THE NEW ARMENIAN MEDICAL JOURNAL
Volume20 (2026), Issue 2, p. 23-29

DOI: https://doi.org/10.56936/18290825-2026.20v.2-23

IN-VITRO EVALUATION OF NANOPARTICLE-REINFORCED ORTHODONTIC ADHESIVES FOR ENHANCED SHEAR BOND STRENGTH AND ANTIMICROBIAL ACTIVITY

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Received 2.12.2025; Accepted for printing 14.05.2026

Abstract

Introduction: *White spot lesions and bracket bond failures remain common complications of fixed orthodontic therapy. Conventional orthodontic adhesives lack sustained antimicrobial activity, allowing biofilm formation by Streptococcus mutans. Incorporation of antimicrobial nanoparticles into adhesive systems may enhance biological performance without compromising mechanical properties.*

Material and Methods: *Eighty extracted human premolars were randomly allocated into four groups (n = 20): Group I (control adhesive), Group II (1% titanium dioxide nanoparticles), Group III (1% silver nanoparticles), and Group IV (hybrid: 0.5%* TiO_2 *+ 0.5% Ag nanoparticles). Shear bond strength was evaluated using a universal testing machine, while antimicrobial efficacy against Streptococcus mutans was assessed using a colony-forming unit assay. Adhesive remnant index scores were recorded to determine failure patterns.*

Results: *Mean shear bond strength values were 21.45 ± 2.31 MPa (control), 22.15 ± 2.84 MPa (*TiO_2*), 17.85 ± 3.15 MPa (Ag), and 20.95 ± 2.62 MPa (hybrid). The silver nanoparticle group demonstrated significantly reduced bond strength compared with the control (*$p < 0.05$*), whereas the* TiO_2 *and hybrid groups showed no significant differences. Antimicrobial testing revealed substantial reductions in Streptococcus mutans growth in the silver (88.4%) and hybrid (82.1%) groups compared with the control (*$p < 0.001$*).*

Conclusion: *Hybrid incorporation of titanium dioxide and silver nanoparticles provides effective antimicrobial activity while maintaining clinically acceptable shear bond strength. This approach represents a promising strategy for reducing white spot lesion development during fixed orthodontic treatment.*

Keywords: *orthodontic adhesives, nanoparticles, shear bond strength, antimicrobial activity, silver, titanium dioxide, white spot lesions*

Cite This Article as:
Alfawzan A.A., Alam M.K., Hajeer M.Y. (2026). In-Vitro Evaluation of Nanoparticle-Reinforced Orthodontic Adhesives for Enhanced Shear Bond Strength and Antimicrobial Activity; The New Armenian Medical Journal, vol.20 (2), 23-29; DOI: https://doi.org/10.56936/18290825-2026.20v.2-23

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Introduction

Ideal orthodontic adhesive has been an outstanding theme in the research of dental materials over decades. Although bracket retention to enamel by mechanical methods has been standardized successfully using acid-etching technology and composite resin, the biological interface of the adhesive and oral environment is a significant issue [*Matasa C, 1989; Bishara S et al., 2008*]. Fixed orthodontic appliances cause a complicated condition that hinders oral health causing the buildup of bacteria plaque and determining it to be a major risk factor in the development of White spot lesions [*Ogaard B, 2008*].

White spot lesions are the initial clinical indicators of carious demineralization and they appear in up to 50% of the orthodontic patients, and in many cases, during the first month of therapy [*Tufekci E et al., 2011*]. This transition zone between the enamel and adhesive resin has been specifically vulnerable to colonization by cariogenic pathogens, most of which are Streptococcus mutans and Lactobacillus species that ferment carbohydrates to form organic acid [*Gorelick L et al., 1982*]. This has led to high demand of orthodontic adhesives with bioactive characteristics that are able to inhibit colonization of bacteria without compromise to mechanical integrity [*Altmann A et al., 2016*].

Traditionally, the glass ionomer cements that release fluoride were introduced to fight demineralization. Nevertheless, they have lower mechanical behavior as they tend to have poorer shear bond strength than composite resins, which results in increased failure rates of brackets [*Rogers S et al., 2010*]. This mechanical and biological efficacy dichotomy has motivated the investigation in nanotechnology [*Allaker R, 2010*]. With an at least one-dimensional dimension smaller than 100 nm, nanoparticles (NPs) have specific physicochemical properties because of the large surface-area-volume ratio [*Hernandez-Sierra J et al., 2008*].

Silver nanoparticles (AgNPs) have received a lot of interest as they possess a wide-spectrum antimicrobial activity. AgNPs cause cell wall disruption and influencing of the bacterial cells DNA replication [*Ahn S et al., 2009*]. Nonetheless, at very high levels of silver the extent of conversion during polymerization is influenced and esthetic discolouration (greying) of the adhesive also results [*Moreira D et al., 2015*]. On the other hand, Titanium Dioxide nanoparticles (TiO_2 NPs) are one that is biocompatible, chemically stable and shows photocatalytic antimicrobial effects [*Sodagar A et al., 2017*]. Additionally, TiO_2 NPs have also been reported to serve as an inorganic filler which could be used to strengthen the polymer and could result in the improvement of fracture toughness and bond strength [*Elsaka S et al., 2011*].

Although these nanoparticles have been investigated individually, lack of studies on the hybridization of metal and metal-oxide nanoparticles in orthodontic adhesives is eminent despite the fact these nanoparticles have been studied on their own. The combination of mechanical reinforcement of TiO_2 and potent antimicrobial effect of Ag could be a hybrid approach, which would reduce the disadvantages of using high concentrations of one agent [*Garcia-Contreras R et al., 2015*].

Thus, this research was designed to combine and analyze a new experimental orthodontic adhesive in which a hybrid of TiO_2, and Ag nanoparticles are reinforced. The null hypothesis was that there would be no significant difference in shear bond strength or antimicrobial effects of the incorporation of these nanoparticles on the antimicrobial effects of a conventional adhesive that did not incorporate these nanoparticles.

Materials and Methods

Study Design and Ethical Considerations: Eighty human maxillary first premolars, extracted for orthodontic therapeutic purposes, were collected.

Sample Size Calculation: Based on a pilot study (effect size f=0.4, α=0.05, power 1-β=0.80), a sample size of n=18 per group was calculated. To account for potential sample loss during testing, n=20 per group was selected.

Sample Selection and Preparation: Inclusion criteria involved teeth with intact buccal enamel, no cracks, no caries, and no prior chemical pretreatment. Teeth with fluorosis, hypoplasia, or visible enamel defects were excluded. The teeth were cleaned of soft tissue debris, stored in 0.1% thymol solution for one week, and then transferred to distilled water. The roots were embedded in cold-curing acrylic resin using cylindrical molds (20 *mm* × 25 *mm*) so that the buccal surface was perpendicular to the mold base. The buccal surfaces were polished with pumice and water using a rubber cup for 10 seconds, rinsed, and dried.

Preparation of Nanocomposites: A commercially available orthodontic light-cure adhesive (Transbond XT, 3M Unitek) served as the control and base material. Silver nanoparticles (AgNPs, greater than 50 *nm*, spherical) and Titanium Dioxide nanoparticles (TiO_2 NPs, greater than 25 *nm*, anatase phase) were obtained from Sigma-Aldrich.

Four experimental groups were established:

- Group I (Control): Unmodified Transbond XT.
- Group II: Transbond XT + 1% (w/w) TiO_2 NPs.
- Group III: Transbond XT + 1% (w/w) AgNPs.
- Group IV (Hybrid): Transbond XT + 0.5% TiO_2 NPs + 0.5% AgNPs.

The nanoparticles were weighed using a precision electronic balance and incorporated into the composite paste in a dark room to prevent premature polymerization. Mixing was performed using a centrifugal planetary mixer (SpeedMixer DAC 150) at 3000 *rpm* for 2 minutes to ensure homogenous dispersion.

Bonding Procedure: The buccal enamel was etched with 37% phosphoric acid gel for 30 seconds, rinsed for 15 seconds, and air-dried until chalky white. A thin coat of primer (Transbond XT Primer) was applied and light-cured for 10 seconds. Stainless steel premolar brackets (0.022-slot, 3M Unitek) were bonded using the designated adhesive for each group. Excess adhesive was removed with an explorer, and the adhesive was light-cured for 20 seconds (10s mesial, 10s distal) using an LED curing light (1200 mW/cm^2). All samples underwent thermocycling (500 cycles, 5°C to 55°C, dwell time 30*s*) to simulate oral aging.

Shear bond strength testing (n=10/group): Specimens were secured in the lower jaw of a Universal Testing Machine (Instron Model 5566). A shearing blade was positioned at the bracket-enamel interface. A compressive load was applied at a crosshead speed of 1 mm/min until bond failure occurred. The maximum load (Newtons) was recorded and divided by the bracket base area (10.6 mm^2) to calculate shear bond strength in Megapascals (*MPa*).

Adhesive remnant index evaluation: Following debonding, the enamel surfaces were examined under a stereomicroscope (10× magnification) to classify the failure mode using the adhesive remnant index score:

- Score 0: No adhesive left on the tooth.
- Score 1: Less than half of the adhesive left on the tooth.
- Score 2: More than half of the adhesive left on the tooth.
- Score 3: All adhesive left on the tooth.

Antimicrobial Assessment (n=10/group): The direct contact test was used to evaluate activity against Streptococcus mutans (ATCC 25175). Composite discs (5 *mm* × 2 *mm*) of each group were fabricated and sterilized under UV light. The discs were placed in 24-well plates containing Brain Heart Infusion broth inoculated with 1×10^6 *CFU/mL* of S. mutans. The plates were incubated at 37°C for 24 hours. Following incubation, 100 μL of the suspension was plated onto blood agar plates. After 24 hours, the number of Colony forming units was counted.

Statistical Analysis: Data were analyzed using SPSS version 25.0. Normality was assessed using the Shapiro-Wilk test. Shear bond strength and Colony forming units data were analyzed using One-way ANOVA followed by Tukey's post-hoc test. adhesive remnant index scores were analyzed using the Kruskal-Wallis test. A significance level of $p<0.05$ was established.

Results

Shear bond strength: The descriptive statistics for the shear bond strength values are presented in Table 1. The Shapiro-Wilk test indicated a normal distribution of data. One-way ANOVA revealed significant differences among the groups ($F=14.22$, $p<0.001$).

The Control group (Group I) exhibited a mean shear bond strength of 21.45±2.31 *MPa*. The highest mean shear bond strength was observed in Group

Table 1.

Shear bond strength (*MPa*) of the study groups.

	Composition	N	Mean	SD	Min	Max
I	Control (0% NPs)	10	21.45	2.31	18.2	25.1
II	1% TiO_2 NPs	10	22.15	2.84	17.9	26.5
III	1% AgNPs	10	17.85	3.15	13.4	22.0
IV	Hybrid	10	20.95	2.62	16.8	24.8

TABLE 2.

Frequency of adhesive remnant index scores.

	Score 0 n(%)	Score 1 n(%)	Score 2 n(%)	Score 3 n(%)	Total
I	1 (10%)	4 (40%)	4 (40%)	1 (10%)	10
II	0 (0%)	5 (50%)	4 (40%)	1 (10%)	10
III	2 (20%)	5 (50%)	3 (30%)	0 (0%)	10
IV	1 (10%)	4 (40%)	4 (40%)	1 (10%)	10

II (TiO_2 1%), recording 22.15±2.84 *MPa*. The Hybrid group (Group IV) showed a mean shear bond strength of 20.95±2.62 *MPa*. Tukey's post-hoc analysis indicated no statistically significant difference between Groups I, II, and IV ($p>0.05$). However, Group III (Ag 1%) demonstrated the lowest bond strength at 17.85±3.15 *MPa*, which was significantly lower than all other groups ($p<0.05$).

Adhesive Remnant Index: The frequency distribution of Adhesive remnant index scores is summarized in Table 2. The Kruskal-Wallis test showed no significant differences in the distribution of failure modes among the groups ($p=0.342$). The predominant failure mode across all groups was cohesive failure within the adhesive (Score 1 and 2), indicating that the bond to the enamel was generally stronger than the internal strength of the adhesive or the bond to the bracket base.

Antimicrobial Activity: The results of the colony count assay against S. mutans are presented in Table 3. ANOVA revealed highly significant differences among the groups ($p<0.001$). The Control group (Group I) showed the highest bacterial growth (148.5×10^5 *CFU/mL*). Group II (TiO_2) showed a moderate reduction in bacterial growth compared to the control (112.3×10^5 *CFU/mL*). Group III (Ag 1%) exhibited the most potent antimicrobial activity (17.2×10^5 *CFU/mL*), representing an 88.4% reduction compared to the control. The Hybrid Group IV also demonstrated high efficacy (26.5×10^5 *CFU/mL*), representing an 82.1% reduction.

TABLE 3.

Mean colony forming units (CFU) of *S. mutans* ($\times10^5$ *CFU/mL*).

	Mean CFU ($\times10^5$)	SD	Reduction (%) vs Control	P-value (vs Control)
I	148.5	12.4	-	-
II	112.3	9.8	24.3%	<0.05
III	17.2	4.1	88.4%	<0.001
IV	26.5	5.5	82.1%	<0.001

DISCUSSION

The current research paper investigated the possibility of the creation of bioactive orthodontic adhesive by adding Titanium Dioxide (TiO_2) and Silver (Ag) nanoparticles. The findings partly gave rise to the null hypothesis rejection; although the nanoparticles had great impact on antimicrobial properties, their impact on shear bond strength differed among different compositions. In particular, the hybrid group showed a positive balance, and the mechanical integrity of the group was not compromised but offered a high level of antimicrobial activity.

Shear Bond Strength Analysis: Orthodontically and masticaturally, an adhesive should be able to withstand forces. Reynolds had proposed that most clinical requirements in the 6-8 *MPa* bond strength is adequate [*Reynolds I, 1975*], but today most composites attain 15-25 *MPa*. In this experiment, all the groups including the nanoparticle-modified ones surpassed the minimum clinical requirement.

When 1 percent TiO_2 (Group II) was added, there was a minimal, but not statistically significant rise in shear bond strength over the control. This is in accordance with other researchers who indicated that metal oxide nanoparticles could serve as inorganic fillers that would decrease the shrinkage in polymerization and strengthen the resin matrix [*Poosti M et al., 2013*]. The TiO_2 particles must have enhanced the load transfer mechanism in the composite.

Group III (1% AgNPs) on the other hand showed a large decrease in shear bond strength (17.85 MPa). This decreasing tendency is congruent with a so-called cluster effect of nanoparticles literature [*Cheng L et al., 2012*]. With increasing concentration, the AgNPs have a tendency of agglomerating, which forms the points of stress concentration that undermine the polymer matrix. Moreover, the transparency of the silver nanoparticles might block the transfer of the light in the process of photo-polymerization, which may reduce the extent of the transformation of the resin monomers [*Yamamoto K et al., 1996*].

The Hybrid Group (IV) was able to overcome this problem. The adhesive was able to sustain the same shear bond strength (20.95 *MPa*) that was statistically comparable to the control by reducing the percentage of silver to 0.5% and adding 0.5%

TiO_2. The TiO_2 must have counteracted the structural drawbacks that the silver had brought in, which can be substantiated by the recent findings of hybrid nanocomposites [*Akhavan A et al., 2013*].

Antimicrobial Efficacy: The antimicrobial test indicated the active effectiveness of silver. In group III and IV, there was a drastic decrease in S. mutans growth (88.4% and 82.1%, respectively). The AgNPs are multimodal, i.e., silver ions (Ag^+) escaping particles interfere with metabolic pathways by binding to thiol groups in bacterial enzymes, and enter the cell wall by interacting with DNA [*Morones J et al., 2005*]. This mechanism of contact-killing is the essential one in the prevention of biofilm formation around the bracket bases.

Group II (TiO_2 alone) recorded a slight decrease of 24.3%. TiO_2 antimicrobial works are mainly photocatalytic; under UV light, TiO_2 can produce reactive oxygen species, which destroy bacterial membranes [*Chorianopoulos N et al., 2011*]. Nevertheless, at intraoral where UV exposure is low, its antimicrobial capacity is less than that of silver, which is the reason behind the reduced effectiveness in Group II.

The Hybrid group showed that a low concentration of 0.5% Ag may be enough to cause potent antimicrobial effect and postulated that there will be non-linear dose-response relationship since even lower concentrations will result in a growth-arresting floor in bacteria [*Degrazia F et al., 2016*].

Clinical Implications: The formation of White Spot Lesions is a medico-legal and aesthetic issue in orthodontic practices [*Chapman J et al., 2010*]. The hybrid adhesive had the capability of reducing the colonization of S. mutans by greater than 80 percent which could be translated clinically to a dramatically less incidence of demineralization. Moreover, since the hybrid group ensured a high bond strength, there would be no need to compromise between appliance retention and preventive health as it would be in glass ionomer cements [*Rix D et al., 2001*].

Limitations: This research was an in-vitro research. It was not a complete simulation of the complicated oral environment, which incorporates variations in pH and salivary enzymes, and a variety of microbial flora. The kinetics of long-term release of the silver ions were not tested, and it cast doubts on the longevity of the antimicrobial effect. Also, although the adhesive remnant index scores showed safe debonding, the enamel surface roughness analysis (profilometry) was not performed. Future research ought to concentrate on the in-vivo models and aging simulations over the long term [*Elnafar, A. et al., 2014*].

CONCLUSION

The following inferences can be made within the constraints of the study that was conducted in-vitro:

- Addition of 1 percent Silver nanoparticles greatly improves antimicrobial property but reduces the shear bond strength of the orthodontic adhesive.
- Inclusion of Titanium dioxide nanoparticles at 1 percent ensures continuity in bond strength but it has poor antimicrobial protection properties without the presence of UV light.
- The best trade off is a hybrid nanocomposite with 0.5% TiO_2 and 0.5% Ag nanoparticles, with a strong antimicrobial effect on S. mutans as well as high shear bond strength that either can be compared to traditional adhesives.
- The hybrid adhesive was a potential item that will help to decrease the risks of White Spot Lesions in fixed orthodontic treatment.

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THE NEW ARMENIAN MEDICAL JOURNAL
Volume20 (2026). Issue 2

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THE NEW ARMENIAN MEDICAL JOURNAL

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