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Original Article

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Antimicrobial and bond strength properties of a dental adhesive containing zinc oxide nanoparticles

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Abstract

Aim: To assess the effect of adding zinc oxide nanoparticles to dental adhesives on their anti-microbial and bond strength properties. **Methods:** 45 human premolars were cut at the cement enamel junction (CEJ) and the crowns were sliced into buccal and lingual halves. The specimens were classified into three groups, etched with 37% phosphoric acid for 15 s and rinsed for 30 s. Single Bond, Single Bond+5% zinc oxide and Single Bond+10% zinc oxide were used in the first, second and third groups. A cylinder of Z250 composite was bonded and cured for 40 s. For anti-bacterial testing, 10 samples of each group were assessed by direct contact test; 10 µL of bacterial suspension was transferred into tubes containing adhesives and incubated for one hour; 300 µL of brain heart infusion (BHI) broth was added to each tube and after 12 h, 50 µL of bacteria and broth were spread on blood agar plates and incubated for 24 h. **Results:** The colony count decreased significantly in the second and third groups compared to the first. **Conclusions:** Incorporation of zinc oxide nanoparticles into dental adhesives increases their anti-microbial properties without affecting their bond strength.

Keywords: Zinc Oxide. Nanoparticles. Dental Cements. Anti-Infective Agents. Dental Caries.

Introduction

Dental caries is a common infectious disease, in which enamel and dentin are demineralized due to the acids produced from the fermentation of carbohydrates by acidogenic bacteria[1]. At present, carious teeth are commonly restored with tooth-colored restorative materials such as composite resins due to their optimal esthetic properties[2]. Occurrence of secondary caries is the most important reason for replacement of composite restorations[3,4].

Nanotechnology is the manipulation of matter on a molecular scale, which has revolutionized the modern medicine[5]. Zinc oxide has innate antibacterial properties. In the form of nanoparticles, it shows significantly higher antibacterial activity due to increased surface/volume ratio[6]. Studies have shown that zinc oxide can inhibit acid production by Streptococcus mutans and Lactobacillus in dental plaque[7]. Moreover, it has confirmed antibacterial effects on Gram-negative and Gram-positive bacteria and is commonly used as an antibacterial agent in dental hygiene products such as toothpastes and mouthwashes[8]. The purpose of this study was to assess the effect of adding zinc oxide

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nanoparticles to Single Bond dental adhesive on its antibacterial and bond strength properties.

Material and methods

This experimental study was conducted on 45 samples prepared from caries-free extracted human premolars. Immediately after tooth extraction, the teeth were rinsed under running water and immersed in 12% formaldehyde for one week. Then, they were stored in 0.9% NaCl solution. Using a high-speed hand piece under air and water spray, the crowns were cut at the CEJ and each crown was sliced into a buccal and a lingual half. The specimen surfaces were ground flat in order to achieve dentin surface covered with smear layer.

The specimens were divided into three groups, etched with 37% phosphoric acid solution for 30 s, rinsed with water jet spray for 30 s and the excess water was removed by a cotton pellet without air spray so that the dentin surfaces remained slightly moist. Bonding was performed using Single Bond (Adper Single Bond Plus; 3M ESPE, MN, USA) in the first group, Single Bond with 5% zinc oxide nanoparticles in the second group and Single Bond with 10% zinc oxide nanoparticles in the third group. Zinc oxide nanoparticles (Pishgaman Nano-Material Co., Mashhad, Iran) had an average particle size of 20 nm with a crystalline structure. They were almost spherical in shape and had 99% purity.

Adhesive resins were applied to the teeth surfaces in two consecutive layers and were mildly agitated for 20 s followed by gentle air-drying for 20 s (in order to evaporate the solvent). Prior to light curing, a cylindrical tube (0.75 mm internal diameter and 1.0 mm high) was placed on the dentin surface. After 20 s of irradiation using a light curing unit (Optilux 501; Kerr, Orange, CA, USA) with a 300mW/cm2 light intensity, verified by a Demetron radiometer (Sybron, CA, USA), each tube was filled with A2 shade of Z250 composite (3M ESPE) and cured at all directions for 40 s.

The samples were stored in water at 37 °C for 24 h. Then, shear bond strength was measured in a universal testing machine (Zwick; Roell, Ulm, Germany). The fracture surfaces were evaluated under a stereomicroscope (Olympus, Japan) in order to determine the mode of failure as adhesive, mixed or cohesive.

For antibacterial testing in each group, 10 samples were evaluated by direct contact method; 10 µL of the bacterial suspension was added to the tubes containing adhesives and then the tubes were placed vertically in an incubator and incubated for one hour. Then, 300 µL of BHI were added to each tube and after 12 h, 5 µL of the bacterial suspension mixture and BHI broth was spread on a blood agar plate. The plate was placed in an incubator for 24 h. Then the number of bacterial colonies was counted and reported as colony forming units per milliliter (CFU/mL). Data were analyzed using SPSS (Release 18; SPSS Inc, Chicago, IL, USA) and ANOVA. A post-hoc test was applied for assessment of inter-group differences. The significance level was set at $p < 0.05$.

Results

Table 1 shows the descriptive statistics of bond strength to dentin in the study groups.

Table 1 - Comparison of the mean bond strength to dentin in the study groups (in MPa).

	Mean±SD	Range	F, p-value
Group 1	26.00±3.59	19.70-31.60	F= 0.575,
Group 2	25.47±2.98	19.50-30.00	p = 0.056
Group 3	24.92±2.30	19.20-29.20	

According to Table 1, the mean bond strength was the highest in the control group (Single Bond) followed by Single Bond + 5% zinc oxide. The minimum bond strength was in Single Bond + 10% zinc oxide. However, the difference among the study groups was not significant ($p > 0.05$).

Stereomicroscopic assessment for determination of the mode of failure showed that out of 45 studied samples, 84% had adhesive failure and 16% had mixed failure. No cohesive failure was observed among the samples (Figure 1).

Table 2 shows descriptive statistics for microbial count in the study groups.

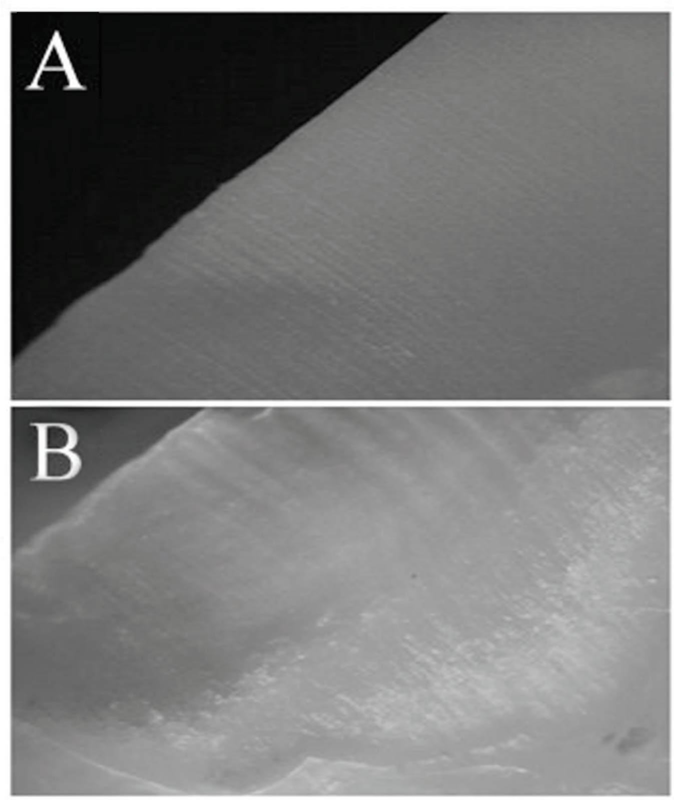

Fig.1. Adhesive Failure (A), Mixed Failure (B).

According to Table 2, the mean colony count was significantly higher in the control group and the minimum colony count occurred in Single Bond + 5% zinc oxide and Single Bond + 10% zinc oxide groups. Inter-group comparison by the post hoc test showed that the control group had a significant difference to Single Bond + 5% zinc oxide and Single Bond + 10% zinc oxide groups ($p < 0.05$). The difference between the two latter groups was not significant ($p > 0.05$).

Table 2 - Comparison of the microbial count among the study groups (CFU/mL).

	Mean±SD	Minimum	Maximum	F, p-value
Group 1	131.65 ± 1020×10^2	800×10^2	1200×10^2	F=568.083,
Group 2	6.68 ± 33.5×10^2	25×10^2	45×10^2	p<0.001
Group 3	4.95 ±18.1×10^2	12×10^2	25×10^2	

Discussion

Considering the extensive use of dental composites due to their excellent esthetic properties and also to the high prevalence of secondary caries as the main reason for failure of composite restorations, attempts are made to confer antibacterial properties to these composites[9].

Because of the suitable antibacterial property of zinc oxide, we assessed the effect of adding zinc oxide nanoparticles in two different concentrations to Single Bond adhesive on Streptococcus mutans count (the main etiologic factor for dental caries) and bond strength to dentin. For antibacterial testing, the agar diffusion test is the most frequently used method for testing the antibacterial activity of dental materials[10]. However, the mechanism of action of this method is based on water-soluble components released from mass substances. Thus, it is not feasible for evaluating the antibacterial efficacy of less water-soluble substances such as zinc oxide[11]. Direct contact test is commonly used for assessment of antibacterial efficacy of solids in soluble substances. In this method, the bacteria are directly exposed to the substance in a controlled fashion and then their growth and proliferation are evaluated after certain time intervals[12]. In our study, this test showed that zinc oxide nanoparticles conferred antibacterial properties to Single Bond, and that increasing concentration of zinc oxide nanoparticles increased the antibacterial activity of the mixture (Table 2).

Tavassoli et al.[11] assessed physical, mechanical and antibacterial properties of flowable composites containing zinc oxide nanoparticles and showed that zinc oxide conferred antibacterial properties to resin and increased concentration of zinc oxide nanoparticles significantly decreased bacterial growth and proliferation, which concurs with the results of the present study.

Zinc oxide nanoparticles have been proven to provide significant antibacterial properties to resin[13,14], as observed in the present study.

Niu et al.[13] found that turbidity test was used to assess the antibacterial properties. But it has a limitation, as it examines both living and dead bacteria in liquid conditions. Instead, in the our study, a colony counting method was used in which only the living bacteria are counted[13]. Spencer et al.[15] showed that adding zinc oxide to a light-cured resin-modified glass ionomer orthodontic bonding agent conferred antimicrobial properties to it without significantly altering the shear bond strength. They also demonstrated that increasing the concentration of zinc oxide, its antimicrobial properties increased and these results were in line with our findings[15]. Moreover, Kasraei et al.[6] showed that the effects of zinc oxide on Streptococcus mutans were significantly greater than those of silver. In terms of bond strength in our study, the mean bond strength was highest in the Single Bond group followed by the Single Bond + 5% zinc oxide and Single Bond + 10% zinc oxide, respectively (Table 1).

Osorio et al.[8] indicated that degeneration decreased collagen in demineralized dentin and the effect of zinc oxide remained for three weeks as opposed to that of chlorhexidine, which was short-term.

Larsen et al.[16] showed that zinc, forming zinc mono hydroxide, links catalytic ions to the lateral chain in the active side of carboxy peptidase A and inhibits it. They showed that zinc not only has a catalytic role for enzymes, but it also plays a role in protein stability. Zinc acts as a matrix metalloproteinase (MMP) competitive inhibitor, a protective effect by bonding to some regions of collagen that are sensitive to cleavage by metal proteinase matrix[16]. Osorio et al.[17], demonstrated that zinc decreased collagen degeneration in Single Bond hybrid layer and had no adverse effect on the bond strength; their results were in accordance with ours.

In their study, Single Bond containing zinc decreased C-terminal telopeptide of type-I collagen concentration (collagen degeneration product) at one and four weeks and the best result was obtained when zinc oxide nanoparticles were added to the bonding agent[17]. Etch and rinse adhesive systems are still the gold standard for bonding to enamel and if bonding by etch and rinse adhesive containing zinc decreases dentin collagen degeneration, a strong bond to dentin can be achieved[18].

In another study[8], bond strength in Single Bond and Single Bond + 10% zinc oxide was higher than the value obtained in the current study after 24 h (37.5 and 36.26 MPa versus 26 and 24.92 MPa, respectively). This difference in results may be ascribed to the fact that they measured micro-tensile bond strength while we measured micro-shear bond strength. Micro-tensile bond strength level is often higher than the micro-shear bond strength[19].

In our study, similar to a previous research[11], the immediate bond strength of Single Bond plus zinc oxide was lower than that of Single Bond alone. This reduction may be attributed to the agglomeration phenomenon or to the increase in the opacity of composite and incomplete curing[11]. As mentioned earlier, the difference in bond strength among the three groups was not significant and all values were within or higher than the acceptable range of bond strength to dentin (20 MPa). Dental restorative materials must be able to provide a strong and durable bond to tooth structure[20,21].

Based on studies on previous investigations[17,20], zinc provides a durable and strong bond at the resin/dentin interface by decreasing collagen degeneration. The results of the current study showed that adding zinc oxide nanoparticles to Single Bond inhibited the formation of Streptococcus mutans colonies without significantly compromising the bond strength. Since 10% zinc oxide had a greater anti-bacterial effect than 5% zinc oxide and considering the insignificant difference in immediate bond strength of these two groups, use of Single Bond + 10% zinc oxide is recommended.

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