Antibacterial activity of composite resin with glass-ionomer filler particles

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The purpose of this study was to examine the antibacterial activity of composite resin with glass-ionomer filler particles versus that of contemporary commercial composite resins. Three composite resins were used: Beautifil II (containing S-PRG filler), Clearfil AP-X, and Filtek Z250. Resin blocks were bonded to maxillary first molars, and plaque accumulation on the resin block surface was examined after 8 hours. For the antibacterial test, the number of Streptococcus mutans in contact with the composite resin blocks after incubation for 12 hours was determined, and adherence of radiolabeled bacteria was evaluated. Less dental plaque was formed on Beautifil II resin block as compared to the other two materials. Antibacterial test revealed that there were no significant differences in the number of Streptococcus mutans among the three composite resins. However, the adherence of radiolabeled bacteria to the saliva-treated resin surface was significantly (p<0.01) lower in Beautifil II than in the other two materials. These results suggested that Beautifil II could reduce dental plaque formation and bacterial adherence, leading to prevention of secondary caries.

Keywords: Glass-ionomer filler, Composite resin, Antibacterial activity

INTRODUCTION

Although the prevalence of primary caries is on the decline worldwide since early 1980s, secondary caries remains an unresolved problem in restorative dentistry. To solve this problem, the resin matrix content and filler of composite resins, which are used for restoration of decayed teeth, have undergone several modifications to the end of providing antibacterial activity to inhibit secondary caries formation3-5.

Amongst the dental restorative materials used in dentistry, the conventional glass ionomer cement (GIC) has been found to have antibacterial effects. It was reported that the population of Streptococcus mutans (S. mutans) on the surface of GIC fillings was lower than on composite resin fillings4-5. The fluoride released from GICs could prevent caries progression by favoring remineralization or by interfering with the growth or metabolism of remaining cariogenic bacteria4-8. In addition, other major advantages of GICs include ion exchange, chemical adhesion to both enamel and dentin, and continuous fluoride release throughout the life of the restoration. However, the mechanical hardness of GICs is considerably lower than that of composite resins6, which makes GICs not clinically applicable in cases where high occlusal loading is expected.

A recent development is a composite resin containing pre-reacted glass ionomer (S-PRG) filler particles. The S-PRG filler particles are formed by an acid-base reaction of fluoroaluminosilicate glass with polyacrylic acid9 and have been found to be capable of fluoride release and recharge10. Similarly, the composite resin containing S-PRG filler also had fluoride release and recharge functions11. Furthermore, our previous studies have highlighted that S-PRG filler exhibited anti-plaque quality as well as characteristics appropriate for caries treatment1-3.

However, the inhibitory effect of composite resin containing S-PRG filler on bacteria remained to be thoroughly clarified. The purpose of this study, therefore, was to determine the antibacterial activity of a composite resin containing S-PRG filler particles as compared to that of contemporary commercial composite resins.

MATERIALS AND METHODS

Composite resins
Materials used in this study are listed in Table 1. Three commercial composite resins, namely Beautifil II (Shofu Inc., Kyoto, Japan), Clearfil AP-X (Kuraray Medical Inc., Okayama, Japan), and Filtek™ Z250 (3M ESPE, USA), were employed in this study. These materials were filled into a metallic mold (4×4×0.7 mm) and covered with a micro-slide glass. After irradiation for 60 seconds using a visible light curing unit (Coltolux 50, Yoshida Corp., Tokyo, Japan), they were gently polished with 2000- and 4000-grit sandpaper sheets. Subsequently, a grinder polisher (Minimet 1000, Buehler, Lake Bluff, IL, USA) was used by adding 6-µm and 0.025-µm diamond pastes (MetaDi II Diamond Paste, Buehler, Lake Bluff, IL, USA) for 5 minutes. Polished specimens were sterilized in ethylene oxide gas and stored in 4°C refrigerator immediately before any test.

Early dental plaque accumulation
The single-blind, randomized study was approved by the Ethics Committee of Asahi University. Three
Healthy volunteers (25 to 26 years of age; one male and two females) were randomly selected among the students at the Asahi University. Written consent to participate in the study was obtained from all volunteers. None of the volunteers had caries or periodontal disease treated with antibiotics.

Three resin blocks were bonded to the buccal surfaces of the maxillary first molars of each volunteer with Liner Bond (Kuraray Medical Inc., Okayama, Japan). Briefly, two resin blocks were bonded on the surface of the right maxillary first molar of each volunteer and the remaining one block on the surface of the left maxillary first molar. After 8 hours of intraoral exposure, the resin blocks were debonded. The debonded blocks were pre-fixed with 2% glutaraldehyde for 2 hours at 4°C, washed twice in a buffer (0.1 M sodium cacodylate) at pH 7.4, fixed with 1% osmium tetroxide for 1 hour at 4°C, and washed twice in a buffer (0.1 M sodium cacodylate) at pH 7.4. Finally, the specimens were dehydrated with alcohol and isoamyl acetate and dried with CO₂ by critical point drying.

The prepared specimens were placed on aluminum stubs with conductive tape, coated with osmium (HPC-1S, Vacuum Device, Ibaragi, Japan) for 10 seconds, and observed under a scanning electron microscope (S-4500, Hitachi, Tokyo, Japan) with secondary electron signal.

**Antibacterial test**

The cariogenic bacteria, *S. mutans* ATCC 25175, was used in the present study. This organism was anaerobically inoculated into 5 ml of Trypticase Soy Broth (BBL, Cockeysville, MD, USA) containing 0.5% yeast extract (Difco Laboratories, Detroit, MI, USA) at 37°C for 10–12 hours. The bacterial strain was adjusted to a cell suspension of 1×10⁹ CFU/ml with reduced transport fluid (RTF). Each sample was immersed in this suspension and anaerobically inoculated for 12 hours. The bacterial suspension was then estimated by culturing on TSBY agar plates, either undiluted or diluted 10-fold, and incubated at 37°C for 4 days. The exact number of colonies was counted in 10 samples for each material.

**Quantitative adherence of radiolabeled bacteria**

*S. mutans* was anaerobically inoculated into 150 ml of Trypticase Soy Broth containing 0.5% yeast extract which included 74 kBq of [6-³H] thymidine (GE Healthcare, USA) and cultured at 37°C for 18 hours. The cells were collected by centrifugation at 8,000 g for 20 minutes with 0.05 M phosphate buffer saline (PBS; pH 7.0), and radiolabeled bacteria were washed three times with PBS. Finally, cells were adjusted in PBS at a concentration of 10⁹ CFU/ml.

Each resin block was suspended in a test tube with 2 ml of the labeled bacterial fluid at 37°C for 2 hours (Fig. 1). To remove the non-adhering bacteria, the resin blocks were removed from the test tubes and immediately washed with PBS three times. Labeled bacteria which adhered to the resin blocks were collected using an automatic sample combustion equipment, and their numbers measured using a liquid scintillation counter (LSC-903, Aloka, Tokyo, Japan).

Energy-dispersive X-ray spectroscopy (EDS)

Resin blocks of 4×4 mm were prepared as described above. The blocks were placed on carbon stubs and

<table>
<thead>
<tr>
<th>Material</th>
<th>Resin type</th>
<th>Filler type</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beautifil II</td>
<td>Bis-GMA, TEGDMA</td>
<td>S-PRG filler, Multifunction glass filler</td>
<td>Shofu Inc.</td>
</tr>
<tr>
<td>Clearfil AP-X</td>
<td>Bis-GMA, TEGDMA</td>
<td>Barium glass, silica</td>
<td>Kuraray Medical Inc.</td>
</tr>
<tr>
<td>Filtek Z250</td>
<td>Bis-GMA, TEGDMA</td>
<td>Zirconia/Silica filler</td>
<td>3M ESPE</td>
</tr>
</tbody>
</table>

**Table 1 Composite resin used in this study**

Bonding material: Clearfil Liner Bond II (Kuraray Medical Inc.)
coated with osmium for 5 seconds. The X-ray microanalysis of the resin blocks was performed using EMAX-7000 (Horiba Ltd., Kyoto, Japan). Spectroscopy data were obtained after 300 seconds of measurement.

Statistical analysis
All the data were tested for normality of distribution (Kolmogorov−Smirnov test) and for uniformity (Bartlett’s test). Differences in measured values among the three composite resins were tested by one-way analysis of variance (ANOVA) with a post hoc test (Bonferroni test) for multiple comparisons. A probability of less than 0.05 for similarity of distribution was considered to be significantly different.

RESULTS

Early dental plaque accumulation
Accumulation of dental plaque was found on the surfaces of the three composite resins, which meant that none of the resins could completely inhibit dental plaque formation. However, the amount of accumulated plaque was lower on the surface of Beautifil II when compared with the other two composites (Fig. 2).

Antibacterial test
Among the three composite resins immersed for 12 hours in the solution with S. mutans, the numbers of colonies were almost similar. No significant differences in the numbers were found among the three composites (Fig. 3).

Quantitative adherence of radiolabeled bacteria
For all the three composite resins, the values of disintegrations per minute (dpm) were significantly ($p<0.01$) lower in the samples soaked in saliva than in the samples soaked in distilled water (Fig. 4). When soaked in distilled water, the dpm values were almost similar among the three composites. When soaked in saliva, the dpm value was significantly ($p<0.05$) lower in Beautifil II than in the other two composites.

EDS
The compositions of the composite resins are shown in Table 2. Based on EDS spectroscopy data, all the materials showed dominant proportions of carbon and oxygen (ca. 30−35%). However, all the materials revealed a specific content not present in the other two composite resins. Fluoride and strontium were

![Fig. 2](image)

Representative SEM photographs of plaque accumulating on the resin surface. Less plaque accumulation was found on the surface of Beautifil II (A), compared to Clearfil AP-X (B) and Filtek Z250 (C). White arrows indicate bacteria mass.
detected in Beautifil II only, while Filtek and Clearfil contained zirconium and barium respectively.

**DISCUSSION**

Dental caries formation has been known to be suppressed by the fluoride release function of GICs. Sá et al. demonstrated that GIC under in vitro pH cycling condition showed significant anticariogenic properties. Similarly, Horiuchi et al. demonstrated that orthodontic adhesive with S-PRG filler resulted in minimal damage of the enamel surface around the bracket after 7-day storage in lactic acid solution. Further, in another in vitro study, the high caries-protection effect of Vitremer, a resin-modified GI, was clearly established when compared with the other fluoride-releasing restorative materials. Although the behavior of fluoride-releasing materials under in vitro cariogenic challenges has been investigated and confirmed by several researchers, the properties of these materials under different caries-like models need to be further investigated to resolve many questions about the pathology and progression of secondary caries. Therefore, the present study was designed to examine the antibacterial activity of composite resin with S-PRG filler particles by using in vitro and in vivo caries-like models.

In our in vivo study, the comparison of dental plaque accumulation among the three composite resins demonstrated that a considerably lower quantity of dental plaque accumulated on the surface of Beautifil II. Dental plaque has been defined as a diverse community of microorganisms found on the tooth surface as a biofilm, embedded in an extracellular polymer matrix of host and bacteria origin. In addition, physicochemical surface properties are important in the formation of biofilms. Hanning reported that early plaque formation on solid surfaces was influenced predominantly by the oral environment rather than material-dependent parameters. Therefore, the difference in dental plaque accumulation among the three composite resins might be due to the fluoride-releasing capability of Beautifil II.

Beautifil II containing fluoride is a fluoride-releasing composite as well as GIC. Consequently, the fluoride released from the composite effected a change in the surrounding environment. In addition, the metal ions uniquely and singularly contained in each composite resin might have influenced their dental plaque inhibitory characteristics. Although all the three composite resins showed dominant proportions of carbon and oxygen, EDS spectroscopy data revealed that metal ions such as strontium, zirconium, and barium were specifically available in only one of the
three composites. Hence, it is necessary to conduct further studies to investigate the effects of different metal ions contained in the resin on its antibacterial ability.

The initial conditioning salivary coat plays an important role in bacterial adhesion to the saliva-coated restorative surface\textsuperscript{22}. In our \textit{in vitro} study, there was reduced oral bacterial adhesion on composite resins coated with human saliva, as compared to samples soaked in distilled water. This result was consistent with previous studies which reported that \textit{Streptococci} bacteria adhesion decreased on a solid surface coated with bovine serum albumin\textsuperscript{23,24}. However, it must be pointed out that although the acquired pellicle itself is free of bacteria\textsuperscript{25}, it is the starting point for microbial colonization on oral hard surfaces whereby the salivary pellicle acts as a receptor for the initial adhesion of bacteria. Indeed, the formation of oral biofilms on hard surfaces is a complex process which begins with salivary pellicle formation and pellicle adsorption to the surface, then progressing on to passive transport of bacteria to the pellicle surface, followed by irreversible adhesion and multiplication of the attached organisms\textsuperscript{26}.

Among the three composite resins tested in this study, bacterial adhesion to Beautifil II was the lowest. This could be attributed to the inhibitory effect of saliva in the oral cavity. Human saliva contains many antibacterial substances, such that the salivary proteins adsorbed on the composite resin surface resulted in decreased bacterial adhesion. In other words, the protein constituents of saliva that adsorbed on Beautifil II might have a role in influencing the results obtained for this composite material. Therefore, it is recommended that an immunological technique be employed to study the salivary proteins which adsorbed on the composite resin surface.

Based on the results obtained in this study, it was clearly shown that Beautifil II exhibited inhibitory effect against bacterial adhesion, suggesting that this composite resin might be effective in suppressing secondary caries formation.

**CONCLUSIONS**

Beautifil II showed a lower quantity of \textit{S. mutans} adherence when the samples were soaked in human saliva. In addition, the adhesion of dental plaque to the surface of Beautifil II seemed to be lower than the other two composite resins. However, there was no significant difference in antibacterial effect among the three composite resins.

**REFERENCES**


