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An *in vitro* Assessment of the Attachment Two Oral Pathogens to Denture Base Materials.

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Abstract

In vitro attachment were determined on a acrylic resin (Meliodent, Heraeus Kulzer GmbH and Co., Germany) and a Co-Cr alloy (CoCr-Modellguß-legierung, Degussa Dental, Germany) denture-base materials using the type strain of two types *C. albicans (*ATCC 26555 and Serotype B Netherland CBS 5983) *and S. mutans* (NCTC 8177)**.** The results of assays showed that there was more attachment of two *C. albicans* to acrylic resin base material than to Co-Cr alloy base material, while there was more attachment of *S. mutans* to Co-Cr alloy than to acrylic resin. Attachment was prevented or reduced considerably in the presence of *Thymus* oil. In spite of this, *Thymus* oil exhibited the most pronounced inhibitory effect against both *C. albicans*, but it did not show inhibitory effect against *S. mutans*. Also, we have observed that the greater hydrophobicity of cells result in the greater attachment to some denture-base material surfaces. Howewer, we haven't determined this relationship between hydrophobicity and attachment in all applications. Additionally, *Thymus* oil effected the attachment of microorganisms mentioned above.

Key words: Denture-base material; attachment; growth inhibition; oral pathogens; *Thymus* oil; hydrophobicity

INTRODUCTION

Dental plaque has been identified as the main aetiological factor in denture stomatitis, dental caries and periodontal diseases [1]. Adherence mechanisms of oral bacteria are essential to bacterial colonization of the oral activity [2]. Adhesive interactions are a prerequisite for species to become part of an oral biofilm and factors involved in oral pathogen adhesion have been described. Dental plaque is a complex and dynamic biofilm that accumulates through the sequential and ordered colonization of over 500 different species of bacteria [3].

Dentures, like teeth, are hard, non-shedding surfaces and accumulate plaque and calculus in a similar way. Surface roughness and surface free energy may contribute to the positively correlated rate of microbial colonization and plaque maturation on surfaces. The effect of material surface roughness has previously been shown to be more significant than surface free energy in the accumulation of supragingival plaque [4].

Candida albicans and other C*andida* species can cause a number of oral diseases and *C. albicans* cells are often found in dental plaque [5,6]. *Streptococcus mutans* is indigenous to the oral cavity [1].

Attachment of microorganisms to a surface may be described as non-specific (hydrophobicity, surface charge) or as specific (receptor-ligand binding) [4]. Some researchers have reported that cell charge and cell surface hydrophobicity influence the strength of microbial adhesion to surfaces [3].

Isolation of natural anti-plaque and anti-caries substances from plants has been reported. Extensive searches have been made for effective anti-plaque agents from a variety of chemical and biological compounds for years. Recently, isolation of natural anti-plaque and anti-caries substances from plants has been reported [1].

The genus *Thymus* is among the aromatic plants belonging to the *Lamiaceae* family. Because of its antiseptic, antispasmodic and antimicrobial properties it is also used for medicinal purposes [7].

The aim of this study was to determine whether (a) the attachment of *Candida albicans* and *Streptococcus mutans* to different denture-base materials, (b) whether a correlation between attachment and hydrophibicity existed, and (c) the antimicrobial effect of *Thymus* oil against these microorganisms.

MATERIALS AND METHODS

Sample preparation

Six mm diameter holes were drilled in a flat metal plate of two mm thickness with a trepanning tool. Melted wax was poured in these holes. Wax replicates were processed against glass plates.

Preparation of acrylic resin discs

Dental stone mixture was poured into the lower part of a dental flask, and wax discs were placed on the surface of stone. After setting the stone was lubricated using petroleum gel. The upper part of dental flask was placed in position, and dental stone was poured into it. Flasks were heated for 10 minutes in boiling water, and the wax was removed using a hot water spray. Flasks were cooled, and then the stone was lubricated using a sealant. A polymethylmethacrylate heat-cured acrylic resin (Meliodent, Heraeus Kulzer GmbH and Co., Germany)

was selected and processed according to the manufacturers' instruction, and applied into the voids in flask. Acrylic resin was polimerisation by heating the flask for 20 min in 100 °C water. After polimerisation, the flask was opened, discs removed and trimmed. Waterproof silicon carbide paper (Waterproof Auto-paper, Kingspor, Germany) of grid size A600 was used to smooth the surfaces. The discs were kept in a dry environment until required.

Preparation of alloy discs

A base metal alloy (CoCr-Modellguß-legierung, Degussa Dental, Germany) was used as Co-Cr alloy denture-base material to fabricate alloy specimens. Wax replicates were casted. After cooling, alloy disc specimens removed from investment and trimmed. Silicon carbide discs were used to polish the surfaces.

Attachment test

Prepared test samples were used for testing by allowing attachment of *Candida albicans* ATCC 2655, *C. albicans* Serotype B Netherland CBS 5983 and *Streptococcus mutans* NCTC 8177 cultures. *S. mutans* and *C. albicans* were subcultured in M17 (Oxoid) and Yeast-peptone-dextrose (YPD) broths (1% w/v yeast extract, 2% w/v peptone, 2% w/v) at 37 ºC for 18 h respectively. The samples that they were prepared as a disc with a total surface area of approximately 94 mm then were sterilized for 2 h at UV a wavelength of 254 nm. They were aseptically placed into a flask containing 10 ml of cell suspension. After 1 h at room temperature, samples were removed, drained and placed into another flask containing 10ml of PBS buffer (pH 7.0), which was vortex for 1 h. After the cells are removed to PBS buffer from sample surfaces, serial dilutions of each sample were plated in duplicated in YPD agar (for *C. albicans*) and M17 agar (for *S. mutans*) [8]. Same process was made for sample discs treated with *Thymus* oil. Plates were incubated at 37 ºC for 48 h. Viable counts of the microorganisms were determined by colony count. Results were expressed as cfu/mm^2. Experiments were repeated tree times and calculated mean values for each specimen.

Anti-microbial activities of *Thymus* Oil

In this study, it was used commercial *Thymus* oil (Kekik Yağı, Kardelen Ltd, Turkey) which was extracted of *Thymus* spp. and includes 68% carvacrol. The determination of the inhibitory effect of *Thymus* oil on two strains of *C. albicans* and *S. mutans* were carried out according to disk diffusion method [9]. Sterile filter discs were treated with 20 µl *Thymus* oil. Inhibition zones measured by slide caliper and expressed as the distance in mm from the edge of the filter disc to the point of normal colony size of the test microorganisms after incubation at 37^{O}C for 24 h.

Cell-surface hydrophobicity

The microorganisms suspended in phosphate buffer (pH 7.0) were adjusted to an optical density (OD) between 0.4 and 0.6 at 600 nm 3 mL volumes and was dispensed into test tubes and mixed with 1 mL of toluene by vortexing vigorously for 30 s. Then phase separation (approx. 30 min) was dispensed in 3 mL volumes into test tubes and mixed with 1 mL of toluene by vortexing vigorously for 30 s. Later phase separation (approx. 30 min) aqueous phase was carefully removed and transferred to clean tubes. OD_{600} was determined, and the percentage of hydrophobicity was calculated from the OD_{600} differences (% hydrophobicity = $OD_{before} - OD_{after}/ OD_{before}$ x 100) [3,8].

RESULTS

The data in Table 1 shows greater attachment of *C. albicans* ATCC 26555 and *C. albicans* Serotype B Netherland CBS 5983 on acrylic resin denture-base material (RDM) (log_{10} 2.9 cfu/mm^2, log_{10} 3.4 cfu/mm^2, respectively) than on Co-Cr alloy denture-base material (ADM) (log_{10} 0.5 cfu/mm^2, log_{10} 1.9 cfu/mm^2, respectively). However, less attachment of *S. mutans* was obtained on RDM (log_{10} 2.0 cfu/mm^2) than on ADM (log_{10} 3.7 cfu/mm^2).

Also, the data in Table 1 shows that attachment of *C albicans* ATCC 26555 strain after treatment with *Thymus* oil was log_{10} 1.1 cfu/mm^2 on RDM, while without *Thymus* oil attachment was log_{10} 2.9 cfu/mm^2. As the attachment of *C albicans* ATCC 26555 strain after treatment with *Thymus* oil was not determined on ADM, without *Thymus* oil attachment was log_{10} 0.5 cfu/mm^2. The attachment of *C. albicans* Serotype

Table1. Attachment and hydrophobicty of microorganisms to different denture-base materials with/withouth *Thymus* oil.

Microorganisms	RDM* (log_{10} cfu/mm^2)		ADM* (log_{10} cfu/mm^2)		Control* (log_{10} cfu/mL)	Hydrophobicity (Toluene)
	A	B	A	B		
Candida albicans ATCC 26555	1.1±0.0	2.9±0.2	-	0.5±0.3	7.7±0,4	%19
Candida albicans Serotype B Netherland CBS 5983	2.3±0,2	3.4±0,0	1.8±0.1	1.9±0.1	6.3±0,1	%49
Streptococcus mutans NCTC 8177	3.0±0.0	2.0±0.1	-	3.7±0.0	8.3±0,5	%14

(-): Not growth; RDM: acrylic resin denture-base material; ADM: Co-Cr alloy denture-base material; A: with *Thymus* oil; B: without *Thymus* oil; *: Values are the means ± standard deviations of triplicate measurements.

B Netherland CBS 5983 strain after treatment with *Thymus* oil was $\log_{10}$ 2.3 and 1.8 cfu/mm^2, respectively on RDM and ADM, while without *Thymus* oil attachment was $\log_{10}$ 3.4 and 1.9 cfu/mm^2, respectively. As the attachment of *S. mutans* NCTC 8177 strain with *Thymus* oil was $\log_{10}$ 3.0 cfu/mm^2 on RDM, without *Thymus* oil attachment was $\log_{10}$ 2.0 cfu/mm^2 on RDM. The attachment of *S. mutans* NCTC 8177 strain with *Thymus* oil was not determined on ADM while without *Thymus* oil attachment was $\log_{10}$ 3.7 cfu/mm^2 on ADM.

In this study, the hydrophobicity (toluene) of two strains of *C. albicans* and *S. mutans* are shown in Table 1. *C. albicans* Serotype B Netherland CBS 5983 showed the most hydrophobicity (49%) followed by *C. albicans* ATCC 26555 (19%) and *S. mutans* NCTC 8177 (14%).

The diameter of inhibition zone *of Thymus* oil against test microorganisms was presented in Table 2 as millimeter. It was obtained while *Thymus* oil exhibited the most pronounced inhibitory effect against *C. albicans* ATCC 26555 (44.6±4.2 mm) and *C. albicans* Serotype B Netherland CBS 5983 (26.3±2.1 mm), it had no inhibitory effect against *S. mutans* NCTC 8177.

Table 2. The antagonistic effect of *Thymus* oil on microorganisms

Microorganisms	**Inhibition zone* (diameter, mm)**
Candida albicans ATCC 26555	44.6±4.2
Candida albicans Serotip B Netherland CBS 5983	26.3±2.1
Streptococcus mutans NCTC 8177	-

(-): No inhibition; *: Values are the means ± standard deviations of triplicate measurements.

DISCUSSION

The attachment of two strains of *C. albicans* and *S. mutans* was studied by using an acrylic resin denture-base material (RDM) and a Co-Cr alloy denture-base material (ADM). It The attachment of *C. albicans* ATCC 26555 and *C. albicans* Serotype B Netherland CBS 5983 was more on RDM than on ADM was determined. In contrast to two strains of *C. albicans*, the attachment of *S. mutans* NCTC 8177 to was more on ADM than on RDM. These results demonstrated that the type of denture-base material could play an important role at the attachment of microorganisms. Some researchers had reported that the attachment of microorganisms to different materials was depent on the surface finishes [10,11]. Eick et al. found no correlation between the surface roughness and the number of viable *S. mutans.* They noticed that *Streptococcus mutans* had relatively high surface energy and is negatively charged; electrostatic forces played the important role on the unspesific adhesion of *S. mutans* [12]. This implied the less attachment of *S. mutans* to on ADM than on RDM.

Cell-surface hydrophobicity has been associated with bacterial attachment to a variety surfaces [8]. The relationship between hydrophobicity and attachment has changed according to used materials. While the greater hydrophobicity of the cells result in the greater attachment to some surfaces. This relationship wasn't observed in all applications. Piette and Idziak have reported that the attachment is influenced by the hydrophobicty [13]. Morgan and Wilson have exhibited differences in hydrophobicity can be responsible for the differing extent of the attachment of microorganisms to the two types of acrylic [4]. In contrast, Marin *et al.* have found that no correlation between hydrophobicity and attachment existed [8].

While *Thymus* oil exhibited the most pronounced inhibitory effect against *C. albicans* ATCC 26555 and *C. albicans* Serotype B Netherland CBS 5983, it did not show inhibitory effect against *S. mutans*. *Thymus* oil is widely used as an antiseptic agent in many pharmaceutical preparations and as a flavouring agent for many kinds of food products [14]. Also, the high antimicrobial effect of *Thymus* oil in several investigations was demonstrated [7,15].

It was found that the attachment of two strains of *C. albicans* on RDM and on ADM reduced or not after treatment with *Thymus* oil. However, the attachment was prevented after treatment with *Thymus* oil of *S. mutans* on ADM but it was not observed on RDM. Additionally, the effect of *Thymus* oil on the attachment of microorganisms on denture-base materials had not been reported. It needs to be done the further investigations to explain differences in the attachment of microorganisms in treatment with *Thymus* oil.

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