Abstract

The addition of ZnO and CuO nanocomposites in adhesive system may protect dentin and improve bonding ability.

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ABSTRACT

Adhesive dentistry has rapidly developed past decades. With continuously improving bonding technology, the adhesive resin restoration strategy is being used more and more often in clinical practice due to the focus of less invasiveness. However, failure of the composite resin and secondary caries still occurs due to dentin deterioration, bacterial infection and degradation of adhesive resins. Therefore, the development of materials with antibacterial and enzymatic inhibitor properties is important, without decreasing the mechanical properties and durability of the adhesives. Zinc (Zn) and copper (Cu) are widely used because of their antimicrobial activity. Several studies found that addition of zinc oxide (ZnO) and Cu nanoparticles had matrix metalloproteinases (MMPs) inhibitory effect and addition of this material in adhesive system had no influence on bond strength. Matsuda et al. introduced novel fluoride containing ZnO and CuO nanocomposites (ZCF) which had high antibacterial activity along with ZnO and CuO nanocomposites without fluoride (ZC). Further investigation of these materials is needed. Therefore, ZC and ZCF was prepared along with low copper concentration, which is ZC low Cu and ZCF low Cu. This study is aimed to evaluate the influence of four ZnO and CuO nanocomposites (ZC, ZC low Cu, ZCF and ZC low Cu) addition in adhesive system on a micro-shear bond strength test, MMP inhibition and antibacterial activity.

To conduct μSBS test, dentin discs were prepared from bovine teeth and a self-etch adhesive with four ZnO and CuO nanocomposites was applied by two different micro-brush following manufacturer's instruction. Then several Tygon tubes were fixed and filled with composite resin, light cured and stored in distilled water at 37°C for 24 h, one month, and three months. After storage tested bond strength using an EZ – Test Machine (Shimadzu, Japan). To evaluate the anti-MMP activity test, previously prepared dentin disc samples were subjected into a 300 μl PBS and incubated at 37°C for 24 h. The supernatant of the dispersion of each nanocomposite was used as a sample. The samples and active MMPs were mixed in 96-well

plates along with 5-FAM/QXLTM 520 fluorescence resonance energy transfer (FRET) and incubated for 1 h. The fluorescence intensity was measured using a LightCycler®96. Antibacterial activity test was conducted on *S. mutans*. Overnight cultures of test bacteria were diluted and adjusted in fresh media to 5 × 105 cells/mL, 500µl of the suspension was placed in each well of 48-well plate with previously prepared dentin disc sample and incubated at 37C degree for 24h. Then supernatants were collected and treated with PMA and checked for qPCR.

There were no significant differences among the bond strengths of the four different nanoparticles applied by the 1 mm micro-brush after 24 h. However, the bonding strength of the materials group tended to increase compared to that of the control group. ZC and ZCF tended to decrease after one month and were not significantly different from the control group. ZCF also decreased after three months, but the difference was not significant. Meanwhile, the bond strength of ZCF and ZCF low Cu slightly decreased after 24 h in the 2 mm micro-brush group, while others tended to increase after 1 and 3 months, but no significant difference was detected. However, the bond strength of all material and control groups significantly decreased after three months compared to 24 h in both the 1 mm and 2 mm groups.

Anti-MMP activity test result showed that ZCF had a significant inhibitory effect on MMP-2, MMP-8 and MMP-9, ZC on MMP-2 and MMP-9 while others had a significant effect only on MMP-9. Next, the antibacterial activity test showed that all four nanocomposites have significant effect on growth of *S. mutans.*

The present study was capable to demonstrate that the addition of ZnO and CuO nanoparticles to adhesive system could provide anti-MMP and antibacterial effect without negative influence on the mechanical properties.