Surface Roughness and Adherence of Candida Albicans on Soft Lining Materials as Influenced by Accelerated Aging

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Abstract

Aim: *Candida albicans (C. albicans)* has been widely associated with the etiology of denture-related stomatitis and has been found on soft denture lining materials. The aim of this study was to examine the surface roughness and adherence of *C. albicans* to saliva coated and non-coated soft lining materials by subjecting them to an *in vitro* accelerated aging test.

Methods and Materials: Samples were prepared from three soft lining materials (Visco Gel, Ufi Gel P, Molloplast B). Surface roughness measurements and adhesion of *C. albicans* were examined before and after an aging process. The stimulated human whole saliva was used to assess its effect on adhesion.

Results: The aging process promotes the surface roughness of soft lining materials. The aging surface roughness of Visco Gel was significantly higher than Ufi Gel P and Molloplast B. No significant difference was observed between non-aged and uncoated materials, but aged and uncoated soft lining materials showed a greater adherence of *C. albicans*. No significant difference was observed between non-aged and saliva coated materials, but aged and saliva coated soft lining materials showed a greater adherence of *C. albicans*.

Conclusions: Candidosis induced by *C. albicans* is the most common fungal infection. Awareness of susceptibility of soft lining materials to the adherence of *C. albicans* is an important factor in their selection. The use of soft lining materials with smooth surfaces minimizes the adherence of *C. albicans*.

Introduction

It is well known candidosis induced by *Candida albicans (C. albicans)* is the most common fungal infection in human beings.[1,2] The most prevalent form of oral candidosis is chronic atrophic candidosis (CAC) occurring as a candidal associated denture stomatitis characterized by erythema of the palate beneath the mucosal surface of the upper denture.[3] The predisposing factors of infections caused by *Candida* species can be divided into systemic and local factors. Some of the systemic factors include age, endocrine diseases, systemic steroids and antibiotics, concurrent infections, and deficiency states. Local factors include salivation reduction, smoking, topical antibiotic or steroid treatment, coexistent oral mucosal disease, and especially the wearing of dentures.[1,2] Prevalence has been reported at 11-67% in complete dentures.[4,5]

Soft denture liners may be defined as soft polymers which may be applied to the fitting or mucosal surface of a denture for the purpose of reducing and more evenly distributing the occlusal load on the underlying mucosal tissues. The use of soft denture liners has increased in recent years in an effort to achieve some measure of redistribution and reduction of locally damaging occlusal forces of dentures on the underlying oral tissues.[6] These materials can be classified as provisional or definitive according to their composition of either silicone rubber or acrylic resin, and they can be either chemically or heat polymerized.[7,8]

The adherence of *C. albicans* to host cells or polymers such as denture acrylic resin and soft lining materials is an essential initial step in the successful colonization and development of an infection.[9,10,11] Soft lining materials have been found to be more prone to microbial adhesion than acrylic resin denture base materials. They have demonstrated the ability to interact with oral microorganisms because of their surface texture and the physical and chemical affinity of the materials.[12,13] Radford et al.[4] reported *C. albicans* adhered in greater numbers to soft lining materials compared with acrylic surfaces. Verran and Maryan[10] showed more fungal adhesion was found on rough surfaces than smooth surfaces and counted more cells on rough silicone than on rough acrylic surfaces.

Only limited data are available on the interactions between soft lining materials and *C. albicans*, however, its growth on these lining materials is thought to be an important factor. Although the lining materials are known to show changes in their physical properties with age, little attention has been paid to the growth of *Candida* when aging of the material has occurred.[14] It is apparent the relationship between the salivary or serum pellicle on denture material surfaces and candidal colonization is a complex subject, particularly when the surface aging of soft lining materials are taken into account.[15]

When wearing a denture, the base becomes colonized with pellicles composed of salivary or serum proteins which may provide receptor

Table 1. Soft denture lining materials used in the study.

Material	Type	Manufacturer
Visco Gel	Cold-cured acrylic resin soft lining material.	Dentsply Ltd., De Trey Division, Weybridge, U.K.
Ufi Gel P	Cold-cured silicone soft lining material.	Voco, Postfach 767, D-27457 Cuxhafen, Germany
Molloplast B	Heat-cured silicone soft lining material.	Detax Karl Huber GmbH, Ettlingen, Germany

sites for the adherence of microorganisms; surface roughness is a contributing factor in the colonizing process.[8,16,17]

Recent investigations have reported the continuous swallowing or aspiration of microorganisms from denture plaque exposes patients to the risks of unexpected infections.[3,11,18] Because of the clinical importance of the growth and adhesion of *C. albicans* on the soft lining materials, the aim of the present study was to determine the surface roughness and *C. albicans* adhesion on saliva coated and non-coated soft lining materials by subjecting them to an *in vitro* accelerated aging test.

Methods and Materials

Sample Preparation
Three different soft lining materials: Visco Gel (Dentsply Ltd., De Trey, Division, Weybridge, UK), Ufi Gel P (Voco, Cuxhafen, Germany), and Molloplast B (Detax Karl Huber GmbH, Ettlingen, Germany) were used in this study. The compositions of the soft lining materials are shown in Table 1.

All samples were processed against glass slides with dimensions of 15 mm X 1.5 mm using an aluminum mold. A clean technique was adopted to reduce contamination of the surface to be assessed for adhesion.

The cold cured soft lining materials (Visco Gel, Ufi Gel P) were processed according to the manufacturers' directions and made to a uniform size (15 mm diameter X 1.5 mm thickness) using an aluminum mold and to a uniform surface roughness by allowing polymerization to occur against identical glass surfaces.

The heat cured soft lining material (Molloplast B) samples measuring 15 mm in diameter were cut from a 1.5 mm thickness of red boxing wax. Each wax sample was mounted onto the same glass surface and placed into the mold with glass slides. Samples were flasked with vacuum spatulated dental stone in the usual manner. All samples were processed according to the manufacturers' instructions.[9,10]

Fifty samples were made from each material. Ten of the 50 samples of each material were used for the measurement of surface roughness, and the remaining 40 samples were used for the adherence assay of *C. albicans*.

Weathering Procedure
After baseline measurements of surface roughness were made, each sample was mounted on a panel that attached to the frame of an accelerated weathering tester[19] (Q-U-V, Q-Panel Company, Cleveland, OH, USA). Adhesion assays were repeated after a second set of samples were subjected to an accelerated aging chamber.[20] The weathering cycle was programmed for each material according to their usage period in the mouth as preferred by manufacturers (Table 2). The samples were continuously exposed to a visible ultraviolet and visible light and sprayed with distilled water for 3.5 minutes at the beginning of every condensation cycle. The humidity in the chamber was maintained at 90ºC, the UV temperature at 60ºC, and the condensation temperature at 40ºC.

Surface Roughness of Soft Lining Materials
Each sample of soft lining materials was boxed using boxing wax and poured using Type IV die stone (Tritech, Deltastone, Germany) to create gypsum samples for indirect measurements.

Table 2. The usage period, aging time, and sample number of the materials used in the study.

Material	Usage Period	Aging Time	Sample Number
Visco Gel	3 weeks	17 hours	30
Ufi Gel P	6 months	138 hours	30
Molloplast B	2 years	600 hours	30

The die stone was mixed according to the manufacturer's instructions with vacuum spatulation equipment (Whip Mix Corp., Louisville, KY, USA).

The surface roughness of the samples was measured with a Surtronic 10 profilometer (Taylor Habson Ltd. Leicester, England) where a stylus traverses across the layer of the surface and an amplified trace of the profile is provided.[21] The surface roughness value (Ra) is the arithmetical average of all departures of the profile through the mean sample length.

Direct surface roughness measurements were not obtained from the soft lining material samples because they were too thin and easily distorted. As a result, indirect measurements on stone models were used. Like impression materials, soft lining materials provide a mirror image of the surface against which it is processed so measurements taken from stone models is indicative of the surface roughness.[22] Each sample of soft lining materials was measured before and after *in vitro* accelerated weathering procedure.

Adhesion Assay
The 40 samples of each material were used for adhesion assay, and 20 of the samples were used after accelerated aging. Ten of the 20 samples of each material were coated with saliva. All samples were sterilized by ethylene oxide and aired for three days to remove residual ethylene oxide.

Fresh stimulated human whole saliva samples were collected from four healthy *Candida*tes and were used the same day. The *Candida*tes had not taken any medication during the preceding three months and had no active periodontal disease or active caries. Saliva secretion was stimulated using paraffin and collected on ice.

The collected saliva was centrifuged at 5000 Xg for 15 minutes and the supernatant fluid was used.[23]

The samples were immersed in the clarified saliva and incubated for one hour at 37ºC then washed in distilled water for 30 seconds to remove unbound and loosely bound substances and allowed to air-dry for 24 hours.

Preparation of Yeast Suspensions
The *C. albicans* ATCC 90028 strain was used as a quality control. This was transferred onto Sabouraud dextrose agar Sigma-Aldrich, Inc., St. Louis, MO, USA) plates to re-grow the yeast colonies. The *Candida* culture was incubated in Sabouraud broth (Sigma-Aldrich, Inc., St. Louis, MO, USA) supplemented with 500 mmol/lt of sucrose. This medium was used because previous studies have shown increased candidal adherence to acrylic resin after culturing in Sabouraud's broth supplemented with sucrose. A loopful of *Candida* culture was incubated on Sabouraud broth for 24 hours at 37ºC (Dedeoglu, Instanbul, Turkey) in a Thermolyne Maxi-mix III Type 65800 horizontal shaker (Barnstead International, Dubuque, IA, USA) in order to grow yeast cells in a stationary phase. The culture was centrifuged at 1700 xg for ten minutes, and the resultant pellet was washed twice with phosphate buffered saline (PBS) solution (0.15 mol/L, pH 7.2). A final yeast suspension of approximately 10^7 yeast/ml was prepared by adding PBS. The number of the yeast per ml was monitored in all the experiments by spectrophotometric counting using a Shimadzu UV-1201 (Shimadzu Precision Instruments, Torrance, CA, USA).[11,24,25]

Using strict aseptic techniques, the sample acrylic strips were placed in the tubes containing 4×10^7 yeasts in one ml of PBS. Tubes were placed in a shaker incubator for three hours at 37ºC with gentle agitation. The strips were then

washed in sterile distilled water, air-dried, and stained using a gram stain. After drying at room temperature, they were mounted on glass slides; the adherent yeasts were quantified using X 40 magnification under a light microscope. Ten fields were randomly counted on each strip, and all experiments were repeated on three separate occasions with duplicate determinations on each occasion (Table 3).

Statistical Analysis

Table 3. Adhesion scores.

Adhesion Score	Cell Number
+	1-25
++	25-50
+++	50-75
++++	75-100

One-way analysis of variance (ANOVA) was used to examine the differences in soft lining materials' surface roughness. Data collected were analyzed for homogeneity of variances using Levene's test. The results were considered significant at the 0.05 level only. All adhesion assay data obtained here were analyzed by Cramer's V.

Results

Surface Roughness

The results are presented as surface roughness values of average surface roughness measurements, and the results for surface roughness of soft lining materials according to the accelerated aging process are shown in Table 4.

As shown in Figure 1, aging essentially promoted the surface roughness of the materials. The aging surface roughness of Visco Gel was significantly higher than Ufi Gel P and Molloplast B.

There was a significant difference between variances (Levene's test= 48.333 and p<0.001). Data collected from three soft lining material samples were analyzed first for homogeneity of variances using Levene's test followed by ANOVA. Homogeneity of variances was rejected

because of the Visco Gel. Therefore, ANOVA was performed to compare Ufi Gel P and Molloplast B.

Molloplast B and Ufi Gel P were compared separately (Figure 2), and Molloplast B produced the smoothest surface after accelerated aging process (F=11.705 ve p=0.003).

Adherence of *C. albicans*

Uncoated samples: The adherence of *C. albicans* between non-aged and uncoated materials was not significant (Cramers V= 0.222, P= 0.56). In the case of aged and uncoated samples the adherence of *C. albicans* was greatest on Molloplast B (Cramers, P= 0.007) (Table 5).

Saliva coated samples: No significant correlation was observed between the adherence of *C. albicans* on non-aged and saliva-coated samples (Cramer's V=0.111, p=0.830). In the case of aged and saliva coated samples the adherence of *C. albicans* was greatest on Molloplast B (Cramer's V= 0.468, p=0.041) (Table 6).

Discussion

The accelerated aging process has been used to examine color change over time as well as changes in physical and mechanical properties of various materials, but little attention has been paid to the colonization of *Candida* when they aged.[19,20,26,27,28,29,30,31]

In this study a simple *in vitro* model was used to compare the surface roughness and the adherence of *C. albicans* with three commercially available soft denture lining materials before and after an aging process. An aging device was used to subject soft lining material samples to both visible and UV light along with a distilled water spray to simulate aging. The manufacturer of the weathering instrument estimates 300 hours of aging are equivalent to one year of clinical service.[31] In the present study the samples were stored in the accelerated aging chamber according to time limits recommended by the manufacturer for each lining material (Table 2). Dootz et al.[20] and Kawona et al.[30] stored the soft lining materials in an aging chamber for 900 hours, but this is not equivalent to their clinic usage time.

Table 4. Mean surface roughness values and standard deviations for surface roughness, before (Ra1) and after (Ra2) accelerated aging and the difference values before and after accelerated aging (Ra1-Ra2).

Materials	Ra1 Mean (SD)	Ra2 Mean (SD)	Ra1-Ra2 Mean (SD)
Visco Gel (n=10)	1.60 (0.2055)	16.67 (3.2428)	15.07 (3.2346)
Ufi Gel P (n=10)	1.47 (0.2452)	1.86 (0.2836)	0.39 (0.1197)
Molloplast B (n=10)	0.94 (0.1578)	1.18 (0.2044)	0.24 (0.0699)

Ra: the arithmetical average of all departures of the profile through the mean sample length.
SD: standard deviation.
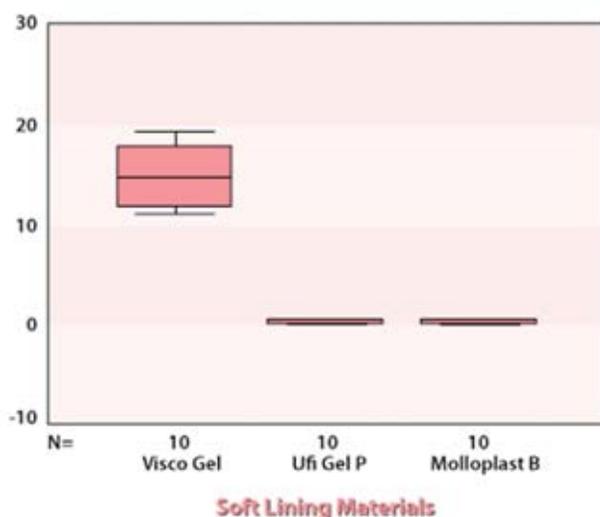**n:** number of samples

Figure 1. Differences on surface roughness values of soft lining materials according to the accelerated aging.
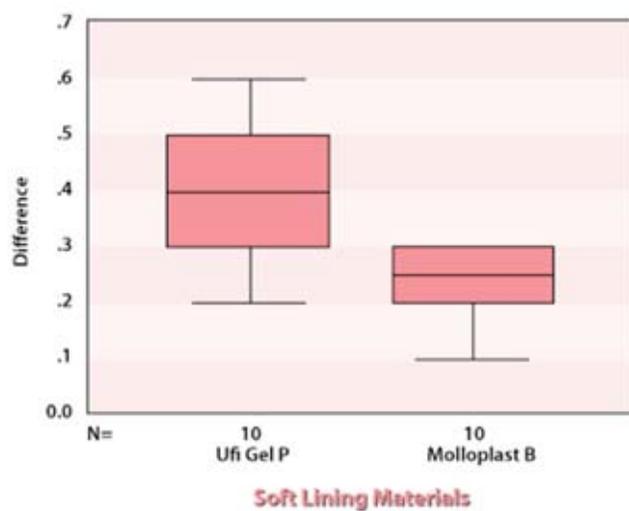

Figure 2. Differences on surface roughness values of Ufi Gel P vs Molloplast B according to the accelerated aging.

Table 5. Adhesion scores for uncoated soft lining materials before accelerated aging (UAS1) and after accelerated aging (UAS2).

MATERIAL	UAS1*(n=30) +	++	+++	UAS2**(n=30) ++	+++	++++
Visco Gel (n=20)	7	2	1	0	8	2
Ufi Gel P (n=20	5	3	2	4	2	4
Molloplast B (n=20)	8	2	0	0	4	6

*Cramer's V=0.222, p=0.560
**Cramer's V=0.483, p=0.007
n: number of samples

Table 6. Adhesion scores for saliva-coated soft lining materials before accelerated aging (CAS1) and after accelerated aging (CAS2).

MATERIAL	CAS1* (n=30)			CAS2** (n=30)		
	+	++	+++	++	+++	++++
Visco Gel (n=20)	8	2	1	7	2	1
Ufi Gel P (n=20	7	3	2	3	2	3
Molloplast B (n=20)	8	2	3	0	2	5

*Cramer's V=0.111, p=0.830
**Cramer's V=0.468 , p=0.041
n: number of samples

The surface roughness of a material used for a removable prosthesis is of importance since it affects, directly or indirectly, retention, staining resistance, plaque accumulation, as well as oral tissue health and patient comfort.[11,32,33,34]

In the present study we examined the surface roughness of soft lining materials before and after an aging process. After aging, all soft lining materials became rougher. The roughest was Visco Gel, and the least rough was Molloplast B before and after the aging process. The surface roughness value for Molloplast B and Visco Gel was found to be lower than reported by Zissis et al.[34] who found a surface roughness value for Molloplast B of 3.5 µm. and by Loney et al.[35] who reported a surface roughness value for Visco Gel of 3.7 µm. This may be because of the different surface textures of the specimens caused by processing against different materials.

The findings in this study for Ufi Gel P samples showed surface roughness values are similar to those reported by Jin et al.[9] This may be because the same indirect technique was used for measurement.

There were several papers suggesting the relationship between surface roughness and fungal adherence to denture materials.[4,10,32] The surface roughness and adhesion of soft lining materials was analyzed using an aging process. In reviewing the pertinent literature no other studies using exactly the same aging process were found, so it is presumed only indirect comparisons are possible.

In this study a number of yeast cells were examined on the samples of each material before and after the *in vitro* accelerated aging process and analyzed the effect of saliva on the adherence of *C. albicans*. Some investigators point out the pellicle of salivary proteins may provide receptor sites for specific adherence of microorganisms.[36] Some investigators have reported a coating of saliva reduced the adherence of *C. albicans* to soft lining materials[4,11,37], while some showed the coating actually increased the adherence.[38] The others have shown no effect of saliva on *C. albicans* adherence.[13,39]

According to the results of this study, adhesion of *C. albicans* to saliva coated and uncoated soft lining materials were not significant. However, aged Molloplast B samples had a higher candidal adherence than did Ufi Gel P and Visco Gel. The

Candidia albicans

difference between saliva coated and non-aged soft lining materials was not significant, however, aged Molloplast B samples had higher candidal adherence than others.

Bulad et al.[5] reported no difference in the adhesion of *C. albicans* between different soft lining materials processed against glass slides, and more adhesion was observed on Molloplast B when processed against dental stone. No significant difference in the adhesion of *C. albicans* between non-aged soft lining materials was observed in the present study. The aging process essentially promoted the fungal adhesion on the uncoated and coated specimens with the greatest occuring on Molloplast B.

Surface roughness is a significant factor in the attachment and adhesion of microorganisms on surfaces with an increase in roughness causing increased retention of cells.[4,10] In the present study the aging process increased the surface roughness of the soft lining materials, and *C. albicans* adhesion was increased on aged Molloplast B. The results of our study may be explained by the effect reported previously.

Saliva coating had no affect on the adherence of *C. albicans* to the non-aged specimens, but after the *in vitro* accelerated aging process fungal adherence to Visco Gel and Molloplast B increased. This could be partly attributed to the reduction in the antifungal effect of soft lining

materials by pellicle coatings. Nikawa et al.[13,24], in their investigations on fungal adherence to soft lining materials with or without a salivary pellicle, showed there was no difference in fungal adherence to saliva coated soft lining materials.

The aging process essentially promoted the fungal colonization particularly on the coated specimens with the greatest being on Molloplast B. Since aging essentially promoted the fungal colonization, this can probably be attributed to the significant reduction in antifungal effects of the soft lining materials during the aging process.

Conclusion
This study has shown, for a given soft lining material, aging leads to increased surface roughness and the adhesion of *C. albicans*. The presence of a salivary pellicle derived from stimulated human whole saliva had no effect on the adhesion of non-aged soft lining materials, but after an accelerated aging process the adhesion of *C. albicans* increased. In many instances this increase was considerable since the aging process promoted the surface roughness of the soft lining materials.

It is important to use soft denture lining materials with smooth surfaces for this will lead to a reduction in the adherence of *C. albicans*, an opportunistic pathogen commonly associated with denture wearing. Awareness of the susceptibility of these materials is an important factor in their selection and use.

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