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Cleaning Efficiency of Alkaline Peroxide Type Denture Cleansers on Silicone-Based Soft Lining Materials Colonized With *Candida Albicans*

SUMMARY

Background. *Silicone-based soft lining materials have been found to be more susceptible to Candidal adhesion. Denture hygiene is essential to maintain the serviceability of the denture, and denture cleaners have been suggested for denture disinfection.*

Purpose. *The purposes of this study were to investigate the Candida albicans adhesion, and determine the effectiveness of peroxide-type denture cleaners in the disinfection of silicone-based soft lining materials.*

Material and Methods. *2 different silicone-based soft lining materials have been used in this study (Molloplast-B and Luci-Sof). For each soft lining materials, 7 specimens have been prepared (10mm×10mm×3mm in diameter). Sterile specimens have been contaminated with Candida albicans and immersed in 4 different denture cleaners (Efferdent, Polident, Steradent, Correga tabs). The reduction in viable, adherent cells have been calculated by comparison with appropriate control specimens that have been treated in same way as test specimens, but without a disinfection regime.*

Results. *Both soft liners showed Candidal adherence, but Luci-Sof soft liner exhibited higher Candidal adherence than Molloplast-B soft liner. Alkaline peroxide-type denture cleaners have been found effective in the disinfection of silicone-based soft liners contaminated with C. albicans.*

Conclusion. *Alkaline peroxide-type denture cleansers can be used in order to maintain effective denture hygiene.*

Clinical Implications. *Heat cured silicone based soft liners materials could be safely disinfected with alkaline peroxide type denture cleansers.*

Keywords: Denture Cleansers; Soft Lining Materials; Candida Albicans

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Introduction

Resilient denture lining materials are widely used in prosthetic dentistry because they can assist the clinician in restoring health to the inflamed and distorted denture supporting tissues[1-5]. However, these materials have some physical and microbial disadvantages. One of the most serious problems has been colonisation and infection of the material surface by *Candida albicans* and related *Candida* species, resulting in the denture induced stomatitis. Also, effective denture plague control is indispensable for clinical use of these materials, because bacterial and yeast plaque is a major factor of denture stomatitis[6-17]. Denture plaque is important factor in the pathogenesis of denture stomatitis[18-20]. Therefore, denture cleansing should include removal of *Candida* organisms that have been reported to be closely related to the disease [6,21].

Chemical cleansing with immersion denture cleansers is suggested as the first choice for plaque control with these soft-lining materials, since brushing is likely to damage the liners, and ultrasonic treatment *per se* is not effective[22-24]. Also, geriatric or handicapped denture wearers can use chemical cleansers more advantageously[24-26].

A variety of experimental approaches have been tested in attempt to examine the efficacy of denture cleansers. Some authors have investigated the minimal inhibitory or minimal bactericidal concentration of these agents[27], while others have examined the fungicidal effects[28,30], Candida lytic effects[28-30], or the agents' ability to remove attached *Candida* cells from acrylic resins [25-27].

Many types of cleansers have been commercialised; sparse data are available on the efficacy of denture cleansing agents. These agents may be classified as alkaline peroxides, neutral peroxide with enzymes, crude drugs, acid detergents, and mouthrinses for dentures[31].

The purposes of this study were to examine: (1) the ability of *Candida albicans* to adhere to 2 permanent soft liners; and (2) the effectiveness of alkaline peroxide-type denture cleansers in the disinfection of long-term soft lining materials contaminated with *Candida albicans*.

Material and Methods

Preparation of Specimens

2 heat-cured soft lining materials (Molloplast-B, Regneri GmbH and Co; and Luci-Sof, Dentsply,) were used in this study (Tab. 1). Square of specimens for each soft liners were prepared (10mm×10mm×3mm) and polymerized according to recommendation of the manufacturer. They were prepared in a stainless-steel mould with highly polished surfaces to produce reproducible results. All specimens were saturated with sterile water for 24 hours at room temperature[32-34]. Specimens for each soft liner were randomly divided into 5 subgroups; 4 of test and 1 of control (n: 7).

Table 1. Type and manufacturer of soft denture materials

Material	Type	Manufacturer
Molloplast-B (Group I)	Heat-cured silicone rubber	Regneri GmbH and Co.
Luci-Sof (Group II)	Heat-cured silicone rubber	Dentsply, IntL, Pa.

Preparation of Candida Albicans

A reference *C. albicans* (ATCC 2091, Istanbul University, School of Medicine, Kükens) was used to investigate the efficacy of disinfection. *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[34]. Candidal growth was harvested after 24 hours by centrifugation (3000 g 15 minutes, 10ºC). The Candidal cells were washed in phosphate-buffered saline solution (PBS), 0.15 mol/L, pH 7.2[34,35]. This procedure was repeated 2 times.

Adherence Assay

The principle of the experiment was to contaminate sterile specimens of Molloplast-B and Luci-Sof soft lining materials with *Candida albicans* and to determine any reduction in count of viable adherent cells after test disinfection regimes. The reduction in viable, adherent cells were calculated by comparison with appropriate control specimens that were treated in the same way as test specimens, but without a disinfection regime[36]. At the commencement of the experiment, specimens were autoclaved at a temperature of 121ºC for 15 minutes. Sterile specimens were deposited in 20 ml yeast suspensions inserting the sterile universal bottles. They were incubated for 1 hour at room temperature to provide the adherence of *Candida albicans*[34,35,37,38]. All specimens were washed with PBS for 1 minute.

Table 2. Type and manufacturers of denture cleansers

Material	Type	Manufacturer
Efferdent (A)	Alkaline-peroxide	Warner-Lambert, NJ
Polident double action (B)	Alkaline-peroxide	Block Drug, NJ
Steradent triple action (C)	Alkaline-peroxide	Reckitt & Colman Ltd, NJ
Correga (D)	Alkaline-peroxide	Stafford-Miller Ltd, UK

Procedure

Control groups were stored in 40 ml sterile distilled water for 2 hours before fixation period. Disinfection regimes were carried out for each test group[34]. 4 denture cleaners (Efferdent - A, Polident duble action - B, Steradent triple action - C , and Correga - D) were used for the disinfection regime. Denture cleaner solutions were prepared according to manufacturers' instructions. The test specimens were immersed in 40 ml of disinfection solutions in sterile universal bottles at 37ºC for 2 hours[39-41] .

All specimens were washed twice with PBS (Phosphate Buffer Solutions) with gentle rocking to remove non-adherent cells after discarding disinfection solutions and sterile water. Excess of PBS solution was drained from specimens. After they 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 mm^2 per field) were enumerated by light microscopy and the results were expressed as yeast cells/mm^2 of material remaining after each cleanser in comparison with the control group[34,39]. Scheffe F-test was used to analyze the data.

Results

Both soft liners showed Candidal adherence. At disinfection period, inhibition was observed for *Candida albicans* for both tested soft lining materials. Table 3 shows the mean and standard deviation values of *Candida albicans* adherence to the silicone-based soft lining materials before and after the disinfection. Before disinfection, adhesion of *Candida albicans* to the Luci-Sof soft liner was found statistically higher than to the Molloplast-B in control groups ($p<0.0001$). There were statistical differences between the soft liners and denture cleansers (Tab. 4). After disinfection, Cleaner B showed the highest cleaning efficiency for the 2 tested lining materials. However, Cleaner D and A was found the least effective for Group 1 and 2, respectively (Tab. 5).

Table 3. Mean and SD of the tested materials

Denture cleanser	Control	A	B	C	D	Totals
Group 1	7 130 (35)	7 30 (26)	7 21(7)	7 32(19)	7 33(26)	35 49
Group 2	7 486 (123)	7 286 (169)	7 141 (76)	7 183 (152)	7 196 (97)	35 258
Totals	14 308	14 81	14 114	14 108	14 158	70 154

Table 4. Two-way ANOVA results

Source	df	Sum of square	Mean square	F-test	P value
Mollo-Luci (A)	1	764836	764836	9	0.0001
Denture cleansers (B)	4	457966	114492	13	0.0001
AB	4	129717	32429	4	0.0079
Error	60	510067	8501		

Table 5. Scheffe-F tests results; comparison denture cleanser and soft lining materials

Denture cleansers	Molloplast-B denture liners	Lucisoft denture liners.
Co. vs B	S	S
Co. vs D	S	S
Co. vs C	S	S
Co. vs A	S	S
B vs D	NS	NS
B vs C	NS	NS
B vs A	NS	S
D vs C	NS	NS
D vs A	NS	NS
C vs A	NS	S

Scheffe-F test: Significant at 95 %, NS: Non-significant

Discussion

Bacterial and yeast plaque on dentures is thought to be an important factor in the pathogenesis of denture stomatitis[18,20]. Since these fungi have been reported to colonize easily and penetrate denture materials[34,42], particularly tissue conditioners, and mechanical cleaning *per se* is insufficient to remove harboured *Candida* and harmful to soft liners[43,44], chemical cleansing is suggested to be indispensable to denture plaque control[43,45]. Therefore, many denture cleansers have been marketed for removal or reduction of denture plaque.

For the past years, resilient denture-lining materials have been widely used in prosthodontic treatment. However, severe deterioration of these materials have been reported to be caused by some denture cleansers[36,43,45] in a relatively short period, depending upon the combination of the soft liners and cleansers. The deteriorated surface of soft liners should facilitate further plaque accumulation. Therefore, denture cleansers used for plaque control with tissue-conditioned dentures should consider both microbial and physical requirements[41].

Plaque control for soft-lining materials has not been stressed by either clinicians or researchers, and there are scant data available on either the material or microbiologic aspects of denture cleansers. This may be a result of the lack of recognition of the importance of plaque control for soft liners, since the prevention of Candida invasion and denture plaque formation may be achieved not only by plaque but also by replacement of the lining materials every few days. However, plaque control is particularly

essential in the clinical use of soft liners and aetiology of denture stomatitis[18,20]. Accordingly, the purposes of this study were to examine the ability of Candida albicans to adhere to 2 permanent soft liners, and the effectiveness of alkaline peroxide-type denture cleansers in the disinfection of long-term soft lining materials contaminated with Candida albicans.

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 free energies of the surfaces and the microorganisms. In addition, the hydrophobicity of the microorganisms has been theorized 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 stereo-chemically to complementary receptors on the surface. This stage is necessary for the tight binding of the microorganisms to the surface, which permits colonization. In addition to tightly binding the microorganisms to the surface, the irreversible interactions are also responsible for the site-specific colonization of the oral microorganisms, which provides a selective advantage for microorganisms that possess relevant adhesins. Adhesins have been postulated to be associated with the microorganisms surface appendages that, by virtue of their small radius, are unable to overcome the energetic barrier of the primary force[37].

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[37] .

In this study, a simple in vitro model was used to compare the adherence of C. albicans with 2 soft lining materials. We aimed to provide a reproducible variables that could be examined in future studies. In light of this, the material surfaces were reproduced in a stainless-steel mould; the variability of surface roughness was therefore not examined. The concentration, viability, and culture conditions of the assay were kept constant. Adhesion was initially carried out on surfaces with no saliva coating to produce a reproducible assay before the introduction of variables. Only one dye - crystal violet - used within the study is commonly used in microbiology. Because crystal violet stains all cells present, with no ghost cells evident[34].

Even though this study did not evaluate the fungicidal effects of denture cleansers, there are many studies that examine the fungicidal effects of denture cleansers. Nikawa et al[28,29] reported that fungicidal effects of some denture cleansers tested was less effective with a soaking period of 30 minutes, as compared with 2-hour incubation periods, in accordance with the conclusions of other investigators[43,46]. Thus, a 2-hour incubation period was used to asses the efficacy of the cleansers[28,29].

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[47,48]. 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 need for further investigations.

The results of the experiment clearly indicated that all peroxide-type denture cleansers were effective in the 2-hour test period. In this study, peroxide-type denture cleansers were used for disinfection regimes, because these cleansers are commonly used by denture wearers and more often found in the markets.

Conclusion

The ability of Candida albicans to adhere to 2 permanent soft liners and the effectiveness of alkaline peroxide-type denture cleansers in the disinfection of long-term soft lining materials contaminated with Candida albicans were examined. The following conclusions may be made:

Both Luci-Sof and Molloplast-B soft liners showed Candidal adherence;

Luci-Sof soft liner exhibited higher Candidal adherence than Molloplast-B soft liner;

2-hour immersion period was found effective to reduce Candidal colonization clinically;

Alkaline peroxide-type denture cleansers can be used in order to maintain effective denture hygiene;

There is a need for further investigations to explain if there is any relation between Candidal adherence and surface free energy of the materials and microorganisms.

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