BASIC SCIENCE RESEARCH

Effect of the Association of Nystatin with a Tissue Conditioner on its Ultimate Tensile Strength

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Purpose: **This study evaluated the ultimate tensile strength of a tissue conditioner without nystatin incorporation (GI—control group) and the same tissue conditioner modified by the addition of nystatin in two concentrations: GII—500,000 International Units (U) and GIII—1,000,000 U, in which each milligram of the medicament corresponded to 6079 U.**

Materials and Methods: **Dumbbell-shaped specimens ($N = 7$) with a central cross-sectional area of 33 × 6 × 3 mm were produced for the three experimental groups. After polymerization following manufacturer's instructions, specimens were immersed in distilled water at 37°C for either 24 hours or 7 days and then tested in tension in the MTS 810 at 40 mm/minute. Data were analyzed by two-way ANOVA followed by Tukey's test, at 95% level of confidence.**

Results: **The means (force-grams (gf) ± standard deviation) of the ultimate tensile strength were: GI—634.29 ± 122.80; GII—561.92 ± 133.56; and GIII—547.30 ± 73.47 for 24-hour storage, and GI—536.68 ± 54.71; GII—467.50 ± 143.51; and GIII—500.62 ± 159.76 for 7-day storage. There were no statistically significant differences among the three experimental groups ($p > 0.05$). The ultimate tensile strength means of all experimental groups after 7 days were significantly lower than those observed after 24 hours ($p = 0.04$).**

Conclusions: **The results of this study suggest that the addition of nystatin into the tissue conditioner investigated in concentrations below 1,000,000 U did not affect its ultimate tensile strength.**

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ORAL CANDIDAL infection is a concern in dentistry. Particularly, denture stomatitis affects 65% of healthy adult mouths.[1] It is an opportunistic infection related to an inflammatory process, which compromises the mucosal surface beneath dentures. In spite of its multifactorial etiology, *Candida albicans* has been established as a primary etiologic agent;[2] however,

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Candida-induced denture stomatitis presents other factors that contribute to the etiology including trauma and poor hygiene.[2]

The adherence and accumulation of microbial plaque on a denture will produce mucosal abnormalities that can compromise the denture-supporting tissues. From the continuous use of prostheses, the denture base and space between mucosa and surface of the denture provide a refuge for any potential pathogen, encouraging its colonization and proliferation.[3] Dentures with poor adaptation can rub the underlying mucosa, contributing to non-vital epithelial cell dislodging. The accumulation of cell debris, food particles, and other organic matters provides a propitious environment for microbial growth to pathogenic proportions even in the absence of general predisposing factors.[3] After microbial accumulation, there is an intense immunological reaction, and denture stomatitis develops.

Effective therapy must focus on the multifactorial etiology of the disease.[4] The treatment plan should include the substitution of old prostheses, elimination of anatomical irregularities, establishment of a non-traumatic occlusion, nutritional restitution, oral hygiene instructions, antifungal treatment, and systemic evaluation.[2] Also, to protect and preserve the integrity of the epithelium, the patient should sleep without dentures. This procedure will temporarily remove large numbers of potential pathogens from the oral cavity and reduce the duration of traumatic effects, including heavy grinding habits, which occurs more often during sleep.[3]

The aim of an antifungal therapy (denture cleaning, disinfection, and topical medication) prior to the construction of new prostheses is the reduction of acute candidal overgrowth to levels that can be controlled by the host's defenses. Nystatin and amphotericin B have been prescribed as the antimycotics of choice for the topical treatment of oral candidosis.[3]

However, the success of topical application of drugs in the oral cavity may be compromised by some factors, such as the absence of the discomfort caused by the infection, the medication expenses for an unperceived need, the continuing use of the dentures during the medication, the unpleasant taste,[5] and the frequency of dosage.[4] Besides, it is impossible to maintain the effective concentration in the infected location due to saliva and deglutition, which quickly dissolve and eliminate any drug from the oral cavity. Thus, the incorporation of an antifungal agent into one tissue conditioner can reduce the limited compliance of patients[5] and the cost of the treatment. Also, the action of the drug becomes prolonged, enabling tissue recovery[6] and providing a more predictable therapeutic outcome.[5]

The incorporation of antifungal agents into tissue conditioners has been shown to be effective and viable in some in vitro and in vivo studies.[3,5,7,8] Carter, Kerr, and Shepherd have shown that the soft denture-liner Viscogel (De Trey, Surrey, England) with antifungal agents is effective against *C. albicans* over a long period.[3] In microbiological assays, the nystatin was able to inhibit the *C. albicans* growth, when this antimycotic was incorporated into a tissue conditioner.[3,6,7] Quinn[6] observed that nystatin (500,000 International Units (U)) added to some tissue conditioners was as effective as the antifungals miconazole and ketoconazole in inhibiting the growth of *C. albicans*. According to Truhlar et al,[5] the dosages of 500,000 and 1,000,000 U were capable of maintaining the drug-leaching amount above the minimum necessary to the fungicidal activity for stomatitis treatment. Schneid[4] demonstrated that a sustained release system, which incorporated four antifungal agents (chlorhexidine, clotrimazole, fluconazole, and nystatin) into a tissue conditioner, significantly inhibited *C. albicans* growth. Nevertheless, this association between tissue conditioner and antifungal agents can modify the physical and mechanical properties of the polymeric materials.

The aim of this study was to evaluate the ultimate tensile strength (UTS) of the tissue conditioner (DuraConditioner) mixed with nystatin after either 24 hours or 7 days of storage in water. As stated by Waters and Jagger,[9] the tensile strength, which provides information on the ultimate strength of a rubber in tension, is one fundamental property of rubber materials. Although some rubbers are only subjected to compression and shear, as with soft lining and tissue conditioner materials, the tensile properties are regarded as a general guide to the quality of rubbers. Clinical significance is then associated with the ability of the material to resist rupture during normal use under detrimental conditions promoted by the wet environment. The null hypothesis is

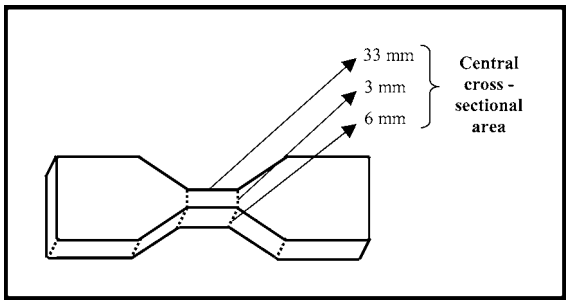

Figure 1. Schematic design of the specimen for the UTS test.

that the incorporation of nystatin does not affect the UTS of the modified tissue conditioner after different periods of water storage.

Materials and Methods

The tissue conditioner DuraConditioner (Batch number 091800, Reliance Manufacturing Co., Worth, IL) used in this study is presented as powder and liquid. The powder is composed of a methyl methacrylate copolymer, and the liquid consists of a solution of methyl methacrylate, dibutyl-*n*-phthalate, and ethanol.

To calculate the proportion of nystatin added to the tissue conditioner powder in milligrams, the conversion of International Units (U) to milligrams was that 6079 U corresponded to 1 mg of nystatin. Therefore, for Group II (500,000 U) the amount of nystatin used was 82.25 mg. For Group III (1,000,000 U) the amount of nystatin used was 164.50 mg. The nystatin powder concentrations of 500,000 (82.25 mg) and 1,000,000 U (164.50 mg) were added to 6 g of the tissue conditioner powder prior to the mixture with its liquid.

Both solid phases (tissue conditioner powder and nystatin) were hand mixed, and the liquid (6 ml) was added when the powder mixture obtained was homogeneous. All mixture was then manipulated according to the manufacturer's instructions (for 15 minutes at 37°C). A control group without nystatin powder (GI) was created using the same procedures described above.

The resin mixtures were applied to a stainless steel mold—producing dumbbell-shaped specimens ($N = 7$) with a central cross-sectional area of 33 × 6 × 3 mm (Fig 1) (ASTM D412).[10] After 15 minutes, the specimens were removed from the mold, and the resin excesses were removed with a scalpel. Afterwards, each specimen was stored for either 24 hours or 7 days in 200 ml of distilled water at 37°C, which was changed every day.

The specimens were subjected to tension in the MTS-810 (Material Test System, Eden Prairie, MN) at a rate of 40 mm/minute. A specific claw was made so the central cross-sectional area could stand exposed, while the ends would stay confined by the claw. The values of UTS were obtained in force-grams (gf).

Data were analyzed by two-way ANOVA and Tukey's test, at 95% level of confidence.

Results

The mean tensile strength ranged from 467.50 gf to 634.29 gf (Table 1). Mean UTS values at 24-hour storage were 634.29 gf for the control, 561.92 gf for the GII, and 547.30 gf for the GIII (Table 1). After 1 week, the mean values were 536.68 gf for the control, 467.50 gf for the GII, and 500.62 gf for the GIII (Table 1). The results of ANOVA revealed that there was no significant difference ($p = 0.25$) in UTS among the three experimental groups (GI, GII, GIII). There was a significant difference in UTS between the two storage periods investigated, in which the mean values of all experimental groups after 7 days were significantly lower than those observed after 24 hours ($p = 0.04$).

Discussion

In the initial mixing of the powder and liquid of the conditioner, the ethanol from the liquid is absorbed into polymer particles, and this absorption leads the powder particles to swell. Polymer chain disentanglements then occur, allowing the higher-sized molecules of the plasticizers to penetrate between polymer chains. The addition of a substance in a tissue conditioner might hinder the plasticizers from penetrating and forming a weak gel.[11] Accordingly, the nystatin incorporated

Table 1. UTS Means (gf ± Standard Deviation) of the Tissue Conditioner in the Three Experimental Conditions for the Two Periods Evaluated

Storage Period	*Nystatin Concentration*		
	Control (GI)	*500,000 U (GII)*	*1,000,000 U (GIII)*
24 hours	634.29 ± 122.80	561.92 ± 133.56	547.30 ± 73.47
7 days	536.68 ± 54.71	467.50 ± 143.51	500.62 ± 159.76

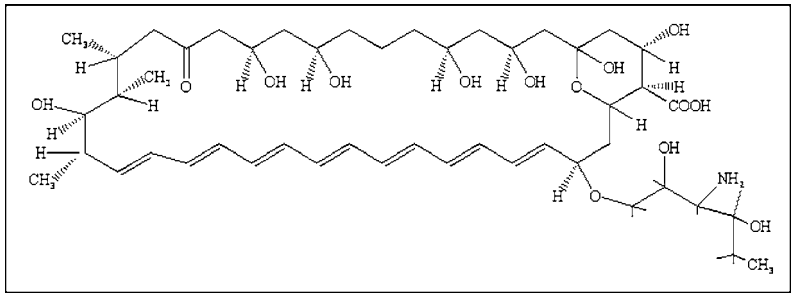

Figure 2. Chemical structure of the antifungal nystatin.

might affect the penetration of plasticizers and change the properties of the tissue conditioner; however, we can suppose that the possible change in gel formation of the tissue conditioner investigated, promoted by the addition of the polymeric structure of nystatin (Fig 2) in the two concentrations evaluated, did not affect its UTS values.

In this study, the UTS test was performed at a high rate as in other studies,[9,12,13] and a different measurement unit was used. The rate of 40 mm/min was determined during previous tests, which demonstrated that the use of such a high rate provides results similar to those obtained when a low rate was applied. The measurement unit used for UTS was gf. The rejection of the megapascal unit (MPa) as a measurement unit is explained by the significant decrease in the cross-sectional area of the specimen during the test because of its low elastic modulus. Moreover, the cross-sectional area measurement at specimen break would not represent the real area due to the material's elastic memory. One limitation of this study is that the percent elongation values could be obtained by attaching an extensometer to the tensile specimen.

Our results corroborate other studies involving the incorporation of fungicidal agents into tissue conditioners. Some studies have shown no clinically significant changes in physical and mechanical properties of materials containing antifungal agents. Evaluating the tensile strength of the autopolymerized acrylic resin Croform (Davis Schottlander & Davis Ltd., London, England) containing chlorhexidine (20 or 30% wt), Thaw et al[14] observed a decrease in the hardness and in the tensile strength, which was not considered by the authors so expressive as to interfere in its clinical use. Schneid[4] demonstrated significantly greater tensile strength of the tissue conditioner Lynal (Dentsply L.D. Caulk Division, Milford, DE), when it was associated with nystatin at amounts of 250 mg and 500 mg, which were higher than the maximum concentration evaluated in the present study (164.50 mg = 1,000,000 U). Therefore, these data agree well with the results obtained in the present study, that the maximum concentration studied (1,000,000 U) did not affect the UTS of the tissue conditioner. In addition, Schneid[4] related that all failures were cohesive when the tensile strength test was applied, indicating that the bond strength of the tissue conditioner (Lynal) to a heat-polymerized denture base acrylic resin was enhanced or unaffected by the presence of nystatin. Moreover, the addition of nystatin did not alter the material's hardness beyond acceptable limits within the expected clinical life of the material. Another limitation of this study is that only one brand of tissue conditioner was used. The results obtained here therefore may not apply to other tissue conditioners incorporated with the same nystatin concentrations.

The 7-day storage period affected the UTS for both the control group and the nystatin groups. The wet environment allows the ethanol and ester plasticizers to be leached into water, which is then absorbed by the polymeric phase of the gel.[15] The loss of plasticizer leads to gradual hardening of the material. Jepson et al[16] demonstrated a significant loss of viscoelasticity of Coe-Soft (GC America, Alsip, IL) over an 8-week period. Ueshige et al[11] did not find changes in the dynamic viscoelastic properties of one tissue conditioner (Shofu Tissue Conditioner, Shofu Inc., Kyoto, Japan) containing silver-zeolite (Zeomic® AJ10N, Shinagawa Fuel Co., Nagoya, Japan) over a 28-day storage time in distilled water or artificial saliva, while the other tissue conditioners investigated demonstrated changes over time, which were associated to the leaching out of ethanol. Therefore, in the present study, we can speculate that the amount

of ethanol or plasticizer released from the Dura-Conditioner through 7 days was high enough to alter the mechanical property tested.

Within the limitations of this in vitro study, the null hypothesis that the incorporation of nystatin after 24 hours or 7 days does not affect the UTS of the tissue conditioner was accepted. Additionally, other research on different brands of tissue conditioners mixed with topical antifungals is being performed to evaluate their mechanical, physical, and biological properties. The results of this future research may demonstrate if tissue conditioners containing antifungal agents can be recommended for candidosis treatment without detrimental effects on their properties.

Conclusion

The results of this in vitro study suggest that the addition of nystatin at the concentrations of 500,000 U and 1,000,000 U (which have been considered by other authors[5] as concentrations above the minimum necessary concentration to the fungicidal activity for stomatitis treatment) into the tissue conditioner investigated did not affect its ultimate tensile strength.

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