Effect of incorporation of surface pre-reacted glass ionomer filler in tissue conditioner on the inhibition of Candida albicans adhesion

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We investigated the effects of incorporation of surface pre-reacted glass ionomer (S-PRG) filler in tissue conditioner (TC) on Candida albicans adhesion. We prepared specimens containing 0, 5, 10, or 20 wt% of S-PRG filler, and measured the amount of C. albicans on the surface using a colony forming unit (CFU) assay and scanning electron microscopic images. In addition, we measured the consistency, penetration depth, and surface roughness (Ra). CFU values for 10 and 20 wt% were significantly lower than that for the control (p<0.05). Hyphal density on the surface was greater in the control. The 10 and 20 wt% specimens showed significantly higher consistency and Ra, lower penetration depth ratio than control (p<0.05). These results suggest that incorporation of S-PRG filler may reduce C. albicans adhesion onto TC surface; however, the optimal amount of filler is dictated by the influence of filler incorporation on mechanical and surface characters of TC.

Keywords: Candida albicans, Denture stomatitis, Tissue conditioner, S-PRG filler

INTRODUCTION

Denture plaque derived from oral microorganisms adhering to the denture surface contains the fungus Candida albicans (C. albicans), which is known as one of the major causes of denture stomatitis1-3. Removal of this fungus may be effective for the treatment and prevention of denture stomatitis3.

Tissue conditioners (TC) are clinically used for the conditioning of denture-bearing mucosa damaged by ill-fitting dentures, functional impression, and temporary lining of immediate dentures and ill-fitting dentures4,5. However, because of higher porosity and viscosity, the TC surface retains more plaque than does polymethyl methacrylate (PMMA) resin6. In addition, the TC surface is sensitive to brushing7. Thus, mechanical cleaning cannot be recommended. Because of these reasons, it is difficult to maintain the TC surface clean.

As alternative cleaning methods, chemical cleaning such as soaking in sodium hypochlorite8 or denture cleansers9 are known to be effective. Sodium hypochlorite is commonly used as a disinfectant as it is highly bactericidal and fungicidal10, however, it decolorizes the denture materials and corrodes the metals11. Immersion in denture cleanser is more effective than washing the TC with water11. However, some denture cleansers deteriorate the TC surface12, thus limiting the practical applicability of chemical cleaning.

Research efforts have been made to find ways to clean TC surfaces with antimicrobial agents. For example, Nystatin and silver-based antimicrobial agents have been demonstrated to inhibit Candida growth13,14. However, indication for these antimicrobials is limited to patients with xerostomia with movement disorders, because there is a risk of developing resistant bacteria15. Thus, they have not been widely used in the clinical setting.

We recently reported that incorporation of surface reaction-type pre-reacted glass ionomer (S-PRG) filler (S-PRG filler, Shofu, Kyoto, Japan) in PMMA resin as a denture base reduced C. albicans adhesion16. S-PRG filler particles are formed by the reaction between the reaction-type pre-reacted glass ionomer (S-PRG) filler and polyacrylic acid. As a result of this reaction, a glass ionomer layer is formed on the surface of the glass core. The glass ionomer layer releases mainly 6 types of ions (Na+, Sr2+, SiO32-, Al3+, BO33- and F−)17. We consider that the antimicrobial action of the released ions is not bactericidal but bacteriostatic10. The filler can reduce the adhesion of not only C. albicans, but also cariogenic bacteria, including Streptococcus mutans, and endodontic bacteria, including Porphyromonas gingivalis, and can prevent plaque formation by inducing mineralization18-20. These studies suggested that S-PRG filler may be effective in preserving a clean TC surface in the clinical setting.

On the other hand, antibacterial substances incorporated in the denture base material have been reported to influence mechanical properties and increase...
Surface roughness affects *C. albicans* adherence\(^{22}\). TC consistency and initial flow determine working time and adaptation of the denture surface to oral mucosa\(^{22}\). Furthermore, optimal change in TC mechanical property after application is required for functional impression. Therefore, it is necessary to investigate the effects of incorporation of S-PRG filler in TC on mechanical properties and surface roughness.

The aim of this study was to investigate the effect of incorporation of S-PRG filler in TC on *C. albicans* adhesion, as a pre-clinical investigation, and on mechanical and surface characters. The null hypothesis was that there is no difference in *C. albicans* adherence, mechanical properties, and surface roughness between TC specimens with/without S-PRG filler.

**MATERIALS AND METHODS**

**Fungal adhesion**

1. Specimen preparation

We mixed the powder component of TC (Shofu Tissue Conditioner II, Shofu) with S-PRG filler at a ratio of 0, 5, 10 and 20 wt%, and then mixed them with a liquid at a powder-liquid (P/L) ratio of 4.8 g/4 mL (per the manufacturer’s instruction) at 25°C (Tables 1 and 2). The specimens were prepared to have 2-mm thickness and 10-mm diameter using glass plates, and they were placed on round PMMA disks (2 mm thick and 10 mm in diameter) (ACRON, GC, Tokyo, Japan) polished using 2000-grit abrasive papers (FUJISTAR, Sankyo, Saitama, Japan). PMMA disks were immersed in distilled water for 24 h after polymerization to remove residual monomers, and then, ethylene oxide gas sterilization was performed. Fifteen specimens were prepared for each S-PRG filler content.

**Table 1** Materials used in this study

<table>
<thead>
<tr>
<th>Material</th>
<th>Brand name</th>
<th>Manufacturer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue conditioner</td>
<td>Shofu Tissue Conditioner II</td>
<td>Shofu, Kyoto, Japan</td>
<td>Powder Polyethyl methacrylate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liquid Di-n-butyl sebacate</td>
</tr>
<tr>
<td>S-PRG filler</td>
<td></td>
<td>Shofu, Tokyo, Japan</td>
<td>Absolute ethanol</td>
</tr>
<tr>
<td>Polymethyl methacrylate</td>
<td>ACRON</td>
<td>GC, Tokyo, Japan</td>
<td>Powder Polyethyl methacrylate</td>
</tr>
<tr>
<td>resin</td>
<td></td>
<td></td>
<td>Liquid Methyl methacrylate</td>
</tr>
</tbody>
</table>

**Table 2** P/L ratio of the tissue conditioner used in this study

<table>
<thead>
<tr>
<th>S-PRG filler (wt%)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(Polyethyl methacrylate)/L ratio (g/mL)</td>
<td>1.20 (4.8/4)</td>
<td>1.14 (4.56/4)</td>
<td>1.08 (4.32/4)</td>
<td>0.96 (3.84/4)</td>
</tr>
<tr>
<td>P(Polyethyl methacrylate+S-PRG filler)/L ratio (g/mL)</td>
<td>1.20 (4.8/4)</td>
<td>1.20 (4.8/4)</td>
<td>1.20 (4.8/4)</td>
<td>1.20 (4.8/4)</td>
</tr>
</tbody>
</table>
Auto Coater sc-701AT, Sanyu Electron, Tokyo, Japan) and observed under a Hitachi S-4500 (Hitachi, Tokyo, Japan).

Consistency and penetration depth (PD)

For assessment of the TC mechanical character, we conducted consistency and PD tests according to JIS T6519:2000 based on ISO 10139-1:1991. In the consistency test, 2 mL of TC mixed at the P/L ratio of 4.8 g/4 mL was placed between glass plates. A 1-kg weight was applied on the upper glass plate for 8 min in an incubator at 37°C. The maximum and minimum width of the TC specimen were measured and averaged for consistency. Four specimens were tested for each S-PRG filler content.

In the PD test, the TC mix was poured into a metal mould with a hole (30 mm in diameter, 3.0 mm in depth) and immersed in water at 37°C for 2 h. Specimens were then set on a device with a Vicat needle and PD was measured 4 times and averaged (PD2h). PD measurement was repeated after 7 days of water immersion (PD7d), and the PD ratio (PD2h/PD7d) was determined. Five specimens were tested for each S-PRG filler content.

Surface roughness

We took images of TC specimens using a 3D measuring laser microscope (LEXT OLS4000, Olympus, Tokyo, Japan) after immersion in YPD medium for 24 h at 37°C. Surface roughness (Ra) was measured at 10 points randomly selected on the image and averaged. Five specimens were tested for each S-PRG filler content.

Determination of the concentrations of released ions

The TC specimens were immersed in ultra-pure water (Milli-Q1 RG, Millipore, Billerica, MA, USA) for 24 h at 37°C. Concentrations of Na⁺, Si⁴⁺, SiO₃⁺, Al³⁺, and BO₃⁻ in the water were measured using inductively coupled plasma atomic emission spectroscopy (ICPS-7000, Shimazu, Kyoto, Japan). F⁻ concentration was measured using a fluoride ion electrode (Orion 9609BBNWP, Thermo Scientific, Waltham, MA, USA) connected to a fluoride ion meter (Orion 4-Star, Thermo Scientific). Five specimens were tested for each S-PRG filler content.

Statistical analysis

We performed statistical analyses using one-way analysis of variance (ANOVA) and Dunnett’s post-hoc test for multiple comparisons. The significance level was set to 0.05. SPSS 21.0 (IBM Japan, Tokyo, Japan) was used for statistical analyses.

RESULTS

C. albicans CFU decreased with increasing S-PRG filler content (ANOVA; p<0.005) (Fig. 1). The CFU were significantly lower for 10 and 20 wt% (p<0.05), but not 5 wt% (p=0.071), than for the control (0% content). In SEM images, we observed more hyphal growth on the TC surface in the control samples (Fig. 2), while the yeast form was prevalent in S-PRG-supplemented specimens.

Consistency, penetration (PD2h, PD7d), and surface roughness (Ra) increased, and PD ratio (PD2h/PD7d) decreased with increasing filler content (ANOVA; p<0.001) (Figs. 3 and 4). Significantly higher consistency, PD2h, and PD7d, and lower PD ratio for 10 and 20 wt% specimens than for the control (p<0.05) were observed.
Fig. 3 S-PRG filler increases consistency and PD. Means and SDs of consistency, PD, and PD ratio (PD2h/PD7d) of TC specimens containing S-PRG filler (0, 5, 10, and 20 wt%) (consistency; n=4, PD; n=5), *p<0.05.

Fig. 4 (a) S-PRG increases TC surface roughness (Ra). Means and SDs of Ra of TC specimens containing S-PRG filler (0, 5, 10, and 20 wt%) (n=5), *p<0.05. (b) Laser microscopic view of TC surface containing S-PRG filler (0, 5, 10, and 20 wt%). In filler content groups, incorporated fillers appeared.
while these changes were not significant for 5 wt% (p>0.05). On laser microscopic images, more S-PRG filler material was observed on surfaces with higher filler content (Fig. 4).

All six types of ions (Na+, Sr2+, SiO32−, Al3+, BO33−, and F−) were released from S-PRG-supplemented specimens, and the concentrations increased with increasing filler content (Fig. 5).

**DISCUSSION**

The results of this study indicated that the adhesion of *C. albicans* on the TC surface was reduced by the incorporation of at least 10 wt% S-PRG filler. Therefore, the null hypothesis in this study was rejected. In addition, the study confirmed that six types of ions (Na+, Sr2+, SiO32−, Al3+, BO33−, and F−) were released from the S-PRG filler. Boric acid prevents the formation of invasive hyphae through the inhibition of the pH-signalling cascade24. Fluoride shows antibacterial effects, such as metabolic disorder and decreased acidity of the dental plaque25. However, the amounts of boric acid and fluoride ions released in this study were lower than the concentrations reported to be effective for inhibiting *C. albicans* growth24. Combinatorial osmotic (Na+) and oxidative stress effectively inhibits and kills *C. albicans*26. Multiple types of ions released from S-PRG fillers may act in the inhibition of bacterial adhesion on the TC surface.

We have reported that PMMA containing S-PRG filler prevents the transition from the yeast to the hyphal form in *C. albicans*16. In this study, we observed a similar trend on the TC surface. A study has shown that *C. albicans* maintains the yeast form in normal environment and transits to the hyphal form in inflammatory environment27. The hyphal form has higher pathogenic potential, as it can penetrate the surface of and invade epithelial tissue28. Another study has reported that inhibition of the transition from the yeast to hyphal form reduces biofilm formation29. Thus, inhibition of hyphal expression on the TC surface may decrease the pathogenicity of *C. albicans* and biofilm formation.

The amount of ions released from TC specimens containing S-PRG filler measured in this study was higher than that from PMMA specimens with same content of S-PRG filler16. However, the least effective content of S-PRG filler to inhibit fungal adhesion for TC (10%) was greater than that for PMMA (5%). This could be explained by the fact that the TC surface is more porous and viscous, and thus, denture plaque formation is higher on TC surface than on PMMA resin30.

In this study, adhesion of *C. albicans* on the TC surface decreased with an increase in filler content of up to 10 wt%, while 10 and 20 wt% had comparable effects. On the other hand, surface roughness increased with increasing filler content up to 20%. It is considered that the surface roughness increased with the increase in filler material being exposed on the TC surface, as observed by laser microscopy. The increase in surface roughness increases the hydrophobicity of the surface that accelerates candida adhesion30. Thus, the surface roughness is an accelerating factor in the adhesion of fungus32. The increased surface roughness at 20 wt% content might have offset the inhibitory effect of the released ions.

In the clinical setting, it is recommended to renew the TC approximately weekly. In this study, we
examined the early stages of fungal adhesion. A study using PMMA containing S-PRG filler showed that the amount of released fluoride decreased on the second day after immersion into distilled water, and further decreased to approximately one tenth at one week after immersion\(^{31}\). Furthermore, TC surface roughness increased with time\(^{22}\). These findings suggest that the effect of S-PRG filler on the inhibition of fungal adhesion will likely decrease over time. On the other hand, fluoride ions could be recharged into PMMA by immersion in a fluoride ionic solution\(^{31}\). Thus, the effects of ion recharge on fungal adhesion over longer periods should be further investigated.

Consistency and PD increased, and PD ratio decreased with an increase in the filler content, indicating that the incorporation of S-PRG filler softened TC; however, they remained within the JPS standard (consistency 25–75 mm, PD2h ≤1.8 mm, PD7d ≥0.18 mm, PD ratio ≤5.0). On the other hand, the consistency and PD ratio for 20 wt% did not fulfill the manufacturer’s indications (consistency: 40.0–54.0 mm, PD ratio: 1.2–2.2). Considering these mechanical properties, 10 wt% may be the most feasible content in the clinical setting.

It has been reported that reduction of P/L ratio increased gelation time and viscoelasticity of TC\(^{5}\). In this study, the P/L ratio was constant across filler-containing specimens, which indicates that the amount of TC polymer reduced with the addition of S-PRG filler. Thus, the ratio of polyethyl methacrylate to liquid decreased with the addition of S-PRG filler, resulting in changes in mechanical character as indicated in consistency and PD.

Clinically, the preservation of TC flexibility is necessary for conditioning of the denture-bearing mucosa. In contrast, high flowability and a large change in hardness of the TC over time are required for functional, dynamic impression. Low flowability and a small change in hardness over time are desirable to maintain adaptation with the oral mucosa and occlusal vertical dimension for temporary relining\(^{39}\). In addition, the surface roughness of TC used for functional impression affects the surface properties of the dental stone model\(^{39}\). Therefore, the increase in surface roughness of the TC by incorporation of S-PRG filler possibly deteriorates the surface character of the working cast. Therefore, TC containing S-PRG filler may not be indicated for dynamic impression.

Residual monomers in PMMA were reported to be cytotoxic\(^{34}\). In this study, because TC was placed on the PMMA disk, the results may indicate not only an effect of TC but also that of PMMA. However, the cytotoxic effect of acrylic resin was greater in the first 24 h after polymerization and decreased with time\(^{35}\). In this study, PMMA disks were immersed in water after polymerization. Therefore, the cytotoxicity due to the residual monomer in the disk seems to be very low.

There are some limitations to this study. We did not add protein, essential to form pellicles in artificial saliva; thus, no denture plaque was formed on the pellicles. Second, we used only C. albicans in the CFU test, while denture plaque contains multiple microorganisms such as cocci, bacilli, and filamentous bacteria. CFU tests including several types of bacteria will have to be conducted in future. Third, it is unclear whether incorporation of S-PRG filler in TC is effective for the prevention of denture stomatitis and mucosal inflammation in clinical settings. Forth, we evaluated C. albicans adhesion onto TC after 24 h of incubation. TC is usually used for 3 to 7 days in clinical settings. Thus, a further study with longer incubation is necessary to confirm antifungal effect of S-PRG filler on TC.

CONCLUSION

Within the limitations of this study, it was suggested that TC containing 10 wt% or more of S-PRG filler may reduce C. albicans adhesion onto the surface of TC. While filler content was limited by its influence on mechanical and surface characters, 10 wt% may be feasible for tissue conditioning of denture-bearing mucosa in practice.

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