

Antimicrobial Effects of Zinc Oxide in an Orthodontic Bonding Agent

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ABSTRACT

Objective: To test the null hypothesis that the addition of zinc oxide (ZnO) has no effect on the antimicrobial benefits and shear bond strength of a light-cured resin-modified glass ionomer.

Materials and Methods: ZnO was added to Fuji Ortho LC to create mixtures of 13% ZnO and 23.1% ZnO. Specimen discs of the modified bonding agent were incubated with *Streptococcus mutans* for 48 hours in a disc diffusion assay that was used to measure zones of bacterial inhibition. In addition, brackets were bonded to bovine deciduous incisors with the modified bonding agents, and shear bond strength was evaluated with a universal testing machine.

Results: The modified samples showed that antimicrobial activity increased as the concentration of ZnO increased. There were significant differences ($P < .05$) in antimicrobial activity. Post hoc tests showed that the antibacterial effects were 1.6 times greater with 23.1% ZnO than with 13% ZnO. There was no difference between Transbond and 0% ZnO (the negative control). After 1 month of daily rinsing, the antibacterial effects of 23.1% ZnO and 13% ZnO decreased 65% and 77%, respectively, but both maintained significant effects over the negative controls. There were no significant differences ($P = .055$) in shear bond strength between any of the mixture comparisons.

Conclusions: The incorporation of ZnO into Fuji Ortho LC added antimicrobial properties to the original compound without significantly altering the shear bond strength. ZnO holds potential for preventing decalcification associated with orthodontic treatment. (*Angle Orthod.* 2008;79: 317–322.)

KEY WORDS: Zinc oxide; Antimicrobial effects; Decalcification; Bond strength

INTRODUCTION

While fixed orthodontic appliances offer a myriad of methods to improve smile esthetics and occlusal relationships, they also pose a challenge to both the patient and the clinician in maintaining a healthy dentition. Decalcification around orthodontic brackets and bands, known as white spot lesions (WSL), is a prob-

lem for every clinician and is a potential risk for patients with poor oral hygiene. Patients undergoing full fixed treatment are at a higher risk for developing caries due to increased levels of *Streptococcus mutans* in plaque and saliva.^{1,2} Various incidences have been reported due to differences in detection, scoring, and reporting methods.² With increased levels of bacteria and inadequate oral hygiene, decalcification can occur in as little as 4 weeks.^{3–6}

While fluoride varnishes, fluoride mouth rinses, and oral hygiene instructions have been employed to inhibit WSL, they rely heavily on patient compliance and provide only intermittent protection against decalcification. Bonding agents that release fluoride show rapidly decreasing levels after the first 24 hours.^{7,8} Bonding agents that provide antimicrobial protection have been evaluated,^{1,9,10} but to date, none are commercially available.

Materials containing zinc oxide have antimicrobial effects and are used in various ways, including pharmaceutical creams or ointments for the treatment of leg ulcers, traumatic wounds, and burns.^{11,12} Dental materials such as endodontic sealers and fixed res-

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toration cements have been utilized for this same reason.^{13,14} Zinc, which serves as an activator of enzymes that can be toxic to microbes at concentrations as low as 0.5 ppm, has been shown to inhibit the growth of plaque at concentrations of 4, 6, and 16 ppm.¹⁵

The goal of this study was to test the antimicrobial properties of zinc oxide when incorporated into an orthodontic bonding material (Ortho Fuji LC) and determine: (1) if zinc oxide exhibits antimicrobial properties; (2) the duration of the antimicrobial effect, if present; and (3) if the adhesive properties of the bonding agent are affected by the zinc oxide.

MATERIALS AND METHODS

Tooth Preparation

Sixty deciduous bovine incisors were collected from a USDA-inspected meat processing plant. All vital pulp tissue was removed with a high-speed handpiece and a diamond bur. The teeth were stored in 0.1% thymol solution. In order to provide a consistently flat, smooth enamel surface, each tooth was fixed in orthodontic resin and polished to a uniform, flat surface on a variable speed grinder (Ecomet 3, Buehler, Lake Bluff, Ill) using 400 and 600 grit wet SiC abrasive papers at 150 rpm.

Bonding Agent Preparation

Three mixtures of Fuji Ortho LC (GC America, Alsip, Ill), a resin-modified glass ionomer (RMGI), were prepared for bonding. One mixture, serving as the negative control, was prepared according to manufacturer's instructions. The second mixture contained 0.3 g of zinc oxide (ZnO) powder per 1 g Fuji powder (23.1% ZnO). The third mixture contained 0.15 g of zinc oxide (ZnO) powder per 1 g Fuji powder (13% ZnO). Each mixture was placed in a separate test tube and mixed for 1 minute using a Vortex mixer to create a uniform powder.

Bonding

For bonding, 30 teeth were randomly assigned to the three RMGI mixtures (negative control, 13% ZnO, and 23.1% ZnO). Each tooth was cleaned with pumice and water for 5 seconds, rinsed for 10 seconds, and air dried to avoid desiccation. Teeth were then etched with 37% phosphoric acid gel for 20 seconds and rinsed for 10 seconds. The bonding material was mixed using one scoop of powder with one drop of liquid for the control group. For mixtures containing zinc oxide, two drops of liquid had to be used in order to prepare a material with the appropriate viscosity. Half of the powder was incorporated into the liquid for 10 seconds followed by the rest of the powder. Total

mixing time was 25 seconds. The RMGI material was incorporated into the base of a lower central incisor SPEED bracket (Strite Industries, Cambridge, Ontario, Canada) using a cement spatula, and the bracket was placed on the enamel surface using a Hollenbeck carver. Excess material was removed from around the bracket, and the remaining adhesive was light cured for 40 seconds (10 from each side) using the 3M Unitek Ortholux LED curing light (Ortholux, 3M Unitek, Monrovia, Calif). Bonded teeth were stored in water at 37°C for at least 24 hours before testing bond strength.

Debonding

A screw-driven universal test machine (model 5567, Instron, Norwood, Mass), with a crosshead speed of 1 mm/min was used to determine shear bond strength. The acrylic cylinder in which the tooth was embedded was secured using a fitted vice. A metal blade was directed parallel to the bracket base which, according to the manufacturer, had a surface area of 10.95 mm². Modified adhesive remnant index (MARI) scores¹⁶ were used to quantify the bonding material left on the bracket base, with 0 = no adhesive left on the bracket, 1 = less than half of the bracket covered with adhesive, 2 = more than half of the bracket covered with adhesive, and 3 = the entire bracket covered with adhesive.

Antimicrobial Testing

Disc Preparation

Three grams of the RMGI were prepared for each of the three mixtures. The first mixture contained no zinc oxide, the second mixture contained 0.45 g ZnO (13% ZnO), and the third mixture contained 0.90 g ZnO (23.1% ZnO). The mixtures were prepared as previously described, along with a fourth mixture consisting of a light-cured resin-base orthodontic adhesive (Transbond XT, 3M Unitek). The 0% ZnO mixture served as the negative control for antimicrobial testing.

Each bonding material was loaded into an empty allergy syringe (1 mL, Becton Dickinson and Company, Franklin Lakes, NJ) and light cured for 4 minutes. The syringes containing the bonding material were then sliced using a precision high-speed saw with water cooling (Techcut 5, Allied High Tech Products, Rancho Dominguez, Calif), creating discs that were approximately 4.6 mm × 2 mm. The sliced discs were prepared at 3200 rpm at a rate of 0.15 inches/min and with low force. Discs were allowed to air dry and were then stored in airtight bags.

Agar Plate Preparation

Brain heart infusion (BHI) plates were prepared for antimicrobial testing using a decreased concentration

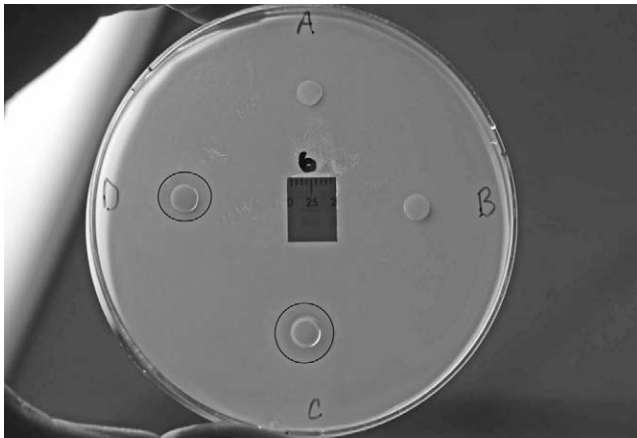


Figure 1. Infusion plates used to evaluate zones of microbial inhibition of the four groups.

of nutrient material, 2.31 g/L BHI, and 16 g/L of Bacto-Agar (Difco, Detroit, Mich). One disc from each mixture was placed on each plate. A soft agar overlay was prepared with 2.3 g/L BHI and 7.0 g/L agar. An overnight *S. mutans* culture (200 μ L) grown in BHI at 37°C statically was mixed with 3.5 mL of soft agar and poured evenly over the plates, surrounding the disks. Following solidification of the overlay, the plates were then incubated at 37°C for 48 hours. A 10-mm plastic ruler was glued on the bottom of the agar plates for calibration. Photographs were taken and magnified twofold so that the perimeter of each zone could be accurately traced. Image Tool (University of Texas Health Science Center at San Antonio Dental School, San Antonio, Tex) software was used to measure the areas (mm²) of the inhibition rings (Figure 1). All measurements were performed twice by the same blinded operator; mean values were used to assess antimicrobial activity.

After initial data collection, the test discs were removed from the plates, rinsed, and placed in 8 ounces of distilled water. The discs were rinsed using fresh, distilled water each day for approximately 20 seconds. After 1 month, the discs were sterilized with ethylene dioxide for 24 hours, and the previously described antimicrobial assay and evaluations were performed.

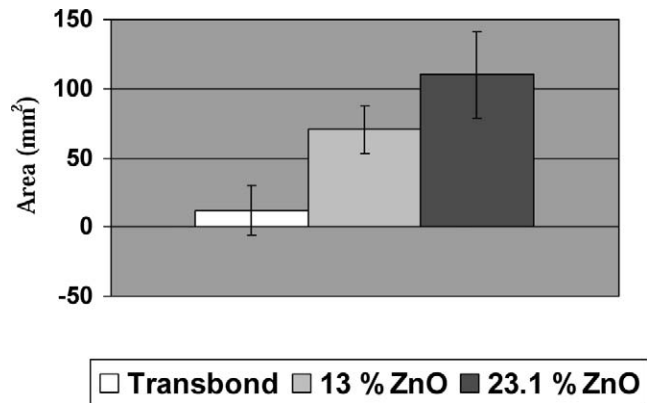


Figure 2. Mean zones of inhibition (\pm 1 SD) of the % ZnO group, the 13% ZnO group, and the 23.1% ZnO group.

Analysis

The data were distributed normally and described with means and standard deviations. One-way analysis of variance (ANOVA) was used to determine mixture differences. Post hoc Bonferroni tests were performed for multiple comparisons. The significance level for all statistical tests was set at $P < .05$.

RESULTS

Disc Diffusion Assay

The Fuji Ortho LC RMGI without zinc oxide served as the negative control and only showed minimal zones of inhibition (Table 1). Antimicrobial activity, as measured by the zones of inhibition, increased as the concentration of ZnO increased (Figure 2). The 13% ZnO mixture had an effect that was six times greater than the negative controls. Samples containing 23.1% zinc oxide exhibited antibacterial effects that were nine times greater than the negative controls. The 23.1% zinc oxide mixture showed a significantly greater (1.6 times) effect than the 13% zinc oxide mixture. The Transbond XT samples also showed a zone of inhibition, albeit smaller than the mixtures containing ZnO. ANOVA identified significant ($P < .001$) differences in the antimicrobial activity. Post hoc Bonferroni tests (Table 2) showed all paired comparisons to be statistically significant ($P < .001$), except the difference be-

Table 1. Mean and Standard Deviation Values for the Area (mm²) of the Zones of Inhibition for All Four Mixtures at the Initial Test and 1 Month After Daily Rinsing With Distilled Water^a

	0% ZnO		13% ZnO		23.1% ZnO		Transbond XT		Mixture Differences
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P Value
Initial	0.00	0.00	70.52	17.33	110.03	31.00	12.02	18.21	<.001
1 Month	0.0	0.0	16.0	11.6	38.3	13.3	0.0	0.0	<.001

Table 2. Post Hoc Comparisons of the Antibacterial Activity of the Four Mixtures Showing Significance Levels for Each^a

Comparison	P Value (Initial)	P Value (1 Month)
0% ZnO vs Transbond XT	.294	N/A
13% ZnO vs Transbond XT	<.001	<.001
23.1% ZnO vs Transbond XT	<.001	<.001
0% ZnO vs 13% ZnO	<.001	<.001
0% ZnO vs 23.1% ZnO	<.001	<.001
13% ZnO vs 23.1% ZnO	<.001	<.001

^a N/A, not applicable.

tween the negative control and Transbond XT ($P = .294$).

After daily rinsing for the 1 month, both the 13% ZnO and 23.1% ZnO mixtures showed significant ($P < .001$) antimicrobial activity compared with the negative control (Table 2). The zones of inhibition for the 13% and 23.1% ZnO mixtures decreased by 77% and 65%, respectively, over the 1-month period (Table 1). Differences between the two zinc oxide mixtures were statistically significant ($P < .001$).

Comparison of Shear Bond Strengths

Bond strengths decreased as the concentration of zinc oxide increased (Table 3; Figure 3). The Kruskal-Wallis test showed that mixture differences approached significant levels ($P = .055$), but the differences were not statistically significant. The mean bond strength of the 13% ZnO mixture was 76% of the negative control, whereas the 23.1% ZnO mixture had a mean bond strength that was 71% of the negative control.

Modified Adhesive Remnant Index Scores

Bond failure occurred most frequently at the bracket/RMGI junction rather than at the enamel surface (Table 4). Most (approximately 82%) of the brackets had little (less than half of the bracket covered) or no adhesive left on the bracket.

DISCUSSION

Zinc oxide showed significant antimicrobial effects on the disc diffusion test, as demonstrated by visible zones of inhibition. Zones of inhibition were seen on 7 of the 22 samples from the 0% ZnO mixture, but mea-

Table 4. Frequency Distribution of Modified Adhesive Remnant Index (MARI) Scores^a

Mixture	MARI Scores			
	0	1	2	3
0% ZnO	4	6	—	—
13% ZnO	2	5	2	—
23.1% ZnO	1	5	1	2

^a Scores: 0 = no adhesive left on the bracket; 1 = less than half of the bracket is covered with adhesive; 2 = more than half of the bracket is covered with adhesive; 3 = all of the bracket is covered with adhesive.

surements were not statistically different when compared with the negative control (Transbond XT). The presence of fluoride ions in the Fuji Ortho LC was the most likely cause of these zones of inhibition. Since the addition of zinc oxide was the only change made to the Fuji Ortho LC material, these data support other studies attributing antimicrobial effects of zinc oxide.^{15,17,18}

The antimicrobial effect of the ZnO was concentration dependent. A 78% increase in the concentration of zinc oxide resulted in a 56% increase in the zones of inhibition. Other studies have reported that antimicrobial effects of other compounds are concentration dependent.^{1,9} These findings agree with Moorer and Genet,¹⁸ who proposed that antimicrobial action from zinc oxide may be a result of a "reservoir" effect. Including larger amounts of zinc into the bonding material would, therefore, make more zinc ions available for mobilization. Bates and Navia¹⁵ suggested that zinc may act by blocking the electron-transport chain or by inhibition of ATP formation. They also note that zinc may interfere with transport mechanisms by binding preferentially to sites on membranes causing conformational changes in proteins or enzymes.

While bond strengths showed no significant differences between the material without zinc oxide and the experimental mixtures, there was a decrease in bond strength with increased concentrations of zinc oxide. The lack of significant differences between the negative control and experimental mixtures may have been due to a lack of statistical power. Mean bond strength for the 0% ZnO mixture in the current study was 6.3 MPa (SD = 1.84), which falls at the low end of the range of bond strengths reported for Fuji Ortho LC.¹⁹⁻²³ Mean bond strengths for the 13% and 23.1% ZnO mix-

Table 3. Comparisons of Shear Bond Strength of the Control and Two Experimental Groups

	Negative Control		13% ZnO		23.1% ZnO		Difference Probability
	Mean	SD	Mean	SD	Mean	SD	
Bond strength, N	68.8	20.2	55.2	12.7	49.9	21.8	.055
Bond strength, MPa	6.3	1.8	5.0	1.2	4.6	2.0	.055

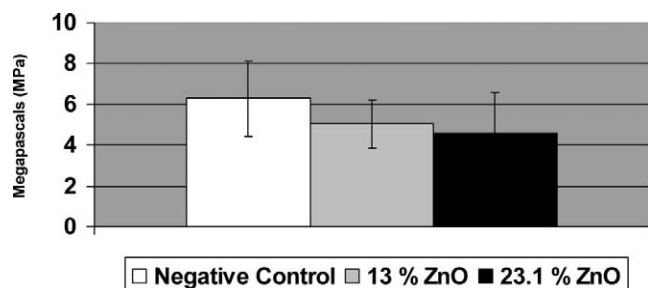


Figure 3. Mean bond strengths (± 1 SD) of negative controls, the 13% ZnO group, and the 23.1% ZnO group.

tures were 5.04 MPa and 4.56 MPa, respectively, which also fall in the low end of the range for orthodontic bond strengths previously reported. Bond strengths obtained in this study (Table 3) were slightly below the adequate range (5.9–7.8 MPa) for orthodontic bonding previously suggested.^{24,25} They were adequate according to McSherry,²⁶ Wheeler and Ackerman,²⁷ and Keizer et al.²⁸ However, the bond strengths obtained in this study are low based on the recommendations (97.88 N) of Maijer and Smith.²⁹

The wide-ranging differences in reported bond strengths are probably due to various methods used to measure bond strength.³⁰ Wire loops, steel rods, and steel plates have all been utilized to determine shear bond strength.³¹ In addition, the shear blade used in this study applied the force closer to the tie wing rather than the base. Changes in the orientation of the shearing force by as little as 15° can decrease bond strength values by 27.4%.³⁰ Moreover, rather simple means of incorporating the zinc oxide were employed, and a larger than normal liquid portion of the RMGI was used, which could have weakened the material. The presence of zinc oxide particles on the surface of the RMGI could have further decreased the bond strength due to reduced contact between enamel and RMGI. Finally, while bovine teeth have been shown to be an adequate substitute for human enamel, they tend to produce lower bond strengths than human teeth.^{32,33}

Future studies should evaluate more refined methods of adding zinc oxide in order to have less of an impact on the physical properties of the bonding agent. Further development of bonding agents utilizing zinc oxide could lead to the prevention of WSL. Caution should be exercised when attempting to extrapolate in vitro results to the clinical setting. Disc diffusion tests are adequate for showing the presence of antimicrobial activity, but they cannot be correlated with intra-oral conditions. Further clinical studies are needed to assess the capabilities of zinc oxide as an intraoral antimicrobial agent.

CONCLUSIONS

- As the concentration of ZnO increases, antimicrobial activity significantly increases.
- Antimicrobial activity of ZnO lasts for at least 1 month, albeit at lesser levels.
- As the concentration of zinc oxide increases, shear bond strength decreases.

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