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Superhydrophilic co-polymer coatings on denture surfaces reduce *Candida albicans* adhesion—An in vitro study

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ABSTRACT

Objective: In this study, we aimed to investigate denture-base-resin coatings prepared with a crosslinkable co-polymer containing sulfobetaine methacrylamide (SBMAm) and the relationship between their surface characteristics and the initial adhesion of *Candida albicans* (*C. albicans*).

Methods: Acrylic resin discs were coated with co-polymers containing various concentrations of SBMAm and *N,N'*-(4,7,10-trioxa-1,13-tridecadiamine) diacrylamide (JDA) as crosslinking agent. Uncoated discs were used as controls. An acquired pellicle was formed on each disc using artificial saliva, and the discs were immersed in a suspension of *C. albicans* (JCM2085) cells. After incubation, tetrazolium salt (XTT-reduction) and colony forming unit (CFU) assays were performed and the morphogenesis of *C. albicans* was examined using scanning electron microscopy (SEM). The surface roughness, film thickness, and the water contact angle of each disc surface were measured.

Results: All coating groups showed significantly lower amounts of adhered *C. albicans* in the XTT-reduction and CFU assays than the control, confirmed by the SEM images. Many wrinkle structures were observed on the surfaces coated with co-polymers containing more than 30% SBMAm. There were no significant differences in surface roughness among all groups. The co-polymer films on the coated discs were less than 5.0 μm in thickness, and these surfaces exhibited significantly lower mean water contact angles than the control.

Conclusion: Crosslinkable co-polymers containing SBMAm can enhance the hydrophilicity of the surface of denture-base resins and reduce the initial adhesion of *C. albicans*.

1. Introduction

Denture plaque that adheres to denture surfaces contains high levels of the fungus *Candida albicans* (Ramage, Tomsett, Wickes, López-Ribot, & Redding, 2004; Redding et al., 2009). *C. albicans* is generally attributed as being the main causative agent of denture stomatitis (DS) (Budtz-Jørgensen, 2000; Martin-Mazuelos et al., 1997); hence, the removal of *C. albicans* biofilms from denture surfaces has been assumed to be effective for the treatment and prevention of DS (Redding et al., 2009; Tan, Tsoi, Seneviratne, & Matinlinna, 2014). The development of denture plaque begins with the adhesion of microorganisms to the denture surface (Nikawa, Hamada, & Yamamoto, 1998). Since there are many micro cracks and irregularities on the denture fitting surface, adhering microorganisms that are deeply embedded there are difficult to remove by mechanical or chemical cleaning (Ramage et al., 2004; Redding et al., 2009). In particular, *C. albicans* adheres easily to the fitting surfaces of dentures; thigmotropism enhances the yeast-to-hypha transition, (Jackson, Coulthwaite, Loewy, Scallan, & Verran, 2014; Mayahara et al., 2014) and the hyphae of *C. albicans* induce the formation of a biofilm (Davies, Stacey, & Gilligan, 1999; Nikawa, Nishimura, Hamada, Makihira, & Samaranayake, 1998). Therefore, one of the most effective methods for preventing DS is to avoid or reduce the initial adhesion of the fungus to the surface.

The adhesion of *C. albicans* to a denture surface is probably mediated by hydrophobic interactions (Minagi, Miyake, Inagaki, Tsuru, & Suginaka, 1985; Nikawa et al., 2003), and the hydrophobicity of the

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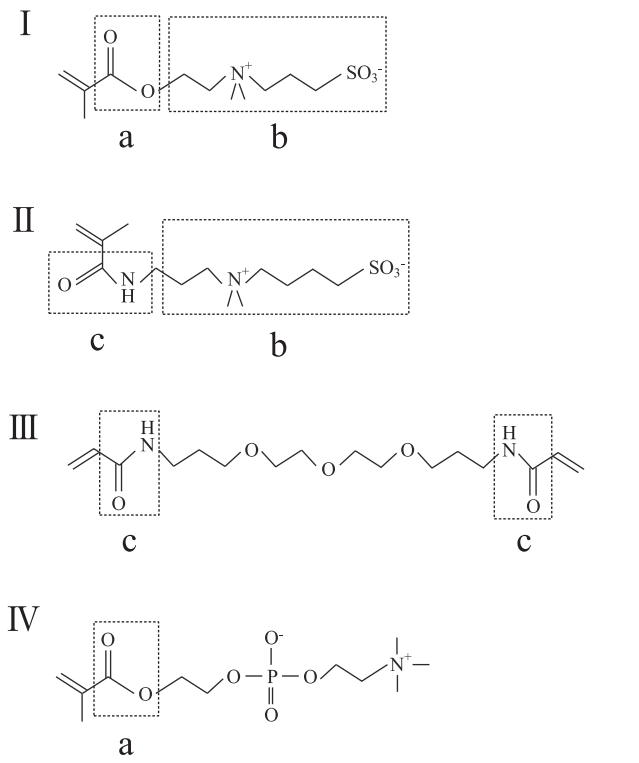

Fig. 1. Chemical structures of each monomer. (I) Sulfobetaine methacrylate (SBMA), (II) sulfobetaine methacrylamide (SBMAm), (III) *N,N'*-(4,7,10-trioxa-1,13-tridecanediamine) diacrylamide (JDA) and, (IV) 2-methacryloyloxyethyl phosphorylcholine (MPC). SBMA contains an ester group (a) and a sulfobetaine group (b) in its molecular structure. SBMAm differs from SBMA in that it contains an amide group (c). JDA is a multifunctional monomer that contains two amide groups (c) in its molecular structure. MPC contains an ester group (a) in its molecular structure.

denture surface is an important factor for the initial adhesion of *C. albicans* (Minagi et al., 1985; Nikawa et al., 2003; Yoshijima et al., 2010). Therefore, altering the characteristics of a denture surface by increasing its hydrophilicity has been thought to reduce *C. albicans* adhesion because of the inhibition of hydrophobic interactions (Hatsuno et al., 2006; Lazarin et al., 2013, 2014; Yodmongkol et al., 2014; Yoshijima et al., 2010). Methods for hydrophilically modifying denture surfaces are not well established, though hydrophilic polymer coatings like 3-hydroxypropyl methacrylate (HPMA), and others containing sulfobetaine methacrylate (SBMA) (Fig. 1, I), which is a zwitterionic monomer, have been reported to improve the wettability of denture surfaces and reduce *C. albicans* adhesion (Lazarin et al., 2013, 2014). These monomers contain ester groups in their molecular structures and it has been suggested that intramolecular ester hydrolysis is likely to occur; however, the stabilities of these polymers have not been clarified (Andres, Pierini, & de Rossi, 2006; Bender, 1951).

Sulfobetaine methacrylamide (SBMAm) (Fig. 1, II) is a zwitterionic monomer containing a sulfobetaine group, which is the same as the hydrophilic group in SBMA (Fig. 1, b); however, SBMAm contains an amide group (Fig. 1, II, c), unlike SBMA, which contains an ester group (Fig. 1, I, a) (Quintana, Gosa, Janczewski, Kutnyanszky, & Vancso, 2013). It is well known that amides are more hydrophilic than esters, while being more stable against hydrolysis; therefore, SBMAm is more hydrophilic and more stable than SBMA (Quintana et al., 2013; Robinson & Tester, 1990). Since SBMAm is a monofunctional monomer, a crosslinking agent is assumed to be required. On the other hand, previous studies indicate that SBMAm coatings on gold and silicon enhance the hydrophilicities of these substrata and reduce the adhesion of proteins and fibroblasts (Cho, Kong, & Choi, 2007; Cho et al., 2010), suggesting that an SBMAm coating on a denture surface can reduce the initial adhesion of *C. albicans*. However, no studies have investigated the effect of reducing *C. albicans* adhesion to the denture base resin coating using SBMAm.

This in-vitro study aimed to investigate denture base resin coatings prepared with crosslinkable co-polymers containing SBMAm and the relationship between their surface characteristics and *C. albicans* adhesion.

2. Materials and methods

2.1. Specimen preparation

An initiator (FAI-101L, FUJIFILM, Tokyo, Japan), SBMAm and *N,N'*-(4,7,10-trioxa-1,13-tridecanediamine) diacrylamide (JDA) (Fig. 1, III), as the crosslinking agent, were dissolved in methanol. The SBMAm-to-JDA ratios in the remaining solutions were: 0:100, 15:85, 30:70, and 50:50 (wt%), and the solutions are referred to as "SM0%", "SM15%", "SM30%" and "SM50%", respectively. A set of 250 heat-polymerising acrylic resin discs (2 mm in thickness and 12 mm in diameter) (Natural resin, Nissin Co., Kyoto, Japan) was prepared and the upper and lower surfaces of each disc in the set were polished using abrasive paper (8000 grit) under dry conditions; 50 discs were used as controls. The remaining 200 discs surfaces were modified by plasma cleaning (EXAM, Shinko Seiki Co. Ltd., Hyogo, Japan) for 60 s at a discharge power of 100 W. After the plasma treatment, NEOSTAN U-600 (Nitto Kasei Co., Ltd., Osaka, Japan) and Karenz (Showa Denko K.K., Tokyo, Japan) primers were applied to each disc, followed by drying for 2 h at 40 °C. The modified discs were immersed in SM0%–SM50% for 10 s. The surfaces were subsequently irradiated with a UV lamp for 27 s (4 J/cm^2). All discs were sterilized under ethylene oxide gas, stored in an incubator at 40 °C for 24 h, and then immediately used for testing.

2.2. Biofilm analysis

2.2.1. Candida growth conditions

Each disc was placed in one of the wells of a 12-well plate (Sumitomo Bakelite, Tokyo, Japan) with 2 mL of 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 (Carvalho & Lussi, 2014)] to form a pellicle. The plates were incubated for 60 min (37 °C on a shaker at 60 rpm) and washed twice with 2 mL of phosphate-buffered saline (PBS; pH 7.2). *C. albicans* (JCM2085) cell suspensions were prepared using the method from our previous study (Tsutsumi, Takakuda, & Wakabayashi, 2016), while yeast cells in the mid-log phase were standardized at 10^7 cells/mL in yeast extract peptone dextrose (YPD) medium. The *Candida* cell suspension (2 mL) was added to each disc-containing well. All well plates were aerobically maintained for 1.5 h at 37 °C during the cell adhesion phase (Chandra et al., 2001), after which all specimens were washed twice with 2 mL of PBS to remove the non-adhered cells. The YPD medium (2 mL) was then added to each well and all the well plates were aerobically incubated for 24 h at 37 °C for subsequent biofilm analyses. All specimens were washed twice with PBS before being analysed.

2.2.2. XTT-reduction assay

A 700-μL aliquot of PBS as added to each well and the *Candida* biofilm was scraped from each disc using a cell scraper (Iwaki Co., Tokyo, Japan). We used an XTT kit (Cosmo Bio, Tokyo, Japan). The reaction solution was prepared in accordance with the manufacturer's instructions, and 350 μL of this solution was added to each well. All well plates were incubated for 3 h in the dark, and colorimetric changes in the solutions were measured using a microplate reader (Chro Mate®, Awareness Technology Inc., Palm City, FL, USA) at 450 nm. Ten discs from each group were used for this assay.

2.2.3. CFU assay

A 1-mL aliquot of trypsin solution (from porcine pancreas 10 × ;