Novel antimicrobial denture adhesive containing S-PRG filler

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The antimicrobial effects of denture adhesives containing novel surface pre-reacted glass-ionomer (S-PRG) fillers were assessed. We prepared denture adhesives containing S-PRG (particle sizes: 1 and 3 µm; quantities: 5, 7.5, and 10 wt%). We evaluated acid buffering capacity, ion release, and antimicrobial effects of denture adhesives with and without S-PRG. Significantly higher pH changes were observed in 1 µm S-PRG adhesives than in 3 µm S-PRG adhesives. Adhesives containing 7.5 and 10 wt% S-PRG exhibited significantly higher ion release than adhesives with 5 wt% S-PRG. The 1µm–10wt% S-PRG denture adhesive exhibited significantly lower colony-forming units on the denture adhesive contact surface than in the control group; additionally, it exhibited excellent acid buffering capacity, ion release properties, and antimicrobial effect against C. albicans, C. glabrata, S. mutans, and A. naeslundii. Longer contact periods resulted in significantly lower adhesion of Candida albicans to the denture base resin treated with denture adhesive.

INTRODUCTION

The increase in global life expectancy has led to an aging population. Consequently, the number of people using dentures, predominantly elderly individuals, is continuously increasing⁶. As a result, cases of severe ridge resorption due to denture use are also increasing.

Denture adhesives are dental materials used mainly to improve dysfunctional dentures⁵ and can be classified as either powder, cream, or sheet types, or as a home reliner (cushion type). They can improve the denture retention and masticatory ability, thereby ensuring patient satisfaction⁶. Coates reported that at least 30% of people using dentures use denture adhesives⁶. While dentists generally believe that denture adhesives are not required when properly manufactured dentures are used⁵, patients with intractable cases (e.g., remarkable ridge resorption, thin mucous membrane, or xerostomia) struggle to use their dentures comfortably. Several researchers have attempted to address this issue while simultaneously combating oral microorganisms by developing denture adhesives with antimicrobial properties⁶–⁸. The effects of different forms of commercially available denture adhesives such as powder, cream, and sheet type, on the growth of oral microorganisms and the oral environment have been reported⁹.

When surface pre-reacted glass-ionomer (S-PRG) fillers are added to dental materials, they release six ions: fluoride, strontium, sodium, aluminum, boric acid, and silicic acid, which impart various properties to the materials, such as antimicrobial effects, tooth remineralization ability, and acid buffering capacity¹⁰–¹⁵. More recently, S-PRG fillers have been introduced clinically in temporary cements, orthodontic resin, and tissue conditioners¹⁴,¹⁶. Thus, in this study, we attempted to add S-PRG fillers to the denture adhesives with the assumption that it may achieve a sufficient antimicrobial effect and acid buffering ability, and improve the oral environment of people using dentures. Specifically, denture adhesives with S-PRG fillers are expected to improve denture stomatitis and exhibit an antimicrobial effect against oral microorganisms such as Candida albicans and Streptococcus mutans¹⁷–¹⁹.

This study aimed to assess the effect of various particle sizes and contents of S-PRG fillers on the acid buffering capacity, ion release, and antimicrobial effect of denture adhesives. The hypothesis was that different sizes and amounts of S-PRG fillers would affect these properties.

MATERIALS AND METHODS

S-PRG filler-containing denture adhesives

Six grades of adhesives containing S-PRG (Shofu, Kyoto, Japan) were prepared, and the detailed compositions are listed in Table 1. The study design is graphically explained in Fig. 1. Two particle sizes (1 and 3 µm) and three amounts of S-PRG (5, 7.5, and 10 wt%) were used, and the samples were labeled as follows: 1µm–5wt%, 1µm–7.5wt%, 1µm–10wt%, 3µm–5wt%, 3µm–7.5wt%, and 3µm–10wt%. Further, a denture adhesive without S-PRG was used as the control. When
preparing the S-PRG-containing denture adhesive, the S-PRG filler content was set to 5, 7.5, and 10 wt% of the total denture adhesive weight. This was achieved by replacing carboxymethylcellulose sodium in the control denture adhesives with the S-PRG filler because carboxymethylcellulose sodium can be used to control viscosity. The viscosity of the denture adhesive increased when the S-PRG filler was added to it. Thus, we replaced carboxymethylcellulose sodium with the S-PRG filler to control the viscosity of the denture adhesives. During a preliminary study, denture adhesives containing high S-PRG contents (>10 wt%) were investigated. However, the thick consistency of these adhesives caused difficulties during their application to the dentures. Thus, the maximum S-PRG content was set to 10 wt%.

Microstructural analysis
Microstructures of the S-PRG fillers with different particle sizes (1 and 3 µm) were evaluated using scanning electron microscopy (SEM; Hitachi S-4500, Hitachi, Tokyo, Japan). The imaging conditions used in SEM were, accelerating voltage: 15 kV, emission: 10 µm, and working distance: 15 mm. Prior to SEM, the specimens were coated with palladium and platinum nanoparticles using an automatic coating machine (Quick Auto Coater sc-701AT, Sanyu Electron, Tokyo, Japan).

Acid buffering capacity
Denture adhesive specimens (0.35 g) were placed in centrifuge tubes (15 mL) filled with demineralization solutions comprising 2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄, 50 mmol/L acetic acid, and 0.02% NaN₃ (pH=4.5, n=5/group). The mixtures were then stirred with a mixer (Vortex-Genie2, IKEDA SCIENTIFIC, Tokyo, Japan) and stored at 37°C for 24 h, after which the pH was measured using a pH meter (F-52, HORIBA, Kyoto, Japan).

Ion release
Specimens of denture adhesive (0.18 g) were immersed in distilled water (8 mL) via a filter (MILLPORE, 0.2 µm, Merck, Darmstadt, Germany). They were then placed in a plastic container and stored at 37°C (n=5/group). After 24 h, the Al, B, Na, and Sr concentrations of the distilled water were measured using high-frequency inductively coupled plasma atomic emission spectroscopy (ICP-AES; Spectro Arcos, Hitachi High-technologies, Tokyo, Japan). A multi-element standard solution (100 ppm, XSTC-22, Seishin Trading, Kobe, Japan) and an Sr standard solution (1,000 ppm, Nakarai Tesque, Kyoto, Japan) were used as standards for the ICP-AES analyses. F⁻ was measured using an ion meter (ORION 4STAR, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a fluoride ion electrode (ORION 9609BN, Thermo Fisher Scientific). Calibration curves for the F⁻ ion electrode were prepared using a series of NaF solutions (0.5, 1, 5, and 10 ppm).

Antimicrobial effect on the contact surface of the denture adhesive
The investigated strains were C. albicans (ATCC18804; clinical isolate), Candida glabrata (ATCC2001; clinical
isolate), S. mutans (ATCC25175; clinical isolate), and Actinomyces naeslundii (ATCC12104; clinical isolate). C. albicans, C. glabrata, S. mutans, and A. naeslundii were inoculated into Sabouraud glucose (SG) agar or Brain Heart Infusion (BHI) plates. Specimens of denture adhesives were placed on agar plates via a filter (MILLPORE, 0.2 µm, Merck) and were incubated at 37°C for 24 h (n=6/group). The specimens were removed with the filter, and the agar underneath the denture adhesive was immediately divided into three pieces. The weight of the agar samples was noted. We then added 500 µm phosphate buffered salt (PBS) to each of the sectioned agar samples. The PBS and agar samples were mixed using a homogenizer. Homogenized fungus liquid was diluted using PBS (C. albicans: 100 times, C. glabrata: 100 times, S. mutans: 10,000 times, A. naeslundii: 1,000 times). The diluted fungus liquid was spread on SG or BHI agar plates, and then incubated at 37ºC for 24 h. Colony-forming units (CFUs) were counted for each specimen. S. mutans and A. naeslundii were evaluated for only denture adhesives containing S-PRG (1µm–5wt%, 1µm–7.5wt%, and 1µm–10wt%) following the results of a pilot test.

Antimicrobial effect against C. albicans on acrylic resin treated with the denture adhesive

The antimicrobial effect experiment described above indicated that the 1µm–10wt% S-PRG filler-containing denture adhesive was the most effective based on the number of CFUs on the contact surface of the denture adhesive. Therefore, the 1µm–10wt% denture adhesive was further investigated to assess the number of C. albicans adhering to acrylic resin. Standardized acrylic resin disks (Urban, Shofu; 10 mm diameter) were prepared by placing the denture adhesive on both sides of an acrylic resin disk via a filter (MILLPORE, 0.2 µm, Merck). Several researchers have reported that the duration of the retentive action denture adhesives ranges from 3 to 12 h. Therefore, the acrylic resin disks with the denture adhesive were stored in a sterilized petri dish in a sterilized environment for 10 h. Then, the denture adhesive was removed with the filter, and the acrylic resin disks were stored in sterile water in a sterilized petri dish in a sterilized environment for 14 h (one cycle). This cycle was repeated for contact periods of 1 day (one cycle), 3 days (three cycles), and 5 days (five cycles). Each acrylic resin disk with the denture adhesives was immersed in artificial saliva comprising NaCl (0.381 g), CaCl$_2$ 2H$_2$O (0.213 g), KCl (1.114 g), KH$_2$PO$_4$ (0.738 g), and mucin (1.1 g) in 1,000 mL of distilled water (pH=7) for 30 min, followed by washing with PBS. The washed acrylic resin disks were exposed to 1.0×10$^7$ CFU/well C. albicans fungus fluid for 1 h at 37°C and 120 rpm, followed by washing three times with 1 mL of PBS for 5 min at 120 rpm. C. albicans that was adhered to the acrylic resin disks was collected using two cycles of ultrasonic cleaning in 2 mL of PBS for 30 s. The collected liquid was diluted by 1,000 times and inoculated into SG agar medium, and the CFU was measured after 24 h (n=6/group).

Statistical analysis

Tables 2–5 summarize the results of one-way analysis of variance (ANOVA). The acid buffering capacity, ion release, and antibacterial effect against various microbes on the denture adhesive contact surface were evaluated using a Shapiro–Wilk test. The obtained data were statistically compared using either one-way ANOVA and Tukey’s post hoc test, or the Kruskal–Wallis test and Dunn’s test. The antibacterial effect against C. albicans that was adhered to the denture adhesive-treated acrylic resin was evaluated using a Shapiro–Wilk test, followed

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### Table 2  Summary of one-way ANOVA (acid buffering capacity)

| Source of variability | Degree of freedom | Sum of squares | F-value | p-value |
|-----------------------|-------------------|----------------|---------|---------|
| Denture adhesive      | 6                 | 1.6093         | 249.7   | <0.0001 |

### Table 3  Summary of one-way ANOVA (B release from the S-PRG filler-containing denture adhesives)

| Source of variability | Degree of freedom | Sum of squares | F-value | p-value |
|-----------------------|-------------------|----------------|---------|---------|
| Denture adhesive      | 6                 | 0.4211         | 2.633   | <0.0001 |

### Table 4  Summary of one-way ANOVA (Na release from the S-PRG filler-containing denture adhesives)

| Source of variability | Degree of freedom | Sum of squares | F-value | p-value |
|-----------------------|-------------------|----------------|---------|---------|
| Denture adhesive      | 6                 | 0.1183         | 15.46   | <0.0001 |

### Table 5  Summary of one-way ANOVA (F release from the S-PRG filler-containing denture adhesives)

| Source of variability | Degree of freedom | Sum of squares | F-value | p-value |
|-----------------------|-------------------|----------------|---------|---------|
| Denture adhesive      | 6                 | 155.03         | 8.132   | <0.0001 |
RESULTS

**S-PRG microstructure**

SEM images of the S-PRG fillers with different particle sizes (1 and 3 µm) confirmed that the 1 µm S-PRG particles were smaller than the 3 µm S-PRG particles (Fig. 2). The filler size distribution of the 1 µm S-PRG filler ranged from approximately 0.1 to 5 µm, whereas that of the 3 µm S-PRG filler ranged from approximately 0.5 to 10 µm.

**Acid buffering capacity**

One-way ANOVA followed by Tukey’s *post hoc* test revealed that the denture adhesives containing S-PRG with 1µm–5.0wt% and 1µm–7.5wt% (*p*<0.001) had a stronger acid buffering capacity than the denture adhesives with 3µm–5.0wt% and 3µm–7.5wt% (*p*<0.001) particles (Fig. 3).

**Ion release**

Al release from 3µm–7.5wt% (*p*=0.01618) and 3µm–10wt% (*p*=0.00860) adhesives was significantly higher than that from the control; further, B release from...
1µm–5.0wt% (p=0.02292), 1µm–7.5wt% (p=0.02050), 1µm–10wt% (p<0.001), 3µm–7.5wt% (p=0.00209), and 3µm–10wt% (p<0.001) adhesives was significantly higher than that from the control (Fig. 4). The highest release of B was from the 1µm–10wt% adhesive. Sr release from 1µm–10wt% (p=0.03761), 3µm–7.5wt% (p=0.01122), and 3µm–10wt% (p=0.00839) adhesives was significantly higher than that from the control. An increased amount of S-PRG facilitated higher B and Sr release. Na release from the 1µm–10wt% (p=0.0227) adhesive was significantly higher than that from the control; however, no significant release of Na above the control was observed for other specimens (p>0.05). This was attributed to the Na already present in the base material of the denture adhesive. F release from the 1µm–10wt% (p<0.001) adhesive was significantly higher than that from other adhesives.

Antimicrobial effect on the contact surface of the denture adhesive

The CFUs of *C. albicans*, *C. glabrata*, *S. mutans*, and *A. naeslundii* on the contact surface of the denture adhesive are shown in Fig. 5. The Kruskal–Wallis test followed by Dunn’s test revealed that the 1µm–7.5wt%, 1µm–10wt%, and 3µm–10wt% adhesives led to significantly lower CFUs of *C. albicans* than in the control adhesive, indicating suppressed growth of *C. albicans*. One-way ANOVA followed by Tukey’s post hoc test indicated that the 1µm–7.5wt%, 1µm–10wt%, 3µm–7.5wt%, and 3µm–10wt% adhesives led to significantly lower CFUs of *C. glabrata* than in the control adhesive. The 1µm–5wt%, 1µm–7.5wt%, and 1µm–10wt% adhesives led to significantly lower CFUs of *S. mutans* than in the control adhesive. The 1µm–7.5wt% and 1µm–10wt% denture adhesives led to significantly lower CFUs of *A. naeslundii* than in the control adhesive. Overall, the 1µm S-PRG particles suppressed microbial growth more effectively than the 3µm particles.

Antimicrobial effect against *C. albicans* on acrylic resin treated with the denture adhesive

The CFUs of *C. albicans* adhered to the acrylic resin are shown in Fig. 6. Two-way ANOVA followed by Tukey's
**DISCUSSION**

The acid buffering capacity of the denture adhesives containing S-PRG with smaller S-PRG particles was better than those with larger particles. Higher S-PRG content resulted in increased release of Al, B, Na, and Sr ion. Both smaller particle size and higher particle content led to more effective suppression of the four representative oral microorganisms. Therefore, the hypothesis that the particle size of S-PRG and the filler particle content in the denture adhesives affect the acid buffering ability, ion release, and antimicrobial effectiveness was accepted.

Denture adhesives exhibit a higher viscosity when stored in water and are often used for ill-fitted dentures. As ill-fitted dentures tend to move in a patient’s mouth, the denture adhesive becomes mixed with saliva. Therefore, the denture adhesives were stirred in the demineralization solutions to evaluate the acid buffering capacity, while the specimens were stored in distilled water to measure ion release. It is difficult to measure the denture adhesives in their gel state using ICP-AES. Therefore, the ion release was measured in specimens stored in water without stirring.

The pH value differs depending on the products, but mostly ranges from 4 to 10. Tajima *et al.* reported that the pH of the oral environment turned acidic after long-term use of denture adhesives, which could induce decalcification of residual teeth. However, Takayama claimed that a change in the pH of the oral environment is unlikely owing to the acid buffering capacity of saliva produced when using denture adhesives.

Shimizu *et al.* reported that a higher S-PRG content in experimental cement facilitated a higher acid buffer capacity. This was also observed in the current study. Stirring of the denture adhesive in the demineralization solutions using a mixer enhanced the ion release from the denture adhesive. Moreover, smaller S-PRG fillers exhibited a higher acid buffer capacity. The promising acid buffering capacity of the denture adhesives containing S-PRG is indicative of the effective improvement in maintaining the oral environment.

The denture adhesives containing S-PRG exhibited a considerably lower ion release than the S-PRG fillers themselves. The other dental materials containing a considerably lower ion release than the S-PRG fillers improved in maintaining the oral environment. S-PRG fillers exhibited a higher acid buffer capacity. This was also observed in the current study. Stirring of the denture adhesive in the demineralization solutions using a mixer enhanced the ion release from the denture adhesive. Moreover, smaller S-PRG fillers exhibited a higher acid buffer capacity. The promising acid buffering capacity of the denture adhesives containing S-PRG is indicative of the effective improvement in maintaining the oral environment.

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The inhibition of microbial proliferation on the contact surface of the denture adhesives was not evaluated using the agar diffusion method, as no clear inhibition circle was formed around the denture adhesives containing S-PRG when the agar diffusion method was used in a preliminary study. This was attributed to the negligible influence of the denture adhesive containing S-PRG on all parts except for the area in direct contact with the adhesive. Consequently, the denture adhesive was applied to the filter (MILLPORE, 0.2 µm) enabled removal of the adhesive as an intact piece, thereby allowing examination of the area that was in direct contact with the agar. After incubation, the specimens were removed with the filter, and the CFUs were quantified. The experimental setup was similar to the agar diffusion method in terms of placing the denture adhesives on the side of the well. However, the use of a filter enabled larger contact area than in the agar diffusion method, thereby augmenting the influence of the antimicrobial effect of the S-PRG filler-containing denture adhesives.

The inhibition of bacterial growth followed similar trends to B, F, and Sr ion release, with the 1µm–10wt% adhesive being effective against all microorganisms. Longer contact periods resulted in significantly lower microorganism adhesion to the denture base resin. Similar results were reported by Hotta *et al.*, where reduced adhesion of microorganisms to a composite resin containing S-PRG was attributed to the formation of salivary pellicles due to the release of BO$_3^{2-}$, Al$^{3+}$, SiO$_2^{2-}$, Sr$^{2+}$, and F$^{-3}$. Unlike the composite resin in the previous study, the denture adhesives containing S-PRG in this study were repeatedly placed in the denture base resin; thus, BO$_3^{2-}$, Al$^{3+}$, SiO$_2^{2-}$, Sr$^{2+}$, and F$^{-3}$ were continuously supplied. Kitagawa *et al.* reported that an F$^{-3}$ concentration of 3.4–13.5 ppm and B concentration of 67.8–271 ppm inhibited the metabolic activity and growth of *S. mutans*.

The highest concentration of F$^{-3}$ in the 1µm–10wt% S-PRG filler-containing denture adhesive was approximately 8 ppm, which was within the range of F$^{-3}$ concentration for *S. mutans* inhibition. The assessment of *C. albicans* adhesion to the acrylic resin revealed that the control group had fewer CFUs after 5 days than after 1 and 3 days. This may be attributed to the amount of residual preservatives in the denture adhesive applied to the acrylic resin. *C. albicans* exhibited less resistance to these preservatives after 5 days, thereby showing reduced adhesion of *C. albicans* and smaller number of CFUs after 5 days than after 1 and 3 days. A minimum S-PRG content of 7.5 wt% was found to be necessary to suppress the growth of *C. albicans*, *C. glabrata*, *S. mutans*, and *A. naeslundii*.

Regarding the cytotoxicity of the S-PRG filler, Ito *et al.* investigated the cytotoxicity of HeLa cells cultured water, where the release of B was generally higher than that of Sr. However, in this study, the release of Sr is higher than that of B. This could be related to the viscosity of the matrix of the denture adhesives; nevertheless, it is necessary to further investigate the reason for this difference.
with the S-PRG filler\textsuperscript{32}. They reported that the six ions released (F, Al, Na, B, Si, and Sr) from the S-PRG filler resulted in low cytotoxicity. Most denture adhesives are adhered to the oral mucosa and denture base resin, and the inflow by saliva is considered to be limited. Therefore, we assume that the negative influence of the denture adhesive containing S-PRG filler is limited. Moreover, we consider Al toxicity. Jalili \textit{et al.} investigated the genotoxicity of Al in rats following oral exposure\textsuperscript{33). They reported that Al did not induce mutations in the chromosomes of rat colons. Provisional tolerable weekly intakes of Al are 1 mgAl/kg/week as per the European Food Safety Authority (EFSA)\textsuperscript{34} and 2.3 mgAl/kg/week as per the Food and Agriculture Organization/World Health of the United Nations\textsuperscript{35}. Thus, it is essential for dentists to properly regulate the amount of denture adhesives containing S-PRG filler. However, even if patients use denture adhesives containing S-PRG filler every day, the sustained release of Al from the denture adhesives containing S-PRG filler would have limited influence on the human body. We estimated the Al intake from denture adhesives containing S-PRG filler in the following manner. Assuming a patient weighs 50 kg, the amount of denture adhesive used once is 2 g. When the released Al is set to 4 ppm, the calculated Al intake would amount to 6.4×10\textsuperscript{-3} mg in 24 h. If the denture adhesives are used for 12 h and all the Al is released into the body, the estimated Al intake would be 3.2×10\textsuperscript{-3} mg. Therefore, even if the denture adhesives containing S-PRG filler are used every day, the estimated Al intake in a week would amount to 22.4×10\textsuperscript{-3} mg. This estimated value is lower than the provisional tolerable weekly intakes of Al reported by EFSA (1 mgAl/kg/week). Thus, we consider that the Al toxicity of the denture adhesive containing S-PRG filler is limited.

The present study revealed that denture adhesives containing S-PRG filler may contribute to improving oral environment of denture wearers. An apparent limitation of this study is that the experimental setups for evaluating the acid buffering capacity and ion release differ, which should be reconsidered in the future. Further, a more clinically suitable approach for assessing the antimicrobial effect would involve applying the denture adhesive directly to the culture medium or acrylic resin. The setup in the current study, \textit{i.e.}, using a filter to hold the adhesive, was chosen because of the nature of the denture adhesive. In the future, it is necessary to conduct experiments in three replicates to investigate the antimicrobial effect for more reliable results. Furthermore, we will conduct systemic toxicity tests, cytotoxicity tests, sensitization tests, mutagenicity tests, and oral mucosal irritation tests to determine whether the denture adhesives containing S-PRG filler can be safely used in a clinical situation. In the continuation of this work, the inhibition mechanism of the denture adhesives containing S-PRG filler against \textit{C. albicans} should be investigated. This novel denture S-PRG filler-containing adhesive should be evaluated in a clinical study.

CONCLUSIONS

This \textit{in vitro} study yielded the following results regarding the denture adhesives containing S-PRG:

1. Denture adhesives containing S-PRG exhibited antimicrobial effects.

2. Denture adhesives containing 1µm–10wt% S-PRG particles exhibited excellent antimicrobial activity against \textit{C. albicans}, \textit{C. glabrata}, \textit{S. mutans}, and \textit{A. naeslundii}.

3. A smaller S-PRG particle size and higher S-PRG content facilitated a better antimicrobial effect.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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