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Peel bond strength of resilient liner modified by the addition of antimicrobial agents to denture base acrylic resin

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ABSTRACT

In order to prolong the clinical longevity of resilient denture relining materials and reduce plaque accumulation, incorporation of antimicrobial agents into these materials has been proposed. However, this addition may affect their properties. Objective: This study evaluated the effect of the addition of antimicrobial agents into one soft liner (Soft Comfort, Dencril) on its peel bond strength to one denture base (QC 20, Dentsply). Material and Methods: Acrylic specimens (n=9) were made (75x10x3 mm) and stored in distilled water at 37°C for 48 h. The drug powder concentrations (nystatin 500,000U - G2; nystatin 1,000,000U - G3; miconazole 125 mg - G4; miconazole 250 mg - G5; ketoconazole 100 mg - G6; ketoconazole 200 mg - G7; chlorhexidine diacetate 5% - G8; and 10% chlorhexidine diacetate - G9) were blended with the soft liner powder before the addition of the soft liner liquid. A group (G1) without any drug incorporation was used as control. Specimens (n=9) (75x10x6 mm) were plasticized according to the manufacturers' instructions and stored in distilled water at 37°C for 24 h. Relined specimens were then submitted to a 180-degree peel test at a crosshead speed of 10 mm/min. Data (MPa) were analyzed by analysis of variance ($\alpha=0.05$) and the failure modes were visually classified. Results: No significant difference was found among experimental groups ($p=0.148$). Cohesive failure located within the resilient material was predominantly observed in all tested groups. Conclusions: Peel bond strength between the denture base and the modified soft liner was not affected by the addition of antimicrobial agents.

Key words: Antifungal agents. Tensile strength. Stomatitis. Denture bases.

INTRODUCTION

The oral candidiasis known as denture stomatitis is related to the use of removable dentures and is considered the most common oral lesion observed (65%)²⁷ in patients wearing removable dentures. Although the etiology of denture stomatitis is multifactorial, infection by *Candida* spp., especially *C. albicans*, is considered the main etiologic factor. Local factors associated with the denture are also related to this pathology, such as: presence of

biofilm^{4,18}, local trauma caused by dentures¹², xerostomia²¹, continuous use of the dentures and alteration in salivary pH¹².

Different treatments for denture stomatitis are available and may include topical antifungal and systemic therapy, care with oral hygiene, denture cleaning and disinfection procedures¹⁸, replacement of old dentures, elimination of anatomic irregularities, re-establishment of atraumatic occlusion, and nutritional restitution³. Furthermore, in order to protect and preserve the integrity of

the mucosal epithelium, patients should sleep without the dentures⁶. The choice of a treatment or association of more than one treatment is an aspect to be individually considered. Re-infection of the treated oral mucosa may occur in up to two weeks post-treatment, and is attributed to the survival of *Candida* spp. due to insufficient concentration of the antifungal agent on the denture surfaces¹⁶. Therefore, it is crucial to adopt methods that reduce or preferably eliminate the microorganisms from denture surfaces.

In addition, resilient materials have been routinely used with the purpose of recovering tissues that are in contact with the denture base²⁴. These materials partially absorb chewing load on the denture during function, thus reducing the energy transmitted to the associated paraprosthodontic tissues¹⁷. However, these materials are easily degradable and susceptible to microbial colonization¹⁴, which may cause different degrees of denture stomatitis.

To prolong the clinical longevity of resilient materials and reduce plaque accumulation, incorporation of antimicrobial agents into these materials has been proposed²⁰. This combination may be a logical therapy in the treatment of denture stomatitis because of several factors: 1. reducing the trauma caused by the internal surface of removable dentures; 2. eliminating contact of the contaminated surface with the oral tissues and consequently, interrupting the cycle of re-infection, and 3. action of antimicrobial agents incorporated into the material on the infected tissues²⁰. In this context, denture stomatitis may be treated before fabricating new dentures, in a relatively short period. The reason is attributed to their gradual degradation and hardening, so it should not take longer than two weeks, which is a period similar to the one required for the treatment with conventional topical antifungal drugs^{20,22}.

The incorporation of antimicrobial agents into resilient materials has shown to be effective and

feasible both in *in vitro* and *in vivo* studies^{6,20,22}. Despite these therapeutic advantages, the incorporation of drugs into polymeric materials, including tissue conditioners and resilient liners, may affect their properties. For the resilient liner to adequately perform its function of recovering the tissues injured by trauma, it should remain bonded to the acrylic base of the removable denture⁵. Peeling of the resilient material from the denture base has been reported as the cause of clinical failure and the bond between the resilient materials and the denture base acrylic resins has been the object of previous investigations^{13,17}. Thus, the aim of this study was to evaluate the effect of the addition of antimicrobial agents (nystatin, miconazole, ketoconazole, and chlorhexidine diacetate) to a resilient liner on its peel bond strength to a denture base acrylic resin. The hypothesis investigated in this study was that the addition of antimicrobial agents to a resilient liner would result in changes in the peel bond strength to a denture base acrylic resin.

MATERIAL AND METHODS

The acrylic materials, manufacturers, batch numbers, compositions, powder/liquid ratios, and polymerization conditions selected for this study are listed in Figure 1. The selected antimicrobial agents were nystatin, miconazole, ketoconazole (Alonatu Farmácia de Manipulação e Cosméticos/Farmácia Dermatol., Maceió, AL, Brazil – Req. 119704-1), and 98% chlorhexidine diacetate (Acros Organics, Morris Plains, NJ, USA).

Specimen preparation

Specimens (n=9)¹⁷ measuring 75x10x3 mm^{13,17} of heat-curing acrylic resin QC 20 (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) were made. For this purpose, stainless steel matrixes measuring 75x10x3 mm were molded using laboratory silicone

Materials	Type	Manufacturer	Batch number	Composition	Powder/liquid ratio	Polymerization conditions
Soft Confort	Resilient liner	Dencril Prod. Odontol., São Paulo, SP, Brazil	Powder (010068)	Poly(ethyl methacrylate), phtalate ester, ethyl alcohol	1.27 g/1 mL	5 min at room temperature
QC 20	Heat-curing acrylic resin	Dentsply Ind. Com. Ltda., Petrópolis, RJ, Brazil	Liquid (503793)	Poly(methyl methacrylate), methyl methacrylate, n-butyl methacrylate	2.3 g/1 mL	1. Heat water to boiling point; 2. Turn off the water bath; 3. Put the flask in and leave it for 20 min; 4. Turn on the water bath; 5. When the boiling point is reached, wait another 20 min.

Figure 1- Materials selected for this study

(Zetalabor, Rovigo, Veneto, Italy) between two glass plates. The mold/matrix set was invested in conventional metal dental flasks in Type III dental stone (Herodent, Vigodent, Rio de Janeiro, RJ, Brazil). The dental flasks were closed and remained under pressure (500 kgf) in a hydraulic press during stone setting time. After this period, the dental flasks were opened and the stainless steel matrixes were removed.

QC 20 was proportioned, mixed according to the manufacturer's instructions (Figure 1), and was inserted into the silicone matrix mold. The dental flask was closed and kept under pressure at room temperature ($23\pm 2^{\circ}\text{C}$) for 30 min. After this period, the test specimens were submitted to the polymerization cycle "B" recommended by resin manufacturer (Figure 1). When the polymerization cycle ended, the dental flasks were bench cooled for 30 min and then under running water for 15 min. The specimens were removed from the molds and stored in distilled water at 37°C for 48 h¹⁰.

After this period, specimens were submitted to surface preparation to receive the modified resilient liner. One of the specimen surfaces was abraded automatically in a polishing machine using #600 silica carbide abrasive paper (Norton Abrasivos, São Paulo, SP, Brazil). The abraded surface was cleaned with detergent for 20 s, washed under running water, and dried. The specimen was then placed in a hollow stainless steel mold with internal measurements of 75x10x6 mm. The specimen area (650 mm²) to not be bonded to the resilient material was covered with a polyester strip.

The antimicrobial powders in each experimental group (Figure 2) were manually mixed with resilient lining powder with a spatula, until a homogenous mixture was obtained^{24,25}. The resilient lining liquid was added to this mixture and the material was mixed in accordance to the manufacturer's instructions (Figure 1). The modified material was inserted into the hollow mold containing the test specimen of the heat-curing acrylic resin prepared for the relining procedure. This set was covered with

glass slide and kept under finger pressure during the resilient liner polymerization time recommended by the manufacturer (Figure 1). The excesses of the modified resilient liner were eliminated and the specimen was removed from the mold. The relined specimens were then stored in distilled water at 37°C for 24 h prior to the peel test.

Peel test

A universal testing machine (Versat 2000, Panambra Ind. Tech. SA, São Paulo, SP, Brazil) was used to perform the peeling bond strength test of the relined test specimens at an angle of 180° . A portion of modified resilient material not bonded to the resin base (65 mm) was folded upwards and fixed onto the top hook of the equipment at 20 mm from the adhesive bond area of the test specimen. The other un-relined portion of the heat-curing acrylic resin was fixed onto the bottom hook of the equipment^{13,17} at the same distance from the adhesive bond area. Each test specimen was submitted to tension to promote peeling of the modified resilient liner from the heat-curing acrylic resin base at a speed of 10 mm/min until failure occurred.

Bond failures were visually observed and classified into three categories: adhesive, when peeling occurred between the modified resilient liner and the denture base acrylic resin; cohesive, when there was tearing (rupture of the resilient liner within the area bonded to the denture base) or snapping (resilient material had stretched and then ruptured away from the bonded area) within the modified resilient liner; and mixed, when regions with two types of failure were observed on the surface of the denture base material^{13,17}.

The results of rupture force were initially obtained in N and transformed into peeling bond strength in MPa and then submitted to one-way ANOVA at a significant level of 5%.

Group	Antimicrobial agent	Amount of drug incorporated
G1	None (control)	None
G2	Nystatin	500,000 U
G3	Nystatin	1,000,000 U
G4	Miconazole	125 mg
G5	Miconazole	250 mg
G6	Ketoconazole	100 mg
G7	Ketoconazole	200 mg
G8	Chlorhexidine diacetate	5%
G9	Chlorhexidine diacetate	10%

Figure 2- Drug dosages incorporated into the resilient liner powder in all experimental groups

Table 1- Peel strength (MPa) at 24 h

Experimental groups	Mean (SD)*	Mode of failure
G1	0.054 (0.017)	80% cohesive; 20% mixed
G2	0.049 (0.017)	All cohesive
G3	0.043 (0.012)	80% cohesive; 20% mixed
G4	0.046 (0.013)	80% cohesive; 20% mixed
G5	0.042 (0.014)	All cohesive
G6	0.046 (0.012)	40% cohesive; 40% mixed; 20% adhesive
G7	0.050 (0.010)	80% cohesive; 20% mixed
G8	0.060 (0.012)	All cohesive
G9	0.049 (0.012)	80% cohesive; 20% mixed

* There was no statistical difference ($p>0.05$) among the experimental groups

RESULTS

The results of peel bond strength are shown in Table 1. There was no significant difference ($p=0.148$) among the experimental groups. Therefore, the incorporation of antimicrobial agents in the concentrations assessed did not affect the peeling bond strength between the resilient liner and the denture base resin after 24 h of immersion in distilled water.

The failure modes obtained after performing the tests are shown in Table 1. The majority of bond failures were cohesive (tearing and/or snapping) within the resilient liner. For the experimental groups G2 (nystatin at 500,000 U), G5 (miconazole at 250 mg), and G8 (5% chlorhexidine diacetate), a mixture of tearing and snapping was observed. Peeling away from the denture base was only observed for groups G6 (ketoconazole at 100 mg) and G9 (10% chlorhexidine diacetate). For the other groups, cohesive and mixed bond failures were observed.

DISCUSSION

The hypothesis investigated in this study that "the addition of antimicrobial agents to the resilient liner would result in alterations in the peeling bond strength to denture base resin" was rejected because there was no difference between the experimental groups assessed in comparison with the control group without the addition of drugs.

During clinical use, the resilient materials are highly subjected to degradation and susceptible to the colonization by microorganisms. If these materials are not regularly replaced, they may act as microorganism reservoirs, causing systemic complications²³. An example of this is the presence in the oral cavity of *Staphylococcus aureus*, a microorganism responsible for respiratory infections¹⁵. The combination between resilient

materials and antimicrobial agents seems to be a logical therapeutic modality for denture stomatitis. This method results in a reduction of the trauma caused by the old denture and tissue reconditioning associated with antimicrobial therapy; important etiologic factors in triggering infection by *Candida* spp. are simultaneously eliminated. In addition, this method favors a relined denture that can more easily be kept clean by the patient²⁰.

Several drugs have shown reduced water solubility, so maximum dose is required to have the effectiveness required for a certain medication⁸. Among the antimicrobial agents assessed, chlorhexidine shows higher solubility in water, followed by nystatin, miconazole, and ketoconazole⁸. Although these medications are soluble in water, they are insoluble in monomers and plasticizers¹. Thus, they could not interfere with the polymerization or plasticization¹ process of these materials. However, their physical presence within the polymer matrix could interrupt the structure of the polymerized materials²¹. Resilient materials containing nystatin showed increased water sorption, and for these materials, this resulted in breaking their morphological structure⁷. According to Addy and Handley² (1981), change in material properties may be consistent with the incorporation pattern of the medication into the polymer matrix. A previous study²⁴ assessed the incorporation pattern of antimicrobial agents into a tissue conditioner with the same concentrations to those investigated in this study, by scanning electronic microscopy (SEM) and energy dispersive spectroscopy x-ray (EDS). The test specimens containing nystatin and miconazole exhibited particles with irregular shapes and sizes distributed uniformly within the tissue conditioner matrix while specimens with chlorhexidine exhibited more irregular particles distributed randomly within the material. However, these alterations would not prevent the incorporation of drugs for release in the oral cavity if they were added to materials to relined

already existent dentures², without necessarily reducing their strength.

Although some soft materials are submitted only to compression and shear, tensile strength is used to measure the quality of the material. The ability of the material to resist tearing is of practical importance. In clinical use, including the cleaning and disinfection procedures, the soft materials are submitted to conditions that start the tearing process. Adequate bonding between denture base resin and soft material is therefore essential. Clinical failure of these materials is frequently attributed to the rupture of this bond, and the measurements of this bond are clinically relevant. Reduced bond between the soft liner and the denture base resin effectively negates any other property considered adequate for this material²⁶. In the peel bond strength test, the stress is confined to a line restricted to the end of the bond, and is considered the most clinically representative of the failure modes²⁶. This is the only method in which the failure proceeds at controlled speed and it is a direct measure of peeling, while it also represents the elastic deformation of the material⁹. The peeling test simulates the lining procedure more precisely, with a uniform and constant distribution of force throughout the bond area²⁶.

The results of this study demonstrated that the addition of antimicrobial agents in all the assessed concentrations did not affect the peeling bond strength of the resilient liner to denture base resin. However, the bond strength values were considered low, since they were approximately 10 times lower than the acceptable value for the clinical use of resilient liners (0.44 MPa)¹¹.

While the methodology in this study was performed, some modifications were made, such as the reduction in bond area and surface roughness of the denture base, to ensure that the methodology evaluated the bonding between materials rather than the cohesive strength of the liner material. If the bond failures observed in this study were predominantly cohesive within the liner material, the peeling bond strength would not be measured⁹. The failure mode of the cohesive type provides information related to the material itself and not to the bond between materials¹⁹. Emmer, et al.⁸ (1995) suggested the term "strength failure" instead of "bond failure" when cohesive failures occur. Predominant cohesive failures, such as those that occurred in this study, indicate poor resistance to tearing of the resilient material. However, mixed and adhesive failures were observed in some samples, indicating that the cohesive strength values of the resilient liner and bond strength values to base resin were similar.

A previous study²⁵ observed that a tissue conditioner (Duraconditioner, Reliance Manufacturing

Co., Worth, IL, USA) modified by the addition of nystatin showed cohesive strength values similar to those of the control group. These values were close, if not similar, to the ones obtained in this study. Therefore, the cohesive strength of the resilient material tested in this study is equivalent to its bond strength to the denture base material. Thus, the material will snap or tear at the bond interface at forces lower than those necessary to cause bond failures.

One of the limitations of this study was that only one brand of the resilient liner was assessed. Moreover, the peeling bond strength could have been assessed after other storage periods. This assessment is also important to observe a possible reduction in bond strength of the modified liner to the denture base material, since it has been reported that plasticizers and alcohols are released from resilient materials after periods of storage in water and this release is responsible for the decrease in the bond strength values between the materials¹³. However, these analyses are object of future investigations.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that it is possible to incorporate any of the antimicrobial agents assessed in the selected concentrations into a resilient liner without changing the bond strength of this material to denture base resin. A clinical study is still needed to determine the therapeutic validity of this alternative treatment modality.

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