Iran J Orthod. 2026 June; 21(1):e1305. doi:10.22034/ijo.2026.2069390.1305.
Published online 2026 June 1 Research Article
Original Article **Open Access**

Physicochemical Properties and White Spot Lesion Inhibition of an Orthodontic Adhesive Containing Zinc Oxide Nanoparticles: An In Vitro Study

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Received: 2025 August 19; **Revised:** 2025 November 03; **Accepted:** 2026 May 19

Abstract

Aim

This study aimed to experimentally synthesize a homogeneous orthodontic adhesive containing zinc oxide (ZnO) nanoparticles and to evaluate its physicochemical properties and its indirect effects on caries-related fluorescence and demineralization associated with *Streptococcus mutans* biofilm activity.

Methods

This in vitro study evaluated 30 extracted premolars that were subjected to bracket bonding with three types of adhesives: (I) experimentally synthesized adhesive without ZnO (basic/control), (II) experimentally synthesized adhesive containing 1.6 wt% ZnO nanoparticles (optimized), and (III) American Orthodontics (AO) adhesive (Gold standard). To assess the antibacterial activity of the adhesives, the teeth were immersed in *Streptococcus mutans* suspension after bracket bonding, and DIAGNOdent was used to assess the formation of white spot lesions (WSLs) at different time intervals (0,7,14,21 and 28 days). The shear bond strength (SBS) and adhesive remnant index (ARI) scores, as well as the water sorption and water solubility of the adhesives, were evaluated. Data were analyzed using one-way ANOVA, repeated-measures ANOVA, Tukey, Kruskal-Wallis, and t-tests (α=0.05).

Results

The optimized adhesive containing ZnO nanoparticles demonstrated clinically acceptable SBS values comparable to those of the control and AO adhesives ($P = 0.086$). DIAGNOdent values were significantly lower in the optimized adhesive group compared with the basic and AO adhesives at all time intervals ($P < 0.001$), indicating reduced caries-related fluorescence and demineralization potential. Water sorption and water solubility of the optimized and basic adhesives were within the acceptable range according to ISO 4049 and were significantly lower than those of the AO adhesive ($P < 0.05$). Zn ion release from the optimized adhesive remained substantially below reported cytotoxic thresholds.

Conclusion

The experimentally synthesized orthodontic adhesive containing 1.6 wt% ZnO nanoparticles demonstrated favorable physicochemical properties and lower DIAGNOdent values associated with reduced cariogenic activity around orthodontic brackets. In addition, Zn ion release remained well below reported cytotoxic levels. Further microbiological and clinical studies are required to confirm the long-term antimicrobial efficacy and clinical performance of this material.

Keywords: Dental bonding, Nanoparticles, Orthodontic brackets, Shear strength.

Background

Orthodontic bracket bonding and the installation of archwires, bands, and other

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attachments create an imbalance in the oral environment, thereby promoting the accumulation of dental plaque and cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacilli*. The presence of orthodontic appliances in the oral cavity impairs the optimal function of oral self-cleaning mechanisms, such as the salivary flow and muscle movements, and complicates oral hygiene practice, resulting in increased demineralization and decreased remineralization. Bacterial activity in dental biofilm attached to the tooth surface creates an acidic environment, which results in the dissolution of enamel prisms and formation of an irregular enamel surface with a chalky white appearance, known as the white spot lesion (WSL) (1–4). WSLs can develop within 4 weeks after the onset of orthodontic treatment, and mainly affect the buccogingival and labial surfaces of maxillary lateral incisor and canine teeth, mandibular canine teeth, and mandibular and maxillary premolars and molars (5–7). Removal of dental plaque and calculus alone is not sufficient for treating WSLs, because minerals in the saliva can only perform superficial remineralization, and deep lesions require additional remineralizing agents. Thus, the best strategy to control WSLs is to prevent their occurrence. Also, in case of development, they should be promptly remineralized to prevent their progression (5). Otherwise, they can lead to the formation of cavitated carious lesions and cause esthetic, periodontal, and functional problems (8).

Fluoride toothpastes, fluoride mouthwashes, chlorhexidine, fluoride varnishes, and casein phosphopeptide amorphous calcium phosphate have been suggested to control WSLs. However, despite their optimal efficacy, the success of all these strategies depends heavily on patient cooperation. To overcome this problem, researchers considered adding antibacterial agents to the formulations of orthodontic adhesives. Several antibacterial agents have been tested for incorporation into orthodontic adhesives and composites to minimize biofilm formation around orthodontic brackets (9–11).

Zinc oxide (ZnO) is a biocompatible metal oxide with favorable antimicrobial properties (12,13). ZnO nanoparticles have superior antibacterial properties compared with larger particles (14). Several studies evaluated the addition of ZnO nanoparticles to dental resin materials to confer antibacterial activity. Some studies added ZnO nanoparticles to commercially available adhesives (11,15,16) or commercially available orthodontic composites (10), while some others formulated ZnO nanoparticles in the composition of adhesives or composites (17–20). Composite resins containing ZnO have shown improved antibacterial activity (14). Also, it was demonstrated that adding small amounts of ZnO nanoparticles (1 wt%) to composite resin did not significantly affect its mechanical properties or bond strength. ZnO nanoparticles have strong antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and prevent biofilm formation even at low concentrations (16). Recent investigations also reported significant antimicrobial activity of ZnO nanoparticles added to dental adhesives or composite resins (9,18,21,22). It appears that adding an antibacterial agent to orthodontic adhesive, which is applied to the tooth surface and around the bracket margins, may effectively prevent bacterial colonization and the formation of WSLs. Nonetheless, it should be noted that adding antibacterial agents to commercial adhesives can cause particle agglomeration or deposition due to non-uniform dispersion, as previous studies did not report a homogeneous distribution of added particles (11,15). Also, commercially available adhesives may have undisclosed ingredients that interfere with ZnO nanoparticles and their antibacterial activity (20).

The presence of 2-hydroxyethyl methacrylate (HEMA) in the formulation of orthodontic adhesives is not justified due to its hydrophilic nature, because in orthodontic treatment, brackets are bonded to the enamel surface, which is hydrophobic. Also, the hydrophilicity of HEMA can enhance bacterial adhesion and neutralize the antimicrobial effects of ZnO. Another shortcoming of HEMA is that it decreases the mechanical properties of the adhesive (23).

It has been demonstrated that adding an inorganic filler, such as ZnO, to composite resins can enhance their dispersion when silanized before incorporation. Optimal dispersion of filler nanoparticles in the resin matrix results in molecular-level mixing. If appropriate coupling is also used, the mechanical properties significantly increase. Improper surface modification of fillers can lead to filler agglomeration, poor attachment to the matrix, and reduction of mechanical properties (24,25).

Considering all the above and the significance of prevention of WSLs, this study aimed to experimentally synthesize a new formulation of an orthodontic adhesive containing silanized ZnO nanoparticles for bracket bonding to enamel and assess its antimicrobial activity, Zn ion release (for assessment of its cytotoxicity), and mechanical and physicochemical properties. Ajeli Bazkiayi et al (26) synthesized an experimental adhesive devoid of HEMA. He added ZnO nanoparticles to the adhesive

to take advantage of their antimicrobial properties. He found that adding ZnO nanoparticles prevented biofilm formation with no adverse effects on the physicochemical properties, such as bond strength, degree of conversion, or hardness of adhesive. However, the water sorption and solubility of the experimental adhesive were higher than those of Transbond XT (Gold standard). Water sorption by the adhesive is not favored, as it can lead to early degradation. Also, increased solubility can comprise adhesive durability, strength, and antibacterial activity, and increase the release of toxic monomers.

Methods

This in vitro study evaluated three orthodontic adhesives using extracted human premolars. The teeth had been extracted for orthodontic purposes and were disinfected and stored in saline solution until use. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1403.070).

Sample Size

To determine the appropriate sample size, specific parameters were considered. The study aimed for a type I error (α) of 0.05 and a type II error (β) of 0.2, which corresponds to a test power of 80%. The required sample size was calculated using the following formula:

$n=2(z\ 1-\alpha/2 + z\ 1-\beta\ )2\ \sigma\ 2\ /\ (\mu1 - \mu2)2$

were

(n) = number of samples per group

(z 1-α/2) = standard normal deviation for a two-tailed test at α = 0.05 (1.96)

(z 1-β) = standard normal deviation for 80% power (0.84)

(σ) = standard deviation (from literature or pilot study)

(μ1 - μ2) = expected difference between group means (effect size)

The mean and standard deviation values were obtained from the literature (26).

Thirty extracted premolars were used for the SBS test, and another 30 extracted premolars were used for white spot lesion/demineralization assessment, with 10 specimens allocated to each group in both experiments. For water sorption and water solubility evaluations, separate disc-shaped specimens were fabricated, with 3 discs prepared for each group.

Synthesis of Experimental Adhesive

Three types of adhesives were evaluated in this study: (I) an experimentally synthesized adhesive without ZnO (basic), (II) an experimentally synthesized adhesive containing ZnO nanoparticles (optimized), and (III) American Orthodontics (AO) conventional orthodontic adhesive (Gold standard) (American Orthodontics, USA).

The basic adhesive without ZnO (6 g) was synthesized using 51.5% bis-GMA (Sigma Aldrich, USA), 24.7% TEGDMA (Sigma Aldrich, USA), 23.6% TMPTA (Sigma Aldrich, USA), 0.1% CQ (Merck, Germany), and 0.1% NN dimethyl aminoethyl methacrylate (Merck, Germany). The ingredients mentioned above were weighed, mixed, and stirred at 30°C in the dark for 1 hour.

The optimized adhesive containing ZnO (6 g) was synthesized using the ingredients mentioned above. Next, active TEGDMA and TMPTA diluents were mixed, and 0.1 g ZnO (1.6 wt%; 10–30 nm; Pishgaman Nano Mavad Iranian, Iran) was silanized (γ-methacryloyloxypropyltrimethoxysilane) and added to the solution. The mixture was ultrasonicated for 10 minutes. Next, bis-GMA, CQ, and amine were added to the mixture, which was stirred on a magnetic stirrer (Pol Ideal Pars, Iran) at 30°C for 1 hour in the dark. To prevent nanoparticle aggregation, a two-step method was employed: surface modification using a silane coupling agent followed by ultrasonic dispersion. The silane coupling agent provided steric stabilization by forming covalent bonds between nanoparticles and the polymer matrix, while ultrasonication ensured uniform dispersion by breaking up agglomerates. This combination was chosen to achieve both chemical compatibility and stable particle distribution in the composite (26,32).

White Spot Lesion Assessment

The 30 extracted teeth were cleaned using a prophylaxis brush and pumice paste, followed by immersion in 10% Betadine solution for 1 hour to reduce microbial contamination. The teeth were then rinsed with deionized water (3×) and stored in deionized water for 24 hours.

All specimens were then placed in sterile capped test tubes (100 × 16 mm) containing 3 mL of brain heart infusion (BHI) broth (Conda, Spain) and autoclaved before inoculation. The samples were incubated at 37°C for 24 hours to confirm sterility, and any contaminated samples were excluded.

The teeth were coded and randomly allocated into three groups (n=10 per group) using a computer-generated randomization method (Excel). All procedures were performed under aseptic conditions in a Class II biosafety cabinet (Sina Tajhiz, Iran).

The buccal enamel surfaces were etched with 37% phosphoric acid for 30 seconds, rinsed with

deionized water for 60 seconds, and dried with oil- and moisture-free air spray. In each group, the assigned adhesive system (three different systems: I, II, and III) was applied as a thin layer on the etched enamel surface, gently air-thinned (15 cm distance), and light-cured for 10 seconds using an LED curing unit (Woodpecker, China).

Three different orthodontic composite resins (I, II, and III) were then applied in standardized amounts to the base of the brackets (Ortho Organizer, USA). The brackets were positioned at the middle third of the buccal surface and seated with gentle pressure using an explorer. Excess adhesive was removed, and final curing was performed from mesial, distal, gingival, and occlusal directions for 10 seconds each.

After bonding, 3 mL of sterile BHI broth was added to each tube, and specimens were incubated at 37°C for 24 hours to confirm the absence of contamination before bacterial inoculation.

Microbial preparation

Streptococcus mutans (ATCC 35668, PTCC 1683) was obtained and cultured on BHI agar (Conda, Spain) using the streak plate method and incubated at 37°C for 48 hours. A bacterial suspension was prepared in sterile BHI broth and adjusted to 0.5 McFarland standard (OD600 = 0.08–0.1), corresponding to approximately 1.5×10^8 CFU/mL.

Infection model and incubation

Each specimen was immersed in the standardized bacterial suspension. Tubes were incubated at 37°C for 28 days. The culture medium was refreshed weekly to maintain bacterial viability. Tubes were vortexed daily to prevent sedimentation, and turbidity was monitored.

Outcome measurement

Enamel demineralization was assessed using DIAGNOdent (KaVo Dental GmbH, Germany) at baseline (0), 7, 14, 21, and 28 days. The device was calibrated before each measurement. Specimens were handled under aseptic conditions, and measurements were obtained from the mesial and distal areas adjacent to the bracket at the middle third of the buccal surface. The higher reading was recorded for analysis. All measurements were performed by a single calibrated operator to ensure measurement consistency, and the values were recorded by a second operator.

Shear Bond Strength (SBS) Testing

Thirty sound human premolars extracted for orthodontic purposes were collected, cleaned, and stored in saline solution at room temperature. The teeth were disinfected by immersion in 1% chloramine solution for 72 hours (26,32). Next, they were polished using a low-speed handpiece and fluoride-free pumice slurry for 10 seconds, rinsed thoroughly, and air-dried. The teeth were then mounted in auto-polymerizing acrylic resin using standardized glass molds.

All specimens were coded, randomly allocated into three groups, and subjected to bracket bonding according to the assigned adhesive group, as previously described. The specimens were then stored in distilled water for 24 hours and subjected to 500 thermocycles between 5°C and 55°C (26).

The shear force required for bracket debonding was measured using a universal testing machine (STM-20; Santam, Iran) equipped with a 50-kg load cell (DBBP-50; BONGSHIN, Korea) at a crosshead speed of 1 mm/min. Shear bond strength (SBS) was calculated using the following formula (27):

SBS (MPa) = Debonding force (N) / bracket base surface area (mm^2)

Adhesive Remnant Index (ARI) Score

To determine the ARI score, the debonded specimens were inspected under a stereomicroscope (ZSX9, Olympus, Japan) at ×10 magnification, and the ARI score was determined as follows (28):

Score 0: no adhesive remaining on the surface

Score 1: less than 50% of adhesive remaining on the surface

Score 2: more than 50% of adhesive remaining on the surface

Score 3: almost all adhesive remaining on the surface

Water Sorption and Water Solubility

Nine disc-shaped specimens (6 mm in diameter and 1.5 mm in thickness) were fabricated from the three adhesive groups (n = 3 per group): basic adhesive, optimized ZnO-containing adhesive, and AO gold-standard adhesive. Immediately after fabrication, the discs were placed in a desiccator containing fresh silica gel and stored at 37°C for 22 hours, followed by 2 hours at 25°C. The discs were then weighed using a digital balance (Sartorius, Germany) with an accuracy of 0.0001 g. This cycle was repeated until a constant mass was achieved, and the obtained value was recorded as the initial mass (m1). The diameter and thickness of each disc

were measured using a digital caliper (Mitutoyo, Japan), and the specimen volume (V) was calculated in mm^3. The discs were subsequently immersed in 10 mL of distilled water at 37°C. At 24 hours and after 7, 14, and 28 days, the specimens were removed from the water, rinsed with distilled water, gently dried with absorbent paper, air-dried for 15 seconds on each surface, and weighed to obtain m2. After 28 days, the specimens were reconditioned in the desiccator at 37°C until a constant mass was reached (m3)(5 days). Water sorption and water solubility were calculated according to ISO 4049 using the following formulae (15,17):

$$\text{Water sorption } (\mu g/mm^3) = (m2 - m3)/V$$

$$\text{Water solubility } (\mu g/mm^3) = (m1 - m3)/V$$

Zn Ion Release

After tooth sterilization and bracket bonding with the optimized adhesive containing ZnO nanoparticles, the teeth were placed in three sterile test tubes containing 30 mL of artificial saliva for 24 hours, 3 days, and 5 days. The solutions were then sent to a laboratory for inductively coupled plasma testing (ZSX9; Olympus, Tokyo, Japan).

Statistical Analysis

Normality of the data was evaluated using the Shapiro-Wilk test. Homogeneity of variances was analyzed using Levene's test. Accordingly, the three groups were compared regarding the SBS, antibacterial activity, water solubility, and water sorption using ANOVA followed by pairwise comparisons with post hoc Tukey tests (for antibacterial activity and water sorption). The Kruskal-Wallis test was used to compare the three groups regarding the ARI score. Zn ion release was analyzed by t-test. All the statistical analyses were conducted using SPSS 24 at a 0.05 level of significance.

Results

SBS

Table 1 presents the measures of central dispersion for SBS of the three groups. As shown, the three groups did not differ significantly in SBS (P=0.086).

Table 1. Measures of central dispersion for SBS of the three groups (n=10)

Group	Mean	Std. deviation	Minimum	Maximum	P value
AO (Gold standard)	14.59	4.7381	7.31	20.74	
ZnO	15.49	4.2463	7.42	20.63	0.086
Basic adhesive (control)	11.17	4.177	5.95	17.81	

WSLs Assessment

Figure 1 shows DIAGNOdent values of the three groups at different time intervals. Repeated-measures ANOVA revealed a significant interaction between time and adhesive type on DIAGNOdent values (P<0.001). Pairwise comparisons of the groups by Tukey tests showed significantly higher DIAGNOdent values in the basic adhesive group compared with the optimized adhesive containing ZnO and the AO gold standard at all time intervals (P<0.001), suggesting greater cariogenic activity and demineralization potential. of the basic adhesive. Also, the AO gold standard adhesive showed significantly higher DIAGNOdent values than the optimized adhesive containing ZnO at all time intervals (P<0.001), indicating greater bacterial activity and lower antimicrobial properties of the gold standard adhesive. Nonetheless, intra-group changes over time were not significant in any group (P>0.05).

ARI Scores

Table 2 presents the frequency of different ARI scores in the three groups. The Kruskal-Wallis test showed no significant difference in ARI scores between the three groups (P=0.470).

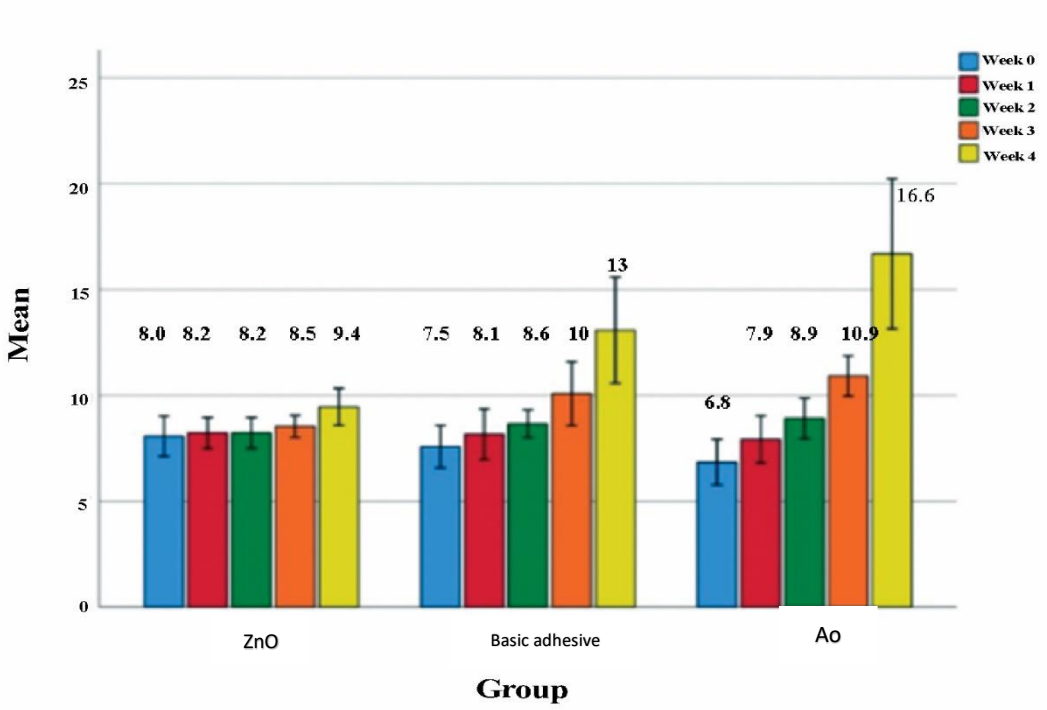

Figure 1: Changes in DIAGNOdent values over time among the experimental groups.

Table 2. Frequency of different ARI scores in the three groups

Group	ARI Frequency (percentage)				Mean rank
	Score 0	Score 1	Score 2	Score 3	
Basic adhesive	6(60.0%)	3(30.0%)	1 (10.0%)	0(0%)	16.80
ZnO	7(70.0%)	2(20.0%)	1(10.0%)	0(0%)	15.20
AO	9(90.0%)	1(10.0%)	0(0%)	0(0%)	12.90

Water Sorption and Water Solubility

Table 3 presents measures of central tendency and dispersion for water sorption and water solubility across the three groups. ANOVA showed a significant difference in water sorption between the three groups (P=0.001). Pairwise comparisons by Tukey tests showed that the optimized adhesive containing ZnO and the basic adhesive had a significantly lower water sorption than the AO gold standard adhesive (P<0.01); however, the difference between the optimized adhesive containing ZnO and the basic adhesive was not significant (P=0.780). ANOVA also showed a significant difference in water solubility between the three groups (P=0.002). Pairwise comparisons by post hoc Tukey tests showed that the optimized adhesive containing ZnO and the basic adhesive had a significantly lower water solubility than the AO gold standard adhesive (P<0.01); however, the difference between the optimized adhesive containing ZnO and the basic adhesive was not significant (P=0.820).

Zn Ion Release

Table 4 presents the Zn ion release from the optimized adhesive at different time points. One-sample t-test showed a significant difference between the mean ion release and the threshold of 50–100 ppm (P<0.001).

Table 3. Measures of central dispersion for the water sorption and water solubility of the three groups (n=9)

Variable	Group	Mean	Std. deviation	Minimum	Maximum	P-value
Water sorption	Basic adhesive	39.1500	1.35000	37.50	41.20	0.001
	ZnO	38.5200	1.30000	36.70	40.10	
	AO	96.9800	2.78000	93.90	100.50	
Water solubility	Basic adhesive	-4.7800	0.65000	-5.50	-4.10	0.002
	ZnO	-4.3500	0.60000	-5.10	-3.80	
	AO	-9.2000	1.40000	-11.10	-7.90	

Table 4. Zn ion release from the optimized adhesive at different time points (n=9)

Number of samples	Time point	Maximum (ppm)	Minimum (ppm)	Mean (ppm)
3	24 hours	0.0748	0.0484	0.0659
3	3 days	0.0715	0.0459	0.0595
3	5 days	0.0753	0.0528	0.0637

Discussion

This study experimentally synthesized a new formulation of an orthodontic adhesive containing silanized ZnO nanoparticles (to reduce water sorption and solubility) for bracket bonding to enamel and assessed its anti-demineralized activity, Zn ion release (to assess its cytotoxicity), and mechanical and physicochemical properties. Spherical nanoparticles were chosen due to their uniform surface energy, ease of synthesis, and good dispersibility in the matrix. Although nanowires and nanoplates can offer higher surface area and potentially stronger antibacterial effects, they tend to aggregate more readily, potentially affecting the mechanical integrity and cytocompatibility of the composite. The Zn ion release findings suggest a potentially favorable safety profile. The present results showed that the optimized adhesive containing 1.6 wt% ZnO nanoparticles exhibited lower DIAGNOdent values compared with the control groups, indicating reduced caries-related fluorescence over time. Although DIAGNOdent does not directly measure antimicrobial activity, its values may indirectly reflect changes in bacterial activity through alterations in caries-related fluorescence and biofilm-associated metabolic byproducts. The mean DIAGNOdent values from 0 to 4 weeks ranged from 7.58 to 8.50 for the optimized adhesive, while these values ranged from 16.30 to 16.40 for the basic adhesive and from 10.02 to 10.10 for the gold-standard adhesive. It has been reported that in aqueous and acidic environments, ZnO releases Zn2+ ions, which replace Mg2+ ions that are imperative for the enzymatic activity of bacterial biofilms and, as such, impair bacterial metabolism. Also, ZnO generates active peroxides that penetrate the bacterial membrane, disrupt the molecular structure of the phospholipid bilayer, and enter the bacterial cytoplasm. They react with the -SH groups of key microbial enzymes, leading to protein denaturation and DNA damage (15,18,20). The high surface-to-volume ratio of ZnO nanoparticles with 10–30-nm size further enhances their antibacterial activity (20). Furthermore, the ZnO nanoparticles were silanized in the present study to ensure their more homogeneous dispersion and dissolution. The optimized adhesive demonstrated lower DIAGNOdent values, which may indirectly reflect reduced cariogenic activity. containing ZNO nanoparticles compared with other groups, consistent with previous findings. Pourhajibagher et al. (10) added 7.5% photo-activated cationic curcumin-ZnO to Transbond XT composite and observed a descending trend in antibacterial activity over 180 days of immersion in artificial saliva. Gutierrez et al. (15) added 5/0.1 and 5/0.2 wt% ZnO/Cu nanoparticles to two commercial universal adhesives and observed a reduced cariogenic activity. Spencer et al. (9) demonstrated antimicrobial activity when ZnO was added to resin-modified glass-ionomer using the agar disc diffusion test. Kachoei et al. (29) added silver and ZnO nanoparticles to an orthodontic composite and showed its optimal antibacterial activity compared with the control group. Aydin Sevinç and Hanley (30) evaluated the antibacterial activity of a

composite containing 10% ZnO nanoparticles and reported an 80% reduction in Streptococcus sobrinus biofilm growth. Tavassoli Hojati et al. (21) reported a direct correlation between the reduction of *Streptococcus mutans* biofilm and increased concentrations of ZnO nanoparticles. Brandão et al. (20) highlighted the antibacterial activity of composites containing 1–10 wt% ZnO nanoparticles. Behnaz et al. (11) also reported an antibacterial effect of adding TiO2 and ZnO to Transbond XT adhesive, as evidenced by a reduction in WSL formation over one month. The difference between the present study and the studies mentioned above is that an experimental adhesive was synthesized in the current study by eliminating HEMA and using silanized ZnO nanoparticles, which were intended to improve nanoparticle dispersion within the adhesive matrix and prevent their agglomeration.

The SBS of the three adhesives was another variable evaluated in the present study. The results showed that the mean SBS of optimized (15.49 MPa) and AO (14.59 MPa) adhesives was higher than that of the basic adhesive (11.17 MPa). However, the difference was not statistically significant (P= 0.086). Nonetheless, all three values exceeded the minimum clinical threshold of 6-8 MPa, indicating optimal SBS for all three adhesives in clinical applications (31). The present results were consistent with the findings of Behnaz et al. (32), who added 1.3 wt% ZnO to a synthesized adhesive with no adverse effect on SBS, Pourhajibagher et al. (10), who added cationic curcumin/ZnO nanoparticles to Transbond XT composite, and Dastjerdi et al. (27), who synthesized a nanohybrid orthodontic adhesive containing an anti-biofilm agent (octafluoropentyl methacrylate). However, others reported the opposite when they added Cu nanoparticles, silver nanoparticles/hydroxyapatite, and 1 wt% ZnO, silver, and TiO2 to adhesives, and observed a significant reduction in bond strength compared with the control groups (33–35). Variations in the reported results can be due to differences in the type of bond strength test (for example, measurement of microtensile bond strength in some studies), the addition of different metal oxides to commercial adhesives, the measurement of resin–dentin bond strength, or variations in the concentration of added ZnO. Some studies reported an inverse correlation between the concentration of added ZnO and SBS (9,36). It appears that adding 1.6 wt% ZnO nanoparticles is suitable to ensure optimal antibacterial activity against *Streptococcus mutans* with no adverse effect on SBS.

The three groups had no significant difference in ARI scores in the current study, which was in contrast to the results of Pourhajibagher et al. (10), who reported the highest frequency of score 0, which is optimal in terms of minimal enamel surface cleaning required after debonding but increases the risk of enamel damage since score 0 indicates debonding at the enamel–adhesive interface (27).

The present results showed that the optimized adhesive containing ZnO nanoparticles and the basic adhesive had a superior performance in terms of water sorption and water solubility compared with AO. The mean water sorption of the optimized and basic adhesive groups was significantly lower than that of the gold standard adhesive and within the standard range according to ISO 4049 (≤40 µg/mm3), suggesting favorable resistance to water uptake. Water solubility of the optimized and basic adhesives was also significantly lower than that of the gold standard adhesive and optimal according to ISO 4049 (≤7.5 µg/mm^3). The slight difference in this regard between the optimized and basic adhesives may be due to the incorporation of silanized ZnO nanoparticles into the optimized adhesive. Silanization of nanoparticles improves the attachment of fillers to the resin matrix and further decreases water sorption. However, this difference did not reach statistical significance due to their highly similar formulation. The superiority of the synthesized adhesives over the gold-standard adhesive (AO) can be attributed to the reduction of TEGDMA and the replacement of DMA-EMA with TMPTA in their formulation. Ajeli Bazkiayi et al (26) used DMA-EMA in the formulation of their adhesive; in contrast, TMPTA was used instead of DMA-EMA in the current study, which decreases water sorption due to its higher hydrophobicity (25).

Another study (15) showed that the Prime & Bond Active universal adhesive containing ZnO/Cu nanoparticles had higher cytotoxicity than the control group, which may be due to greater water sorption and solubility, and release of ZnO and Cu ions and their combined toxic effects. However, Amber universal adhesive containing ZnO/Cu nanoparticles exhibited no significant difference in cytotoxicity from the control group, which may be due to its lower water sorption and solubility, and consequently lower release of toxic ions. Unlike the present results, Barcellos et al. (17) reported no significant difference in water solubility and water sorption between a dentin adhesive containing ZnO and zinc methacrylate with the control group. In a study by Brandão et al. (20), the composite containing 10% ZnO nanoparticles showed the highest solubility. Since ZnO is a water- and alcohol-

insoluble amphoteric oxide, this behavior can only be attributed to a low degree of conversion at this specific concentration. This finding highlights the significance of reducing ZnO concentration as much as possible (while preserving antimicrobial activity) to decrease the adverse effect of the degree of conversion on solubility. The manufacturer of AO does not precisely disclose its ingredients; thus, this difference is likely due to general differences in the formulation of AO compared with the synthesized adhesives. The present results highlighted the possibly higher durability of the synthesized adhesives compared with AO.

The toxic properties of nanomaterials depend on various parameters, including surface charge, chemical composition, concentration, solubility, shape, and surface area (31). Seker et al. (31) showed that ZnO nanoparticles in 50–100-µg/mL concentration were significantly cytotoxic for the initial cultures of periodontal ligament fibroblasts and mouse dermal fibroblasts after 48 hours; while in the present study, the maximum amount of released ZnO was 0.0753 µg/mL within 5 days. Therefore, the Zn ion release levels observed in the present study were substantially lower than previously reported cytotoxic concentrations.

Consistent with the present results, Gutierrez et al. (15) showed that adding 5.02 wt% ZnO/Cu nanoparticles did not change the cytotoxicity of the adhesive. Leitune et al. (37) demonstrated that adding ZnO nanoparticles did not affect the cytotoxicity of the adhesive compared with the positive control, and Thi Minh Thu Nguyen et al. (14) found that ZnO spherical nanoparticles were compatible with mammal cells. Also, Barcellos et al. (17) showed that adding ZnO nanoparticles decreased the cytotoxicity of the adhesive.

The main strengths of the present study included the following: changing the concentration of ZnO from 1.5% to 1.6% and reducing TEGDMA content and its replacement with TMPTA to improve hydrolytic resistance compared with the study by Ajeli Bazkiayi (26), and the evaluation of Zn ion release to ensure biocompatibility and safety of the synthesized adhesive. To the best of our knowledge, limited studies have evaluated caries-related fluorescence changes using DIAGNOdent in orthodontic adhesives containing ZnO nanoparticles over an extended 28-day period. Unlike previous studies that mainly relied on colony counting or diffusion-based methods, this approach provides a non-destructive and clinically relevant method to monitor changes in caries-associated activity over time. However, it should be noted that since DIAGNOdent primarily detects fluorescence associated with enamel demineralization and bacterial metabolites, its findings should be interpreted as indirect indicators of cariogenic activity rather than direct measurements of antimicrobial efficacy or bacterial viability. Therefore, the observed changes in DIAGNOdent values should be interpreted cautiously as indirect reflections of caries-related fluorescence rather than direct evidence of antibacterial effects. Future studies should incorporate direct microbiological assays, such as CFU counting or biofilm viability tests, to confirm the antimicrobial efficacy of the material.

Limitations

The antimicrobial activity of the synthesized adhesive was investigated only against *Streptococcus mutans*, while a wide range of cariogenic microorganisms are present in the oral environment. Therefore, future studies are recommended to assess the antibacterial activity of the synthesized adhesive against other cariogenic microorganisms, such as lactobacilli. Moreover, this study had an in vitro design, and oral clinical conditions such as thermal and pH alterations, as well as the effects of saliva, diet, and oral hygiene practices, were not considered, which limits the generalizability of the findings. Thus, clinical studies are required to verify the present findings. No significant change in DIAGNOdent values for the experimental adhesive in the present study indicated its relative stability. Nonetheless, long-term studies are required to assess and ensure its optimal durability over longer periods of time. Moreover, the microhardness and degree of conversion of the synthesized adhesive should be investigated in future studies. Due to the unavailability of Transbond XT adhesive in Iran, AO adhesive was used as the gold standard in the present study. Further studies are required to compare the synthesized adhesive with Transbond XT, the gold standard.

Conclusion

The experimentally synthesized orthodontic adhesive containing 1.6 wt% ZnO nanoparticles demonstrated favorable physicochemical properties and lower DIAGNOdent values associated with reduced cariogenic activity around orthodontic brackets. In addition, Zn ion release remained substantially below previously reported cytotoxic concentrations. Further microbiological and clinical studies are required to confirm the long-term antimicrobial efficacy and biocompatibility of the material.

Acknowledgement

None.

Conflict of Interests

The authors declare no conflict of interests.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

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