Effect of an antifungal denture liner on the saliva yeast count in patients with denture stomatitis: a pilot study

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SUMMARY **Although *in vitro* studies on the release of antifungal agents from tissue conditioners have been done, no *in vivo* research on the topic could be found. The purpose of this study was to determine the *in vivo* effect of an antifungal agent released from a tissue conditioner on the salivary yeast count. Forty edentulous patients with denture stomatitis caused by *Candida albicans* were divided in two groups. Group 1 (control) was treated with a tissue conditioner only. Group 2 was treated with a tissue conditioner incorporating 500 000 U nystatin. Oral rinses were performed by both groups before treatment and every second day during treatment for a period of 14 days. Total yeast counts of the oral rinses were performed and the averages and standard deviations for both groups calculated and logarithm-transformed data of the counts over time were statistically analysed using the Wilcoxon signed-rank test. The average oral rinse yeast count of the control group decreased up to day 4. Thereafter, the count increased till the end of the test period. At day 14, the oral rinse yeast level was higher than the pre-treatment level. The average yeast count of the test group decreased up to day 7. Thereafter, the count increased but remained significantly lower ($P = 0{\cdot}01$) than the control group and did not return to its pre-treatment level. A nystatin-containing short-term denture liner significantly decreases the salivary yeast count of patients with denture stomatitis compared with a liner without nystatin.**

KEYWORDS: denture stomatitis, *Candida albicans*, oral rinse, denture liner, nystatin

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Introduction

Denture stomatitis is a common and recurring problem of complete denture wearers with a prevalence ranging between 11% and 67% (1). The aetiology of denture stomatitis is complex. Exogenous and endogenous factors have been implicated: trauma (2), yeast infection (3, 4), general predisposing factors such as decreased salivary flow, medication, endocrinopathies, nutritional and metabolic factors (5, 6), defects in host defence (1), and local predisposing factors such as poor denture hygiene, denture retention and stability (7). The unique microenvironment provided by a denture may promote yeasts to proliferate without any other predisposing factors present (7). Although *Candida albicans* is often isolated in significant quantities from individuals with generalized simple or granular denture stomatitis, other organisms might be involved as well (4, 8, 9).

Yeast-associated denture stomatitis is often asymptomatic. There are, however, reasons for providing treatment. Denture stomatitis might lead to soft tissue hyperplasia, negatively affecting denture support. Angular cheilitis might develop which is disfiguring and sometimes painful. The infection can become more severe, even life-threatening, during temporary or permanent immunosuppression (1).

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 (10–12).

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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 (13, 14). Therefore, it could be speculated that the incorporation of an antifungal agent in a short-term denture liner may be beneficial. It has the additional benefit of antifungal action even if dentures are worn at night and it is independent of patient compliance in using medication (15).

The purpose of this study was to investigate the efficacy of an antifungal agent incorporated in a short-term denture liner in reducing the salivary yeast count of edentulous denture wearers with clinical signs of denture stomatitis.

Materials and methods

Edentulous denture-wearing patients requesting prosthetic treatment at the Tygerberg Oral Health Centre, University of the Western Cape (UWC), were informed about the study and entered into the trial on the basis of: (i) a clinical diagnosis of generalized simple or granular denture stomatitis, (ii) microbiological confirmation for the presence of yeasts and (iii) the understanding and signing of an informed consent. The project was approved by the institution's ethical committee. Forty consecutive patients were selected and allocated to one of the two groups. The test group was treated with Visco-gel®* and Mycostatin®† and new dentures. The control group was treated with Visco-gel® only and new dentures. Patients on or with a recent history of topical or systemic antibiotic or antifungal treatment, and anti-inflammatory drugs were excluded from the study.

During the initial visit (day 0), an oral rinse, at least 1 h after last meal or drink, was collected after the removal of the denture from the patient's mouth. These samples were used for the confirmation of the presence and the baseline count of yeasts. Each subject performed an oral rinse for 1 min using 10 mL of sterile phosphate-buffered saline (PBS) before expectorating the fluid back into the container. The latter was centrifuged at 1520 ***g*** for 15 min. The pellet was re-suspended in 1 mL sterile PBS to concentrate the solution and 100 μL of this suspension was plated in duplicate onto Sabouraud Dextrose Agar plates* (16–18). The petri dishes were incubated aerobically at 37 °C for 24–48 hours and the colony-forming units (CFU) on the plates were counted.

After collection of the first oral rinses, the patient's denture was prepared to receive a Visco-gel® liner. For the control group, only a liner was applied without antifungal agent added to it. The liner was applied adhering to the manufacturer's instructions. For the test group, one Mycostatin® tablet of 500 000 U, the dosage recommended by Thomas and Nutt (19), was thoroughly pulverized, incorporated in the measured Visco-gel® powder before the Visco-gel® liquid was added and mixed into a homogenous gel and applied to the denture surface. All patients were instructed on the proper care of the tissue conditioner and to wear the denture at night.

Consequently, oral rinses were performed on days 2, 4, 7, 9, 11 and 14 and CFU were counted for each specimen. The averages and standard deviations for each sample were calculated and the logarithm-transformed data of the counts were compared over the different time intervals. The Wilcoxon unpaired signed-rank test was used to compare the control and test groups and the Wilcoxon paired signed-rank test was used to compare the counts at each time interval to that of the initial count (day 0) for each group. A *P*-value of 0·05 was considered as statistically significant.

The nystatin activity was determined at every visit by removing 4-mm-diameter liner specimens, placing them on Sabouraud Dextrose Agar plates inoculated with 0·1 mL of a standardized suspension containing 10^8 CFU mL^{-1} of *C. albicans*. The plates were incubated at 37 °C for 48 h and the presence of inhibition was noted.

Results

Of the total number of 40 patients with clinical signs of denture stomatitis, nine tested negative for yeasts and one patient was excluded because of deliberate premature removal of the liner. The remaining 30 patients were spread equally over the two groups, with only four male patients in total, two in each group. Age ranged between 36 and 77 years, with a mean age for the control group of 55 years and for the test group of 52 years. In the control group, all patients reported to

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have been wearing dentures at night, five patients reported being smokers, four patients were diabetic and four used medication for asthma. In the test group, all patients except one have been wearing the dentures at night, nine were smokers, four were diabetic and one suffered from asthma.

The descriptive statistics of the logarithm of the yeast counts of the oral rinses for both groups over time are shown in Table 1 and a graphic representation thereof in Fig. 1. The average pre-treatment (day 0) yeast count was of the same order for both groups. The average yeast count of the control group decreased until day 4, while the yeast count of the test group decreased until day 7. After 4 and 7 days respectively the average yeast counts started to rise again until the end of the test period on day 14. At the end of the test period, the yeast count for the control group was higher than the pre-treatment level at day 0. However, at the end of the test period, the yeast count for the test group was significantly lower ($P = 0{\cdot}01$) than the pre-treatment level of day 0. The yeast count of the test group remained lower than the control group during the complete test period; however, the mean yeast counts of the test group at the different time intervals were statistically significantly different ($P < 0{\cdot}05$) than the control group only from day 7 onwards. When the mean counts for the different time intervals of the control group were compared with the mean count at day 0, no statistically significant difference was observed ($P < 0{\cdot}05$) for any of the time intervals. However, when the mean counts for the different time intervals were compared with the mean count at day 0 for the test group, a statistically significant difference ($P < 0{\cdot}01$) was observed for all the time intervals.

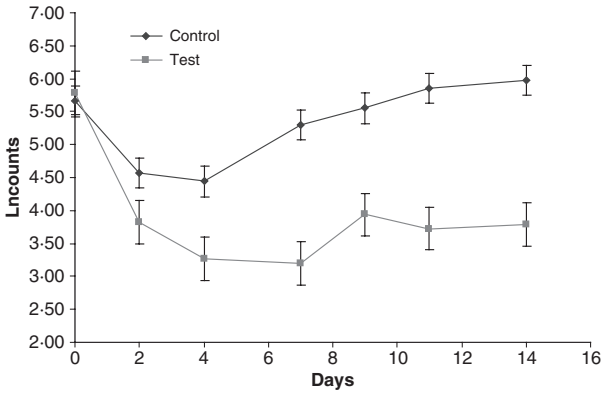

Fig. 1. Graphic representation of the logarithm of the yeast counts for both groups over time.

In Table 2, the number of subjects who had a reduction in their yeast counts is provided from one visit to the next. The highest number of subjects who had a reduction in their yeast counts, 11, occurred during the period day 0 to day 2. This was the same for both groups. The total number of occasions where a reduction has taken place for a particular individual is slightly more for the test group (42 occasions) compared with the control group (39 occasions). As a

Table 1. Descriptive statistics of the logarithm of the yeast counts for the baseline measurements and the six follow-up visits

Day	Data	Control	Test
0	Average	5·66	5·79
	Standard deviation	1·64	0·99
	Minimum	1·10	4·62
	Maximum	7·56	7·41
2	Average	4·57	3·83
	Standard deviation	2·01	1·95
	Minimum	0·69	0·00
	Maximum	8·01	5·97
4	Average	4·44	3·27
	Standard deviation	2·09	1·89
	Minimum	0·69	0·00
	Maximum	6·96	6·12
7	Average	5·30	3·20
	Standard deviation	1·58	2·08
	Minimum	1·95	0·00
	Maximum	7·60	6·03
9	Average	5·55	3·94
	Standard deviation	1·65	1·93
	Minimum	2·77	0·00
	Maximum	7·78	6·17
11	Average	5·85	3·73
	Standard deviation	1·84	1·84
	Minimum	2·08	0·00
	Maximum	8·71	6·10
14	Average	5·97	3·79
	Standard deviation	1·77	2·04
	Minimum	2·30	0·00
	Maximum	8·70	6·31

Table 2. Frequency table of the number of subjects who had a reduction in their *Candida. albicans* counts from one visit to the next

	Day					
	0–2	2–4	4–7	7–9	9–11	11–14
Control group	11	7	5	6	6	4
Test group	11	10	6	4	8	3

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whole, the two groups were very similar with respect to their reduction of the yeast counts between sequential visits.

The liner specimens retrieved from the test group showed *in vitro* antifungal activity throughout the 14-day test period, by exhibiting an inhibition area on a Sabouraud plate inoculated with a *C. albicans* suspension. No inhibition area was ever found around liner specimens from the control group.

Discussion

Since the 1980s, the imidazoles became increasingly more popular and seem to have replaced, to a large extent, the polyenes for the treatment of oral thrush. Miconazole and ketoconazole were found to be effective in *in vitro* denture liners, but they are more expensive and toxicity of ketoconazole is a problem (20). Rapid relapse (21, 22), resistance and cross-resistance between the azoles have also been reported, particularly in association with immunosuppressed individuals (23).

The reasons for use of nystatin over other drugs in the treatment of fungal denture stomatitis still remain: Johnson *et al.* (24) in a clinical study showed nystatin (pastille) to be effective in reducing or eliminating the *Candida* organism associated with denture stomatitis. Contrary to amphotericin B, the activity of nystatin incorporated in an acrylic soft denture liner is not diminished (15, 19, 20, 25). So far, no nystatin-resistant *C. albicans* has been reported (26, 27). Last but not least, nystatin is cheap.

The selection of the Visco-gel/nystatin combination for this study was based on unpublished results of an *in vitro* pilot study comparing the two liners Visco-gel® and GC Soft Liner®* that are most frequently used in the Oral Health Centre of the UWC. These results showed that Visco-Gel® produced a more predictable release of several antifungal agents. These findings are in line with an *in vitro* study by Thomas and Nutt (19) who found that a Visco-gel/nystatin mixture had strong antifungal properties. Truhlar *et al.* (25) reported that nystatin in Visco-gel® was more fungicidal than the other tested combination.

In the present *in vivo* study, a sharp drop in oral rinse yeast count was observed within the first 2 days for both the control group and the experimental group. The reason for the drop in the control group might be: (i) the cleaning of the prosthesis before the application of the liner, (ii) the liner covering the infected intaglio surface of the old denture and (iii) an altered denture hygiene routine by the increased awareness of the patient. The drop for the test group was bigger, suggesting a combined effect of mechanical cleansing and release of the nystatin from the liner.

Walker *et al.* (10) reported that mechanical cleansing of dentures was as effective as topical treatment with antifungal drugs in treating *Candida*-associated denture stomatitis. However, assuming that the effect of the mechanical cleansing and increased denture hygiene awareness for both groups was the same, the results of this *in vivo* study show an additional beneficial effect on the oral rinse yeast count if nystatin was incorporated in the denture liner. It would have been ideal if patients who were not informed about their *Candida*-induced denture stomatitis were included in the study to determine the effect of these possible actions. This would have been problematic in obtaining informed consent of the patients and ethical clearance.

It is important to notice that, for the control group, an increase of the oral rinse yeast count occurred from day 4 to reach a level at day 14 higher than the pre-treatment level. The effect of the isolated infected intaglio surface of the denture was neutralized by day 4 and combined with the fact that the liner itself may promote yeast growth, caused a count higher than the pre-treatment level. Fungal growth promotion on liners has been shown in previous studies (13, 14).

Truhlar *et al.* (25) showed that antifungal activity of nystatin in liners decreased with time. In the test group, the effect of the nystatin on the oral rinse yeast count was most pronounced during the first 4 days and diminished thereafter. This is also in agreement with Chow *et al.* (28) who reported an *in vitro* peak antifungal function at 3 days.

Thomas and Nutt (19) showed that an *in vitro* Visco-gel/nystatin mixture had strong antifungal properties for a period of up to 28 days. However, they warned that the clinical situation differs from the *in vitro* scenario and that nystatin may be exhausted earlier under the influence of the intake of fluids and food. Indeed, in the present study, the saliva count started to rise again after 7 days suggesting that the nystatin started to get exhausted.

Truhlar *et al.* (25) showed that *in vitro* fungicidal activity was proportional to the concentration of nys-

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tatin used. Therefore, it could be speculated that an increase in the antifungal concentration in the liner *in vivo* might lead to proportionally lower oral salivary yeast counts. However, under clinical conditions, higher nystatin content in the liner negatively affected the quality and longevity of the liner, relative to the Visco-gel-only liner. This would make comparison with the control group problematic. A poor quality liner might influence the release of the drug over time in the oral environment. It was therefore decided to limit this study to a concentration of 500 000 U nystatin, which produced a clinically acceptable liner with a longevity of about 2 weeks. A maximum period of 14 days was selected because unacceptable deterioration of the nystatin/Visco-gel liner occurred beyond a period of 14 days.

The changes in the clinical appearance of the denture stomatitis during the 14-day period were not monitored. The time intervals between specimen collections as well as the complete trial period were too short to do this. Johnson *et al.* (24) showed that clinical improvement lags behind mycological improvement by at least 1 week.

Truhlar *et al.* (25) showed insignificant inherent *in vitro* antifungal activity of Visco-gel®. Others also reported that Visco-gel® alone is ineffective (19, 28). Observations from the present study confirm this. No inhibition areas were noticed around the Visco-gel-only specimen on the cultured Sabouraud plates. In contrast to this, the Visco-gel/nystatin specimens did show inhibition areas over the 14-day test period.

Elimination of the *C. albicans* from saliva was not achieved by either treatment regimen, although lower oral rinse counts were achieved with the Visco-gel/nystatin liner. It can be speculated that the replacement of the Visco-gel/nystatin liner after 7 days will result in continued decrease of the *C. albicans* count. This could be investigated further.

Because the average count increased again after 7 days for the Visco-gel/nystatin group and after 4 days for the Visco-gel-only group, it is recommended that the liners are replaced after these respective time intervals. A weekly replacement routine was also recommended by Könsberg and Axéll (7) in a clinical study with a topical miconazole and lacquer. Several other studies of antifungal therapy in the treatment of denture stomatitis showed the best mycological improvement after 1 week of treatment (29, 30).

It should be kept in mind that denture stomatitis is a multifactorial disease. A single remedy is not sufficient to cure the condition and recurrence rates after antifungal treatment are high (22, 31, 32). A relapse of 50% has been reported after 12 weeks by Bissell *et al.* (21). Treatment should be geared towards re-establishing mucosal health, elimination of plaque on the denture and mucosa, increasing host resistance and establishing the presence of predisposing factors (e.g. treatment with antibiotics, systemic steroids, immunosuppressive drugs, inhalers for asthma, xerostomia). The aim of antimycotic treatment is to reduce the acute candidal overgrowth to levels that can be controlled by the host's defences. Bergendal and Isacsson (33) reported that nystatin does not cure denture stomatitis and recolonization of the yeast occurs after cessation of drug therapy. Therefore, drug therapy must be coupled with appropriate prosthodontic treatment and oral and denture hygiene. Salonen *et al.* (34) recommends combining antifungal treatment with renewal of dentures, as a result of some positive effect of new dentures on healing. In the present study, all patients were provided with new complete dentures.

Conclusion

This clinical study demonstrated a sharp drop in the yeast count of oral rinses after the placement of a short-term soft denture liner. This decrease lasted for 4 days if the liner did not include the antifungal agent nystatin and lasted 7 days if nystatin was included. Furthermore, the decrease in yeast count was more pronounced if nystatin was incorporated in the liner.

It is therefore recommended that, in the treatment of a candidal denture stomatitis, a nystatin-containing short-term denture liner is used for a maximum period of 7 days. As candidal denture stomatitis is a multifactorial disease, antifungal therapy should be supported by appropriate additional treatment strategies in order to achieve long-term success and to prevent recurrence. A formal trial is recommended, with increased power, to fully determine the clinical significance of the findings of this pilot study.

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