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The adherence of *Candida albicans* to acrylic resin reinforced with different fibers

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Abstract One important aethiological factor in the pathogenesis of chronic atrophic candidosis is the presence of *Candida albicans* on the fitting surface of the dentures. Fibers may come into contact with oral mucosa during the finishing procedures of acrylic resins. The exposed fibers may provide mechanical retention for yeast cells at the interface of the components. The effect of two different glass fibers and two different environments were evaluated in respect of *Candida albicans* adhesion to the acrylic surface. Half of the acrylic samples reinforced with two different fibers (Sticknet and Eversticknet) were pretreated with phosphate-buffered saline (PBS) and the rest with unstimulated saliva. The test specimens were placed in yeast suspension. The adhered cells were examined with a scanning electron microscope. The amount of adhered cells in PBS was lower for Eversticknet but the difference was not significant ($p > 0.05$). The number of yeast cells decreased in saliva for both groups and the difference was statistically significant for the samples reinforced with Eversticknet ($p < 0.01$). The use of Sticknet or Eversticknet as reinforcing material for poly(methylmethacrylate) had no effect on surface topography due to the same adhesion state of *Candida albicans*. The presence of a salivary pellicle derived from unstimulated saliva reduced adhesion of *Candida albicans*.

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Introduction

Despite its inefficiency in fulfilling the mechanical requirements of a prosthesis, the material most commonly used for the fabrication of complete dentures is poly(methylmethacrylate) (PMMA). The causes of denture fractures may be mechanical, due to a multiplicity of factors leading to the failure of the denture base material. In general terms, there are three methods which have been developed to improve the impact properties of PMMA: the search for, or development of, an alternative material to PMMA, the chemical modification of PMMA and the reinforcement of PMMA with other materials [1].

Alternative materials to PMMA have been introduced only to be later withdrawn. Over the years various types of fibers or beads, such as carbon [2, 3], polyethylene [4, 5], glass [6, 7], aramid [8, 9] and poly(methylmethacrylate) [10, 11] have been added to the acrylic resin in an attempt to improve its mechanical properties. Metal inserts in the form of wires, meshes and plates have been incorporated into dentures in an attempt to reinforce areas potentially vulnerable to fracture [12, 13].

The reinforcement of PMMA with various fibers has increased in dental practice. The entire denture base can be reinforced with a fiber weave or a fiber reinforcement can be accurately placed in the weak region of the denture.

The colonization of the mouth by Candida species has a long recorded history. *Candida albicans* is the most prevelant yeast isolated from the human body as a commensal or as an opportunist pathogen [14] and has been

widely associated with the aethiology of denture related stomatitis. These yeasts adhere to the denture surface and act as a reservoir of microorganisms. Several investigators have analyzed the adherence of *C. albicans* to acrylic surfaces [15]. Consequently it has been shown that adhesion of *C. albicans* to acrylic surfaces could be influenced by various factors, such as presence of other microorganisms and diets rich in sucrose [16]. Furthermore, significant increase in retention of *C. albicans* to the rough surfaces has been reported in previous studies [17, 18]. The aim of this in vitro study is to determine the adherence of *C. albicans* to the surfaces of PMMA reinforced with different fibers and to evaluate the effect of salivary pellicle on this process.

Materials and methods

Preparation of acrylic resin specimens

Heat-polymerized acrylic resin (Paladent 20, Heraus Kulzer GmbH & Co, Germany) was used as the denture base and preimpregnated woven glass fibers were used as reinforcing materials (Sticknet and Eversticknet, Stick Tech Ltd., Turku, Finland). Before the application of the fibers, each was wetted with a mixture of polymer powder and monomer liquid as poor wetting of the fibers within the acrylic resin is believed to destroy the layer of resin on the surface of the fibers and decrease the bond between the fibers and the monomer [1].

The wax sheets (Modelling wax, De Trey S. A., Bois Colombes, France) were prepared and invested in a flask and boiled out. Reinforcing fibers (Sticknet and Eversticknet) were embedded seperately into the rectangular acrylic sheets which were processed against stone during flasking. After polymerization, 20 samples of the size of $10 \times 10 \times 2$ mm were cut from the acrylic sheets with a precision cutter (Micracut, Metkon, Bursa, Turkey). Ten of the samples were reinforced with Sticknet and the remainder with Eversticknet and all samples were stored in sterile distilled water for 72 h. The test sides of the polymerized acrylic resin samples were not polished in order to give as accurate a representation as possible of the tissue surface of the dentures.

Adherence assay procedures

In the present study, initial adhesion and colonization of *C. albicans* on the surface of the samples was evaluated by performing candidal adherence assays on early pellicle which did not contain bacteria and their products [19].

Unstimulated saliva was collected from the same single donor over ice and centrifuged at 20,000*g* for 30 min. Ten reinforced acrylic specimens (5 Sticknet, 5 Eversticknet) were pretreated with unstimulated mixed saliva, and the remainder (5 Sticknet, 5 Eversticknet) were pretreated with 0.15 M phosphate-buffered saline (PBS) at 37 °C for 1 h.

After removal from the medium, the test specimens were placed in yeast suspension (*C. albicans*) and incubated at 37 °C for 1 h, then washed by being dipped gently 10 times in sterile PBS to remove non-adherent cells. The remaining adherent cells were fixed by immersion in methanol and left to dry.

The samples were coated with gold–palladium (20 nm) and examined in a scanning electron microscope (SEM) with a magnification of ×500 (LEO 438 VP, Oberkochem, Germany). The number of yeast cells was counted by using 70 SEM fields from each test specimen.

The statistical analysis of the mean number of yeast cells on the reinforced polymer surfaces were compared using Mann–Whitney-*U* test.

Results

The results of the adherence of *C. albicans* cells to reinforced acrylic resin are shown in Table 1. No significant differences were observed between the adherence of *C. albicans* on PMMA surface reinforced with Sticknet and Eversticknet pretreated with PBS ($p > 0.05$) (Figs. 1, 2). Saliva pretreatment reduced the adherence of *C. albicans* to both type of fibers. The reduction was particularly significant in the Eversticknet group ($p < 0.01$) (Figs. 3, 4).

Discussion

In the pathogenesis of denture stomatitis, the growth of large numbers of Candida on the fitting surface of the denture and the following acid production by grown yeasts are known to be one of the most important factors [20], through direct cytotoxicity, activation of acid proteinase and phospholipase produced by the yeasts and promotion of Candida adherence [21, 22].

The ability of *C. albicans* to adhere to polymeric surfaces is due to attractive London–van der Waals and electrostatic forces. The contribution of electrostatic and

Table 1 The effect of different environments on *C. albicans* adherence by mean numbers and standard deviations of adherent cells per mm^2 of fiber reinforced PMMA

Acrylic resin	PBS	Saliva	*p*
Sticknet reinforced	291.400 ± 138.997	30.200 ± 15.853	NS
Eversticknet reinforced	239.400 ± 95.582	3.000 ± 2.144	<0.01
p	NS	NS	

NS: Non-significant, *n*: 5

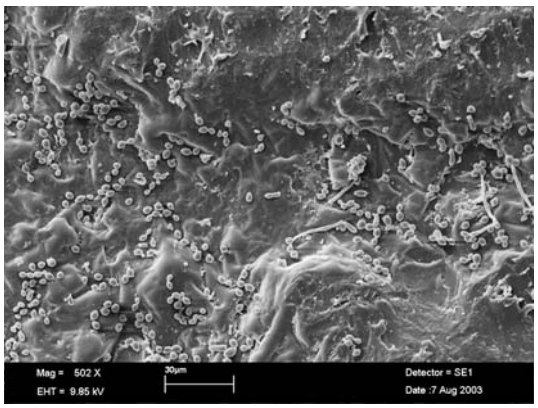

Fig. 1 *C. Albicans* on the surface of acrylic resin reinforced with Sticknet and pretreated with PBS. (x 502 SEM)

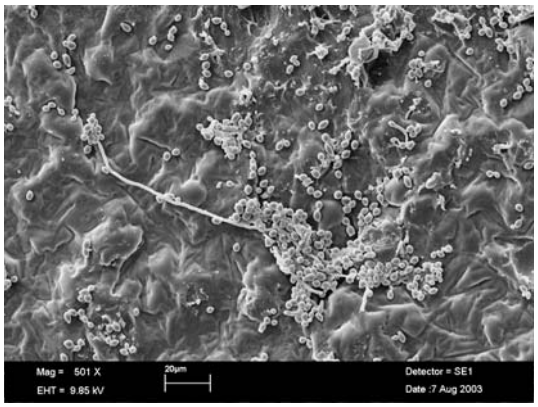

Fig. 2 *C. Albicans* on the surface of acrylic resin reinforced with Eversticknet and pretreated with PBS. (x 501 SEM)

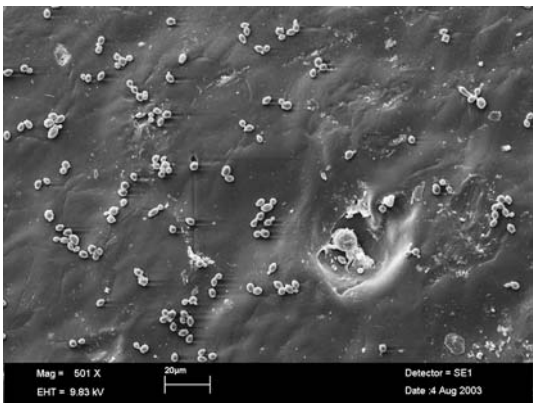

Fig. 3 *C. Albicans* on the surface of acrylic resin reinforced with Sticknet and pretreated with unstimulated saliva. (x 501 SEM)

hydrophobic forces to the adherence process varies between substrates and environments. However, these forces are important in the initial adherence of yeasts and they offer an opportunity for further bonding and denture plaque formation. The presence of saliva, serum and other microorganisms as well as differences in surface topography and chemistry may affect this complicated process [23].

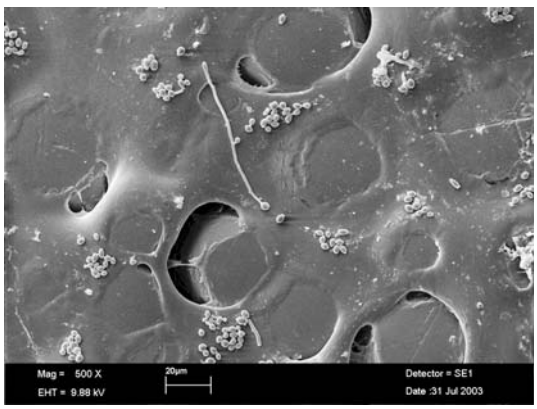

Fig. 4 *C. Albicans* on the surface of acrylic resin reinforced with Eversticknet and pretreated with unstimulated saliva. (x 500 SEM)

The successful combination of fiber reinforcement with highly viscous denture base resins requires polymer pre-impregnation of the glass fibers as introduced by Vallittu [24]. Fiber reinforcements in the denture base are either partial or total fiber reinforcement. With total reinforcement, the entire denture base is reinforced with fibers, but with partial fiber reinforcement only the weakest part of the denture base is reinforced. The reinforcement fibers may be exposed during the finishing of the denture, especially with total fiber reinforcement. This may lead to a situation in which the fibers come into contact with the oral mucosa and it has been reported in a previous study that the permanently exposed fibers may provide mechanical retention for yeast cells at the interface of the components [23].

Sticknet and Eversticknet have the same composition properties. However, there are microcavities in the matrix structure of Eversticknet which increase the penetration ability of acrylic resin. Thus, in addition to chemical bonding, a micromechanical bonding mechanism occurs between Eversticknet and the acrylic resin. In spite of this difference in composition, the adherence affinity of *C. albicans* to Eversticknet was not significantly less than to Sticknet in both PBS and saliva**.**

Some researchers have demonstrated that pretreatment of acrylic strips and yeast cells with whole saliva decreased the initial adherence of *C. albicans* to denture acrylic [16], whereas some found that coating the denture with whole saliva enhanced Candida adhesion [25, 26]. Conflicting reports have been published regarding the role of the material's surface free energy on the degree of microorganism adhesion. It has been reported in previous studies that the higher the surface free energy of the substrata, the higher the amount of adhesion of microorganisms [27]. However, although surface energies play a role in explaining the adhesion of yeast to denture surfaces, the more predominant factor is likely to be selected salivary components coating the material surfaces specifically

mediating adhesion [28]. For instance, salivary mucins are claimed to provide one regulatory mechanism of *C. albicans* colonization of surfaces in the oral cavity [25]. The results obtained from the present study coincide with the those of studies which found an increase in the prevalence of intraoral *C. albicans* in patients with Sjogren's syndrome [29] and in hyposalivatory rats [30], in that all suggest a protective effect of saliva.

The number of the cells on the surface of the acrylic resin reinforced with Sticknet decreased with the presence of saliva but the difference was not statistically significant. However, the decrease in the number of the cells with the presence of saliva in the Eversticknet reinforced specimens was statistically significant ($p < 0.01$). As the number of the cells adhered to both surfaces in PBS was comparable, the difference in *C. albicans* adherence between experimental groups following saliva treatment may be explained in several ways. First, an important factor affecting the Candida colonization is the surface free energy of the material as this may interfere with the components adsorbed to form an acquired pellicle. That is, an acquired pellicle containing anti-Candida salivary components such as lysozymes, histatins, lactoferrin, and IgA interact with the Candida species, decreasing its adherence to the surface [31, 32]. In contrast, other components including mucins, statherin and proline-rich proteins have been reported to increase the adhesion of *C. albicans* [31, 33, 34]. In the current study, the significant decrease in the number of the yeast cells in saliva for the Eversticknet group may be due to the minor advantageous difference of the fiber in respect of bonding ability to acrylic resin [35]. The surface chemistry of the Eversticknet reinforced samples, one of the determinants of the surface free energy might have led to the formation of a pellicle supressing the adherence of *C. albicans* as compared with the samples reinforced with Sticknet.

Another factor influencing the adherence of the yeasts to the acrylic surfaces is the hydrophilicity of the microorganism. It has been reported that *C. albicans*, being a relatively hydrophilic species among other yeasts, adheres to the surfaces in larger amounts as the surface wettability (surface energy) increases [15]. Therefore, it may be speculated that saliva coated Sticknet reinforced samples, establishing higher surface energy due to the composition of acquired pellicle might have promoted *C. albicans* adherence.

When the surface tension of the microorganism is larger than that of the suspending medium, it has been shown that adhesion is more extensive to hydrophilic substrata than to hydrophobic substrata. When the surface tension of the suspending medium is larger than that of the microorganism, the reverse occurs. It has also been shown that a higher degree of *C. albicans* adhesion is seen to a substrata whose surface energy is closer to that of the yeast [36]. Further investigation for the characterization of the surface properties of the microorganisms, the surface tensions of the suspending medium and the surface energies of the substrata is required to establish more useful relations.

Conclusions

Within the limitations of this study,

- The use of Sticknet or Eversticknet as a reinforcing material for PMMA had no effect on surface topography due to the same adhesion state of *C. albicans.*
- The presence of a salivary pellicle derived from unstimulated saliva reduced adhesion of *C. albicans*. It can be emphasized that salivary pellicle formation is effective in modifiying the surface energy of reinforced acrylic resin, particularly when reinforced with Eversticknet. However, several factors should be taken into account regarding the adherence of *C. albicans* to the PMMA surface, such as the effect of the salivary components and the presence of bacteria.

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