In Vitro Evaluation of Candida Albicans Adherence to Silicone-Based Soft Lining Materials

SUMMARY

Colonization of soft lining materials with microorganisms, especially Candida albicans, is a common clinical problem. Silicone-based soft lining materials have been found to be particularly susceptible to Candidal adhesion. This study investigated the Candida albicans adhesion to 6 silicone-based soft lining materials (Molloplast-B, Mollosil, Ufigel P, Ufigel C, Soft Liner and Luci-sof). For each soft lining material, 7 specimens (10mm×10mm×3mm) were prepared. Sterile specimens were contaminated with Candida albicans. Adherent cells were fixed in methanol and stained with crystal violet and calculated by light microscopy. Scheffe F-test and ANOVA were used to analyze the data (p=0.001).

The results of this study showed that adherence of C. albicans occurred for all silicone-based soft lining materials. Significant differences were found in the Candidal adhesion among soft liners. Silicone-based soft lining materials tested have been found to exhibit particular Candidal adhesion.

Keywords: Soft Lining Materials; Candida Albicans

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Introduction

Denture stomatitis is an erythematous pathogenic condition of the denture-bearing mucosa that is mainly caused by microbial factors, especially Candida albicans1-3. Candida albicans is recovered more often and in high numbers from the fitting surface of the denture than the palate, indicating that the dentures act as a reservoir of infection and that yeast adhesion to the denture surface is a normal prerequisite for colonization of the palate4-8. Plaque formation is believed to be initiated by microbial adhesion to the surfaces of teeth or dental materials; subsequent microbial colonization may occur either by division of these adherent organisms or by cohesion of floating cells to adherent ones9. It has been reported that the higher the surface free energy of the substrata, the higher the amount of adhesion of microorganisms10,11.

Permanent soft denture liners have been a valuable asset for dentists and, because of their visco-elastic properties, they act as shock absorbers and reduce and distribute the stresses on the denture-bearing tissues. Their use for patient comfort and the treatment of the atrophic ridge, bone undercuts, bruxism, xerostomia, and dentures opposing natural teeth has been known to be clinically beneficial12-16. However, these materials have some physical and microbial disadvantages. One of the most serious problems has been colonization and infection of the material surface by C. albicans, resulting in denture stomatitis17-28. Colonization may reduce the intraoral life of the soft lined denture, but little is known about the degree of adhesion in relation to soft lining materials.

Soft lining materials can be categorised into 5 main types: natural rubbers, vinyl copolymers, acrylic-based soft lining materials, silicone-based soft lining materials and fluoropolymers. Acrylic-based and silicone-based soft lining materials are more popular in clinical use. Both type are available in auto-polymerizing and heat-polymerizing forms, differing in the percentage of plasticizers, cross-linking agents, catalysts and fillers. In general, silicone-based soft lining materials are considered to be more successful clinically because of colour stability28,29, compatibility with denture cleansers18,30, low water absorption and hardness changes15,27,28,31,32. However, Candidal adhesion of silicone-based soft lining materials is contradictory. While some researches have shown that silicon-based soft lining materials did not support Candidal adhesion20,25,26,33, others claimed that silicone-based soft lining materials were found to be more prone to microbial
adhesion because of their rough surface texture. The aim of this study was to evaluate the *Candida albicans* adhesion to silicon-based soft lining materials.

**Material and Methods**

**Preparation of Specimens**

Table 1 lists the soft lining materials used in this study. The tested liners were fabricated according to manufacturers’ recommendations. 7 specimens were prepared for each soft liner. Squares of soft liner materials with a 10 mm length, 10 mm width and 3 mm thick, were prepared in a stainless-steel mould and polymerized. They were saturated with a sterile water for 24 hours at room temperature.

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autopolymerized</td>
<td>VOCO, Cuxhaven, Germany</td>
</tr>
<tr>
<td>Autopolymerized</td>
<td>DETAX GmbH &amp; Co. Ettingen, Germany</td>
</tr>
<tr>
<td>Autopolymerized</td>
<td>PROMEDICA Neumünster/Germany</td>
</tr>
<tr>
<td>Autopolymerized</td>
<td>VOCO, Cuxhaven, Germany</td>
</tr>
<tr>
<td>Autopolymerized</td>
<td>DETAX GmbH &amp; Co. Ettingen, Germany</td>
</tr>
<tr>
<td>Heat- polymerized</td>
<td>DENTSPLY International Inc. York, USA</td>
</tr>
</tbody>
</table>

**Preparation of Candida**

A reference *C. albicans* (ATCC 2091, Istanbul University, School of Medicine, Kükens) was used to investigate adhesion of the soft liners. Candida strains were incubated in Sabouraud’s broth supplemented with sucrose 500 mmol/L overnight at 37°C. This medium was used because previous studies have shown increased Candidal adherence to acrylic resin after culture in Sabouraud’s broth supplemented with sucrose. Candidal growth was harvested after 48 hours by centrifugation (3000 g, 15 minutes, 10°C). The Candidal cells were washed in phosphate-buffered saline solution (PBS), 0.15 mol/L, pH 7.2. This procedure was repeated 2 times.

**Adherence Assay**

The principle of the experiment was to contaminate sterile specimens of the tested soft lining materials with *Candida albicans* and to determine the count of viable adherent cells.

At the commencement of the experiment, soft lining material specimens were autoclaved (15 minutes/121°C/PSI) to ensure that the specimens were sterile. Sterile specimens were deposited in 20 ml of yeast suspension in sterile universal bottles. The materials were incubated for 1 hour at room temperature. After contamination, the suspensions were discarded and the specimens washed twice with PBS with gentle rocking to remove non-adherent cells. Excess PBS solution was drained from specimens. After materials were dried, adherent cells were fixed in methanol, stained with crystal violet and examined by light microscopy. Adherent cells in 30 fields of view (0.25 mm² per field) were enumerated and the results were expressed as yeast cells/mm² of the material.

**Results**

Figure 1 shows the mean adherence and standard deviation values of *Candida albicans* to the silicone-based soft lining materials. For all the tested materials, the adhesion was observed for *Candida albicans*. The adherence of *C. albicans* was the highest with Ufigel P lining material, and the lowest with Ufigel C lining material.

One-way ANOVA results are shown in table 2. Statistically significant differences were found among soft liners by means of Candidal adhesion. The results of comparisons between materials tested are shown in table 3.
Table 2. One-way ANOVA results

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of square</th>
<th>Mean square</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>5</td>
<td>147274,690</td>
<td>29454,938</td>
<td>8.656</td>
</tr>
<tr>
<td>Within groups</td>
<td>36</td>
<td>122495,714</td>
<td>3402,659</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td>269770,405</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p=0.001

Table 3. The results of comparisons between materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Mean (Standard deviation) (yeast/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>65.14 (56.24)</td>
</tr>
<tr>
<td>MB</td>
<td>141.29 (31.49)</td>
</tr>
<tr>
<td>SL</td>
<td>89.86 (54.03)</td>
</tr>
<tr>
<td>UP</td>
<td>253.29 (103.95)</td>
</tr>
<tr>
<td>ML</td>
<td>146.29 (38.63)</td>
</tr>
<tr>
<td>LS</td>
<td>131.43 (32.33)</td>
</tr>
</tbody>
</table>

* Vertical rods shows statistical significance

Discussion

The adherence of a microorganism to a surface is classically considered to be a 2-stage process. The initial interactions between the 2 surfaces are non-specific and reversible, although the secondary phase is caused by specific intermolecular interactions. Many approaches have been used to explain the initial adherence of microorganisms to surfaces, including the thermodynamic approach to adhesion, which describes the adhesion of microorganisms to surfaces in terms of the surface free energies of the surfaces and the microorganisms. In addition, the hydrophobicity of the microorganisms has been theorised as a reason for high adherence and also for electrostatic interactions between surfaces. The second phase of the adhesion process involves specific adhesin-receptor interactions. The microorganism carries adhesins that bind stereochemically to complementary receptors on the surface. This stage is necessary for the tight binding of the microorganisms to the surface, which permits colonisation. In addition to tightly binding the microorganisms to the surface, the irreversible interactions are also responsible for the site-specific colonisation of the oral microorganisms, which provides a selective advantage for microorganisms that possess the relevant adhesins. Adhesins have been postulated to be associated with the microorganism’s surface appendages that, by virtue of their small radius, are unable to overcome the energetic barrier of the primary force.

Other factors associated with the adherence of yeast to surfaces include surface roughness, presence of salivary proteins, presence of other adherent microorganisms, strain variability, concentration, viability of yeast cells, and culture conditions.

Because plaque formation is believed to be initiated by microbial adhesion to the surfaces of teeth or dental materials, subsequent microbial colonization may occur either by division of these adherent organisms or by cohesion of floating cells to adherent ones.

In this study, a simple in vitro model was used to compare the adherence of C. albicans with silicone-based soft lining materials. It was aimed to provide a reproducible assay for comparison of materials in which further variables could be examined in the future studies. For this reason, the material surfaces were reproduced in a highly polished stainless-steel mould in order to eliminate the variability of surface roughness. The concentration, viability, and culture conditions were kept constant. Adhesion was initially carried out on surfaces with no saliva coating to produce a reproducible assay before the introduction of variables. Crystal violet dye was used in this study, because crystal violet stains the adherent cells only. For all the tested materials, the adhesion was observed for Candida albicans. The highest C. albicans adherence was observed for Ufigel P, whereas the lowest for Ufigel C. Although both materials have similar chemical composition, results were very surprising. These differences may be their consistency and mixing procedure. In this study, surface roughness, and concentration, viability, and culture conditions of the assay were kept constant, except surface free energy and chemical properties of the materials tested. Conflicting reports have been published regarding the role of the materials’ surface free energy on the degree of microorganism adhesion. It has been reported that the higher the surface free energy of the substrata, the higher the amount of adhesion of microorganisms. This unclear situation highlights the importance of the surface properties of the lining materials and surface tensions of the suspending denture cleansing medium, both not measured. Therefore, there is a need for further investigations.

Conclusion

The ability of Candida albicans to adhere to 6 silicone-based soft liners was examined. The following conclusions may be made:

All the tested soft liners showed some degree of Candidal adherence;

The adherence of C. albicans was the highest with Ufigel P, and the lowest with Ufigel C;

There were statistically significant differences among soft liners for Candidal adhesion.
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References


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