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Microleakage of Orthodontic Fixed Retainers Bonded with Zinc Oxide- and Silver Nanoparticle-Modified Adhesives: An in Vitro Study

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Abstract

Aim: Fixed orthodontic retainers are widely used to maintain post-treatment dental alignment; however, microleakage at the adhesive-enamel interface can compromise their long-term performance. Incorporation of nanoparticles into orthodontic adhesives has recently gained attention as an approach to modify adhesive properties and influence microleakage behavior. This study aimed to compare microleakage in fixed orthodontic retainers bonded with conventional adhesives versus adhesives modified with 1% zinc oxide (ZnO) and 1% silver (Ag) nanoparticles.
Methods: Sixty extracted human mandibular central incisors were randomly allocated into six subgroups (n = 10 per subgroup): ZnO-modified Transbond XT (group 1a), ZnO-modified Enlight (group 1b), Ag-modified Transbond XT (group 2a), Ag-modified Enlight (group 2b), unmodified Transbond XT (group 3a), and unmodified Enlight (group 3b). A 0.0175-inch multistranded stainless steel wire was bonded to each lingual surface, and specimens underwent 1500 thermocycles (5°C-55°C). Microleakage was assessed using a stereomicroscope at 10x magnification.
Results: ZnO-modified groups recorded the lowest mean microleakage (0.0385 ± 0.0556 and 0.0314 ± 0.1223). The data were non-normal with unequal variances, so the Kruskal–Wallis test was the primary analysis and showed a significant difference across groups (H = 12.96, p = 0.023). Dunn's post hoc test (Bonferroni) showed both ZnO groups had significantly lower microleakage than the Ag-modified and unmodified groups (adjusted p = 0.01–0.04).
Conclusion: The ZnO-modified groups had significantly lower microleakage at the adhesive-enamel interface than the Ag-modified and unmodified adhesives. Studies with larger samples are required to confirm whether ZnO modification improves the marginal seal of bonded orthodontic retainers.

Keywords: fixed retainers; microleakage; nanoparticles; orthodontic adhesives; silver; zinc oxide

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Background

Fixed bonded retainers are a recognized standard in post orthodontic retention, particularly in the mandibular anterior region where spontaneous alignment relapse is well documented (1). Long-term success depends on the mechanical integrity of the adhesive interface between enamel-composite and the retainer wire. A persistent concern in clinical practice is microleakage at this interface.

Microleakage is defined as the ingress of bacteria, fluids, molecules, or ions between a restorative material and the tooth surface (2). Clinically, this manifests as enamel demineralization, white spot lesion formation, and secondary caries complications that have been repeatedly documented in patients wearing fixed retainers(3,4). The retainer wire itself impedes oral hygiene, promoting plaque accumulation and creating conditions conducive to microbial activity around the bonded site.

Transbond XT (3M Unitek, Monrovia, CA, USA) and Enlight (Ormco, Glendora, CA, USA) are among the most frequently used orthodontic composite adhesives, valued for their handling characteristics and reported bond strengths. Despite this, their capacity to maintain an adequate marginal seal under simulated intraoral conditions particularly thermal cycling and moisture exposure has been questioned (5,6). Several investigations have documented microleakage with both systems, regardless of the bonding protocol employed.

The application of nanotechnology to dental materials has attracted considerable attention over the past two decades. Nanoparticle-modified composites have demonstrated improvements in antimicrobial activity, mechanical strength, and resistance to degradation(7). Zinc oxide (ZnO) and silver (Ag) nanoparticles have attracted particular interest because of their broad-spectrum antibacterial properties and capacity to suppress biofilm formation (8,9), attributes of clear relevance in orthodontics, where appliance-related plaque accumulation is a recognized clinical challenge.

ZnO nanoparticles have been reported to improve the physicochemical properties of dental composites while delivering antibacterial activity without substantial adverse effects on material performance(10). Silver nanoparticles are similarly recognized for potent antimicrobial action and have been incorporated into a range of dental materials to reduce bacterial colonization(11). However, the influence of these nanoparticles on the marginal sealing of orthodontic adhesives remains uncertain, with existing studies yielding inconsistent findings.

Most published investigations have examined either a single nanoparticle type or a single adhesive system, constraining the clinical applicability of their conclusions. Direct comparisons across multiple commercially available bonding systems under fixed retainer bonding conditions are lacking (12).

The present in vitro study therefore aimed to evaluate and compare microleakage associated with Transbond XT and Enlight following incorporation of ZnO and Ag nanoparticles at 1% concentration, and to compare these modified formulations with unmodified control groups.

Null hypothesis: Incorporation of ZnO or Ag nanoparticles into Transbond XT and Enlight adhesives at 1% by weight would not produce a statistically significant difference in microleakage compared with unmodified adhesives.

Materials and Methods

Sample Selection

Sixty extracted human mandibular central incisors were obtained from patients referred for extraction for periodontal or other therapeutic indications. Teeth with visible enamel cracks, active caries, or existing restorations were excluded. All specimens were cleansed of soft tissue debris and stored in 0.5% chloramine solution for 1 week at 4°C. Teeth were randomly allocated to the six groups (n = 10 each). Randomization was done using www.randomizer.org

Sample Size

The sample size was calculated for a one-way ANOVA across six groups (α=0.05, power 80%) with an expected effect size of Cohen's f=0.54, derived from the microleakage data reported by Jazi et al. (13) and corresponding to a large effect (Cohen's f ≥ 0.40). This gave a minimum total of 50 specimens, which was rounded up to 10 per group (N=60); the achieved power for the omnibus test with N=60 is 89%. Because the design is a 2 × 3 factorial, the calculation was repeated for the interaction and nanoparticle main effect (numerator df=2): 37 specimens give 80% power and N=60 gives 96% power for the same effect size. The primary analysis is non-parametric; the Kruskal–Wallis test has an asymptotic relative efficiency of at least 0.864 compared with ANOVA, so the requirement rises to about 58 specimens for the omnibus test (50 ÷ 0.864), which N=60 still meets. With N=60, the

smallest effect detectable with 80% power is f=0.49 for the omnibus test and f=0.42 for the interaction.

Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee, Kempegowda Institute of Medical Sciences (Approval No. and University Research Code: KIMS/IEC/D242/D/2023). Informed consent was not obtained for the use of the extracted teeth. All procedures were conducted in accordance with the ethical standards of the responsible institutional committee.

Preparation of Nanoparticle-Modified Adhesives

The adhesive systems used in the study are illustrated in Figure 1. ZnO nanoparticles and Ag nanoparticles (Nano Research Lab, India) (Figure 2) were each incorporated into Transbond XT and Enlight composites at a concentration of 1% by weight (10 mg nanoparticles per 990 mg composite, yielding 1000 mg per formulation). The modified adhesive formulations following nanoparticle incorporation are shown in Figure 3. The primers (Transbond XT Primer and Ortho Solo) were used as supplied and were not modified with nanoparticles; only the composite adhesives were modified.

According to the manufacturer's certificates of analysis, the ZnO nanoparticles (Nano Research Lab, Jamshedpur, India; CAS 1314-13-2) had a purity of 99.5%, an average particle size of 30–50 nm, and a nearly spherical shape. The Ag nanoparticles (Nano Research Lab, Jamshedpur, India; CAS 7440-22-4) had a purity of 99.9% (metal basis), a particle size of 30–50 nm, and a spherical shape.

A vortex mixer and an IKA® T25 digital ULTRA-TURRAX® homogenizer were used to incorporate the nanoparticles (1.0% ZnO and 1.0% Ag, weight/weight) into the adhesives. The mixtures were homogenized at 3400 rpm for 2 minutes using a rotor-stator mechanism. The control adhesives (groups 3a and 3b) did not undergo homogenizer mixing.

Specimen Grouping

Specimens were randomly allocated into three main groups with two subgroups each (n = 10 per subgroup):

Group 1 - ZnO nanoparticles: Group 1a: Transbond XT + ZnO; Group 1b: Enlight + ZnO

Group 2 - Silver nanoparticles: Group 2a: Transbond XT + Ag; Group 2b: Enlight + Ag

Group 3 - Control (unmodified): Group 3a: Transbond XT; Group 3b: Enlight



Figure 1. Primer systems (Ortho Solo and Transbond XT) and adhesive systems (Enlight and Transbond XT) used in the study.



Figure 2. Zinc oxide nanoparticles (left) and silver nanoparticles (right) used for adhesive modification.



Figure 3. Adhesive systems (Enlight and Transbond XT) following incorporation of ZnO and Ag nanoparticles.

Bonding Procedure

Lingual enamel surfaces were acid-etched with 37% phosphoric acid gel for 30 seconds (Figure 4), rinsed with water for 10 seconds, and air-dried for 15 seconds to achieve a frosty white appearance. A thin layer of primer (Ortho Solo / Transbond XT Primer) was applied according to each manufacturer's instructions. A 0.0175-inch multistranded stainless-steel wire was cut to standardized length and positioned at the junction of the incisal and middle thirds of the lingual surface. A mini-mold technique was used to standardize both the volume and shape of composite placed at each bonding site (Figures 5 and 6). Each specimen was light-cured for 40 seconds with a Coltene S.P.E.C. 3 LED curing light (1600 mW/cm², at a distance of 3 mm). Following bonding, all specimens were subjected to thermocycling to simulate clinical conditions (1500 cycles; 5°C to 55°C; dwell time 30 seconds per bath).

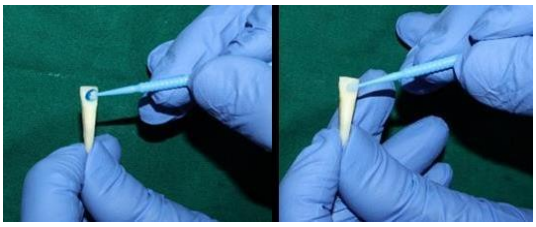


Figure 4. Application of 37% phosphoric acid etchant followed by bonding primer.



Figure 5. Placement of adhesive using the mini-mould wire bonder technique.

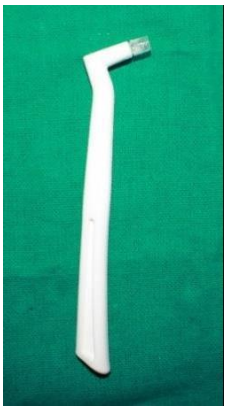


Figure 6. Mini-mould wire bonder used to standardise adhesive placement on the lingual tooth surface.

Microleakage Assessment

Root apices were sealed with sticky wax, and all external tooth surfaces were coated with two layers of nail varnish, with a 1 mm window left exposed around the bonded retainer interface (Figure 7). Specimens were immersed in 0.5% basic fuchsine dye solution for 24 hours at room temperature, then rinsed and dried (Figure 8). Each specimen was sectioned in a transverse plane tangent to the retainer wire using a water-cooled low-speed diamond disc. One section per specimen was examined. Microleakage was evaluated using the dye penetration technique. The specimens were examined under a Zeiss stereomicroscope (Carl Zeiss Microscopy, Germany) (Figure 9) at 10 x magnification, and the captured images were analyzed using ZEN 2011 software (Carl Zeiss Microscopy, Germany). The length of dye penetration along the composite-enamel interface from both the mesial and distal margins was measured in millimeters (Figure 10). The microleakage was calculated by summing the dye penetration lengths on the mesial and distal surfaces, then dividing this total by the overall interface length, defined as the length of the composite-enamel interface between the mesial and distal margins on the section. All specimens were evaluated independently by two calibrated examiners blinded to group allocation. Specimens were coded by a person not involved in the measurements, and the code was broken only after all measurements were complete. Inter-examiner reliability was assessed using the intraclass correlation coefficient (ICC; two-way random effects, absolute agreement, single measures) and was good (ICC = 0.87). Disagreements were resolved by discussion, and the mean of the two examiners' values was used for analysis.

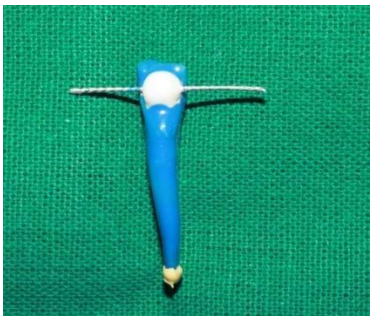


Figure 7. Sealing of root apices with sticky wax and application of nail varnish, with a 1 mm window exposed around the bonded area.

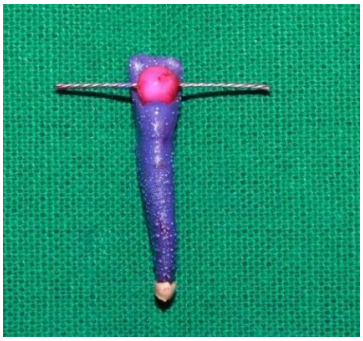


Figure 8. Specimens after 24-hour immersion in 0.5% basic fuchsine dye.

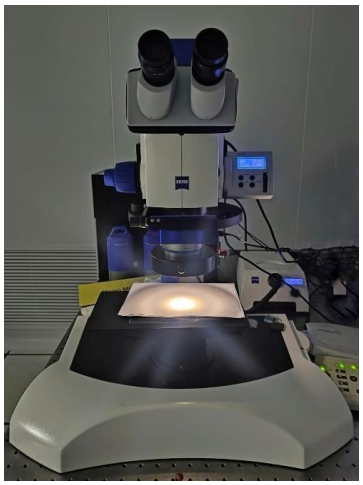


Figure 9. Stereomicroscope used for evaluation at 10x magnification.

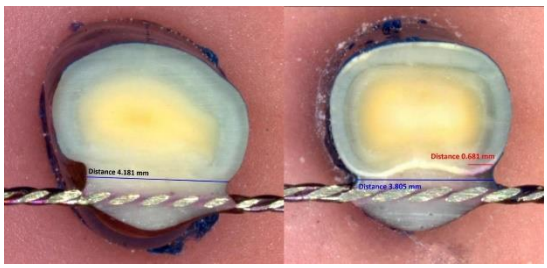


Figure 10. Representative sections demonstrating absence and presence of dye-penetration microleakage at the enamel-adhesive interface.

Statistical Analysis

Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Microleakage was expressed as a proportion. Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test. The data were positively skewed, deviated from normality in two groups (Shapiro–Wilk $p < 0.05$), and showed markedly unequal dispersion (SD range 0.056–0.292; Levene's $p = 0.06$). Because the assumptions of parametric analysis were not satisfied, the Kruskal–Wallis test was used as the primary analysis to compare the six groups, followed by Dunn's post hoc test with Bonferroni adjustment for the 15 pairwise comparisons (Table II). To examine the 2 × 3 factorial design (adhesive system × nanoparticle type), the main effects of adhesive system and nanoparticle type and their interaction were analyzed using a two-way aligned rank transform (ART) ANOVA, which permits interaction testing on non-normally distributed data (Table III). Data are presented as mean ± SD and median (IQR) (Table I).

Results

Descriptive statistics for all groups are presented in Table I. Among the ZnO-modified groups, group 1a (Transbond XT + ZnO) recorded a mean microleakage of 0.0385 ± 0.0556, and group 1b (Enlight + ZnO) recorded 0.0314 ± 0.1223. The Ag-modified groups recorded 0.1106 ±0.0924 for group 2a (Transbond XT + Ag) and 0.1306 ± 0.1460 for group 2b (Enlight + Ag). The unmodified control groups recorded 0.1510 ± 0.2919 for group 3a, which had the highest mean microleakage, and 0.1318 ± 0.1063 for group 3b. Group 1b showed the lowest mean value, followed by group 1a; both ZnO-modified groups had lower mean values than every Ag-modified and unmodified group. Table I provides the median (IQR) values for all groups.

The Shapiro–Wilk test indicated deviation from normality in group 1a ($p = 0.004$) and group 3a ($p = 0.001$), whereas the remaining groups were normally distributed ($p > 0.05$). The data were positively skewed with markedly unequal dispersion (SD range 0.056–0.292; Levene's test p =

0.06). The Kruskal-Wallis test, used as the primary analysis, revealed a statistically significant overall difference among the six groups (H = 12.96, df = 5, p = 0.023). In the two-way aligned rank transform ANOVA, the adhesive system (F(1, 54) = 6.73, p = 0.012), the nanoparticle type (F(2, 54) = 4.23, p = 0.019) and their interaction (F(2, 54) = 3.82, p = 0.028) each had a significant effect (Table III). Because the interaction was significant, the main effects should be interpreted with caution, as the effect of nanoparticle type depended on the adhesive system.

Dunn's post hoc test with Bonferroni adjustment for the 15 pairwise comparisons showed that both ZnO-modified groups differed significantly from the Ag-modified and unmodified groups: group 1a differed from groups 2a (adjusted p = 0.04), 2b (p = 0.02), 3a (p = 0.01) and 3b (p = 0.04), and group 1b differed from groups 2a (p = 0.01), 2b (p = 0.03), 3a (p = 0.02) and 3b (p = 0.03) (Table II). No significant difference was found between groups 1a and 1b (p = 0.34), among the Ag-modified and unmodified groups (adjusted p = 0.056–0.56), or between the two base adhesive systems within any nanoparticle condition. The largest mean difference was observed between group 1b and group 3a (mean difference = −0.1196; adjusted p = 0.02), followed by group 1b versus group 3b (−0.1004; p = 0.03) and group 1a versus group 3a (−0.1125; p = 0.01).

Table I. Descriptive statistics for microleakage across experimental groups

Group	Adhesive system	Nanoparticle	n	Mean microleakage	SD	Median (IQR)
1a	Transbond XT	ZnO	10	0.0385	0.0556	0.0216
1b	Enlight	ZnO	10	0.0314	0.1223	0.0821
2a	Transbond XT	Ag	10	0.1106	0.0924	0.0796
2b	Enlight	Ag	10	0.1306	0.1460	0.0681
3a	Transbond XT (control)	None	10	0.1510	0.2919	0.0821
3b	Enlight (control)	None	10	0.1318	0.1063	0.0980

SD = standard deviation; IQR = interquartile range (first quartile – third quartile); ZnO = zinc oxide nanoparticles; Ag = silver nanoparticles. Microleakage is expressed as a proportion. The distribution is positively skewed (SD exceeds the mean in groups 1a, 1b, 2b and 3a), so median (IQR) is the primary descriptive summary.

Table II. Post hoc pairwise comparisons (Dunn's test with Bonferroni adjustment) following the Kruskal–Wallis test

Group (I)	Group (J)	Mean rank difference (I–J)	Standardized test statistic (z)	Adjusted p value	Significance
1a	1b	0.0071	3.090	0.340	Insignificant
1a	2a	−0.0721	3.004	0.042	Significant
1a	2b	−0.0921	3.209	0.020	Significant
1a	3a	−0.1125	3.403	0.010	Significant
1a	3b	0.0933	3.004	0.042	Significant
1b	2a	−0.0792	3.403	0.010	Significant
1b	2b	−0.0992	3.090	0.031	Significant
1b	3a	−0.1196	3.209	0.020	Significant
1b	3b	−0.1004	3.090	0.031	Significant
2a	2b	−0.0200	3.588	0.560	Insignificant
2a	3a	−0.0404	3.403	0.140	Insignificant
2a	3b	−0.0212	3.209	0.270	Insignificant
2b	3a	−0.0204	2.935	0.056	Insignificant
2b	3b	0.0012	3.090	0.210	Insignificant
3a	3b	0.0192	3.004	0.380	Insignificant

Dunn's test with Bonferroni adjustment for 15 pairwise comparisons. Adjusted p < 0.05 is considered statistically significant. Exact p-values are reported to three decimal places (p < 0.001 where applicable).

Table III. Omnibus test and factorial (adhesive system × nanoparticle type) analysis of microleakage

Analysis/source of variation	df	Test statistic	p value
Kruskal–Wallis test (six groups; primary analysis)	5	H = 12.96	0.023
Two-way aligned rank transform ANOVA: adhesive system	1, 54	F = 6.73	0.012
Nanoparticle type (ZnO, Ag, none)	2, 54	F = 4.23	0.019
Adhesive system × nanoparticle type (interaction)	2, 54	F = 3.82	0.028

Design: 2 (adhesive: Transbond XT, Enlight) × 3 (nanoparticle: ZnO, Ag, none) factorial, n = 10 per cell. The aligned rank transform (ART) procedure permits interaction testing on non-normal data. $p < 0.05$ is considered statistically significant.

Discussion

This study examined the effect of incorporating ZnO and Ag nanoparticles at 1% by weight into two orthodontic adhesive systems, Transbond XT and Enlight, on microleakage beneath bonded fixed retainers. Microleakage differed significantly among the six groups (Kruskal–Wallis H = 12.96, p = 0.023), and the null hypothesis was therefore rejected. Both ZnO-modified groups (1a, Transbond XT + ZnO, mean 0.0385; 1b, Enlight + ZnO, mean 0.0314) showed significantly lower microleakage than Ag-modified and unmodified groups, whereas the Ag-modified groups did not differ significantly from the unmodified controls.

The lower microleakage with ZnO is supported by earlier work on other resin-based materials. Teymoornezhad et al. (14) added 1% and 3% by weight of ZnO nanoparticles to two flowable composites. Microleakage in the Filtek Z-350 group fell significantly as nanoparticle content increased, whereas the Clearfil group showed no significant change. A review of ZnO in dentistry also lists decreased microleakage in a flowable composite containing ZnO nanoparticles (15). In an orthodontic model, Toodehzaeim et al. (16) bonded brackets with Transbond XT containing 1 wt% ZnO, the same base adhesive and concentration as in the present study. Microleakage occurred in 7.8% of the ZnO group compared with 28.1% of the control group, although the difference between groups was not statistically significant.

The significantly lower microleakage observed with ZnO-modified adhesives differs from the findings of Jazi et al. (13), who reported that ZnO incorporation did not significantly alter microleakage in their model, while noting improvements in mechanical parameters. Physicochemical interactions between nanoparticles and resin matrices can vary considerably between formulations, and differences in adhesive composition, nanoparticle characteristics, mixing protocol, and test model may account for the discrepancy.

Within each nanoparticle condition, Transbond XT and Enlight did not differ significantly (1a vs 1b, p = 0.34; 2a vs 2b, p = 0.56; 3a vs 3b, p = 0.38). This is consistent with Ulker et al.(17), who found similar microleakage outcomes between orthodontic composites under controlled conditions. ZnO produced a similar reduction in both adhesives, whereas Ag lowered the mean in Transbond XT (0.1106 vs 0.1510) but not in Enlight (0.1306 vs 0.1318), although neither Ag difference was statistically significant.

The Ag-modified groups showed no statistically significant difference in microleakage compared with the unmodified groups and had significantly higher microleakage than the ZnO-modified groups, despite silver nanoparticles being well established as potent antimicrobial agents (8,11). Antimicrobial capacity does not necessarily translate into improved marginal sealing, and silver incorporation did not improve the marginal seal of either adhesive in this study. Microleakage may be influenced by polymerization shrinkage, adhesive penetration depth, and the quality of interfacial adaptation, none of which was assessed. Silver nanoparticles are metallic and may tend to agglomerate; in other resin systems, agglomerates have been described as potential stress concentrators and voids rather than reinforcement, and silver has been reported to increase resin viscosity with increasing concentration(18). Higher viscosity could, in theory, impair wetting of etched enamel and promote air entrapment. Incorporation of silver nanoparticles into Transbond XT primer has also been reported to reduce both the degree of conversion and shear bond strength(19), and silver may discolor the resin matrix (20). A lower degree of conversion could weaken the interface and might theoretically contribute to the higher microleakage of the Ag-modified groups relative to the ZnO-modified groups; however, none of these mechanisms was assessed here.

ZnO is an inorganic oxide that can be incorporated into resin-based composites as an additional filler phase. Improved filler distribution

within the resin, a reduction of residual microvoids, or attenuation of polymerization shrinkage stress could theoretically contribute to lower microleakage, but none of these was examined; dispersion was not verified. Previous studies indicate that low concentrations of ZnO can be incorporated without substantially compromising certain material properties. Tavassoli Hojati et al. reported no significant differences in the degree of conversion among flowable resin composites containing different concentrations of ZnO nanoparticles, while several mechanical properties remained unaffected(10). A more recent study evaluating 0.5 to 2 wt% ZnO found that the final degree of conversion after 24 hours was independent of ZnO concentration, although depth of cure decreased with increasing nanoparticle content(21). These findings suggest that incorporating 1 wt% ZnO would not necessarily compromise the final degree of conversion, although degree of conversion was not assessed in the present study. Any effect of nanoparticle modification may also depend on concentration, particle size, surface treatment and the homogeneity of dispersion, and whether other concentrations would alter microleakage, and with what effects on handling and mechanical properties, would require systematic evaluation.

Regarding bonding technique, Yagci et al.(22) reported no significant difference in microleakage between direct and indirect bonding protocols. Since the present study employed direct bonding exclusively, technique variability is unlikely to account for the intergroup differences observed.

Transbond XT and Enlight are light-cured materials, so working time is not governed by a chemical setting reaction, and a 1% nanoparticle addition is unlikely to shorten it. Nevertheless, nanoparticle addition can increase paste viscosity,18 which may affect handling. In the present study the nanoparticles were incorporated mechanically using a rotor–stator homogenizer, which standardized the mixing; chairside use would require a similarly validated, standardized protocol. Bond strength is the more important concern and was not evaluated in this study. Saeed et al. found that the control adhesive had the highest shear bond strength, with 0.5 wt% ZnO giving the lowest value, which nevertheless remained within a clinically acceptable range (23).

Limitations: This in vitro study cannot fully replicate the complex intraoral environment. Only a single nanoparticle concentration was tested, and significant findings need confirmation in larger samples. Bond strength and nanoparticle dispersion were not assessed, and the composite-wire interface was not evaluated. In addition, the control adhesives (groups 3a and 3b) were not subjected to homogenizer mixing. Future studies should use larger samples and multiple nanoparticle concentrations, and should progress to prospective clinical evaluation to establish translational relevance.

Conclusion

Within the limitations of this in vitro investigation, the following conclusions are drawn:

1. Incorporation of 1% ZnO nanoparticles into Transbond XT and Enlight was associated with significantly lower microleakage than the Ag-modified and unmodified adhesives, and the null hypothesis was therefore rejected.
2. Pairwise comparisons showed no significant difference in microleakage between Transbond XT and Enlight within any nanoparticle condition.
3. Studies with larger samples, different nanoparticle concentrations and in-vivo evaluation are needed to determine whether ZnO modification improves the marginal seal and clinical performance of bonded fixed orthodontic retainers.

Authors' Contributions

Sushmitha N: conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing—original draft

Vedavathi H K: Data acquisition, analysis and interpretation

Bharath Reddy P, Sowmya K S, Megha N: conceptualization, methodology, validation, supervision, writing—review and editing

All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of Interest

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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