CASE REPORT

EFFECT OF ADDITION OF ANTIFUNGAL AGENTS ON THE ULTIMATE TENSILE STRENGTH OF TEMPORARY SOFT DENTURE LINERS

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ABSTRACT

Aim: To investigate the ultimate tensile strength of temporary soft denture liners modified by minimum inhibitory concentrations (MICs) of antifungal agents for Candida albicans biofilm (SC5314) determined in previous microbiological research.

Materials and Methods: Dumbbell-shaped specimens (n = 7) with a central cross-sectional area of 6 x 3 x 33 mm were produced by Softone and Trusoft, without (con-trol) or with incorporation of drugs in powder form at MICs for C. albicans biofilm (per g of material powder): nystatin (0.032 g), chlorhexidine diacetate (0.064 g), ketoconazole (0.128 g), miconazole (0.256 g), and itraconazole (0.256 g). After plas-ticization, specimens were immersed in distilled water at 37°C for 24 hours, 7 or 14 days, and then tested in tension in a universal testing machine at 40 min/min. Data of tensile strength (MPa) and elongation percentage (%) were submitted to 3-way ANOVA and Tukey's test (a = 0.05).

Results: At the end of 14 days, the tensile strength for both materials was significantly lower in the groups modified by miconazole and itraconazole compared to the other groups (p < 0.0001), which showed no significant difference between them (p > 0.05). After 7 and 14 days in water, miconazole and itraconazole added into both materials resulted in significantly lower elongation percentages compared to the other antifungal agents and control (p < 0.0001), which were similar to each other (p > 0.05).

Conclusions: The addition of the nystatin, chlorhexidine, and ketoconazole at MICs for C. albicans biofilm resulted in no harmful effects on the tensile strength and elongation percentage of the temporary soft denture liner materials up to 14 days.

INTRODUCTION

Species of the *Candida* genus live in commensalism in different parts of the body of most healthy individuals. Under certain environmental conditions, these microorganisms may act as opportunistic pathogens and colonize mucosa and tissues, causing local and systemic infections.[1] Oral candidosis is the most common fungal infection in humans, especially in the elderly and complete denture wearers. Denture stomatitis is the most common type of oral candidosis and the most frequent mucosal alteration in the elderly. Despite its acknowledged multifactorial etiology, infection by *Candida,* especially *Candida albicans,* has been indicated as the main factor associated with denture stomatitis, affecting 93% of individuals.[1-4]

Topical and/or systemic antifungals are effective in minimizing the signs and symptoms of denture stomatitis.[2,5] However, these drugs cannot reach a therapeutic antifungal concentration on the denture surfaces,[6,7] and therefore mucosal reinfection occurs rapidly after treatment completion.[2] An

effective treatment of denture stomatitiswould ideally require a therapy based on the sustained release of antifungal drugs that may reach sufficient therapeutic concentrations to eliminate the *Candida* from both the supporting tissues and infected denture surfaces.[5]

The feasibility of using a drug delivery system by incorporation of antifungal agents in denture base materials has been evaluated.[8,9] This system is even more advantageous when used in temporary soft denture liners, which are easily degradable and highly susceptible to microbial colonization.[10] This occurs because the drugs gradually released on the infected tissues treat the fungal infection and prevent the accumulation of prosthetic biofilm on these biomaterials, avoiding reinfection via prosthesis.[11,12] The incorporation of antifungals in short-term resilient liners allows these materials, besides aiding the treatment of denture stomatitis, to perform their functions of favoring the recovery of injured periprosthetic tissues, and promoting comfort to the individual during their life cycle,[13] which corresponds to a similar period as the treatment with a conventional topical antifungal (14

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days).[11,12] Despite these promising results, the addition of drugs at commercially available concentrations into denture base materials may affect the material's changes at the surface17 and properties such as tensile strength,[11] hardness,[11,18] and roughness.[18] In the mixture of powder and liquid of the resilient denture liner, the ethanol in the liquid is absorbed by the polymer particles, which in turn are swollen. This disarranges the polymeric chains, allowing the greater molecules of plasticizers to penetrate between them. The addition of a substance in a resilient material might impair the plasticizers to penetrate in the polymeric chains and form a softened gel.[19] With this change in the gel formation, the properties of these materials may be affected.

In the search for antifungal concentrations compatible with the properties of short-term soft liners, Bueno *et al*[20] determined the minimum inhibitory concentrations (MICs) for *C. albicans* biofilm of antifungal agents (nystatin, miconazole, ketoconazole, itraconazole and chlorhexidine) incorporated in temporary resilient liners. The authors observed that all drugs incorporated in both materials were able to inhibit 90% or more of *C. albicans* biofilm for up to 14 days of incubation; however, before this protocol is clinically recommended 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.

For the performance evaluation of resilient liners, their physical, mechanical, and biological properties including tensile strength, hardness, roughness, sorption and solubility, biocompatibility, and peel bond strength to denture base must be considered.[21] The tensile strength refers to the maximum material strength under tension, being considered a fundamental property for rubber materials.[22,23] However, while rubbers as tissue conditioners and resilient liners are subjected only to compression and shear forces, the tensile properties are relevant in the overall analysis of performance and quality of these materials, since they are clinically related to the material ability to resist rupture when the relined denture is in function, under deleterious oral humidity conditions.[24] Elongation is the maximum plastic deformation resulting from the application of tensile forces. The greater the material flexibility, the greater the capacity to absorb impacts; however, this same flexibility acts in the tensile movement, in the same path yet in the opposite direction.[22,23] Thus, ideally the elongation of a resilient liner should not be too low to avoid significant increase in hardness and loss of functions of comfort and impact absorption, and simultaneously it should not be too high to interfere with elastic deformation of the material.[22,23]

Despite this importance, the authors have identified no information in the literature regarding the effects of drug incorporation at MICs on these properties of temporary soft materials. Therefore, this study evaluated the effect of addition of antifungal agents at MICs on the ultimate tensile strength and elongation percentage of short-term resilient liners during their life cycle. The alternative hypothesis was that drug incorporation at MICs affects the properties of both materials up to 14 days of water immersion.

METHODOLOGY

Dumbbell-shaped specimens (n = 7) with a central crosssectional area of 6 × 3 × 33 mm (ASTM D412)25 were produced with a tissue conditioner and a temporary resilient liner,without (control) or with incorporation of five drugs

according to the study groups: nystatin-Ny, chlorhexidine diacetate-Chx, ketoconazole-Ke, miconazole-Mi, and itraconazole-It. For each specimen, material powders were mixed with the drugs in power form at their MICs: 0.032 g for Ny, 0.064 g for Chx, 0.128 g for Ke, 0.256 g for Mc, and 0.256 g for It per each g of material powder. Afterwards, liquids of each material were added to the mixture of powders for insertion in a matrix positioned between two glass slabs until plasticization of soft liners, following the manufacturers' instructions (6 to 7 min). Each specimen was carefully separated from the mold, and the edges were smoothed. Specimens were then individually immersed in distilled water at 37°C for 24 hours, 7 or 14 days prior to the tensile strength tests.

The tensile strength test was performed in a testing machine at 40 mm/min.24 An extensometer was connected to the testing machine during the tests to measure the elongation of specimens. The tensile strength values were obtained in megapascals (MPa), and the elongation values were obtained in percentage in relation to the specimen length (33 mm).
Data were statistically analyzed by 3-way ANOVA ("material," "antifungal agent," and "time") and Tukey HSD test (α = 0.05). Post hoc power analysis was performed for statistical analysis of tensile strength and elongation data using personal statistical software (SPSS 22; SPSS Inc., IBM Co., Armonk, NY).

RESULTS

For the number of specimens used for the tensile strength test (n = 7), this study was adequately powered (Trusoft-95%, Softone-96%; a = 0.05). ANOVA showed significance (p < 0.0001) for the interaction "material x drug x time."

Table 1 shows the mean values and standard deviations of tensile strength for the experimental conditions. Table 1 shows that in all evaluation periods there was significant reduction of the mean tensile strength for groups modified by Mc and It in relation to the control (p < 0.0001), which was statistically similar to the other groups (p > 0.05). Comparison of the tensile strength did not reveal statistically significant difference between the materials analyzed, considering the same experimental condition (time x drug) (p > 0.05). Table 1 also shows that the tensile strength did not vary significantly between periods of 24 hours, 7 and 14 days for the same experimental condition (material x drug) (p > 0.05).

Power analysis also demonstrated adequate sample size (n = 7) for elongation percentages, revealing a sample power of 95% for both resilient materials (a = 0.05). ANOVA detected statistical significance in the interaction "material x drug x time" (p = 0.0057). The mean values and standard deviations of elongation percentages for the study groups are exhibited in Table 2. Table 2 demonstrates that for Trusoft in all evaluation periods the addition of Mc and It caused significant reduction in the mean percentage of elongation (p = 0.0004) in relation to the other study groups, which were not statistically different from each other (p > 0.05). This result was also observed for Softone at 7 days of water immersion. After 24 hours and 14 days of evaluation, the lowest tensile strength values for Softone were observed with addition of Mc (p < 0.0001). In these periods, the It also reduced the mean tensile strength of Softone in relation to the other study groups, which were not different from each other (p > 0.05). Comparison of the

elongation percentage did not reveal statistically significant difference between materials, considering the same experimental condition (time x group) (p > 0.05), except for groups modified by Mc (p = 0.0011) and It at 14 days (p = 0.0002), which presented the lowest values for Softone.

Table 1 Tensile strength (MPa) ± standard deviations for the experimental conditions

	Groups	Trusoft	Softtone
	Control	0.146 ± 0.034	0.124 ± 0.030
	Ny	0.140 ± 0.028	0.145 ± 0.044
	Mc	0.071± 0.006	0.078 ± 0.003
24 hours	Ke	0.157 ± 0.025	0.131 ± 0.013
	ChK	0.131 ± 0.024	0.132 ± 0.028
	It	0.089 ± 0.010	0.077 ± 0.008
	Control	0.122 ± 0.025	0.131 ± 0.016
	Ny	0.118 ± 0.021	0.135 ± 0.035
	Mc	0.081 ± 0.012	0.076 ± 0.007
	Ke	0.140 ± 0.014	0.155 ± 0.028
7 Days	Chx	0.141 ± 0.026	0.133 ± 0.019
	It	0.073 ± 0.005	0.066 ± 0.009
	Control	0.129 ± 0.01	0.167 ± 0.038
	Ny	0.131 ± 0.015	0.168 ± 0.026
	Mc	0.073 ± 0.010	0.074 ± 0.007
14 Days	Kg	0.142 ± 0.019	0.179 ± 0.030
	ChK	0.135 ± 0.025	0.1B5 ± 0.041
	It	0.085 ± 0.011	0.069 ± 0.006

Vertically, for each layer of each evaluation period, different capital letters indicate significant differences between the groups for the same material (*p* < 0.05); horizontally, for each group within the same period, different lowercase letters indicate significant differences between the materials (*p* < 0.05).

Table 2 Elongation percentages (%) ± standard deviations for the experimental conditions

	Groups	Trusoft	Softtone
	Control	331.7 ± 51.7	262.9 ± 32.7
	Ny	338.7 ± 35.7	292.6 ± 82.3
	Mc	185.2 ± 19.9	141.2 ± 11.4
24 hours	Ke	314.0 ± 32.2	269.6 ± 30.1
	ChK	318.1 ± 38.5	255.0 ± 46.4
	It	256.8 ± 34.6	211.2 ± 22.5
	Control	370.5 ± 82.7	266.3 ± 37.7
	Ny	367.0 ± 89.7	318.0 ± 60.4
	Mc	212.0 ± 16.9	175.9 ± 16.9
	Ke	298.7 ± 39.6	295.2 ± 51.8
7 Days	Chx	380.4 ± 91.1	332.6 ± 37.0
	It	257.6 ± 29.3	217.8 ± 45.7
	Control	434.3 ± 42.5	364.3 ± 57.4
	Ny	418.4 ± 66.7	404.9 ± 47.8
	Mc	212.5 ± 33.7	148.8 ± 29.0
14 Days	Kg	367.7 ± 52.1	359.2 ± 40.1
	ChK	431.4 ± 64.4	398.0 ± 48.6
	It	306.1 ± 19.9	228.6 ± 42.1

Vertically, for each layer of each evaluation period, different capital letters indicate significant differences between the groups for the same material (*p* < 0.05); horizontally, for each group within the same period, different lowercase letters indicate significant differences between the materials (*p* < 0.05).

DISCUSSION

The alternative research hypothesis stating that antifungal incorporation affects the properties of temporary resilient liners was partially accepted because only the addition of Mc and It at their MICs for the *C. albicans* biofilm changed the tensile strength and elongation of both materials over a 14-day immersion period in distilled water. There is scarce information on the effect of addition of drugs at commercially available concentrations on the tensile strength of tissue conditioners.[11,24] Moreover, there are no studies available on the properties of tensile strength and elongation percentage after modification of temporary soft liners by drugs at MICs, which precludes comparison with the present results.

Urban *et al*[24] observed that the incorporation of Ny at concentrations up to 1,000,000 U (0.164 g) did not interfere with the tensile strength of a tissue conditioner (Dura Conditioner) in up to 7 days of water immersion, corroborating the results obtained in this study for this drug. At the 14-day interval, Schneid[11] observed only cohesive failures when the tensile strength test was applied at the union between a heat-polymerized denture base acrylic resin and a tissue conditioner modified by commercial concentrations of Chx (0.250, 0.500, and 1 g) and Ny (0.125, 0.250, and 0.500 g). That is to say, the tensile bond strength of the material was not altered or was even improved by addition of these drugs,[11] in agreement with the present findings for Ny and Chx. Thus, it is probable that the MICs of Ny, Chx, and Ke (0.032, 0.064, and 0.128 g, respectively) were insufficient to cause changes in the tensile strength and elongation of short-time resilient materials evaluated in the present study. These MICs, which are much lower than the drug concentrations tested in the investigations of Urban *et al*[24] and Schneid,[11] inhibited the fungal biofilm of materials tested in 90%, which may be advantageous for maintenance of other properties of the modified polymeric matrix.

Conversely, in the present study, the incorporation of Mc and It in all study periods promoted reduction of tensile strength and elongation percentage of both materials analyzed compared to the other study groups. This result may be basically assigned to the quantity of drug incorporated to these materials, since the MICs of Mc and It (0.256 g) were much higher compared to the other antifungals tested. During specimen fabrication, there was difficulty in incorporating Mc and It to the material powders, as well as in the manipulation of liners modified by these drugs, thus delaying the plasticization. Srivatstava *et al*[26] observed incomplete gelling of a tissue conditioner after the incorporation of origanum oil, which resulted in significant lower tensile strength values. Also, according to the authors, the lower content of plasticizer with incorporation of antimicrobial agent might have reduced the disentanglement of polymer beads, yielding a weak cohesion among the polymer chains.[26] Schneid[11] also observed decrease of mean tensile strength with increasing fluconazole concentration into a tissue conditioner. Thus, it is probable that the higher concentration of Mc and It tested in this study interfered with formation of the polymeric matrix, reducing the tensile strength of soft liners. Conversely, reduction of the elongation percentage caused by Mc and It observed in this study may be discussed only by indirect comparisons, since the authors identified no information in the literature regarding this property for resilient liners modified by drugs. It is possible that materials modified by higher MICs of Mc and It became more rubbery, leading to lower percentage of specimen length under tension upon rupture.

The divergent outcomes found in this study for It and Mc may also be explained by the different distribution of each drug in the material matrix and by the distinct size of their particles.[17] The Mc presents lower molecular weight than Ny, Ke, and Chx,[17] and its small particles present greater diffusibility inside the polymeric matrix, leading to a higher level of

solvation.27 Therefore, it may be assumed that the greater solvation caused by Mc may have resulted in lower resilience and consequently lower tensile strength for the resilient materials analyzed in this study. The results obtained with It may be associated with processing of this drug, which is commercially available as pellets. Though ground and processed in an ultrafine sieve, it is probable that the final itraconazole powder still presented remnants of pellets, favoring the reduction in tensile strength. Other aspects that may explain the results obtained for Mc and It are associated with fragility of the modified polymeric matrix and material porosity after incorporation of these drugs with higher MICs.28 Future investigations should be conducted to evaluate these hypotheses following the conditions adopted in this study.

Even though previous studies established clinically acceptable mean values for the tensile bond strength between resilient liners to denture base resins, there are no references of adequate values for the tensile strength only of the material, nor for its elongation. Therefore, additional studies on these properties, especially for short-term resilient liners, are still required.

The results demonstrated that, for most experimental conditions (drug x time), the tensile strength and elongation percentages were not statistically different between the two temporary soft liners analyzed. Despite the similar composition of these materials, according to the literature,29,30 it is expected that Softone, which is a tissue conditioner, presents a greater quantity of plasticizer than Trusoft, which is a temporary resilient liner. This would cause significant differences between their properties, as observed for Shore A hardness in a previous study.11,18

CONCLUSION

It can be concluded from the study that among the drugs tested for the treatment of denture stomatitis, the addition of Ny (polyene antifungal), Chx (antimicrobial), and Ke (azole antifungal) at their MICs for the *C. albicans* biofilm did not adversely affect the tensile strength and elongation percentage of the temporary resilient liners during their life cycle (14 days); Moreover, the results of the present in vitro investigation should be carefully applied to clinical conditions, since relevant factors such as oral environment and denture base design were not considered in this methodology.

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