

## Original Research

# Antibacterial effects and Shear bond strength of Orthodontic Composite containing Zinc Oxide

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### ABSTRACT

**Objective:** To test efficacy of zinc oxide (ZnO) as the antimicrobial agent and shear bond strength of a light-cured resin-modified glass ionomer used in orthodontic treatment. **Materials and Methods:** ZnO was added to Fuji Ortho LC to create mixtures of 12% ZnO and 24% 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 human extracted premolars 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 ( $p$  value  $< 0.05$ ) in antimicrobial activity and shear bond strength between the groups. **Conclusion:** 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.

**Key words:** Zinc oxide, Antimicrobial effects, Shear Bond strength.

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### INTRODUCTION

A significant increase in the prevalence of white spot lesions has been reported in orthodontic patients ranging from 72.3 to 96%.<sup>1,2</sup> Enamel demineralisation occurs due to specific alterations in the oral environment, which includes prolonged bacterial plaque retention in the irregular surfaces of orthodontic appliances and lower pH conditions produced by cariogenic bacteria.<sup>3</sup> In order to improve the antimicrobial activity of orthodontic bonding systems, some studies have suggested the incorporation of antimicrobial agents into orthodontic bonding systems to prevent demineralisation through bactericidal and bacteriostatic action. *S. mutans* is one of the most common microorganisms found in cariogenic plaque on dental hard tissues. In the pH below, 5.5 this microorganism has anaerobic activity, which produces organic acids. A similar microorganism that is common in cariogenic plaque is *Lactobacillus acidophilus* which helps the demineralization

of dentinal tissues. On the other hand, the microorganism found in non-cariogenic plaque at pH equal to 5.5 is *Streptococcus sanguis* and its presence in the plaque is an indication of low cariogenic activity of the plaque.<sup>4</sup> Among the substances with potential for bacterial inhibition, it has been reported that methacryloyloxydodecylpyridinium bromide (MDPB), glutaraldehyde silver nanoparticles, chlorhexidine triclosan and benzalkonium chloride (BAC) have an antimicrobial effect. Studies have suggested that these antimicrobial agents are selectively toxic to oral *Streptococci*, and their incorporation in orthodontic bonding systems helps to prevent demineralisation of the enamel without compromising mechanical adhesion properties. Because nano-sized composites are expected to be more effective in penetrating and disrupting bacterial cell membranes, nano chitosans are also effective against a variety of organisms, and if added silver salts or other

functional antimicrobial agents to chitosan nanoparticle composites, antimicrobial activity enhances.<sup>5</sup>

Zinc oxide (ZnO) is listed as “generally recognized as safe” (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). As a food additive, it is the most commonly used zinc source in the fortification of cereal-based foods. Because of its antimicrobial properties, ZnO has been incorporated into the linings of food cans in packages for meat, fish, corn, and peas to preserve colors and to prevent spoilage. Nano-sized particles of ZnO have more pronounced antimicrobial activities than large particles, since the small size (less than 100 nm) and high surface-to-volume ratio of nanoparticles allow for better interaction with bacteria.<sup>6</sup>

Thus, the objective of the present study was to test the antimicrobial effect of zinc oxide when incorporated into an orthodontic bonding material and its duration of antimicrobial effect. The effect of addition of zinc oxide on the shear bond strength of the bonding material was also tested using universal testing machine.

## MATERIALS AND METHOD

The study was divided into 2 parts: The antimicrobial testing and the shear bond testing.

### Three groups of bonding material were made:

Group 1: Bonding with Fuji Ortho LC GIC (light cure glass ionomer cement)

Group2: Bonding with Fuji Ortho LC GIC mixed with 12% zinc oxide powder

Group3: Bonding with Fuji Ortho LC GIC mixed with 24 % of zinc oxide powder.

### Bonding material preparation

The bonding material for Group 2 and 3 was prepared as follows. For Group 2 the bonding material was prepared by mixing 12 g of zinc oxide powder to 100 g of Fuji Ortho LC GIC. For Group 3 bonding material was prepared using 24 g of zinc oxide powder to 100 g of Fuji Ortho LC GC. Each mixture was mixed for 2 minute using a vibrator to create a uniform powder.

### Antimicrobial testing

For the antimicrobial testing test discs of all the four bonding material were prepared (one disc per group). Discs that were approximately 2 mm × 2 mm were prepared. The bonding material was loaded onto the discs and light cured for 40 s. The discs were stored in airtight containers.

### Agar plate preparation

Two different brain heart infusion (BHI) plates were prepared for antimicrobial testing using a decreased concentration of nutrient material, 2.31 g/L BHI, and 16 g/L of bacto-agar (hi media). One disc from each mixture was placed on each plate. An overnight *S. mutans* culture (200 µL) grown in BHI at 37°C was mixed with 3.5 mL of soft agar and poured evenly over the plates, surrounding the

discs. Following solidification of the overlay, the plates were then incubated at 37°C for 48 h. The zones of inhibition were measured after 48 h using a zone of inhibition measuring scale.

### Shear bond testing

For the shear bond testing 40 freshly extracted human premolar teeth were collected and stored in a solution of 0.2% (weight/volume) thymol. The teeth were embedded in acrylic resin. After mounting the teeth were cleaned and polished with pumice. Metallic first premolar brackets were used. The samples were divided into the above mentioned four groups each containing 10 teeth.

### Bonding procedure

Each tooth was cleaned with pumice and water and air dried to avoid desiccation. Teeth were then etched with 37% of phosphoric acid gel for 20 s and rinsed for 10 s. The bonding material was mixed using one scoop of powder with one drop of liquid for all the groups. The material was incorporated into the base of the premolar bracket using a cement spatula and the bracket was placed on the enamel surface. Excess material was removed from around the bracket and the remaining adhesive was light cured for 40 s (10 from each side) using the 3 M Unitek Ortholux LED curing light. Bonded teeth were stored in water at room temperature for 24 h before testing bond strength.

### Evaluation of Shear Bond Strength (SBS)

An universal testing machine(UTM) was used to measure the SBS. The crosshead of UTM moved at the uniform speed of 1 mm/min. The acrylic block was positioned in the lower crosshead with the crown portion of teeth facing upward. The debonding force was applied in a direction parallel to the bracket base. A loop made of 0.8 mm stainless steel was attached to the upper crosshead to apply shear force to debond the bracket.

### Statistical analysis

The data were analyzed by SPSS software<sup>16</sup>, Descriptive analysis was used for finding the mean and standard deviations of the samples tested for shear bond strength and antimicrobial assay. A paired student t-test was done for comparison of the various groups used for the shear bond testing and a post hoc test and t-test was done for the comparison of groups used for antimicrobial testing.

## RESULTS

The Fuji Ortho LC GIC without zinc oxide and conventional light cure composite showed no zones of inhibition. Antimicrobial activity, as measured by the zones of inhibition, increased as the concentration of Zn O increased. The 24% of zinc oxide mixture showed a greater zone of inhibition than the 12% of zinc oxide mixture [Table 1] and the difference in the antimicrobial activity

was statistically significant ( $P < 0.05$ ). The data obtained is tabulated and the mean values for the shear bond strength with their standard deviations are determined. The mean value of shear bond strength of the resin modified GIC was  $11.44 \pm 0.056$ , GIC + 12% Zn O is  $9.34 \pm 0.033$  and GIC + 23.1% Zn O is  $8.54 \pm 0.045$ . [Table 2]

**TABLE 1: MEAN AND STANDARD DEVIATION OF THE SAMPLES OF ANTIMICROBIAL TESTING**

Group	MEAN	Standard Deviation	p value
Group 1	0.00	0.00	<0.05
Group 2	9.000	0.077	
Group 3	18.000	0.088	
Total	7.88	0.015	

**TABLE 2: MEAN AND STANDARD DEVIATION OF THE SHEAR BOND STRENGTH**

Material	Mean	Standard Deviation	p value
GIC	11.44	0.056	<0.05
GIC+12% ZnO	9.34	0.033	
GIC+24%ZnO	8.54	0.045	
Total	10.11	0.992	

**DISCUSSION**

In the present study, we evaluated the mechanical and biological properties of orthodontic bonding agents containing ZnO and determined the antibacterial and effects on bond strength of these agents. ZnO nanoparticles have a broad spectrum of antibacterial activities. At concentrations higher than 0.24 mg/ml, they inhibit the growth of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Enteritidis.<sup>7,8</sup> An inhibitory effect of ZnO nanoparticles on *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Enterococcus faecalis* has been reported as well.<sup>9</sup> Nano silver particles have antimicrobial effect against refractory bacteria. Nano silver and nano silica filler containing composites prevent enamel demineralization around orthodontic brackets. There are studies which have illustrated the higher antimicrobial properties of 1% weight nano titanium oxide containing composite compared to the control group.<sup>10</sup>

It is necessary to understand the mechanism of action of ZnO against bacteria, but to date, the process underlying their antibacterial effect is not clear. However, a few studies have suggested that the primary cause of the antibacterial function might be from the disruption of the cell membrane activity. Another possibility could be the induction of intercellular reactive oxygen species, including hydrogen peroxide ( $H_2O_2$ ), a strong oxidizing agent harmful to bacterial cells.<sup>[11,12]</sup>

The antimicrobial effect of the ZnO was concentration dependent. Other studies have reported that antimicrobial effects of other compounds are concentration dependent. These findings agree with Moorer and Genet,<sup>13</sup> 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 Navia<sup>14</sup> suggested that zinc may act by blocking the electron-transport chain or by inhibition of ATP formation. They also noted that zinc may interfere with transport mechanisms by binding preferentially to sites on membranes causing conformational changes in proteins or enzymes.

The shear bond strength of the brackets bonded with light cure composite was greater than the shear bond strength of the brackets bonded with RMGIC and RMGIC with 13% and 23.1% zinc oxide. Shammaa et al.<sup>15</sup> in 1999 concluded that the bracket debonding force of RMGIC (Fuji Ortho LC GC America, Alsip, Ill) in wet and dry conditions was found to be significantly lower than the conventional resins. The shear bond strength of the RMGIC group was found to be 10.2683 MPa ( $\pm 0.192$ ), which was in the range of shear bond strength recommended for RMGIC.<sup>16,17</sup> Klocke and Kahl-Nieke<sup>18</sup> demonstrated that variation of the direction of debonding force significantly influences shear bond-strength measurements. Changes in orientation of the shearing force by as little as 15° can decrease bond strength values by 27.4%.

Spencer et al. found that as the concentration of ZnO increases, SBS decreases.<sup>19</sup> Mean bond strengths for the 12% and 24% ZnO mixtures were observed to be 9.34 MPa and 8.54 MPa, respectively in our study. Jatania and Shivalinga also noted increased SBS with decreased concentration of ZnO.<sup>20</sup>

**CONCLUSION**

The present study has shown that zinc oxide powder when supplemented with GIC produces antimicrobial effect, which increases as the concentration of zinc oxide is increased. Incorporation of various nanoparticles into adhesive materials in nominal amounts can affect the SBS which may lead to the failure of bracket or adhesive. Optimization of the concentration of zinc oxide in GIC can bring the desired results where not just the antimicrobial properties of zinc oxide can be used but also the bond strength can be monitored.

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