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Reduction of *Candida* biofilm adhesion by incorporation of prereacted glass ionomer filler in denture base resin

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ABSTRACT

Objectives: This study investigated the influence of surface reaction-type prereacted glass ionomer (S-PRG) fillers on *Candida albicans* adhesion on denture base resin.
Methods: Discs were prepared by incorporating the S-PRG filler into the polymer powder of a polymethyl methacrylate (PMMA)-based, heat-polymerizing resin at 0 (control), 5%, 10%, and 20% (w/w). The surface roughness of all disc surfaces was measured. Elemental analysis of released Na^+, Sr^{2+}, SiO_3^{2-}, Al^{3-}, BO_3^{3-}, and F^- was performed after water immersion. Each disc was placed in a well with artificial saliva to form acquired pellicle, incubated, washed with phosphate-buffered saline, and immersed in a *C. albicans* (JCM2085) cell suspension standardized at 10^4 cells/ml. After aerobic incubation at 37 °C for 24 h, the metabolic mitochondrial activity, total biofilm biomass, and biofilm thickness were evaluated. The morphogenetic transition of *C. albicans* in the early culture stage (1 and 3 h) was observed.
Results: There was a slight but significant increase in the surface roughness with an increase in the filler content. The metabolic activity and total biomass volume were significantly lower in all filler groups than in the control group, although there were no significant differences among the filler groups. Groups with at least 5% filler content exhibited a thinner biofilm compared with the control group. All filler groups showed hyphal forms at 3 h, with the length of the hyphae being lesser than those in the control group.
Conclusions: Although the incorporation of S-PRG filler slightly increases the surface roughness of denture base resin, it reduces the adhesion of *C. albicans*.
Clinical significance: The S-PRG filler has the potential to reduce *Candida albicans* adhesion on denture base resin and may lower the risk of denture stomatitis. However, filler incorporation can increase the surface roughness of heat-polymerizing denture base resin.

1. Introduction

Denture stomatitis often leads to oral mucosal pain, discomfort, and altered taste sensation, resulting in poor nutritional intake [1–3]. Overgrowth of the fungus *Candida albicans* is one of the major causes of denture stomatitis, also known as *Candida*-associated denture stomatitis (CADS). It is predisposed by poor denture plaque control, often in association with trauma from the denture base or the systemic condition of the patients. If trauma from the denture base does not appear to be a significant contributing factor to stomatitis, inhibition of *C. albicans* biofilm formation on denture bases is the first step toward the prevention of CADS [1,4,5]. Oral antifungal agents such as amphotericin B, nystatin, and miconazole and systemic antifungal drugs have been used, although their effects are reportedly limited [6–8]. The therapeutic strategy for CADS has not been definitely established [9,10], although preservatives and disinfectants [9,11,12] and microwaves [10] have been occasionally used for the removal of plaque present on denture surfaces.

Surface reaction-type prereacted glass ionomer (S-PRG) filler particles are formed by an acid–base reaction of fluoroaluminosilicate glass with polyacrylic acid, and the filler is capable of releasing six types of ions (Na^+, Sr^{2+}, SiO_3^{2-}, Al^{3-}, BO_3^{3-}, and F^-) [13,14]. The filler can reduce the adhesion of cariogenic bacteria, including *Streptococcus mutans*, and can prevent plaque formation because of its ability to induce mineralization [15–17]. A clinical study demonstrated that a resin composite containing this filler showed inhibitory effects on the growth of dental plaque on the restored tooth surface [18]. On the other hand, previous bacteriological studies indicated that fluoride and boric acid have

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Table 1
Composition of the denture base resin used in the study.

		Control	Filler groups			Lot no.	Manufacture
Powder	S-PRG filler (wt%)	0%	5%	10%	20%	130731	Shofu, Kyoto, Japan
	PMMA	100%	95%	90%	80%	021302	
	Colorant (clear light pink)						
Liquid	MMA, EGDMA, others					031359	

S-PRG: surface reaction-type prereacted glass ionomer; PMMA: polymethyl methacrylate; EGDMA: ethylene glycol dimethacrylate.

inhibitory effects on *C. albicans* adhesion [19–23], suggesting that the S-PRG filler has the potential to reduce *C. albicans* adhesion on denture surfaces if released from the denture base resin. However, the inhibitory effects of this filler incorporated into a polymethyl methacrylate (PMMA)-based, heat-polymerizing denture base resin on fungal growth have not been supported by evidence. Before the implementation of a clinical trial, the potential antifungal effects of S-PRG incorporated in denture base resin and the effective filler content should be understood in an *in vitro* study. Therefore, we conducted this study to investigate the influence of S-PRG filler incorporation in heat-polymerizing denture base resin on the growth and adhesion of *C. albicans* on the resin surface.

2. Materials and methods

2.1. Specimen preparation and measurement of released ions

Experimental acrylic resin discs were prepared by adding S-PRG filler (Shofu, Kyoto, Japan) to the polymer powder of a commercially available, PMMA-based, heat-polymerizing denture base resin (Urban, clear light pink, Shofu) at 5%, 10%, and 20% (w/w; Table 1). The original powder without the S-PRG filler was used as a control. The polymer powder and resin monomer were mixed with a powder–liquid ratio of 10 g/5 mL at 25 °C in accordance with the manufacturer's instructions. When the mixture reached a dough consistency, it was poured into a cylindrical mold measuring 10 mm in diameter and 80 mm in length and covered with a polyvinyl chloride plate sealed with a polyethylene film. The mold was immersed in a heat-retention bath filled with hot water of 70 °C, and the temperature was increased to 100 °C at the rate of 1 °C/min. The temperature was maintained for 45 min, followed by cooling to room temperature. Each specimen was taken out from the mold, polished using a lathe machine (YS-550V, Towa Seiki, Tokyo, Japan), and sectioned into 2.5-mm-thick discs (IsoMet Low speed, Buehler, ITW, Lake Bluff, IL, USA). The upper and lower surfaces of each disc were polished using abrasive papers (250–2000 grit) under dry conditions and mirror-polished to a thickness of 2 mm using a polishing machine (Union Optical, Tokyo, Japan). The surface roughness of all disc surfaces was measured using a profilometer (Surfcom Flex, Tokyo Seimitsu, Tokyo, Japan). Discs that did not meet the requirements of Ra < 0.10 μm, Rz < 1.0 μm, and Rzmax < 1.0 μm were excluded from the study. Moreover, the surface characteristics of all disc surfaces were examined using 3D measuring laser microscope (LEXT OLS4000, Olympus, Tokyo, Japan). All discs were sterilized in ethylene oxide gas, stored in an incubator at 40 °C for 24 h, and immediately used for testing.

Each of five disc samples from each group was immersed in hyper-pure water (Milli-Q® RG, Millipore, Billerica, MA, USA). Elemental analysis of the five ions (Na^+, Sr^{2+}, SiO_3^{2-}, Al^{3-} and BO_3^{3-}) released from the specimens was performed using inductively coupled plasma atomic emission spectroscopy (ICPS-7000, Shimadzu Co., Kyoto, Japan). The release of F^- was analyzed using a fluoride ion electrode (Orion 9609BNWP, Thermo Scientific Inc., Waltham, MA, USA) connected to a fluoride ion meter (Orion 4-Star, Thermo Scientific Inc., Waltham, MA, USA). The concentrations of all ions were measured in water and analyzed at 24 h after immersion.

2.2. Candida *growth conditions and yeast suspensions*

Each disc was placed in one of the 24-well plates (Sumitomo Bakelite, Tokyo, Japan) with artificial saliva [500 μl; 1.25 mM Ca$(NO_3)_2 \cdot 4H_2O$, 0.90 mM KH_2PO_4, 129.91 mM KCl, 59.93 mM Tris buffer, and 2.2 g/L porcine gastric mucin; pH 7.4] [24]. The plates were incubated for 60 min (37 °C on a shaker at 60 rpm) and washed twice with 1 ml of phosphate-buffered saline (PBS; pH 7.2).

C. albicans (JCM2085) were grown in yeast–peptone–dextrose (YPD) medium (Difco Laboratories, Detroit, MI, USA) for 5 h at 30 °C and on a shaker at 180 rpm before experimentation. The yeast cells in the mid-log phase were standardized at 10^4 cells/ml in YPD medium using the UV–vis spectrophotometer (V-630, JASCO, Tokyo) and a counting chamber (One cell counter, Bio Chemical Science, Tokyo, Japan). This *Candida* cell suspension (1 ml) was added to each well with a disc as described above. To assess the influence of YPD medium on the spectrometry findings, five wells of YPD without the cell suspension were also prepared for all following biofilm analyses in each filler group. All the well plates were aerobically incubated for 24 h at 37 °C for all subsequent biofilm analyses except that described in Section 2.3.4, wherein the plates were incubated for 3 h. All specimens were washed twice with PBS before analyses. As a preliminary calibration, one examiner (first author) accomplished the calibration procedures to confirm the consistency in the amount of biofilm on the basis of evaluation by means of SEM observation. Thereafter, the same examiner performed all the final experiments.

Table 2
Mean (standard deviation) concentrations of released ions (ppm).

	Control	5%	10%	20%
F^-	0.02 (0.02)	0.62* (0.30)	0.78* (0.12)	1.08* (0.06)
Sr	0.00 (0.00)	0.62* (0.06)	1.39* (0.11)	2.29* (0.19)
Si	0.00 (0.00)	0.10* (0.01)	0.17* (0.02)	0.25* (0.00)
B	0.00 (0.00)	0.06* (0.07)	0.18* (0.02)	0.74* (0.09)
Al	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.01 (0.00)
Na	0.08 (0.04)	0.15* (0.04)	0.38* (0.03)	0.56* (0.10)

The asterisk (*) indicates a significant difference between the filler group and control group for each released ion ($n = 5$, $^*p < 0.05$).

Table 3
Mean surface roughness values for the discs used in the study (μm).

	Control	5%	10%	20%
Ra	0.03 (0.00)	0.05 (0.01)	0.05 (0.01)	0.07* (0.08)
Rz	0.25 (0.10)	0.42* (0.13)	0.44* (0.10)	0.48* (0.09)
Rzmax	0.42 (0.22)	0.62* (0.20)	0.63* (0.17)	0.67* (0.16)

The asterisk (*) indicates a significant difference in the roughness value between the filler group and control group ($n = 85$ for each group; $^*p < 0.05$). Discs that did not meet the requirements (Ra < 0.10 μm, Rz < 1.0 μm, and Rzmax < 1.0 μm) were excluded from the study.