Brazilian Dental Journal (2017) 28(2): 158-164
http://dx.doi.org/10.1590/0103-6440201701266

ISSN 0103-6440

Surface Properties of Temporary Soft Liners Modified by Minimum Inhibitory Concentrations of Antifungals

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Evaluating the addition of minimum inhibitory concentrations (MICs) of antifungals for *Candida albicans* biofilm on the hardness and roughness of temporary denture soft liners. Specimens (n=8; 36×7×6 mm) of tissue conditioner (Softone) and resilient liner (Trusoft) were produced either without (control) or with incorporation of drugs at MICs: nystatin (0.032 g/mL), chlorhexidine diacetate (0.064 g/mL), ketoconazole (0.128 g/mL), miconazole (0.256 g/mL) and itraconazole (0.256 g/mL). Specimens were stored in distilled water at 37 °C for 24 h, 7 days and 14 days prior to the hardness/roughness measurements. Data were analyzed by 3-way ANOVA and Tukey HSD test (α=0.05). The addition of the antifungals into both materials demonstrated no evident hardness change or decrease of this property compared with the control, except for miconazole in Softone, which increased the hardness after 14 days ($p=0.003$). The addition of nystatin into both materials, chlorhexidine in Trusoft and ketoconazole in Softone resulted in no significant changes of roughness compared with the control, after 7 days and 14 days ($p>0.05$). In these periods, itraconazole increased the roughness of both materials ($p<0.001$). The addition of all antifungals, except for the miconazole in Softone, resulted in no deleterious effects on the materials' hardness over the evaluation time. The MICs of nystatin in both temporary soft lining materials, ketoconazole in Softone and chlorhexidine in Trusoft resulted in no deleterious effects for roughness up to 14 days.

Key Words: denture stomatitis, denture liners, *Candida albicans*, anti-infective agents, hardness, surface properties

Introduction

Denture stomatitis has been considered the most common injury among users of removable dentures (1). Despite being an infection of multifactorial etiology, denture stomatitis has as main etiological factor the colonization of denture-bearing mucosa and acrylic bases by species of *Candida* spp., especially *Candida albicans* (1).

Treatments targeted to denture stomatitis are varied, including topical antifungal therapy, systemic antifungal medication, oral hygiene care, procedures for cleaning and disinfecting the prostheses, replacing old dentures, elimination of anatomical irregularities, restoration of nontraumatic occlusion, nutritional recovery and overnight denture soaking in disinfectant solutions (2,3). The choice of treatment or the association of more than one of them is an aspect to be considered individually; however, success depends on the methods that reduce microorganisms from the infected mucosa and the inner denture surfaces.

To enhance the treatment of denture stomatitis, it has been recommended to eliminate the contact between the denture biofilm and infected tissues, thus avoiding a reinfection cycle (4). This is possible through the use of a denture reline, especially with soft lining materials (4), which also results in the recovery of injured tissues and patient comfort (5). The greatest disadvantage of the soft lining materials, particularly the temporary ones, is the difficulty in keeping them clean because they are significantly softer and less resistant to brushing compared with denture-based acrylic resins, as well as being porous and incompatible with certain denture cleaning solutions, even in short periods of immersion (6). Thus, the soft lining materials are easily degradable and susceptible to microbial colonization (7).

The incorporation of antifungal agents to temporary soft lining materials has demonstrated to be effective and viable in order to extend their longevity and reduce biofilm accumulation (8). The association between antifungal agents and soft lining materials may be a logical therapy for denture stomatitis before replacing the old dentures and in a relatively short period of time, because it is a method of drug release in the oral cavity which simultaneously treats the injured periprosthetic tissues and *Candida* infection, with limited frequency of application and without relying

on the patient's cooperation (9). Because the temporary soft lining material's life cycle is approximately 14 days, the treatment period of denture-induced stomatitis using these drug-modified materials is similar to the period required for conventional topical antifungal agents (10). However, it has been shown that the incorporation of drugs at commercially available concentrations in soft liners may affect its morphological structure (11) and properties such as tensile strength (9,12), water sorption (13), modulus of elasticity, and weight (9), hardness (9,14) and roughness (14). In the search for antifungal concentrations compatible with the properties of these materials, Bueno et al. (15) determined the minimum inhibitory concentrations (MICs) for *C. albicans* biofilm of antifungal agents (nystatin, miconazole, ketoconazole, itraconazole and chlorhexidine) incorporated in temporary resilient liners (Trusoft and Softone). The authors observed that all drugs added to both materials were able to inhibit 90% or more of *C. albicans* biofilm in up to 14 days of incubation. Nevertheless, before the recommendation of this protocol clinically for the treatment of denture stomatitis, it is necessary to evaluate the effect of addition of drugs at MICs on the properties of the modified polymeric matrix.

Information on the effects of drug incorporation at MICs on the relevant properties of temporary resilient materials is lacking. A recent study demonstrated that after 14 days of water immersion, the addition of nystatin did not affect water sorption of two temporary soft lining materials (16). Despite these promising results, the authors identified no information in the literature regarding the effects of drug addition at MICs on other important properties of temporary soft lining materials.

Among the several properties to be evaluated in a polymer, hardness is regarded as one of the fundamental properties for rubbers because it represents a simple method to determine its modulus of elasticity (17). The hardness provides information about the quality of the material, because a rigid material is not suitable to be used as a base for resilient relining removable dentures (18). The greater the amount of plasticizers in the material, the softer it becomes (19). In this context, a major disadvantage of acrylic-based soft lining materials is the rapid loss of plasticizer, leading to gradual hardening (20). Another important property refers to the surface roughness, which is linked directly or indirectly to relevant factors related to removable dentures, such as retention, resistance to staining, microbial adhesion, health of oral tissues and patient comfort (21). Release of alcohol and plasticizers from acrylic-based soft lining materials to the liquid medium results in increased roughness (7). The rougher the surface of the liner material, the greater the biofilm formation, thus favoring the emergence/maintenance of oral pathologies (21).

The proposal of this study was to evaluate the effect of addition of antifungal agents at MICs on the Shore A hardness and surface roughness of two temporary soft lining materials. The hypotheses tested in this study were that both properties of the temporary soft lining materials would be adversely affected by the incorporation of antifungal agents at MICs for *C. albicans* biofilm.

Material and Methods

The analyses were performed on two soft lining materials: Softone (tissue conditioner) and Trusoft (resilient denture liner) (Bosworth Co, Midland, TX, USA). These materials are composed of a powder of prepolymer of polyethyl methacrylate and a liquid of a mixture of ethyl alcohol and an aromatic ester that acts as a plasticizer (22,23). The antifungal agents selected for this study were nystatin (Galena Química e Farmacêutica Ltda, Campinas, SP, Brazil), ketoconazole, miconazole, itraconazole (Pharma Nostra Comercial Ltda, Anápolis, GO, Brazil), and chlorhexidine diacetate 98% (Acros Organics, Morris Plains, NJ, USA).

Both materials were tested without drug incorporation (controls [Ct]), or with the incorporation of nystatin (Ny) 0.032 g, miconazole (Mc) 0.256 g, ketoconazole (Ke) 0.128 g, itraconazole (It) 0.256 g, or chlorhexidine diacetate (Chx) 0.064 g, each per gram of soft liner powder.

The specimens (n=8; 36 ×7×6 mm) (ASTM; 2002) were made using a stainless steel mold. Material powder (6 g) and drug power in their MIC were proportioned and mixed homogeneously. The liquid material was added to the mixture of powders and mixed according to the manufacturer's instructions. After mixing, the materials were poured into a mold placed on a glass slab. Then, another glass slab was placed over each filled mold and maintained under digital pressure until final plastification, as recommended by the manufacturer. After this, the specimens were separated from the molds and the edges were carefully smoothed with a scalpel (14). Specimens were then individually immersed in 17.5 mL of distilled water at 37 °C (MA 0324; Marconi Equipamentos para Laboratórios Ltda., Piracicaba, SP, Brazil) for 24 h, 7 days or 14 days (12,24) prior to the hardness/roughness measurements. Since the hardness measurement test does no allow reusing the samples, only independent specimens were fabricated in each evaluation period of the present study.

The hardness was measured on one side of specimen (14) using a Shore A durometer (GSD 709A; Woltest Indústria, Comércio, Importação e Exportação Ltda., São Paulo, SP, Brazil). The test was performed at a constant load of 10 N/s, and the results were obtained in Shore A units on a scale from 0 to 100 (14). Five measurements were carried out with a 6 mm distance between them (14) and the mean

was obtained for each specimen at each evaluation period.

The surface roughness was measured on the opposite side of each of the specimens using a surface roughness tester (Surftest SJ-301; Mitutoyo Corporation, Kanagawa, Japan) calibrated with a 0.8 mm cut-off and speed of 0.5 mm/s, resulting in a distance of 4.0 mm. Five measurements of surface roughness were performed, and the mean value (Ra) was obtained for each specimen at each evaluation period (14).

Since the study had 2 resilient materials, 5 drugs plus control group and 3 evaluation intervals, a total of 288 independent specimens were produced. The data obtained from the Shore A hardness and surface roughness were statistically analyzed using a 3-way analysis of variance (ANOVA) ("material," "antifungal agent" and "time") followed by the Tukey HSD test (α=0.05), using statistical software (SAS 8.0 for Windows; SAS Institute Inc., Cary, NC, USA). Post hoc power analysis was performed for statistical analysis of hardness and roughness data using personal statistical software (IBM SPSS 19, SPSS Inc., IBM Company, Chicago, IL, USA).

Results

For the number of specimens used for the hardness tests (n=8), this study was adequately powered (100%; α=0.05). ANOVA detected statistical significance ($p<0.001$) for the factors "material," "drug," and "time", and for the interaction among all factors. Table 1 shows the mean values and standard deviations of hardness for the evaluated groups.

The addition of drugs in both temporary soft lining materials resulted in no significant increase of Shore A hardness compared with the control group, except for Softone specimens with miconazole after 14 days ($p=0.0035$). In general, no significant changes in hardness of both materials were observed with the addition of nystatin, miconazole, or ketoconazole after 7 days of water immersion ($p>0.05$) compared with the control group. All groups modified by the incorporation of drugs in Trusoft liner presented significant reduction of Shore A hardness after 14 days ($p<0.001$) compared with the control group. The addition of itraconazole significantly reduced the hardness values of both materials ($p<0.05$), except for Softone after 24 h, which was not different from the control group ($p=0.577$). The addition of chlorhexidine caused a reduction in hardness values only for Trusoft liner after the evaluation at 7 ($p=0.041$) and 14 days ($p<0.001$).

During the 14-day period, Softone liner showed no significant increase of Shore A hardness ($p>0.05$). Addition of miconazole in Softone resulted in a significant increase of hardness only after 14 days compared with 24 h ($p<0.001$). Trusoft material demonstrated a progressive increase of Shore A hardness along the 14 days of water immersion ($p<0.001$). Similar behavior was observed for the groups modified by this material ($p<0.001$). However, for nystatin ($p=0.009$) and chlorhexidine ($p=0.002$), there was increase of hardness values compared with the 24-h period only after 14 days.

The comparison between the hardness of the two soft lining materials showed no statistically significant difference for the groups modified by addition of ketoconazole and chlorhexidine ($p>0.05$). There was statistically significant difference between the materials in the control group and with itraconazole only after 14 days ($p<0.001$), the group containing miconazole after 7 days ($p=.0004$), and the group containing nystatin after 7 days ($p=0.005$) and 14 days ($p<0.001$) of water immersion. In all these situations, Softone hardness values were lower than those of Trusoft.

For the number of specimens used for the surface

Table 1. Shore A hardness and standard deviation of Softone and Trusoft after drug incorporation over 14-day water immersion

Material	Time	Antifungal agents					
		Ct	Ny	Mc	Ke	It	Chx
Softone	24 h	14.6 (1.7) aA	14.3 (2.1) aA	16.1 (2.2) bA	10.2 (2.1) cA	11.1 (2.8) aA	15.8 (1.4) aA
	7 d	19.2 (1.6) aA	17.4 (1.7) aB	18.1 (1.3) bB	19.0 (2.3) aA	12.7 (1.4) aA*	17.1 (1.0) aA
	14 d	17.5 (1.9) aB	15.3 (2.5) aB	23.1 (3.6) aA*	16.3 (4.2) bA	8.7 (3.0) aB*	17.9 (2.0) aA
Trusoft	24 h	15.6 (4.0) cA	18.6 (1.4) bA	17.0 (1.2) bA	11.0 (2.2) bA	6.8 (1.7) bA*	15.3 (1.6) bA
	7 d	22.8 (2.6) bA	22.8 (1.5) bA	24.3 (1.0) aA	21.0 (1.9) aA	17.0 (1.6) aA*	18.0 (0.9) bA*
	14 d	31.4 (2.7) aA	23.9 (4.7) aA*	24.3 (2.1) aA*	19.9 (3.9) aA*	17.2 (4.1) aA*	21.1 (2.6) aA*

Ct, control; Ny, nystatin; Mc, miconazole; Ke, ketoconazole; It, itraconazole; Chx, chlorhexidine diacetate. Within each material in the same group, means (SD) followed by the same lowercase letter are not significantly different ($p>0.05$) from 24 h. Within the same group at the same time, means (SD) followed by similar uppercase letters are not significantly different ($p>0.05$) for the comparison between the materials. Within each material at the same time, means (SD) followed by asterisk are significantly different ($p<0.05$) from the control group.

roughness test, the present study was adequately powered (96.8%; α=0.05). The results showed statistically significant difference for the factors "drug" (p<0.001), "time" (p=0.005), and the interaction between the factors "material," and "time," and "drug," and "time" (p<0.001), as well the interaction between all the factors (p=0.002). Table 2 shows the mean values and standard deviations of surface roughness (μm) for the evaluated groups.

After 24-h water immersion, there was no statistically significant difference in surface roughness values of both materials in all modified groups compared with the control (p>0.05). The addition of nystatin in both materials, ketoconazole in Softone and chlorhexidine in Trusoft, also resulted in no changes in surface roughness values compared with the control group after 7 and 14 days of immersion (p>0.05). There was significant increase in surface roughness of both materials with the addition of itraconazole and chlorhexidine after 7 and 14 days compared with the control group (p<0.05). After 7 days, both materials containing miconazole and Trusoft containing ketoconazole showed significant increase of surface roughness compared with the control (p<0.05).

Compared with the 24-h period, a significant reduction of roughness was observed for Trusoft (p=0.001) and Softone (p=0.027) after 7 and 14 days of water immersion, respectively. For Trusoft, there was a significantly lower surface roughness with the addition of nystatin, miconazole and ketoconazole after 14 days, compared to those at 24 h (p<0.05). However, Softone containing itraconazole and chlorhexidine showed a significant increase of roughness in both the 7- and 14-day evaluation periods compared with the 24-h period (p<0.05). For the other groups at both 7 and 14 days, there was no change in roughness values when compared with the initial values (24 h) (p>0.05).

Comparing the temporary resilient liners, there was no statistically significant difference between the control groups after 24 h and 7 days of water immersion (p>0.05). Softone control demonstrated significantly lower surface roughness than Trusoft control only after 14 days (p<0.001). No difference was observed between the roughness values of both materials containing ketoconazole or chlorhexidine in all evaluation periods (p>0.05). After 7 days in distilled water, Softone groups modified by nystatin or miconazole demonstrated significantly lower surface roughness than the same Trusoft groups (p<0.05). However, for Softone, nystatin and itraconazole resulted in higher surface roughness in the 14-day evaluation period (p<0.001).

Discussion

Antifungal addition in Softone and Trusoft temporary resilient liners did not change hardness values compared with the controls, except for miconazole in Softone. The addition of nystatin in Softone and Trusoft, ketoconazole in Softone, and chlorhexidine in Trusoft resulted in no significant differences in roughness values compared with the control groups after 7 and 14 days of water immersion. Therefore, the alternative research hypothesis stating that antifungal incorporation detrimentally affected the hardness and surface roughness of temporary soft lining materials was partially accepted.

During clinical use, the soft lining materials are susceptible to hardness changes, which determine the ability of a material to cushion the impacts and is related to its modulus of elasticity (17). Therefore, it is desirable that these materials have a low hardness (17). Maintaining a satisfactory hardness is one of the most complicated factors for denture acrylic lining materials, because they are not stable in an aqueous medium (24). In their life cycle, these materials are immersed in saliva, food, water and hygiene solutions, which are responsible for leaching of

Table 2. Surface roughness (μm) and standard deviation of Softone and Trusoft after drug incorporation over 14-day water immersion

Material	Time	Antifungal agents					
		Ct	Ny	Mc	Ke	It	Chx
Softone	24 h	4.30 (1.21) aA	3.69 (0.40) aA	4.68 (0.43) aA	4.03 (0.42) aA	4.74 (0.86) bA	3.17 (0.51) bA
	7 d	3.26 (0.39) aA	3.55 (0.45) aB	4.60 (0.38) aB*	3.65 (0.59) aA	6.89 (0.77) aA*	4.55 (0.58) aA*
	14 d	2.98 (0.58) bB	3.84 (0.66) aA	3.81 (0.43) aA	3.61 (0.45) aA	6.25 (0.72) aA*	5.04 (0.53) aA*
Trusoft	24 h	4.48 (1.06) aA	5.04 (0.63) aA	5.16 (0.50) aA	4.45 (0.28) aA	5.81 (0.97) aA	3.24 (0.71) aA
	7 d	2.94 (0.48) bA	3.72 (0.63) abA	4.66 (0.50) aA*	4.63 (0.95) aA*	6.34 (1.30) aA*	4.29 (0.67) aA
	14 d	3.61 (0.94) aA	3.20 (0.35) bB	3.64 (0.79) bA	3.19 (0.54) bA	5.74 (0.61) aB*	4.03 (0.76) aA

Ct, control; Ny, nystatin; Mc, miconazole; Ke, ketoconazole; It, itraconazole; Chx, chlorhexidine diacetate. Within each material in the same group, means (SD) followed by the same lowercase letter are not significantly different (p>0.05) from 24 h. Within the same group at the same time, means (SD) followed by similar uppercase letters are not significantly different (p>0.05) for the comparison between the materials. Within each material at the same time, means (SD) followed by asterisk are significantly different (p<0.05) from the control group.

components or sorption of fluids (3). The balance between the loss of components and fluid sorption affects the performance and dimensional stability of resilient liners, because these qualities are associated with expansion, distortion, increased hardness and roughness, unpleasant odor, microbial colonization and color changes (18). The hardness of temporary soft lining materials increases in a short time, which leads to a gradual loss of the cushioning effect (20,23). Loss of viscoelasticity of soft the lining materials may irritate the supporting tissues and accelerate their deterioration (23).

The addition of antifungal agents like nystatin (13) and chlorhexidine (9,16) on resilient liners may also increase their level of water sorption. However, in this study, the addition of all drugs at MICs did not produce significant differences or significantly reduce Shore A hardness values of both temporary resilient liners, except for miconazole in Softone after 14 days water immersion. These results are probably due to the molecular weight of miconazole, which is lower than those of nystatin, ketoconazole, and chlorhexidine (11). The small particles of miconazole present greater diffusibility within the polymer matrix. This leads to a greater degree of solvation (23), which may have contributed to increase the hardness of Softone modified with miconazole in the 14-day period.

While the plasticizer concentrations of the materials used in this study are not stated by the manufacturer, it is expected that Softone, being a tissue conditioner, presents a greater amount of plasticizer that Trusoft, which is a temporary resilient denture liner (20). The plasticizers reduce the glass transition temperature of the polymer, making softer the material. The higher the amount of plasticizer, the lower the material hardness (20). These data corroborate partially the results of the present study, because a gradual increase was observed in Trusoft hardness during the water immersion period and, when there was a difference between the materials under the evaluated conditions, Softone hardness was lower than Trusoft. It may be hypothesized that the amount of plasticizer of Softone is greater compared with Trusoft.

Softone liquid contains ethyl alcohol and plasticizers (dibutyl phthalate and butyl benzoate). Because of their low molecular weight, aromatic esters such as dibutyl phthalate readily leach from the resilient material (22). For this reason, despite the tissue conditioners' high initial softness, they become hard after a few days (19). Therefore, the tissue conditioners are ideally recommended for 3 or 4 days of use and should not exceed two weeks (22). Trusoft liquid has only alkyl phthalate and it is known that this liquid in temporary soft lining materials may contain 25% to 50% plasticizer (23). According to the manufacturer, Trusoft liquid contains ethanol and a low plasticizer concentration, which provides adequate softness for up to 6 months.

There are no limitations for clinically acceptable values for the Shore A hardness of temporary soft lining materials. However, a variation from 13 to 49 Shore A units in 24 h is regarded as acceptable for clinical use of these materials (5). A Shore A hardness of 20–25 units without changes during the life cycle of a resilient material is considered clinically suitable, as reported by Gonzalez (10). The highest hardness values observed for both materials of this study within 14 days (23.1 and 31.4 Shore A units for Softone and Trusoft, respectively) are below the highest mean values postulated for 24 h (5), and close to those suggested in the literature as appropriate for their clinical use (10). The mean hardness of Trusoft modified by drugs after 14 days is also lower than an autopolymerized long-term resilient denture liner not modified after 24 h, 7 days, and 30 days of water immersion (30 units Shore A) (24). Yilmaz et al. (2) found higher Shore A hardness values (60–78 units) for three temporary soft lining materials similar to those tested in this study. Based on the methodology and results of this *in vitro* study and previous studies, it may be assumed that regardless of the modification by the addition of antifungal agents, the hardness changes observed by both soft lining materials would not be considered significant enough to interfere with their clinical use. Thus, it is likely that the addition of the drug MICs in Softone and Trusoft did not result in clinically observable deleterious effects within the period recommended for the treatment of denture stomatitis (14 days).

Although the surface roughness is not the only property related to microbial attachment, rougher surfaces generally present higher microbial adherence, resulting in greater biofilm retention and resistance to the shear forces of toothbrushing. Thus, the material should present a smooth surface to facilitate its cleaning, preventing biofilm formation and consequent inflammation of the oral mucosa (21). The surface smoothness of the specimens from the present study was produced by glass slides, as used in a similar research (21). Yet, this methodology does not mimic the clinical conditions, as the used glass slab results in a more polished surface compared to supporting tissues during the chairside denture reline technique.

For both temporary soft lining materials used in this study, there were significant differences found in roughness between the groups modified by the addition of antifungal agents compared with the control groups after 24 h water immersion. It is known that surface roughness of soft lining materials increases after longer water immersion times, due to the release of alcohol and plasticizers (7). Furthermore, the release of these components associated with water sorption results in loss of surface integrity of the soft lining materials (22). However, in this study after 14 days, the

surface roughness of the temporary soft lining materials generally decreased or was not altered by incorporation of the drugs, except for Trusoft and Softone modified by itraconazole and Softone modified by chlorhexidine. The reduction of surface roughness with water immersion may be related to the composition and inherent characteristics of the materials. The loss of soluble constituents after water immersion may result in formation of voids and pores (6). With time, these pores increase, resulting in craters; the edges of these craters may decrease, making the specimens smoother (6). Future studies are required to confirm this hypothesis within the conditions of this research.

The increase in surface roughness of Softone modified by chlorhexidine concurs with a previous study with the scanning electron microscopy analysis showing higher values for auto-polymerizing denture base resins with time after addition of this drug (8). Chlorhexidine incorporated into an acrylic soft lining material is scattered as particles within the polymer matrix (11) and is successfully released in therapeutic levels for extended periods of time, even in low amounts (8). Yet, the pattern of incorporation of chlorhexidine into the polymer matrix increased the porosity, particularly on the surface (8). This may be the reason for the higher surface roughness values obtained with the addition of chlorhexidine in Softone.

With the incorporation of itraconazole, at 7 days and 14 days, an increase in surface roughness in both Trusoft and Softone was observed compared with the controls. This may be associated with its processing in pellet form rather than powder. The results may also be explained by the different distribution of each drug in the material matrix (11) and the different size of its particles. Other aspects that may have interfered with Softone surface integrity is related to the release of itraconazole, which also depends on the fragility of the polymer matrix, amount of the drug and material porosity (11). Further investigations are advised to evaluate these hypotheses under the conditions of the present study.

In this study, the mean surface roughness values were lower than previously observed (5.04 µm to 6.00 µm) (14,21) in temporary soft linig materials without modification. Similar mean values of surface roughness for auto-polymerizing soft lining materials (2.8 µm to 4.2 µm) and tissue conditioners (1.3 µm to 7.9 µm) were also previously described (22). These variations of roughness values may be ascribed to differences among the used experimental conditions. *In vivo* studies are required using the same protocol of the present assess whether the found surface roughness values are clinically suitable with the temporary soft lining materials modified by the drugs for up to 14 days.

The addition of the nystatin at MIC to inhibit the formation of *C. albicans* biofilm did not affect adversely the Shore A hardness and surface roughness in both temporary soft lining materials up to 14 days water immersion. Nystatin is considered the first-line agent for topical treatment of uncomplicated cases of oral candidiasis because of its both fungistatic and fungicidal effect *in vitro* against a wide variety of yeasts and yeast-like fungi, nevertheless, this antifungal antibiotic exhibits no appreciable activity against bacteria. The complex denture biofilm is formed not only by fungi, but also bacteria, which favor the adhesion of fungal cells to the internal surfaces of dentures by co-aggregation. Thus, in spite of the favorable results observed in this study for nystatin, the addition of drugs with a wide antimicrobial action in temporary liners and tissue conditioners should be considered for denture stomatitis treatment. In the present study, chlorhexidine diacetate resulted in no harmful changes for the Shore A hardness of both temporary liners and the roughness of Trusoft. As the temporary soft lining materials are used for short periods, it has been suggested that small changes in physical and mechanical properties (as observed for the roughness of Softone modified with chlorhexidine) do not contraindicate their modification by antifungal agents (9,12,16). This protocol is important for improving the health of the soft tissues prior to the fabrication of new dentures. Also, it is important to consider that chlorhexidine presents significant substantivity, resulting effectively in longer evaluation periods, especially if used in powder form (25), as observed in this study. Nevertheless, the results of this *in vitro* research should be cautiously applied to *in vivo* conditions, because the oral environment and the denture base design were not considered. Furthermore, only one brand of each type of material (tissue conditioner and temporary soft lining material) was evaluated, so the findings should not be extrapolated to other trademarks. In order to safely indicate the addition of antifungal agents in temporary soft lining materials for denture bases, further *in vitro* studies are required to evaluate other factors such daily drug release and drug pattern of incorporation. In addition, during their life cycle, the temporary soft lining materials may clinically undergo thermal changes, pH variations and deformation by occlusal load.

Resumo

Avaliar a adição de antifúngicos nas mínimas concentrações inibitórias (MCIs) para o biofilme de *Candida albicans* sobre a dureza e rugosidade da reembasadores resilientes temporários. Foram confeccionados corpos de prova (n=8; 36×7×6 mm) a partir de um condicionador de tecido (Softone) e um reembasador resiliente (Trusoft), sem (controle) ou com a incorporação de fármacos nas MCIs: nistatina (0,032 g/mL), diacetato de clorexidina (0,064 g/mL), cetoconazol (0,128 g/mL), miconazol (0,256 g/mL) e itraconazol (0,256 g/mL). Os corpos de prova foram armazenados em água destilada a 37 °C durante 24 h, 7 dias e 14 dias antes das mensurações de dureza e rugosidade. Os dados foram analisados por

ANOVA 3-fatores e teste de Tukey HSD (α=0,05). A adição dos antifúngicos em ambos os materiais não demonstrou nenhuma alteração evidente na dureza ou diminuiu esta propriedade em comparação com o controle, exceto para o miconazol no Softone que aumentou a dureza após 14 dias (p=0,003). A adição de nistatina aos dois materiais, clorexidina no Trusoft e cetoconazol no Softone não resultou em alterações significativas de rugosidade em comparação com o controle após 7 e 14 dias (p>0,05). Nestes períodos, o itraconazol aumentou a rugosidade de ambos os materiais (p<0,001). A adição de todos os antifúngicos, exceto para o miconazol no Softone, não resultou em efeitos deletérios sobre a dureza dos materiais ao longo do tempo de avaliação. As MCIs de nistatina em ambos os materiais reembasadores resilientes temporários, cetoconazol no Softone e clorexidina no Trusoft não produziram efeitos deletérios para a rugosidade em até 14 dias.

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Received August 26, 2016
Accepted February 20, 2017