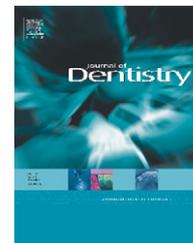


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Biofilm formation of *Candida albicans* on implant overdenture materials and its removal

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ABSTRACT

Objectives: The purposes of this study were to clarify the surface characteristics of various implant overdenture materials and the capabilities of *Candida albicans* adherence and biofilm formation on these surfaces, and to investigate the role of salivary mucin in biofilm formation.

Methods: Seven commonly used implant and restorative materials were assessed. The surface roughness averages of all materials were limited to 0.07–0.10 μm. Contact angles and salivary mucin absorption were measured. After 90-min initial adhesion and 2-day biofilm formation, the amounts of *C. albicans* were determined by counting colony-forming units and the morphological characteristics were observed by scanning electron microscopy (SEM). The effects of saliva coating and the influences of material surface property on initial adhesion, biofilm formation and its removability were analysed by univariate two-way analysis of variance and multiple linear regression analysis.

Results: Surface contact angle of materials, the index of hydrophobicity, was found to be correlated positively with initial adhesion and biofilm formation of *C. albicans*. A negative correlation between mucin absorption and removability of *Candida* biofilm indicates that mucin plays an important role in biofilm formation and its rigidity. SEM observation also revealed fewer *Candida* cells on saliva-coated Ti than on saliva-coated hydroxyapatite or acrylic resin.

Conclusions: The materials with different hydrophobic property and compositions display diverse manners of salivary mucin absorption, initial adhesion and biofilm formation. The hydrophobic materials encourage enhanced initial adhesion, subsequently resulting in the active biofilm formation. Mucin has decisive effects on *Candida* immobilization and biofilm development on the materials.

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Abbreviations: Ra, roughness average; PBS, phosphate-buffered saline; CFU, colony-forming units; SEM, scanning electron microscopy; PET, polyethylene terephthalate; HA, hydroxyapatite.

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Clinical significance: Surface hydrophilic property and composition of materials and salivary proteins, especially mucin, affect the process of *Candida* biofilm formation and influence the amount and rigidity of formed biofilm. The present data may be applied as a reference for selecting materials in implant overdenture treatment from a microbiological point of view.

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1. Introduction

The introduction of osseointegration and implant-retained prostheses has led to a revolutionary shift in the therapeutic paradigm for the treatment of edentulous patients, in particular, implant overdenture has become an optional and recommendable prosthodontic treatment for mandibular edentulism.^{1–3} Implant overdenture has been considered to provide stable, retentive, and functionally comfortable prostheses. Meanwhile, it is plunged into the same infectious problems as the conventional overdenture and implant treatment, such as denture-related stomatitis and peri-implantitis. In different clinical situations, various materials are applied for the components of implant overdenture, such as implants, attachment system and overdenture. These restorations, however, are plagued with long-term mucosal contact, which will result in cleaning and plaque control difficulties.

Candida albicans is the most prevalent fungus in the oral cavity and has been considered the major pathogenic microorganism associated with denture-related stomatitis.^{4,5} The initial and essential stage in the pathogenesis of oral candidosis entails the attachment of *C. albicans* to a host surface or an implanted device, such as dentures and dental implants.^{5,6} The surface properties of materials, such as surface roughness, hydrophilicity, and chemical properties, are reported to significantly influence the amount and characteristics of fungal adhesion.^{6,7} The relationship between these factors and *Candida* adhesion and subsequent biofilm formation still remains controversial. The previous study by Chandra et al.⁸ suggested that the correlation between contact angle, an index of hydrophobicity, and *Candida* biofilm formation was observed for polyetherurethane substrates but not for polyethylene terephthalate (PET) biomaterials. Other studies reported that no conclusive correlation was found between surface roughness, hydrophobicity, and the amount of *C. albicans* adhesion to resin materials.^{9,10} As for the effect of saliva, conflicting results have been reported by different groups. In an in vitro study, saliva had little effect on either *Candida* adhesion or biofilm formation on polystyrene.¹¹ Nikawa et al.^{12,13} reported saliva promoted *Candida* colonization on denture lining materials and type-I collagen. In contrast, Moura et al.¹⁰ found that the adherence of *Candida* species including *C. albicans* was significantly decreased by saliva. Although efforts have been made to evaluate the roles of various determining factors on *Candida* adhesion and biofilm formation, the current data are inconsistent and confusing. Therefore, an understanding of the relationships between these factors and biofilm formation will help to develop a preventive measure against biofilm-associated infection involving implant overdenture.

The main purpose of this study was to clarify the surface characteristics of various restorative materials used in implant overdenture and the capabilities of *C. albicans* initial adhesion and biofilm formation on these surfaces. We also investigated the role of salivary mucin in the process of biofilm formation and the removability of *Candida* biofilm formed on various material surfaces.

2. Materials and methods

2.1. Preparation of implant overdenture materials

Seven implant overdenture-related materials prepared as a round disc (13 mm in diameter and 2 mm in thickness, 12 discs for each material) were polished using waterproof silicon carbide paper until grit p2400c (Table 1). Type-I collagen coated polystyrene was also included as a control since earlier studies used collagen surfaces to address the characteristics of *C. albicans* biofilm formation.^{14,15}

2.2. Measurement of surface properties

For the measurement of surface properties, the surface roughness average (*R_a*) was measured with a stylus instrument (Handysurf E-35A, Tokyo Seimitsu Co., Japan), and the surface contact angles of materials, an index of hydrophilicity, were measured using a contact angle-measuring apparatus (Imoto Machinery Co., Kyoto, Japan).¹⁶

2.3. Mucin absorption

Mucin absorption on the material surfaces was measured. After immersion in 10 mg/mL mucin from bovine submaxillary glands (Sigma-Aldrich, Co., St. Louis, MO, USA) for 90 min, specimens were rinsed with phosphate-buffered saline (PBS, pH 7.2), then stained with alcian blue solution (Wako Pure Chemical Industries, Osaka, Japan). Immediately after rinsing with PBS and 30% hydrogen peroxide (Wako Pure Chemical Industries), the optical density of the supernatant (200 μ L) was measured spectrophotometrically at 595 nm.

2.4. Initial adhesion and biofilm formation of *C. albicans*

Unstimulated whole saliva, collected from 4 healthy adult volunteers, was incubated on ice for 10 min, pooled, and centrifuged at 35,000 $\times g$ for 20 min at room temperature. The resulting supernatant was extracted and pipetted 50 times to shear the long mucin chains, sterilized with a 0.22 μ m polyethersulphone filter (MN Sterilizer PES, Macherey-Nagel, Düren, Germany), and then stored at 4 °C until used.

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