistically. Because nanoparticles are embedded in the matrix of cured adhesive, it seems that release of nanoparticles in the agar plate does not increase as the concentration of nanoparticles increases in the adhesive. Argueta *et al.* [12] incorporated copper nanoparticles into bonding agent. They report that the bonding agent, containing nanoparticles showed bactericidal effect against all three studied bacteria, including S.mutans. In addition, incorporating copper nanoparticles in 0.0100 wt.% concentration increased the shear bond strength significantly. [12] These results are in line with our study, in which in addition to antimicrobial effect against S.mutans, CuO nanoparticles increased shear bond strength of adhesive.

In our view, nanoparticles probably can act as nano-fillers and enhance shear bond strength of adhesive. It might happen within restriction of crack propagation through the nano-filler reinforced matrix of adhesive. Tabrez *et al.* [6] showed that ZnO and CuO nanoparticles had strong antimicrobial effect on microbial plaque growth and colonization, consistent with the results obtained in our study. Eshed *et al.* [13] evaluated the formation of microbial biofilm on teeth coated with CuO and ZnO nanoparticles using sonochemical method. They reported that CuO and ZnO nanoparticles decreased biofilm formation 70% and 80% respectively. [13] This result can probably be explained with anti-adhesion effect of nanoparticles. Poosti *et al.*, [14] studied shear bond strength and antimicrobial properties of orthodontic composite containing titanium oxide nanoparticles. They reported that there was no significant difference in the shear bond strength compared to control group, but antibacterial effects of the Nano-composite group showed significant difference both instantly and after 30 days. [14]

Akhavan *et al.* [15] reported that incorporating silver and hydroxyapatite nanoparticles to adhesive at a concentration of 10% reduced the shear bond strength, but it was not significant at lower concentrations of 1% and 5%. It seems that in low concentrations, incorporating nanoparticles probably does not interfere in light-cured composite polymerization. [15] It is reported that polymerization of adhesive might be affected in higher concentrations of nanoparticles. [12] This can be attributed to the fact that increase in concentration of some nanoparticles result in darker shades of adhesive and reduction of translucency which in turn could reduce light penetration depth and interfere complete adhesive curing. Several other studies verified the antimicrobial effects of nanoparticles, for example, Mirhashemi *et al.* [16] reported that incorporating chitosan and ZnO nanoparticles to orthodontic adhesives significantly improved the antimicrobial effects. Since the antimicrobial effect of nanoparticles is because of their very small size and high surface area to volume ratio and as a result of high surface free energy, rather than the nature of material, consistent results of different studies in antimicrobial effect of nanoparticles incorporated adhesives are not unexpected. [17] As a limitation of clinical usage of the result of this study, it is reported that CuO nanoparticles exhibit toxic effects. It is recommended that toxic effects of metal nanoparticles should be reduced to non-significant level through controlling nano particle diameter and surface modification. [18] Before clinical application, further clinical studies are needed to evaluate the probable cytotoxicity or tissue reactions of adhesives containing CuO nanoparticles.

## Conclusion

The results obtained from this study shows that Transbond XT adhesive (3M Unitek, USA) containing CuO nanoparticles in all three concentration of 0.01, 0.5 and 1.0 wt.% have antimicrobial properties and can inhibit the growth of S. mutans in the medium. Incorporating CuO nanoparticles in all three concentrations of 0.01, 0.5 and 1.0 wt.% has no negative effect on shear bond strength of Transbond XT adhesive(3M Unitek, USA).

## Conflict of Interest

There was no conflict of interest to declare.

## References

[1] Yudovin-Farber I, Beyth N, Weiss EI, Domb AJ. Anti-

bacterial effect of composite resins containing quaternary ammonium polyethyleneimine nanoparticles. J Nanoparticle Research. 2010; 12: 591-603.

[2] Kasraei S, Sami L, Hendi S, Alikhani MY, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silverand zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. Restor Dent Endod. 2014; 39: 109-114.

[3] Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009; 25: 206-213.

[4] Borzabadi-Farahani A, Borzabadi E, Lynch E. Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. Acta Odontol Scand. 2014; 72: 413-417.

[5] Allaker RP. The use of nanoparticles to control oral biofilm formation. J Dent Res. 2010; 89: 1175-1186.

[6] Khan ST, Ahamed M, Al-Khedhairy A, Musarrat J. Biocidal effect of copper and zinc oxide nanoparticles on human oral microbiome and biofilm formation. Materials Letters. 2013; 97: 67-70.

[7] Bishara SE, Ostby AW. White spot lesions: formation, prevention, and treatment. Semin Orthod. 2008; 14: 174–182.

[8] Ramazanzadeh B, Jahanbin A, Yaghoubi M, Shahtahmassbi N, Ghazvini K, Shakeri M, Shafaee H. Comparison of Antibacterial Effects of ZnO and CuO Nanoparticles Coated Brackets against Streptococcus Mutans. J Dent (Shiraz). 2015; 16: 200-205.

[9] Horowitz AM. A report on the NIH Consensus Development Conference on Diagnosis and Management of Dental Caries ThroughoutLife. J Dent Res. 2004; 83 Spec No C: C15-C17.

[10] Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. J Periodontol. 1996; 67: 78-85.

[11] Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. Am J Orthod Dentofacial Orthop. 2010; 138: 188-194.

[12] Argueta-Figueroa L, Scougall-Vilchis RJ, Morales-Luckie RA, Olea-Mejía OF. An evaluation of the antibacterial properties and shear bondstrength of copper nanoparticles as a nanofiller in orthodontic adhesive. Aust Orthod J. 2015; 31: 42-48.

[13] Eshed M, Lellouche J, Matalon S, Gedanken A, Banin E. Sonochemical coatings of ZnO and CuO nanoparticles inhibitStreptococcus mutans biofilm formation on teeth model. Langmuir. 2012; 28: 12288-12295.

[14] Poosti M, Ramazanzadeh B, Zebarjad M, Javadzadeh P, Naderinasab M, Shakeri MT. Shear bond strength and antibacterial effects of orthodontic composite containing TiO2 nanoparticles. Eur J Orthod. 2013; 35: 676-679.

[15] Akhavan A, Sodagar A, Mojtahedzadeh F, Sodagar K. Investigating the effect of incorporatingnanosilver/ nanohydroxyapatite particles on the shear bond strength of orthodontic adhesives. Acta Odontol Scand. 2013; 71: 1038-1042.

[16] Mirhashemi AH, Bahador A, Kassaee MZ, Daryakenari GH, Ahmad Akhondi MS, Sodagar A. Antimicrobial Effect of Nano-Zinc Oxide and Nano-Chitosan Particles in Dental Composite Used in Orthodontics. J Med Bacteriol. 2013; 2: 1-10.

[17] Jandt KD, Sigusch BW. Future perspectives of resin-based dental materials. Dent Mater. 2009; 25: 1001-1006.

[18] Chang YN, Zhang M, Xia L, Zhang J, Xing G. The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials. 2012; 5: 2850–2871.