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Effect of experimental photopolymerized coatings on the hydrophobicity of a denture base acrylic resin and on *Candida albicans* adhesion

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ARTICLE INFO

Article history:
Accepted 7 October 2012

Keywords:
Adhesion
Candida albicans
Hydrophilic
Prosthesis
Surface modification

ABSTRACT

Objective: This study investigated the effect of experimental photopolymerized coatings, containing zwitterionic or hydrophilic monomers, on the hydrophobicity of a denture base acrylic resin and on *Candida albicans* adhesion.
Methods: Acrylic specimens were prepared with rough and smooth surfaces and were either left untreated (control) or coated with one of the following experimental coatings: 2-hydroxyethyl methacrylate (HE); 3-hydroxypropyl methacrylate (HP); and 2-trimethylammonium ethyl methacrylate chloride (T); and sulfobetaine methacrylate (S). The concentrations of these constituent monomers were 25%, 30% or 35%. Half of the specimens in each group (control and experimentals) were coated with saliva and the other half remained uncoated. The surface free energy of all specimens was measured, regardless of the experimental condition. *C. albicans* adhesion was evaluated for all specimens, both saliva conditioned and unconditioned. The adhesion test was performed by incubating specimens in *C. albicans* suspensions (1×10^7 cell/mL) at 37 °C for 90 min. The number of adhered yeasts were evaluated by XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[{phenylamino}carbonyl]-2H-tetrazolium-hydroxide) method.
Results: For rough surfaces, coatings S (30 or 35%) and HP (30%) resulted in lower absorbance values compared to control. These coatings exhibited more hydrophilic surfaces than the control group. Roughness increased the adhesion only in the control group, and saliva did not influence the adhesion. The photoelectron spectroscopy analysis (XPS) confirmed the chemical changes of the experimental specimens, particularly for HP and S coatings.
Conclusions: S and HP coatings reduced significantly the adhesion of *C. albicans* to the acrylic resin and could be considered as a potential preventive treatment for denture stomatitis.

1. Introduction

In spite of its multifactorial etiology, *Candida albicans* infection has often been associated with denture-induced stomatitis.[1] In addition to its high incidence in denture wearers, there is a concern that *Candida* species from the oral cavity may colonize the upper gastrointestinal tract in immunosuppressed patients and lead to septicemia.[2]

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.10.005

Candida spp. are more frequently isolated from the fitting surface of dentures when compared to the corresponding region of the oral mucosa.[1] Therefore, the treatment of denture-induced stomatitis should include denture cleansing and disinfection in addition to topic or systemic antifungal drugs. Although these treatments do show some efficacy, they aim at inactivating the microorganisms after denture surface colonization. As the adhesion of microorganisms to denture surfaces is a prerequisite for microbial colonization,[3,4] the development of methods that can reduce *C. albicans* adhesion may represent a significant advance in the prevention of denture-induce stomatitis.

The use of polymers containing zwitterionic groups such as phosphatidylcholines and sulfobetaines,[5–10] which originate from the simulation of biomembranes,[9,11] has been proposed to modify the surface of biomaterials.[12–14] A significant reduction in protein adsorption has been demonstrated[5,8–10,12–18] and attributed to the formation of a hydration layer on the material surface[5–7,9–14,16,17,19] that prevents the conformational alteration of these proteins.[9,11,13,14,19] Previous researchers[7,13,16,20,21] reported that sulfobetaine application on substrate surfaces reduced bacterial adhesion. These results suggest that sulfobetaine-based polymers may be used to modify the surface of acrylic materials used in the fabrication of removable dentures and reduce microbial adhesion.[6] However, the effectiveness of this surface modification on *C. albicans* adhesion remains to be investigated.

Surface modification by deposition of polymer coatings such as parylene has been reported to improve the wettability of a silicone elastomer and reduce *C. albicans* adhesion and aggregation on its surface.[22] Hydrophilic polymers have also been investigated in biomaterial research.[19,23,24] The hydration state of hydrophilic polymers is different from that of zwitterionic polymers, and the free water fraction on polymer surface is lower in the former.[19] Despite these differences, hydrophilic polymers have been used to modify the surface of biomaterials and reduce bacterial adhesion.[23,24] The adsorption of proteins to neutral hydrophilic surfaces is relatively weak, while their adsorption to hydrophobic surfaces tends to be very strong and practically irreversible.[25,26] Therefore, altering the characteristics of the inner surfaces of dentures by increasing their hydrophilicity could reduce colonization by pathogenic microorganisms, including *Candida* spp. It has been reported that substratum surface properties, such as surface free energy, may influence *C. albicans* adhesion to polymers, where hydrophobic interactions play a role.[27–29]

The purpose of this study was to evaluate the effect of experimental photopolymerized coatings, containing zwitterionic or hydrophilic monomers, on the hydrophobicity of a denture base acrylic resin and on *C. albicans* adhesion. The hypotheses were that the coating application would decrease the surface hydrophobicity and reduces *C. albicans* adhesion, and that there would be differences among coatings.

2. Material and methods

2.1. *Specimen fabrication*

Disc-shaped silicone patterns (13.8 mm × 2 mm) were obtained from metallic matrices. Half of the silicone patterns were inserted between two glass plates and the other half were inserted in dental flasks directly in contact with the stone. These two methods of specimen preparation were used to obtain smooth and rough surfaces that simulate the outer and inner surfaces of the dentures, respectively. The silicone patterns were then removed, and the surfaces were coated with a layer of separating medium (Vipi Film; VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda Pirassununga, SP, Brazil). A colourless microwave-polymerized denture base acrylic resin (Vipi Wave; VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda., Pirassununga, SP, Brazil) was mixed according to the manufacturer's instructions at a mixing ratio of 1 g powder to 0.47 mL of liquid for each specimen. The moulds were filled with the acrylic resin, a trial pack was completed, and excess material was removed. A final pack was performed and held for 15 min. The denture base acrylic resin was processed in a 500 W domestic microwave oven (Brastemp; Brastemp da Amazônia SA, Manaus, AM, Brazil) for 20 min at 20% power followed by 5 min at 90% power. After polymerization, the flasks were allowed to cool at room temperature, the specimens were deflasked, and the excess was trimmed with a sterile bur (Maxi-Cut; Lesfils de August Malleifer SA, Ballaigues, Switzerland). A total of 468 disc-shaped specimens were fabricated by a single operator wearing a mask, gloves and protective clothing.

2.2. *Surface roughness measurements*

Considering the possible influence of roughness on the adhesion of microorganisms to substrate surfaces,[3,30] the surface roughness of the specimens was measured using a profilometer (Mitutoyo SJ 400; Mitutoyo Corporation, Tokyo, Japan) accurate to 0.01 μm. The cutoff length was 0.8 mm, the transverse length was 2.4 mm, the stylus speed was 0.5 mm/s and the diamond stylus tip radius was 5 μm. Four measurements were made on the surface of each specimen and averaged to obtain the Ra value (μm). All measurements were recorded by a single operator.

2.3. *Experimental photopolymerized coatings*

After roughness reading, the specimens were randomly assigned to 13 groups of 36 specimens each; 18 specimens had smooth surfaces and 18 specimens had rough surfaces. In the control group (C), the specimens did not receive any surface treatment. In each experimental group, all specimen surfaces were coated with a layer of one of the experimental photopolymerized coatings. Four coating formulations were evaluated: 3 coatings containing hydrophilic monomers: 2-hydroxyethyl methacrylate (HEMA) – HE, 2-hydroxypropyl methacrylate (HPMA) – HP, and 2-trimethylammonium ethyl methacrylate chloride (TMAEMC) – T, and 1 coating containing a zwitterionic monomer (sulfobetaine methacrylate) – S. These monomers were used at concentrations of 25%, 30% and 35% of the total composition in mmol which resulted in 12 experimental coatings (HE25; HE30; HE35; HP25; HP30; HP35; T25; T30; T35; S25; S30; S35). In addition to the above monomers, all coatings contained the monomer methyl methacrylate, two crosslinking agents (triethylene glycol