Tetrapod-like Zinc Oxide Whisker Enhancement of Resin Composite

INTRODUCTION

The polymerization shrinkage of composite resin may lead to microleakage between the cured composite resin restoration and the tooth structure, which accordingly results in secondary caries and the failure of prosthetic restorations (Mjör and Toffenetti, 2000). Moreover, compared with other restoratives, composite resin restorations are likely to support more bacterial adhesion, thus leading to a greater possibility of caries recurrence (Svanberg et al., 1990) and gingivitis (Van Dijken and Sjöström, 1991). Therefore, more and more attention has been paid to the antibacterial activity of composite resins. Although several attempts have been made to develop antibacterial composite resins by the addition of antibacterial agents such as chlorhexidine (Jedrychowski et al., 1983), fluoride-containing fillers (Yap et al., 1999), and silver-loading materials (Kawashita et al., 2000), they frequently resulted in compromised mechanical properties (Jedrychowski et al., 1983), a short life expectancy (Wilson and Wilson, 1993), and discoloration (Syafiuddin et al., 1997), which has restricted their clinical application (Imazato et al., 1994). Therefore, antibacterial agents that could avoid these shortcomings are urgently required (Imazato, 2003).

Tetrapod-like zinc oxide whisker (T-ZnOw) is a new type of inorganic antibacterial agent with good comprehensive properties (Zhou et al., 1999). T-ZnOw has many advantages compared with other antibacterial agents, including better antibacterial effects, low decomposability, a long life expectancy, non-discoloration, high safety, and low cost (Fang et al., 2007). Above all, it can reinforce materials at the same time (Hu et al., 2009). Our previous study showed that the incorporation of T-ZnOw provided an experimental dental self-etching primer with the highest antibacterial activity against Streptococcus mutans (S. mutans) among any of the inorganic antibacterial agents tested (Fang et al., 2007). However, to the best of our knowledge, there has been no report on the use of T-ZnOw as an antibacterial agent for incorporation into resin composites. We hypothesized that T-ZnOw fillers could improve both the antibacterial activity and the mechanical properties of a two-component dental composite resin.

MATERIALS & METHODS

Materials

A two-component dental composite resin (cis-butenedioic anhydride modified epoxy dimethacrylate resin) was used in this study (Appendix). The powder/liquid ratio of the resin was 7:3. T-ZnOw was used as the filler for the resin (Appendix).
Broth Dilution Test

The minimal growth inhibitory concentration and minimal killing concentration of the T-ZnOw, the powder and liquid components of the resin, and the powder of cured composite resin against \textit{S. mutans} ATCC 25175 were evaluated as described previously (Fang et al., 2006; Mojtahedzadeh et al., 2008). Briefly, we prepared 10 mg/mL suspensions of the 4 samples by suspending the samples in distilled water. The stock suspensions were shaken vigorously until homogeneous, and then were diluted two-fold serially with brain-heart infusion (BHI, Difco, Detroit, MI, USA) broth to a concentration of 9.7 µg/mL, respectively. An overnight culture of \textit{S. mutans} ATCC 25175 was adjusted to 2.0 x 10^{6} colony-forming units (CFU)/mL in BHI broth, and a 1 mL quantity of the bacterial suspension was inoculated into 1 mL of a series of the 4 sample suspensions mentioned above. All the tubes were assessed for visible turbidity after 24 hrs of anaerobic culture at 37°C and were referenced to negative and positive growth control tubes. The negative control was free of bacterial inoculum, and the positive control did not contain the antibacterial agent. The lowest concentration at which growth inhibition was detected was accepted as the minimal growth inhibitory concentration. A 0.1 mL aliquot from each non-turbid test tube was inoculated onto BHI agar plates. The minimal killing concentration was determined as the lowest concentration that yielded negative subcultures. All tests were conducted in triplicate.

Direct Contact Test

The antibacterial activity of cured composite resin specimens against \textit{S. mutans} was evaluated by the direct contact test. We established 3 experimental groups by incorporating T-ZnOw fillers into the powder component of the resin at 3%, 5%, and 10% w/w (the final concentrations of the T-ZnOw fillers in the composite resin were 2.0%, 3.29%, and 6.37%, respectively). Eighteen specimens (10 mm diameter, 2 mm thick) were prepared for each group. All specimens were polished with the use of a polishing machine and wet abrasive papers (grit 1000, 2000, and 4000). Nine specimens from each group were stored in sterilized artificial saliva at 37°C for 24 hrs and served as the non-aged subgroups, and the other 9 specimens were stored under the same conditions for 3 mos and served as the aged subgroups. The artificial saliva was changed every 24 hrs as previously described (Ehara et al., 2000). Composite resin without T-ZnOw was used as a blank control, and polytetrafluoroethylene film (10 mm diameter) with good biocompatibility served as the positive control. Before the experiment, all specimens were sterilized with alcohol and dried on a superclean bench.

A 15-µL quantity of \textit{S. mutans} ATCC 25175 suspension at 1.0 x 10^{7} CFU/mL was added to each specimen, and a sterilized polyethylene film was used to cover the surface. After anaerobic incubation at 37°C for 24 hrs, bacteria on the specimen and the cover film were collected, and the number of viable cells was determined as CFU by a standard spread-plate technique (Fang et al., 2007). The antibacterial rate was calculated as: $r\% = \frac{[b-c]-b}{b} \times 100\%$ (r%, antibacterial rate; b, the bacterial count recovered from the blank control group; c, the bacterial count recovered from the experimental group).

Measurements of Mechanical Properties

Three experimental groups and 1 blank control group were established as described above. The flexural strength, compressive strength, and diametral tensile strength of the cured composite resins were evaluated as described previously (Christensen, 1993; ISO, 2000). Briefly, resin specimens in cuboid (25 mm x 2 mm x 2 mm), cylindrical (4 mm diameter, 6 mm high), and disk (6 mm diameter, 3 mm high) shapes were prepared for evaluation of the 3 strengths, respectively, with 10 specimens for each group in each test. After polymerization, the specimens were stored in distilled water at 37°C for 24 hrs, and then the 3 strengths were tested in a universal testing machine (AGS-10KN, Shimadzu Corp., Tokyo, Japan) at a crosshead speed of 1 mm/min.

Morphological Observations

The fracture surfaces of the specimens from the flexural strength test were observed by scanning electron microscopy (Philips XL 30 ESEM, Eindhoven, The Netherlands).

In addition, freshly prepared composite resin mixtures from the 3 experimental groups and the blank control group were cast between 2 glass plates and pressed into membranes with a thickness of 200 µm (n = 10) (Wang et al., 2003). Then, the distribution of whiskers in the resin was observed by means of an inverted microscope (BX60, Olympus, Tokyo, Japan).

Statistical Analysis

For the broth dilution test, the modal numbers of the results were used. For the direct contact test, we used two-way ANOVA analysis to compare differences at the 0.05 confidence level, using the SPSS 11.5 software package. One-way ANOVA was performed ($\alpha = 0.05$) on the measurements of mechanical properties, followed by the SNK-q test.

RESULTS

Broth Dilution Test

The minimal growth inhibitory concentration and minimal killing concentration of T-ZnOw against \textit{S. mutans} were 156.25 and 312.50 µg/mL, respectively; for the resin components and cured-composite resin powder, they were all higher than 5000 µg/mL.

Direct Contact Test

The main effects of the aging treatment and concentration of the whiskers, and the interaction of these two factors, were significant (all $p < 0.01$). In non-aged groups, the antibacterial rate of the 10% subgroup was significantly higher than that of the other 2 subgroups, and the antibacterial rate of the 5% subgroup was significantly higher than that of the 3% subgroup ($p < 0.05$). In aged groups, the antibacterial rate of the 3% subgroup was significantly lower than that of the other 2 subgroups ($p < 0.05$), and there was no significant difference between the 5% and 10% subgroups ($p > 0.05$). In addition, the antibacterial rate in the
Measurements of Mechanical Properties

The effects of incorporating T-ZnOw into the experimental composite resin were significant (all p < 0.05) for all mechanical parameters, with the values peaking in the 5% group and then decreasing in the 10% group. In the 3% group, the flexural strength and diametral tensile strength were significantly higher than those in the control group (p < 0.05). In the 5% group, the flexural strength and diametral tensile strength were both significantly higher than those of the other groups (both p < 0.05), whereas compressive strength was significantly higher than that in the control group (p < 0.05). In the 10% group, only diametral tensile strength was significantly higher than that of the control group (p < 0.05), with flexural strength and compressive strength staying at the same level (p > 0.05) (Table).

Morphological Observations

The optical micrographs showed that, in the 3% and 5% groups, the whiskers were dispersed uniformly in the resin. Networks of T-ZnOw were formed in the 5% group, but not in the 3% group (Figs. 2b, 2c). Agglomeration of the whiskers was observed in the 10% group (Fig. 2d).

DISCUSSION

This study investigated the antibacterial activity and mechanical properties of a composite resin containing different concentrations of T-ZnOw. The results revealed that T-ZnOw could endow the resin with antibacterial activity, which was enhanced as the concentration increased. The antibacterial activity of the composite resin was significantly lower in the 10% aged subgroup, but not in the 3% or 5% aged subgroups, compared with their corresponding non-aged counterparts. There was no statistical difference in the antibacterial rate between the 5% and 10% aged subgroups. All mechanical parameters were significantly higher in the 5% group compared with those in the control group, whereas the flexural strength and diametral tensile strength were significantly lower in the 10% group compared with those in the 5% group.

The results of antibacterial experiments indicated that neither the 2 components of the resin nor the cured resin exhibited antibacterial activity against S. mutans, as shown in previous studies (Hansel et al., 1998; Yap et al., 1999; Kawai and Tsuchitani, 2000). The resin showed significant antibacterial activity against S. mutans only after the incorporation of T-ZnOw. However, the antibacterial mechanism of T-ZnOw has not yet been clarified. Since the main component of T-ZnOw is ZnO, the antibacterial mechanism of ZnO may also be applicable to T-ZnOw. It has been reported that the primary antibacterial factors of ZnO may be the hydrogen peroxide (H₂O₂) and reactive oxygen species (O₂⁻ and ·OH) (Sawai et al., 1998; Yamamoto, 2001) released by the following reactions: ZnO + hν → e⁻ + h⁺; H₂O + h⁺ → ·OH + H⁺; O₂ + e⁻ → O₂⁻; O₂⁻ + H⁺ → ·HO₂; ·HO₂ → H₂O₂ + O₂; and H₂O₂ + O₂ → OH⁻ + O₂ + OH⁺ (Lin et al., 2005). These H₂O₂ and reactive oxygen species may react with the cell envelope components (e.g., protein cysteinyl residues) or the free ions in the cell to form hydroxyl radicals, which will inhibit the growth of bacteria or kill them (Storz and Imlay, 1999; Ashby, 2008). In addition, the zinc ions, which have antibacterial effects similar to those of silver,

The tetrapod shape of T-ZnOw was clearly visible under SEM (Fig. 3a). The fracture surface of the 5% group was rougher than that of the other groups, with many whiskers, whisker pull-outs, and multiple cracks and fractured pieces of the matrix (Figs. 3b-3e). In the 10% group, bubbles were frequently found (Fig. 3e). The resin remnants on the whisker pull-outs showed the strong bonding interface between T-ZnOw and the resin matrix (Fig. 3f).

Table. Comparison of Mechanical Properties (MPa) of Composite Resin Specimens in Different Groups (values represent means ± SD, n = 10)

<table>
<thead>
<tr>
<th></th>
<th>0% Group</th>
<th>3% Group</th>
<th>5% Group</th>
<th>10% Group</th>
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<tr>
<td>Flexural strength</td>
<td>97.29 ± 18.71a</td>
<td>117.16 ± 14.90b</td>
<td>134.61 ± 18.26c</td>
<td>110.58 ± 16.40ab</td>
</tr>
<tr>
<td>Compressive strength</td>
<td>175.53 ± 21.94a</td>
<td>189.92 ± 17.56ab</td>
<td>200.85 ± 19.85b</td>
<td>193.93 ± 19.05ab</td>
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<tr>
<td>Diametral tensile strength</td>
<td>40.07 ± 5.15a</td>
<td>49.79 ± 5.08bd</td>
<td>56.75 ± 4.60c</td>
<td>51.44 ± 5.34bd</td>
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Different lower-case letters in rows indicate statistically significant difference at 5%.
ions, may also contribute to the antibacterial activity of ZnO (Tam et al., 2008). Further studies are indeed required to clarify the antibacterial mechanism of T-ZnOw.

The significant decrease in antibacterial activity in the 10% aged subgroup may be due to the abundant release of T-ZnOw during the aging treatment. Since, in the 10% subgroup, the excess fillers may cause the agglomeration of T-ZnOw and reduce the effective bonding between T-ZnOw and the matrix, bubbles and inner flaws were formed in the composites, which became the invasion target of water during the aging treatment. These effects may lead to the increased release of T-ZnOw in the 10% subgroup. Since there were no significant differences in the antibacterial activity of the 5% and the 10% aged subgroups, there was no need to increase the T-ZnOw concentration to 10% in view of the long-term effectiveness.

Besides strong antibacterial activity, satisfactory mechanical properties are also essential to the success of resin restorations, especially in situations in which occlusal stresses are concentrated (Xu et al., 1999). Previous studies reported that T-ZnOw could act as a useful reinforcement when added to Al matrix composite and graphite fiber-reinforced bisphenol A dicyanate (2,2'-bis (4-cyanatophenyl) isopropylidene resin) composite (Ren et al., 2006; Guo et al., 2008). The present study revealed that T-ZnOw significantly reinforced the composite resin, especially in the 5% group. The improvement in the mechanical properties may be due to the following:

1. The incorporation of T-ZnOw increased the filling content and particle diameter distribution width of the composite resin, and accordingly reduced the porosity and enhanced the strength (Xu, 2000).

2. The reinforcement mechanisms of whiskers deflecting, branching, bridging, and resisting the cracks, as observed in previous studies (Thompson et al., 1994; Xu, 2000), should also exist in the present study.

3. One-dimensional whiskers are usually easily entangled and “ball up” during mixing, although a previous study (Xu et al., 2002) developed a method of nano-sized silica particle fusion to avoid this drawback. In the present study, the three-dimensional structure of T-ZnOw appeared to have helped the whiskers to disperse relatively uniformly in the resin. Besides, it is beneficial to transfer the stress spatially, which provides the isotropic properties of the resin (Ren et al., 2006).

Compared with the 5% group, the weaker mechanical properties in the 10% group may be attributed to the excess fillers, resulting in the increase of both resin mixture viscosity and the amount of bubbles in the cured resin (Xu, 2000). Moreover, excess whiskers tend to agglomerate, and thus inner flaws may be formed in the composite, leading to stress concentration and weaker mechanical properties.

In summary, the incorporation of T-ZnOw fillers improved both the antibacterial activity and the mechanical properties of the experimental composite resin. The incorporation of 5% T-ZnOw into the resin powder was optimal to give appropriate antibacterial activity, long-term antibacterial effectiveness, and mechanical properties. Thus, T-ZnOw fillers may be promising candidates for the antibacterial modification and reinforcement of the composite resins tested.
ACKNOWLEDGMENTS

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REFERENCES


