Candida albicans biofilms and MMA surface treatment influence the adhesion of soft denture liners to PMMA resin

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Abstract: The effect of *Candida albicans* biofilms and methyl methacrylate (MMA) pretreatment on the bond strength between soft denture liners and polymethyl methacrylate (PMMA) resin was analyzed. Specimens were prepared and randomly divided with respect to PMMA pretreatment, soft liner type (silicone-based or PMMA-based), and presence or absence of a *C. albicans* biofilm. Samples were composed of a soft denture liner bonded between two PMMA bars. Specimens (n = 10) were incubated to produce a *C. albicans* biofilm or stored in sterile PBS for 12 days. The tensile bond strength test was performed and failure type was determined using a stereomicroscope. Surface roughness (SR) and scanning electron microscopy (SEM) analysis were performed on denture liners (n = 8). Highest bond strength was observed in samples containing a silicone-based soft liner and stored in PBS, regardless of pretreatment ($p < 0.01$). Silicone-based specimens mostly underwent adhesive failures, while samples containing PMMA-based liners predominantly underwent cohesive failures. The silicone-based specimens SR decreased after 12 days of biofilm accumulation or PBS storage, while the SR of PMMA-based soft liners increased ($p < 0.01$). The PMMA-based soft liners surfaces presented sharp valleys and depressions, while silicone-based specimens surfaces exhibited more gentle features. *In vitro* exposure to *C. albicans* biofilms reduced the adhesion of denture liners to PMMA resin, and MMA pretreatment is recommended during relining procedures.

Descriptors: *Candida albicans*; Denture Liners; Tensile Strength; Polymethyl Methacrylate.

Introduction

Soft denture liners are used to form a comfortable interface between denture and soft oral tissues, reducing traumatic transmission of occlusal forces to severely resorbed alveolar ridges and areas recovering from surgical procedures.[1] However, failure often occurs in adhesive bond between soft liner and denture base resin, resulting in tearing and material loss during clinical use.[2] This damage can increase surface roughness and create irregularities that act as sheltered sites where oral biofilms may accumulate over time.[3] These biofilms are mainly composed of *C. albicans* and may cause denture-induced stomatitis[4] or accelerate wear and aging of soft liner and denture base.[5] In order to prevent these problems, several denture base surface treatments have been proposed to increase the bond strength between these materials.[6]

When compared to other denture resin pretreatments,[6-8] methyl methacrylate (MMA) surface pretreatment increased tensile bond strength[8] and reduced microleakage between the denture base and silicone-based soft liners.[7] In order to evaluate the durability of bonds between soft liners and pretreated denture resins, previous studies have subjected specimens to distilled water storage,[9] accelerated aging in hot water,[10] or thermocycling procedures[8,11] prior to bond strength testing.

More recently, it was hypothesized that *in vitro* exposure of composites to oral biofilms results in clinically relevant surface degradation.[5] Although there have been reports concerning the bond strength of soft liners to denture base resins and the effects of various pretreatment methods, until now there have been no studies considering the potential damaging effect of oral biofilms on the interface between soft liners and pretreated denture resins. Therefore, the influence of *C. albicans* biofilms on the tensile bond strength between soft liners and denture resin, with or without MMA pretreatment, was analyzed. The principal hypothesis was that biofilms can cause degradation of the denture liner–PMMA interface, decreasing the bond strength.

Methodology

Experimental design

An *in vitro* study with blind analysis was performed, in which specimens were prepared and randomly divided according to PMMA surface treatment (MMA pretreatment or no treatment), denture liner type (silicone-based or PMMA-based), and presence or absence of a *C. albicans* biofilm. Denture liners were applied between two treated PMMA bars, and specimens (n = 10) were subjected to biofilm accumulation, or phosphate buffered saline (PBS) storage, in order to simulate conditions experienced by dentures in clinical applications. Tensile bond strength was measured and the nature of failure (adhesive, cohesive, or mixed) was determined using a stereomicroscope under 10× magnification. Surface roughness (SR) and scanning electron microscopy (SEM) analyses were performed on denture liner discs (n = 8) for surface characterization.

Specimen preparation

Microwave-polymerized PMMA (Vipi Wave, VIPI, Pirassununga, Brazil) resin bars (25.0 × 5.0 × 5.0 mm; n = 160), silicone-based (Ufi Gel SC, VOCO, Cuxhaven, Germany) and PMMA-based soft liner discs (Coe Soft, GC, Coe Laboratories Inc., Chicago, USA; 10.0 mm diameter × 2.0 mm thick; n = 32) were prepared according to manufacturers' recommendations using metal master patterns.

The PMMA bars were trimmed and finished in a polishing machine (APL-4 Model; Arotec, Cotia, Brazil), using abrasive paper (320, 400, and 600 grit, Carbimet; Buehler, Lake Bluff, USA). Specimens were ultrasonically cleaned (Thornton T740, Thornton-Inpec Eletrônica Ltda., Vinhedo, Brazil) and immersed in distilled water at 35°C for 48 h for residual monomer release.[12]

The square faces of PMMA bars were either treated with MMA (180 s) or left without surface treatment prior to adhesion of the 2 mm-thick denture liner.[8,13,14] Specimens were stored in 100% relative humidity before testing.

Soft liner specimens were ultrasonically cleaned and maintained in 100% humidity at 35°C for 24 h prior to biofilm accumulation or PBS storage. The cleaning procedure consisted of sonication for 10 minutes in 0.5% sodium hypochlorite and 10 minutes in sterile water.[12] Discs were used for surface roughness evaluation and SEM analysis before and after PBS storage or biofilm accumulation.

Biofilm formation and PBS storage conditions

Candida albicans (OMZ 110) was reactivated in yeast nitrogen base (YNB) medium containing 50 mM glucose, and the biofilm inoculum was standardized at an optical density of 0.25 in YNB containing 100 mM glucose. After allowing 90 minutes for initial adhesion, specimens were transferred to new tubes containing 7.0 mL of sterile YNB with 100 mM glucose for biofilm development.[15] Control specimens were immersed in 7.0 mL of PBS. Both sets of samples were stored at 35°C with agitation. PBS solution and biofilm culture medium were changed daily. After 12 days, the specimens were ultrasonically cleaned and prepared for testing.

Tensile bond strength evaluation

The tensile strength test was performed in a universal testing machine (4411, Instron Corp., Canton, USA) using a crosshead speed of 5.0 mm/min. Samples were tested until failure. The tensile strength, in MPa, was determined by multiplying the stress (Kgf) at the time of failure by a constant (9.8) and dividing this result by the surface area of adhesion (mm²). Failures were examined using a stereomicroscope at 10× magnification and classified as adhesive (total separation at the interface between the liner and resin), cohesive (tearing within the soft liner), or mixed failures (both adhesive and cohesive).[8,16]

Surface roughness evaluation

Surface roughness was used to identify changes in the soft liner surface occurring during biofilm accumulation or PBS storage. Measurements were obtained using a profilometer (Surfcorder SE1700; Kosaka Laboratory Ltd., Tokyo, Japan) with 0.01 mm resolution and adjusted for a 0.8 mm sample length, 3.2 mm percussion of measurement, and 0.5 mm/s stylus speed. Reported roughness values are the average of three measurements performed on each specimen (n = 8).[12]

Scanning electron microscopy evaluation

To evaluate the effect of biofilm accumulation, or PBS storage, on soft liners surface, specimens were ultrasonically cleaned and prepared for SEM. Samples were examined using an acceleration voltage of 15 kV at 2000× magnification.[17,18] The surfaces were evaluated at baseline (n = 3) and after biofilm accumulation (n = 3) or PBS storage (n = 3).

Statistical analysis

Statistical analysis was performed using the SigmaPlot 12 software (SigmaPlot v. 12.3, Systat Software Inc., San Jose, USA) at 5% significance. Tensile strength results were evaluated using three-way analysis of variance (ANOVA) and Holm-Sidak's test. Soft liner surface roughness results were evaluated using one-way ANOVA and the Bonferroni test.

Table 1 - Tensile bond strength (mean ± SD, MPa) of soft liners to untreated (NT) or surface treated (MMA) PMMA following biofilm accumulation or PBS storage.

		Biofilm accumulation	PBS
Silicone-based liner	MMA	3.60 ± 0.47 ^{Aa*}	5.92 ± 0.70 ^{Ab§}
	NT	3.21 ± 0.78 ^{Aa*}	4.03 ± 0.70 $^{Ab+}$
PMMA-based liner	MMA	1.24 ± 0.19 $^{Bc¥}$	1.11 ± 0.15 $^{Bc¥}$
	NT	1.31 ± 0.39 $^{Bc¥}$	1.14 ± 0.18 $^{Bc¥}$

Different uppercase letters indicate statistical difference between soft liners. Different lowercase letters indicate statistical difference between PMMA surface treatments. Different symbols indicate statistical difference between biofilm accumulation and PBS storage.

Results

The highest tensile bond strength (Table 1) was observed in the groups with silicone-based soft liners stored in PBS ($p < 0.01$), regardless of pretreatment. *C. albicans* biofilm accumulation resulted in a bond strength decrease for silicone-based specimens ($p < 0.01$).

Silicone-based specimens generally underwent adhesive failures while PMMA-based groups experienced predominantly cohesive failures (Table 2). Cohesive failures were mainly observed in the group receiving MMA pretreatment and stored in PBS (Table 2).

The silicone-based liners surface roughness decreased after biofilm accumulation or PBS storage ($p < 0.001$, Table 3), while PMMA-based liners surface roughness increased after PBS storage, and even more after biofilm accumulation ($p < 0.001$).

SEM observations of PMMA-based samples revealed a smooth porous surface (Figure 1A). After PBS storage (Figure 1B) or biofilm accumulation (Figure 1C), surfaces exhibited smaller pores as well as the formation of crests and valleys. Surface modifications may be due to material swelling, which causes pore constriction and creates wrinkles on the surface. At baseline, silicone-based samples presented smooth surfaces without visible pores, but with sharp crests and depressions (Figure 1D). After PBS storage or biofilm accumulation, material swelling

Table 2 - Failure distribution (%) for liner-PMMA bonds.

Failure type	Silicone-based liner				PMMA-based liner			
	MMA		NT		MMA		NT	
	Biofilm	PBS	Biofilm	PBS	Biofilm	PBS	Biofilm	PBS
Adhesive	90	70	100	80	10	0	40	30
Mixed	10	30	0	20	20	20	30	30
Cohesive	0	0	0	0	70	80	30	40

Table 3 - Surface roughness (mean ± SD, μm) of soft liners at baseline and after biofilm accumulation or PBS storage.

	Baseline	Biofilm accumulation	PBS storage
Silicone-based	4.55 ± 0.27 ^{Aa}	2.72 ± 0.19 ^{Ba}	2.58 ± 0.28 ^{Ba}
PMMA-based	6.76 ± 0.25 ^{Ab}	7.98 ± 0.15 ^{Bb}	7.29 ± 0.33 ^{Cb}

Different uppercase letters indicate statistical differences between baseline, biofilm accumulation, and PBS storage groups. Different lowercase letters indicate statistical differences between liner types.

made the depressions less pronounced, resulting in a smother surface than that observed at baseline (Figure 1E and 1F).

Discussion

The observation that soft liner surfaces are generally rough and covered with a biofilm[2] motivated our evaluation of the effect of *C. albicans* biofilms on tensile bond strength between soft liners and untreated or MMA-pretreated PMMA resin. However, we found that the effect of biofilms on adhesion was important mainly in samples employing silicone-based liners.

Our principal hypothesis was accepted in the case of silicone-based liners, in which the presence of *C. albicans* biofilms resulted in significantly lower bond strength. This result is in accordance with a previous study[5] demonstrating that *in vitro* exposure to oral biofilms leads to clinically relevant aging of dental materials.

However, no statistical difference was observed for bond strengths of specimens employing PMMA-based liners, even with MMA pretreatment. There was a relationship between failure type and MMA pretreatment, with better adhesion being associated with more mixed failures in both silicone and PMMA liner groups, as previously reported in the literature.[19]

In spite of the fact that other surface pretreatments had previously been reported ineffective in improving bond strength during a hard chairside reline using PMMA acrylic resin,[6] we found MMA pretreatment to be effective in increasing the bond strength between silicone-based soft liners and PMMA resin stored in PBS. Considering that there is no chemical interaction between silicone-based liner and PMMA acrylic resin,[8] the increase in bond strength may be due to the ability of MMA to dissolve PMMA surface layer and increase the bonding surface area.[18]

For PMMA-based groups there were no significant differences in bond strength due to the presence of biofilm or following resin pretreatment. However, this result should be interpreted with caution, since the high number of cohesive failures in PMMA-based soft liners may be due to the fact that the bond to PMMA resin is stronger than the denture liner tensile strength itself,[16,17,19] inducing failure in soft liner before debonding from PMMA resin occurs. However, all of the soft liners demonstrated bond strengths to denture base resin above the minimum acceptable bond strength for clinical use (0.45 MPa).[11,14]

Besides to the degradation between soft liners and PMMA resin interface, *C. albicans* biofilm accumulation led to a greater overall degradation. This is probably related to the ability of *C. albicans* hyphae to adhere and penetrate into soft liners,[20,21] as well as the production of proteases and phospholipases.[22,23] Thus, it is important to consider the degradation of the soft liner itself, which makes the material more susceptible to tearing.

Storage in aqueous solutions such as PBS or growth medium promotes the release of soluble compounds and plasticizers as well as water infiltration,[11,24] both of which may contribute to deg-

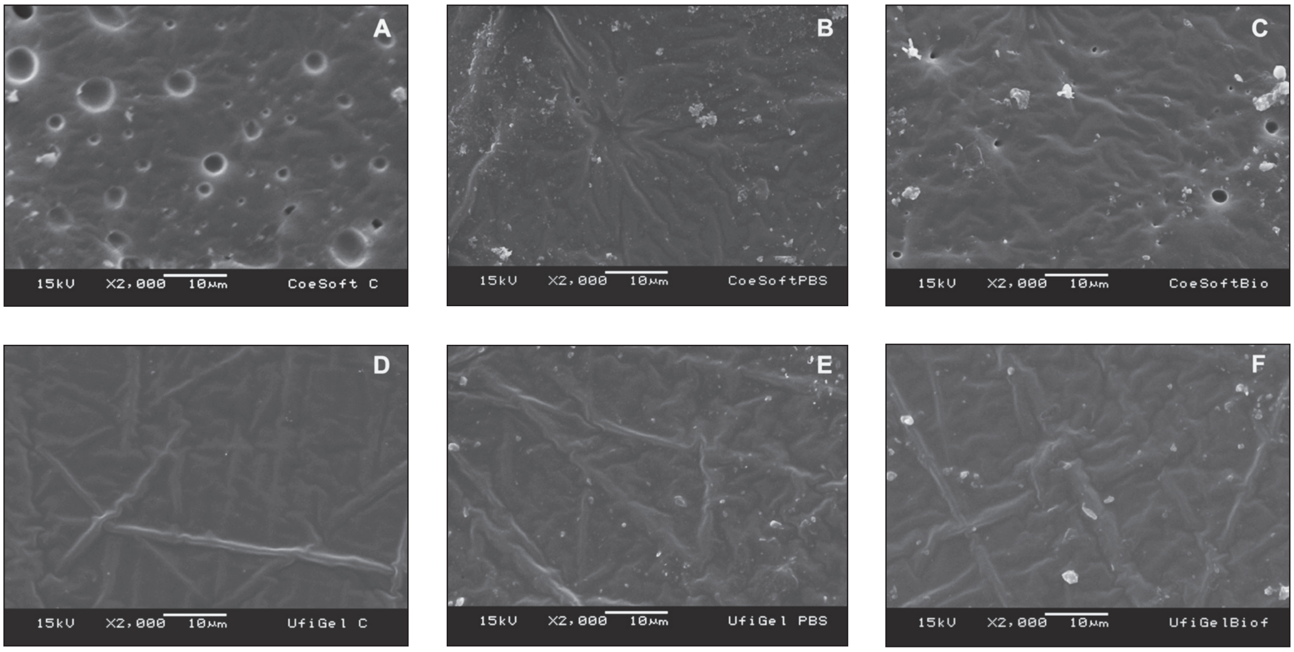

Figure 1 - SEM images of PMMA-based (**A** to **C**) and silicone-based (**D** to **F**) denture liners at baseline (**A** and **D**), after 12 days of storage in PBS (**B** and **E**), and after accumulation of a biofilm (surface cleaned before imaging; **C** and **F**).

radation and surface modification of soft liner materials.[11] A large number of crests and valleys in PMMA-based soft liners and surface modifications in silicone-based liners were evident in SEM images of the liners obtained after storage. However, more studies are necessary to confirm the effects of water uptake, and evaluations of other soft liner types should be undertaken, including other PMMA and silicone-based materials.

When immersed in MMA solution, a decrease in flexural strength is sometimes observed in PMMA resins;[8] however, this would not be expected in clinical practice since the MMA pretreatment involves only surface application, as was performed in the present study. The use of MMA pretreatment may result in better clinical performance and greater prosthetic survival. Biofilm accumulation seems to play an important role in degradation of the adhesive interface and should be avoided.[5] Although the results are based on an *in vitro* study, clinical application of these recommendations may contribute to a higher-strength interface with a smoother surface and less biofilm accumulation.[23] However, in patients with candidiasis, material selection alone may not influence the *C. albicans* biofilms growth, particularly when oral hygiene measures are correctly applied.[25]

Several factors are expected to affect the bond strength between soft liners and denture base resins, including aging in water[9,10] and thermocycling.[8,11] This list may now also include biofilm accumulation, the use of a bonding agent, and the composition of the soft liner. Additional studies incorporating longer periods of biofilm accumulation must be conducted with the purpose of assessing material degradation and structural failures under these conditions.

It is also important to consider that aging of soft liners in the oral cavity involves more than exposure to biofilms: temperature variations and immersion in water or acidic fluids from foods may also contribute to clinical aging.[5] Future *in vitro* studies should attempt to simulate as many of these conditions as possible.

Conclusion

In vitro exposure to *C. albicans* biofilms reduced the adhesion of soft liners to PMMA resin, and MMA pretreatment of denture bases is recommended during relining procedures.

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