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# Augmentation of *Candida albicans* Adhesion to Denture Materials Influenced By Surface Topography and Tobacco Components

## Abstract

The aim of this study was to investigate the influence of tobacco components, tobacco extract (TE) nicotine and Cotinine on fungal colonization and biofilm formation on two acrylic denture resins. Surface topography of the denture materials and alteration of fungal susceptibility to an anti-fungal agent, Nystatin of both planktonic and biofilm fungal cells grown on the acrylic resins were investigated. Ivocap and Lucitone 199 polished and roughened acrylic resin discs were fabricated and randomly assigned to control, tobacco extract-, nicotine-, and Cotinine-treated groups. *Candida albicans* biofilm was prepared on the resin discs in the presence or absence of tobacco components. The relative number of viable fungal cells was determined by the MTT assay. Quantization of biofilm growth was performed by dry weight analysis. Nystatin susceptibility [minimum inhibitory concentration (MIC)] was determined by microdilution method. Statistical significance was evaluated using ANOVA followed by Fisher's test ($p<0.05$). The study showed that the roughened discs had significantly greater numbers of *C.albicans* than the polished discs, Lucitone 199 acrylic discs promoted significantly more fungal biofilm growth than Ivocap discs, and the presence of tobacco components significantly enhanced biofilm formation on both types of acrylic resin. Dry weight analysis showed similar results. The Nystatin susceptibility assay showed that cells treated with tobacco components were more resistant to this antifungal agent.

**Keywords:** Tobacco components; Nicotine; Cotinine; Candida albicans: Biofilm; Ivocap; Lucitone 199; Nystatin

Research Article

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## Introduction

Denture stomatitis, a common oral disease, can induce pathologic changes to the denture-bearing tissues. These tissue changes can vary from localized hyperemia to diffused erythema to papillary hyperplasia [1,2]. Patients with severe denture stomatitis may experience pain, itching, and/or burning sensation [3-5]. Studies have shown that the prevalence of denture stomatitis in the edentulous population ranges from 15% to over 70% with more cases found in the elderly, women, smokers, and immunocompromised [6-12]. *Candida albicans* adherence and colonization of denture prostheses has been shown to induce denture stomatitis [13,14]. In general, *C.albicans* species exist as commensal organisms in about 33% of the adult dentate patients and about 75% of the complete denture patients. Smears obtained from the intaglio surface of dentures often demonstrate the presence of the invasive filamentous hyphal form [8-17]. Denture base resins are susceptible to fungal colonization in the oral environment. Surface roughness is one of the factors that aids in the initial fungal attachment. Surface defects such as scratches, cracks, and porosities serve as protective surfaces for microorganisms to bind [18,19]. One of the more common denture resins used today is polymethylmethacrylate (PMMA), a polymer developed in the 1930s [20]. PMMA can be classified as heat-activated or chemically-activated resin based on the method of polymerization. Heat-activated PMMA can be processed by compression-molded, injection-molded, or microwave-processed techniques [21]. Physical characteristics of PMMA are dependent on the type of resin as well as the processing technique. Manufacturers of the injection-molded resin claim that the "cross-linked, high impact thomopolymer" obtained after polymerization offers excellent polishability and helps reduce plaque build-up [22]. It has been reported that use of tobacco products induces fungal denture stomatitis in susceptible patients [23]. According to a study by Arendorf et al. there are higher numbers of *Candida* in tobacco smokers as compared to non-smokers [24]. Salivary nicotine and cotinine are widely used in clinical and epidemiological smoking studies [25]. Nicotine is a weak base with a pKa value that approaches 8. When salivary pH increases, the non-ionized form of nicotine is absorbed across the buccal and nasal membranes [26]. Cotinine, the major metabolite of nicotine, has been reported to have a pKa of 5 [27]. The concentration of cotinine in saliva is variable depending on an individual's nicotine metabolism and the salivary pH. Salivary concentrations of nicotine and cotinine can be affected by many factors such as cigarette brand, length of cigarette, gender, puffing behavior, and testing methods [28]. Robson et al. [29] reported that salivary nicotine concentrations ranged from 0.36μg/ml to