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Comparison of Microleakage of Orthodontic Brackets Bonded with Copper Oxide and Zinc Oxide Nanoparticles-Containing Composites

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Abstract

Background and objectives: Creating a white spot on the enamel around orthodontic attachments is not unexpected, and nearly half of orthodontic patients experience enamel demineralization. The aim of this study was to investigate the effect of nanoparticles on microleakage between composite and enamel.

Materials and methods: Orthodontic brackets were adhered by orthodontic adhesives in three groups on 48 extracted human premolar teeth. Three groups were studied: control and adhesive containing copper oxide and zinc oxide nanoparticles. Microleakage between adhesive-bracket and adhesive-tooth in the occlusal and gingival area of the study groups was determined by dye penetration method and statistically analyzed.

Results: Among 192 samples in three groups, microleakage was observed in 28.1% of the control group, 7.8% of zinc oxide group, and 10.9% of copper oxide group. Overall, microleakage occurred more frequently at the tooth-adhesive junction than at the bracket-adhesive junction, which was the only statistically significant factor in the GEE (Generalized Estimating Equations) analysis (P = 0.002). Differences in microleakage between occlusal and gingival margins, as well as between treatment groups, were not statistically significant.

Conclusion: Microleakage was not significantly affected by copper oxide and zinc oxide nanoparticles. No significant difference in microleakage was found between occlusal and gingival margin. Significantly greater microleakage was observed at the tooth-adhesive junction than at the bracket-adhesive junction.

Keywords: Microleakage, Zinc oxide nanoparticles, Copper oxide nanoparticles, Orthodontic adhesives.

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1. Introduction

White Spot Lesion (WSL) and demineralization are one of the common side effects of orthodontic treatments (1). Orthodontic appliances can reduce self-cleaning of teeth, change microbial flora, and increase the amount of acidogenic microbial plaque during treatment (2-4). It has been reported that 50-70% of orthodontic patients experience enamel demineralization around the bracket (5), and despite education on oral hygiene, WSL remains a clinical challenge in fixed orthodontic treatment (6).

There are various preventive strategies, such as mechanical plaque removal and fluoride application, that depend on patient cooperation (7,8). Recently, nanoparticles combined with composite are used in orthodontics due to their antimicrobial and anti-adhesion properties, which help reduce biofilm formation and enamel demineralization (9).

Reducing the size of the material from micrometers to nanometers significantly changes their characteristics, including hardness, active surface, chemical activity, and biological activity (10). The biocidal effects of metal nanoparticles are attributed to their high surface-to-volume ratio, which enhances their interaction with microbial

membrane and broadens their antimicrobial properties beyond just metal ion release (11).
Composites containing silver and zinc oxide nanoparticles have shown greater anti-inflammatory and anti-microbial effects against Streptococcus mutans and Lactobacillus compared to the control group (12). Additionally, copper oxide and zinc oxide nanoparticles have a strong anti-microbial effect in preventing the colonization and growth of microbial plaque (13).
While adding nanoparticles of zinc oxide and copper oxide to the composite increases the antimicrobial efficacy, it is crucial that other important mechanical bonding properties, such as shear bond strength and microleakage between composite and enamel not get adversely affected. Experimental studies have demonstrated that increasing the concentration of zinc oxide nanoparticles in orthodontic adhesives enhances their antimicrobial properties while maintaining clinically acceptable levels of bond strength (14).
The purpose of the present study is to investigate the effect of nanoparticles on microleakage between composite and enamel.

2. Materials and methods

This study was designed to evaluate the microleakage of bonded orthodontic brackets using different materials and a scoring scale.
Inclusion criteria: Human premolar teeth extracted for orthodontic purposes.
Exclusion criteria: Teeth with any caries, restorations, enamel hypoplasia, abrasion, attrition, cracks, or developmental defects. 48 human premolar teeth were used in this study. The remaining soft tissue, calculus, and plaque were removed using a scaler and pumice powder. The teeth were then disinfected in a 0.05% chloramine T solution for 24 hours and stored in normal saline until use. The samples were randomly divided into three groups of 16 each. Nanocomposite containing 1% weight concentration of copper oxide and zinc oxide nanoparticles prepared by weighing 0.01 grams of nanoparticles and mixing them with 0.99 grams of Transbond XT composite. This mixture was homogenized in a semi-dark environment using a shaker vortex device for 15 minutes.
The buccal surfaces of the teeth were brushed for 15 seconds, rinsed with water for 20 seconds, and dried for 15 seconds. Edgewise slot 22 brackets used, and the buccal surfaces of the teeth were etched with 37% phosphoric acid gel for 30 seconds. Then they were rinsed with water for 30 seconds and dried with oil-free air for 20 seconds until a chalky white appearance was achieved. Primer was applied to the etched areas, and adhesive was used to coat the bracket bases. (Control group: plain adhesive, Group 2 and 3: adhesives containing copper oxide and zinc oxide). The brackets were positioned on the teeth, and excess adhesive was removed before curing with an LED light-curing device at a wavelength of 450 nm for 40 seconds. The samples were then stored in distilled water for four weeks.
To simulate clinical conditions, the samples underwent 1500 thermocycling cycles between 5°C and 55°C, with each cycle lasting 30 seconds. Microleakage was assessed using the dye penetration method. The apex of each tooth was sealed with wax, and two layers of nail polish were applied around the bracket area, leaving a 1 mm margin. The samples were immersed in 0.5% basic fuchsine solution at room temperature for 24 hours, then rinsed and cleaned. Longitudinal sections were made using a diamond bur, and dye penetration was scored under a stereomicroscope at 16x magnification. The scoring system ranged from 0 for no microleakage, 1 for less than 1 mm, 2 for 1 to 2 mm, and 3 for beyond 2 mm microleakage between adhesive-enamel and adhesive-bracket.
Data were entered into SPSS software for statistical analysis. Microleakage was initially recorded using an ordinal scoring system ranging from 0 to 3. However, because only one sample received a score of 2 and no samples received a score of 3, the outcome variable showed a highly skewed distribution. Therefore, the scores were dichotomized into two categories: absence of microleakage (score 0) and presence of microleakage (scores 1 and 2).
Descriptive statistics, including frequencies and percentages, were calculated for each study group. Since multiple observations (occlusal/gingival and tooth-adhesive/bracket-adhesive interfaces) were obtained from each tooth, the observations were considered correlated. To account for this intra-sample correlation, Generalized Estimating Equations (GEE) with a binary logistic link function were used to evaluate the effects of treatment group, margin location, and bonding site on the probability of microleakage. Odds ratios (ORs) and their corresponding 95% confidence intervals (95% Cis) were calculated. A two-sided P-value of less than 0.05 was considered statistically significant.

3. Results

In this study, 192 samples were analyzed in three treatment groups (each group consisting of 64 samples). Although microleakage was initially assessed using a four-point ordinal scale (0 to 3), the vast majority of observations were limited to scores 0 and 1. Only a single sample received a score of 2, and no samples were assigned a score of 3. Given the extremely skewed distribution of the data, with nearly all samples falling into the lowest two categories, the outcome variable was dichotomized to facilitate a more robust statistical analysis. For this purpose, scores were grouped as follows: score 0 indicated the absence of microleakage, while scores 1 and 2 were considered indicative of the presence of microleakage. This decision was made to simplify the analysis, improve accuracy, and avoid unstable statistical modeling. Accordingly, binary analysis was performed using a GEE model.
This binary classification of the outcome variable laid the groundwork for subsequent statistical analysis. Given the repeated nature of the observations within each tooth and group, a Generalized Estimating Equations (GEE) model was employed to compare the overall probability of microleakage across the groups, while controlling for area and site as potential confounders. This approach enabled valid inference while accounting for within-sample correlation, without requiring separate subgroup analyses. Descriptive results

showed that in the control group, out of 64 samples, 18 samples (28.1%) exhibited microleakage. In contrast, in the adhesive group containing zinc oxide nanoparticles, only 5 samples (7.8%) showed microleakage, and in the group containing copper oxide nanoparticles, 7 samples (10.9%) exhibited microleakage. Overall, without considering the treatment group, microleakage was more frequently observed in the gingival areas and at the adhesive–tooth interface than in the occlusal areas and at the bracket interface (Table 1). At the bracket–adhesive interface (gingival side), the microleakage percentage was 37.5% in the control group, 12.5% in the zinc oxide group, and 12.5% in the copper oxide group. At the occlusal surface of the same interface, microleakage was reported as 12.5% in the control group, 0% in the zinc oxide group, and 6.3% in the copper oxide group (Table 1). At the adhesive–tooth interface (occlusal side), microleakage was 43.8% in the control group, and 18.8% in both the zinc oxide and copper oxide groups. At the gingival surface of this interface, microleakage was 18.8% in the control group, 0% in the zinc oxide group, and 6.3% in the copper oxide group. Overall, the incidence of microleakage in all evaluated areas was lower in the groups containing nanoparticles compared to the control group (Table 1).

In the regression analysis using the Generalized Estimating Equations (GEE) model, the bonding site variable was identified as the only factor significantly associated with microleakage occurrence. Specifically, the odds of microleakage were significantly higher at the tooth-adhesive interface compared to the bracket-adhesive interface (OR = 4.425, 95% CI: 1.710 - 11.453, $P = 0.002$) (Table 2). Although microleakage was more common in the gingival area compared to the occlusal area (OR = 0.695), this difference was not statistically significant ($P = 0.096$). Additionally, after controlling for the variables Margin Location and Bonding Site, the likelihood of microleakage in the group with zinc oxide nanoparticles was lower than the control group (OR = 0.680, 95% CI: 0.109 - 4.252, $P = 0.680$), but this difference was also not statistically significant. The group with copper oxide nanoparticles showed a non-significant increase in the odds of microleakage compared to the control group (OR = 3.489, 95% CI: 0.630 - 19.317, $P = 0.152$) (Table 2).

4. Discussion

Microleakage, caused by gaps between enamel and adhesive, allows bacteria and oral fluids to enter, leading to the formation of white spot lesions under and around the brackets. Increased microleakage has been linked to a higher risk of caries (15). Therefore, the present study aimed to evaluate microleakage in orthodontic adhesives containing nano-

Table 1. Frequency and percentage distribution of microleakage cases by treatment group, region, and site among the study samples.

Group	Subgroup	Sample size	Frequency (percentage)	
			No microleakage	Microleakage
Control	Bracket-adhesive (Gingival)	16	10 (62.5)	6 (37.5)
	Bracket-adhesive (Occlusal)	16	14 (87.5)	2 (12.5)
	Adhesive-tooth (Occlusal)	16	9 (56.3)	7 (43.8)
	Adhesive-tooth (Gingival)	16	13 (18.8)	3 (18.8)
	Total	64	46 (71.9)	18 (28.1)
Zinc oxide	Bracket-adhesive (Gingival)	16	14 (87.5)	2 (12.5)
	Bracket-adhesive (Occlusal)	16	16 (100)	0
	Adhesive-tooth (Occlusal)	16	13 (81.3)	3 (18.8)
	Adhesive-tooth (Gingival)	16	16 (100)	0
	Total	64	59 (92.2)	5 (7.8)
Copper oxide	Bracket-adhesive (Gingival)	16	14 (87.5)	2 (12.5)
	Bracket-adhesive (Occlusal)	16	15 (93.8)	1 (6.3)
	Adhesive-tooth (Occlusal)	16	13 (81.3)	3 (18.8)
	Adhesive-tooth (Gingival)	16	15 (93.8)	1 (6.3)
	Total	64	57 (89.1)	7 (10.9)

Table2. Effects of treatment group, margin location and bonding site on microleakage.

Variable		Beta	Std error	OR (95% CI)	P-value
Margin location	Occlusal	-0.363	0.218	0.695 (0.453-1.067)	0.096
	Gingival			Reference	
Bonding site	Tooth	1.487	0.485	4.425 (1.710-11.453)	0.002
	Bracket			Reference	
Group	Copper oxide	1.250	0.873	3.489 (0.630-19.317)	0.152
	Zinc oxide	-0.386	0.935	0.680 (0.109-4.252)	0.680
	Control			Reference	

particles. The descriptive results indicated that both nanoparticle-containing adhesives were associated with a lower incidence of microleakage compared to the conventional adhesive. Specifically, microleakage was observed in 28.1% of samples in the control group, while this rate dropped to 7.8% in the zinc oxide group and 10.9% in the copper oxide group. These findings suggest a potential role for metal oxide nanoparticles in reducing microleakage of orthodontic bonding materials. Regression analysis using the Generalized Estimating Equations (GEE) after adjusting for margin location and bonding site showed that only the bonding site variable has a statistically significant association with microleakage. The adhesive-tooth interface, exhibited a significantly higher odds of microleakage compared to the bracket–adhesive interface (OR = 4.425, 95% CI: 1.710 - 11.453, *P* = 0.002). Similar findings were reported by Alkis et al (16), Moosavi et al (17), Toodehzaeim et al (18), and Yagci et al (19). who also observed higher microleakage at the tooth-adhesive junction compared to bracket-adhesive junction. This finding emphasizes the critical role of anatomical site in microleakage occurrence. The tooth-adhesive junction is more susceptible to contamination, microleakage, and marginal breakdown, which may explain the elevated risk observed in this study. Regarding the nanoparticle groups, although the zinc oxide adhesive demonstrated a lower odds of microleakage compared to the control group (OR = 0.680), the difference was not statistically significant (*P* = 0.680). Conversely, the copper oxide group showed a non-significant increase in microleakage odds relative to the control group (OR = 3.489, *P* = 0.152). This apparent contradiction between descriptive and analytical findings might be explained by confounding effects of bonding site variability and the limited sample size, which could mask subtle group-level effects in multivariate models.

Zinc oxide nanoparticles have been widely studied for their antimicrobial, anti-inflammatory, and remineralizing properties (20,21). Their incorporation into dental adhesives may enhance marginal integrity by reducing bacterial colonization and matrix degradation (22,23). The present findings, although not statistically significant, support a favorable trend in reducing microleakage, in line with previous studies (24,25).

Previous studies have shown that copper oxide nanoparticles possess antimicrobial properties and may enhance the mechanical characteristics of dental materials (13,26,27). The findings of the current study suggest that while copper oxide nanoparticles may not significantly increase microleakage, they also do not provide a statistically proven advantage in microleakage reduction under the tested conditions.

Overall, while both nanoparticle groups showed promise in reducing microleakage descriptively, only zinc oxide exhibited a consistently beneficial trend across locations. The lack of statistical significance highlights the need for further studies with larger sample sizes and more controlled variables to better understand the true impact of metal oxide nanoparticles on microleakage in orthodontic bonding systems.

5. Limitations

1) *In-vitro* study limitations: Since this study was conducted *in vitro*, the conditions do not fully replicate the complex oral environment, such as salivary flow, pH fluctuation, and mechanical forces from mastication.
2) Limited aging process: The study did not stimulate long-term aging factors like thermal cycling, prolonged exposure to intraoral fluids, or mechanical stresses over extended periods.
3) Microleakage assessment method: While dye penetration is a common method for evaluating microleakage, other advanced techniques like micro-CT or SEM analysis could provide more detailed insights into leakage pattern.

6. Conclusion

The study showed that adding zinc-oxide nanoparticles to orthodontic adhesives slightly reduces microleakage. However, this difference was not statistically significant. Similarly, copper oxide nanoparticles did not significantly affect microleakage under orthodontic brackets, as neither descriptive analysis nor GEE modeling showed statistically significant differences among groups. Additionally, the amount of microleakage in the gingival area was more than the occlusal area in all three groups. However, the difference was not statistically significant. The only statistically significant finding was that the tooth-adhesive junction exhibited higher microleakage compared to bracket-adhesive junction. These findings suggest that incorporating nanoparticles into orthodontic adhesives can improve antimicrobial properties without compromising adhesive performance.

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Ethics
This experimental laboratory study was approved by the Ethics Committee of the Faculty of Dentistry, Shahid Sadoughi University, Yazd (ethical code: IR.SSU.REC.1397.015).

Using artificial intelligence (AI)
It is declared by the authors of this manuscript that no generative artificial intelligence (AI) or AI-assisted technologies were used to generate content, ideas, or theories during the writing process of this work.

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Conflict of interest
The authors declare no conflict of interest.

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