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ORIGINAL RESEARCH

In vitro Antifungal Activity of Two Tissue Conditioners Combined with Nystatin, Miconazole and Fluconazole against *Candida albicans*

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ABSTRACT

Aim: To determine and compare antifungal activity of two tissue conditioners combined with nystatin, miconazole and fluconazole against *Candida albicans*.

Materials and methods: Two tissue conditioners Viscogel and GC Soft combined with nystatin, miconazole and fluconazole were tested against *Candida albicans* using agar core inhibition diameter assay. One-way analysis of variance followed by Tukey's post-hoc test was used to test the intergroup difference. p-value < 0.05 was considered statistically significant.

Results: Maximum inhibition was seen in the fluconazole groups followed by miconazole and the least inhibition was seen in case of nystatin group.

Conclusion: Tissue conditioners when mixed with antifungal agents showed satisfactory inhibition of *Candida albicans*.

Clinical significance: Incorporation of antifungal drugs into tissue conditioners shows good inhibition of *C. albicans* and can be recommended for clinical use.

Keywords: Tissue conditioners, Nystatin, Miconazole, Fluconazole, Antifungal, Denture stomatitis.

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INTRODUCTION

Denture-induced stomatitis is multifactorial in origin.[1] Denture stomatitis is an inflammatory condition involving the tissue covered by the denture base. It is commonly found in the maxillary arch, rarely in the mandibular arch and it is not uncommon under removable partial denture bases in the maxillary arch. *Candida albicans* infection and trauma are significant causes of denture stomatitis. Predisposing factors include inadequate denture hygiene, denture wearing habits, xerostomia, medications and nutritional factors.[2-4]

The condition is managed by: (i) Improvement of denture hygiene, (ii) correction of the adaptation of the denture with a tissue conditioner and (iii) topical application of an antifungal agent when the presence of yeasts has been confirmed.[5-7] Tissue conditioners have been used to improve adaptation of the denture and to allow recovery of denture bearing tissues.[8] Although the use of short-term denture liners to improve the adaptation of the denture in cases of denture stomatitis is part of routine treatment, it has been shown that these liners also promote or support *in vivo Candida* colonization.[4,7-10] *In vitro* studies covering longer time periods show that the use of soft liners might intensify the formation of fungal biofilms.[4,11] Colonization of soft liners by *Candida albicans* is favored by the presence of saliva and serum pellicles. Fungal adhesion to material surfaces is the first step of colonization. Fungi can then penetrate into the material.[9,12-14] Douglas and Walker (1973) had the idea of combining the therapeutic effects of a tissue conditioner and an antifungal agent. This had the advantages that the action of the drug was prolonged, the cost was low and tissue recovery from trauma was encouraged.[1] It could be speculated that the incorporation of an antifungal agent in a short-term denture liner may be beneficial.

AIMS AND OBJECTIVES

To evaluate and compare *in vitro* antifungal activity of two tissue conditioners combined with nystatin, miconazole and fluconazole against *Candida albicans*.

MATERIALS AND METHODS

Procurement of Candida Growth

Sabourauds dextrose agar medium was prepared and poured into a sterile plate. After drying, the plate was inoculated with *C. albicans* obtained from American type culture collection (ATCC, strain no 10231). After inoculation, the plate was incubated for 24 hours at 37ºC. Five distinct colonies of approximately 1 mm were picked up from this culture using a sterile inoculation loop. Colonies are suspended in 5 ml of sterile saline (0.9%). The resulting suspension was centrifuged and turbidity was adjusted to 0.5 McFarland standard.

Susceptibility Test Procedure

Plates were prepared with Mueller-Hitton agar and 2% glucose with 0.5 µg/ml methylene blue dye.The prepared medium was poured into the plates to an approximated depth of 4 mm. The plates were allowed to dry for 15 minutes in the incubator. A sterile cotton swab was used to streak the agar plates with the inoculum. Four plates served as the indicator of pure growth of *C. albicans*. In another 12 plates antifungal test disks, i.e. nystatin (NY), miconazole (MZ), fluconazole (FZ) were placed (4 plates each) using sterile tweezers.

Tissue conditioners were mixed in a sterile dappen dish following the recommended water powder ratios by the manufacturers and the sterile disks were completely embedded in the mix. The disks were carefully lifted up from the mix with the help of a sterile tweezer and gently placed over the agar plate such that the disk is completely embedded in the mix from all the sides. Care was taken to not disturb the shape of the disk while handling. A total of eight plates, i.e. four each for a tissue conditioner were prepared in this manner.

The remaining plates were divided into six equal groups:

1. Viscogel with nystatin (VN),
2. Viscogel plus miconazole (VM),
3. Viscogel plus fluconazole (VF)
4. GC Soft plus nystatin (GN)
5. GC Soft plus miconazole (GM).
6. GC Soft plus fluconazole (GF)

Using the same technique the antifungal susceptibility test disks were completely embedded in the tissue conditioner mix and gently placed on the agar plates such that the disk is completely covered by the mix from all the sides. Care was taken to use an aseptic technique in all the steps. All the plates were incubated at 37ºC for 24 hours. Inhibition diameters were noted using a metallic scale and a divider.

STATISTICAL ANALYSIS

Mean and standard deviation of the zone of inhibition was calculated. One-way analysis of variance followed by Tukey's post-hoc test was used to test the intergroup difference. p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 shows the mean zone of inhibition of the different test materials against *Candida albicans*. Maximum inhibition was seen in the fluconazole groups followed by miconazole and the least inhibition was seen in case of nystatin group. The tissue conditioners did not show any antifungal activity when tested. GC Soft groups showed slightly more antifungal activity as compared to the Viscogel group. Figure 1 shows the comparative antifungal activity of the different test materials.

DISCUSSION

The presence of *C. albicans* on the upper fitting surface of the denture is a major causative factor in denture-associated chronic atrophic candidiasis (denture stomatitis), the most common form of oral candidiasis. The relatively acidic and anaerobic environment under the denture provides an ideal habitat for fungal growth. Treatment of *Candida* in denture wearers should include treatment of the appliance. More recently, it was found that incorporating antimicrobial agents into biomaterials is feasible.[9,15,16]

The tissue conditioners used in the present study are the ones which are commercially available. In the present study, an agar core inhibition diameter assay was devised to investigate the fungicidal effects of antifungal agent and tissue conditioner combinations, *C. albicans* was grown on the petri dish prior to the insertion of antifungal agents and tissue conditioner mixture.

Table 1: Zone of inhibition of the different test materials against *Candida albicans*

Gr. no.	*Material*	*Zone of inhibition*	
		Mean (in mm)	*Standard deviation*
1	Viscogel	0	0
2	Viscogel with nystatin (VN)	10.42	0.227
3	Viscogel plus fluconazole (VF)	23.09	0.376
4	Viscogel plus miconazole (VM)	18.1	0.272
5	GC Soft	0	0
6	GC Soft plus nystatin (GN)	10.5	0.223
7	GC Soft plus fluconazole (GF)	24.11	0.376
8	GC Soft plus miconazole (GM)	18.69	0.363

- ANOVA F: 5343.987; p-value: 0.000; Tukey's post-hoc: 773787476 = 271 = 5

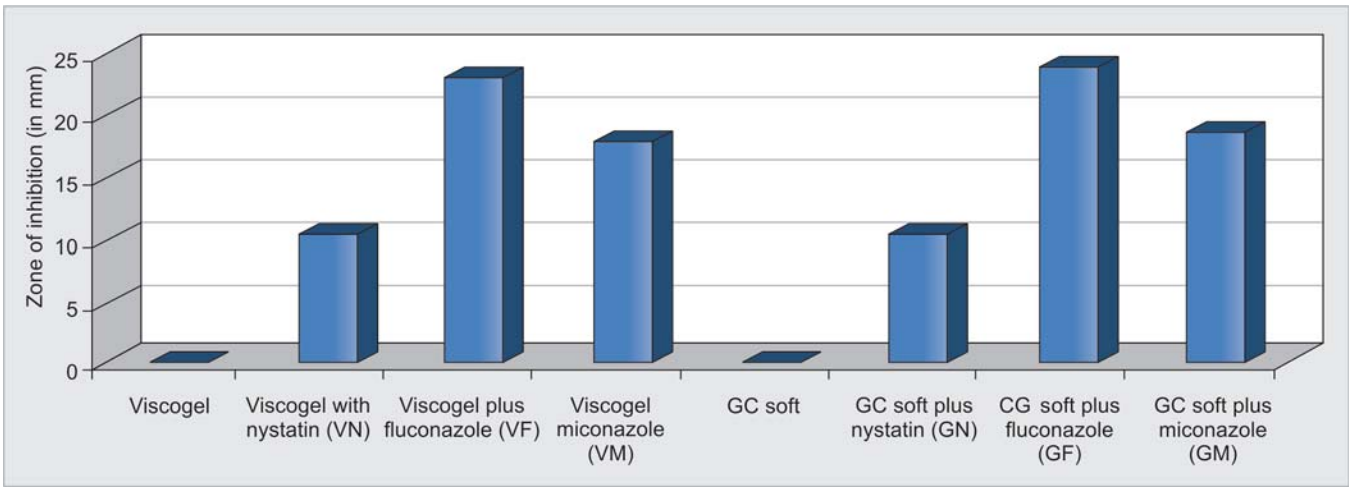

Fig. 1: Comparative antifungal activity of the different test materials

Nystatin was selected since topical nystatin is the current standard treatment for denture stomatitis. The rationale for choosing fluconazole was that it is a common systemic alternative to topical nystatin treatment. It is most commonly given to immunocompromised patients.[17] Miconazole lacquer was effective for treatment of denture stomatitis as shown by a study conducted by Konsberg and Axell in 1994.[18]

The results of the present study showed that maximum inhibition of *Candida albicans* was seen in fluconazole, the groups followed by miconazole, and the least inhibition was seen in case of nystatin group. The tissue conditioners did not show any antifungal activity when tested. GC Soft groups showed slightly more antifungal activity as compared to the Viscogel group.

In this study, there is absolutely no inhibition of *C. albicans* seen in Viscogel and GC Soft controls. This result confirms with the results of a study conducted by Kanathila et al[16] in India and previous study by Thomas et al,[19] who observed that Viscogel alone was completely inert and, therefore, would not be beneficial without antifungal agents in the treatment of denture stomatitis. Also, in an *in vitro* study conducted by Chow et al[8] to know the efficacy of antifungal agents in tissue conditioners in inhibiting *C. albicans*, samples containing only tissue conditioners did not exhibit significant fungicidal activity as compared to combinations of antifungal agents plus tissue conditioners.[16]

The effectiveness of miconazole and fluconazole as antifungal agents is not altered when they are combined with any of the tissue conditioners tested. Nystatin is also unaffected by the tissue conditioners, confirming the work of Thomas and Nutt (1978).[19] The antibacterial effect of miconazole described by MacFarlane et al (1978) could be helpful in treating the angular cheilitis often associated with denture stomatitis.[20]

In the present study, fluconazole showed the highest antifungal activity while nystatin showed the least activity. This observation could be due to the fact that fluconazole is more potent antifungal agent than miconazole and nystatin, since MIC for fluconazole range between 0.125 and 0.5 μg/ml while MIC for miconazole and nystatin range between 0.1 to 10 μg/ml and 1 to 30 units/ml respectively.[17]

Miconazole and nystatin exhibited antifungal activity when incorporated into denture liners. These results are in accordance with the findings of previous studies conducted by various authors.[17,21,22]

CONCLUSION

The following conclusions can be drawn from the present study:

1. Maximum inhibition was seen in the fluconazole groups followed by miconazole and the least inhibition was seen in case of nystatin group.
2. The tissue conditioners did not show any antifungal activity when tested.
3. GC Soft groups showed slightly more antifungal activity as compared to the Viscogel group.

CLINICAL SIGNIFICANCE

Denture stomatitis is an inflammatory condition involving the tissue covered by the denture base. Although, good oral and denture hygiene habits can be of great help in avoiding denture stomatitis, many patients fail to maintain these conditions. Incorporation of antifungal drugs into tissue conditioners shows good inhibition of *C. albicans* and can be recommended for clinical use.

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