Evaluation the Effect of Nystatin or Fluconazole Combined with Tissue Conditioner on Colonization of Canida albicans in vitro over Time

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Recived: Aug 2013        Accepted: Jan 2014

ABSTRACT

Background and Aim: A thin layer of tissue conditioner is usually used to control denture stomatitis, but it can provide a susceptible environment for colonization of Candida, which can be controlled by addition of antifungal. The aim of current study was to evaluate the stability of nystatin or fluconazole incorporated with tissue conditioner on colonization of Candida over time in an in vitro condition.

Materials and Methods: In present study by addition of nystatin or fluconazole with tissue conditioner, thin circular disks and also disks without antifungal as control were prepared. By immersing the disks in artificial sterile artificial saliva, the inhibitory effect of remaining antifungal in disks in 24, 48, 72 hours and 5 day intervals, over spread culture of Candida were evaluated using disk diffusion test. The mean diameter of inhibitory zone at different times was analyzed using ANOVA and Tukey statistical tests.

Results: Combination of nystatin or fluconazole with tissue conditioners ultimately was effective for first 3 days and in the first 24 hours of immersion in artificial saliva, the average diameter of inhibitory zone of Candida colonization around disks containing nystatin and fluconazole were 11.2 and 8.6 mm respectively. There was also revealed that only a statistically significant differences in the average diameter of inhibitory zone around both antifungal disks was seen in 24 (P=0.001) and 48 hours (P=0.013).

Conclusion: According to the results of present study, incorporated nystatin or fluconazole with tissue conditioner immersed in artificial saliva, wasn’t stable and had a statistical significant effect for controlling of Candida colonization on the first 3 days. Topical nystatin showed a higher in vitro inhibitory effect in compare with fluconazole.

Key words: Tissue conditioner, Candida, Colonization, Nystatin, Fluconazole, Time

INTRODUCTION

Different species of bacteria and fungi are seen in human oral cavity as normal flora, which may be changed to pathogenic form in terms of the use of denture.¹ Candida species particularly Canida albicans is known as one of the most common oral microorganisms, which as a reservoir in mucosal denture surface caused denture stomatitis.² There are several factors prepared predisposing condition to this Candida infection including mucosal trauma (such as inappropriate denture), smoking, use of antibi
otics (caused alteration in the oral normal flora), patients with cancer and endocrinological disorders \cite{3,4}.

One of the major types of oral candidiasis is chronic inflammatory condition of the palatal and alveolar mucosa recognized as denture stomatitis in denture users, which can result systemic and more live threaten infections in diabetic or immunosuppressed elderly having complete denture and poor oral hygiene \cite{5}. Control and treat of denture stomatitis may be achieved by removal of denture on nights and also its daily disinfection using common mouth washes \cite{6,7}. In cases, where removing of denture from the mouth is impossible, using a thin layer of tissue conditioner under denture mucosal surface can be used as a buffer for prevention of trauma \cite{8}.

However this layer of tissue conditioner improved denture adaptation, unfortunately this lining material may prepare a suitable surface for the attachments and colonization of oral Candida and intensified denture related disorders \cite{8,9}.

Although in these cases, topical antifungal therapy and elimination of denture’ defect is recommended, but due to leaching and swallowing of antifungal by saliva, preserving a fixed oral dose of the drug is impossible. Besides using topical antifungal drugs in elderly patients due to undesirable taste, loss of memory and physical motion is extremely difficult \cite{10}. Incorporation of topical antifungal with tissue conditioner can inhibited the microbial plaque formation particularly candidal biofilm and is useful for prevention of denture stomatitis \cite{11}.

By adding silver nanoparticles to GC Soft-Liner tissue conditioner and preparation of disks, Nam could inhibit colonization of fungi such as candida (with 0.5 wt% of silver nanoparticles) and bacteria such as streptococcus mutans and staphylococcus aureus (with 0.1 wt% of silver nanoparticles) \cite{12}.

Radnai et al also in an investigation by combining the different percentages of Chlorhexidine and Miconazoles’ gel with Viscogel tissue conditioner, made disks and studied the colonization of Canida albicans, which in their study only combination of miconazoles’ gel with tissue conditioner was reported effective \cite{13}.

With due attention to the usefulness of antifungal combination with tissue conditioner and also its irrigation by constant washing of saliva in mouth of denture users, the present study was aimed to evaluate the maximum effective duration on inhibition of Canida albicans colonization by adding nystatin or fluconazole with tissue conditioner in an in vitro condition.

**Material and Methods:**

A – Disk preparation

In this experimental (Lab-trial) study, the GC reline tissue conditioner (Soft liner-Japan) was prepared according to manufacturer’s instruction by addition of Nystatin (5%wt/wt TC, equivalent to 100,000IU ) or fluconazole (10%wt/wt ) with tissue conditioner powder \cite{16}, and flattened to 1mm thickness on a sterile surface. Circular thin disks with 5mm diameter and 1mm thickness (measured and controlled by a caliper) were finally prepared using a sterile cutter \cite{14}. Similar drug free tissue conditioner disks were also prepared as control (figure 1).

Disks were then immersed in separate sterile containers containing 100 ml of artificial saliva, set on a reciprocal shaker (100rpm) at 37°C, and the saliva were regularly changed every day until used for susceptibility testing. In present study, according to similar studies \cite{15,16}, and based on the type of study (lab-trial) seven repetition for each antifungal containing tissue conditioner disks and duration of incubation were used.

B - Candida cell suspension preparation
To prepare Candida cell suspensions, by cultiva-
tion of Canida albicans (ATCC10231) on a
fresh Sabouraud Dextrose agar and incubation
at 30°C for 48 hours, fresh Candida colonies
were prepared. A single colony was used for
preparation of Candida cell suspensions (con-
taining 1×10³CFU/ml) in a sterile physiological
serum (NaCl 0.85%), using a haemocytom-
eter slide and microscope.

C – Antifungal Susceptibility testing
The agar diffusion test was used for antifungal
susceptibility testing. One hundred μl of Candida
cell suspensions was firstly spread onto sev-
eral Sabouraud dextrose agar plates (streaking
technique). Tissue conditioner disks containing
antifungal and drug free control disks were put
on the surface of plates using sterile forceps by
a sterile forceps 17.

Cultures were incubated at 30°C for 48 hours
and finally the growth inhibitory diameter
around the disks was measured using caliper.
This was repeated 7 times for every tissue con-
ditioner disks containing nystatin, fluconazole
and control drug free disks, for 1, 2, 3 and 5
day intervals.

By repeating the procedures for 7 times for each
of disks containing antifungal drugs in different
times, the diameters of inhibitory zones on Sa-
bouraud dextrose agar were measured using a
caliper and were recorded in tables.

The average growth inhibitory diameters
around disks in different duration of immer-
sion in saliva were analyzed with SPSS15 soft-
ware using statistical ANOVA and Tukey test
for multiple and pairwise comparisons respec-
tively. P value of less than 0.05 was considered
statistically significant in current study.

Results:
The anti-Candida properties of nystatin or flu-
conazole added with the tissue conditioner over
different times were evaluated using the agar
diffusion method (Agar diffusion test). Hereby
tissue conditioner disks containing 100,000 IU
nystatin (with 5% wt of tissue conditioner) as
well as disks containing 10%wt of tissue condi-
tioner with fluconazole 16 were used for evalua-
tion of C. albicans growth and colonization
inhibitory effects.

Antifungal containing tissue conditioner disks
immersed in saliva were inhibited the growth of
C. albicans maximum up to 3 days (equivalent
to 72 hours). However, the inhibitory effect of
nystatin was more in compare with fluconazole
at all studied incubated times.(Table 1)

<table>
<thead>
<tr>
<th>Time</th>
<th>Combined antifungal</th>
<th>Diameter of inhibitory zone (SD±Average)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours 24</td>
<td>Nystatin</td>
<td>1.07±11.2</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>8.6±1.01</td>
<td></td>
</tr>
<tr>
<td>hours 48</td>
<td>Nystatin</td>
<td>2.7±0.7</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>1.7±0.56</td>
<td></td>
</tr>
<tr>
<td>hours 72</td>
<td>Nystatin</td>
<td>1.2±0.07</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>0.71±0.39</td>
<td></td>
</tr>
<tr>
<td>days 5</td>
<td>Nystatin</td>
<td>0.15±3815</td>
<td>0.416</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

By comparing the average diameter for inhibi-
tory zone, around disks including nystatin or
fluconazole at different immersing times with ANOVA statistical test showed only statistically significant differences at 24 (P=0.0001) and 48 hours (P=0.023). But after 72 hours and after 5 days these differences weren’t statistically significant. (P = 0.1 and 0.4) (Table 1). By using Tukey statistical test for pairwise comparisons between the average diameters of inhibitory zone, around disks combined with antifungal, it revealed that the statistically significant differences exist between all studied paired times (Tables 2 and 3).

**Discussion:**
The efficiency of nystatin or fluconazole incorporated with tissue Conditioner on the growth of C. albicans over different times was evaluated in present in vitro study. Results showed that nystatin or fluconazole combined with tissue conditioner in presence of saliva was effective maximum up to third day and maximum effect was shown in first 24 hours. In addition, nystatin showed more topical inhibitory in vitro effect in compare with fluconazole. Several studies indicated a protective and inhibitory roles for nystatin or fluconazole added to tissue conditioner for control and inhibition of Candida colonization, which are in agreement with the results of present study, although fluconazole responded a slightly weaker than nystatin.

In another study, Falah et al, the inhibitory effect of different weight percentages of nystatin and fluconazole added with tissue conditioner, such as 1, 2.5, 5 and 10%wt were studied. Their results showed that even addition of nystatin equal to one percent of tissue conditioner weight, completely inhibited the growth of Candida, whereas only addition of fluconazole equivalent to ten percent of tissue conditioner, caused complete control of in vitro Candida colonization. Their study also showed the

<table>
<thead>
<tr>
<th>Pairwise comparison</th>
<th>Diameter of inhibitory zone (Average±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 and 48 hours</td>
<td>2.7±0.7</td>
<td>0.001</td>
</tr>
<tr>
<td>24 hours and 5 days</td>
<td>0.15±0.38</td>
<td>0.001</td>
</tr>
<tr>
<td>48 hours and 5 days</td>
<td>0.15±0.38</td>
<td>0.01</td>
</tr>
<tr>
<td>72 hours and 5 days</td>
<td>0.15±0.38</td>
<td>0.015</td>
</tr>
<tr>
<td>and 24 hours 48 hours</td>
<td>1.7±0.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>and 72 hours 5 days</td>
<td>0.39±0.7</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table 3: Comparison the average and SD of the diameters of inhibitory zone, around disks containing fluconazole at paired studied times

<table>
<thead>
<tr>
<th>Pairwise comparison</th>
<th>Diameter of inhibitory zone (Average±SD)</th>
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<td>and 24 hours 48 hours</td>
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<td>0.0001</td>
</tr>
<tr>
<td>and 72 hours 5 days</td>
<td>0.39±0.7</td>
<td>0.003</td>
</tr>
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</table>
higher efficiency of nystatin in compare with fluconazole. However contrary to present study the role of time wasn’t studied and all results were evaluated after 48 hours, which are comparable with the results of 48 hours in current study.

The study of El-charkawi et al also showed that the addition of 3, 5, and 10 wt% tissue conditioner with nystatin was useful to inhibit the growth and colonization of Candida, and there is a direct relationship between antifungal weight percentage and their effective time. and the effect of 10 wt% drug is longer than the rest, and also the weight percent of drug does not have any effects on tissue conditioner properties.

Thomas et al in a study compared the effect of nystatin and amphotericin B in combination with tissue conditioner, reported nystatin more effective than amphotericin B for inhibition of Candida colonization.

In present study, by comparison the inhibitory effect of nystatin in compare with fluconazole combined with tissue conditioner at 1, 2, 3 and 5 days, results indicated that each drug containing disks had the ability for controlling of C. albicans colonization maximum up to 3 days (equivalent to 72 hours) in presence of artificial saliva. It seems that nystatin or fluconazol included with tissue conditioner weren’t stable and reduced by saliva irrigation, to be finished after three days. Chow et al in an in vitro study, by combination of 5% wt/wt nystatin, fluconazole and itraconazole with Coe soft tissue conditioner, and placing on a C. albicans culture, their inhibitory effect on colonization of Candida were investigated at different times. Maximum growth inhibitory effect of these antifungal was reported till 3 days. They also reported that their inhibitory effect reach to minimum after 8.2 days, which contradicted with the results of present study. These contrary results can be as a result of differences in methods. In Chow study tissue conditioner disks containing antifungal were directly placed on the surface of cultures and the culture plates changed every day. But nystatin and fluconazole contained tissue conditioner disks were immersed in artificial saliva, incubated on rotator shaker (100 rpm) at 37ºC with daily changing of artificial saliva in current study, comparable with the human oral cavity condition which can wash out nystatin and fluconazole faster than tissue conditioner, and reduce their effects.

**Conclusion:**

Results of present study indicated that the nystatin or fluconazole incorporated with tissue conditioner isn’t stable inside the saliva and their concentration gradually reduced over time, showed efficient inhibitory effect against Candida colonization maximum up to 3 days. Meanwhile, incorporated nystatin with tissue conditioner revealed more topical inhibitory properties than fluconazole in an in vitro condition. Performing an in vivo study using a layer of tissue conditioner containing nystatin or fluconazole, which fixed under the denture’s mucosal surface in patients with denture stomatitis, over time will suggested. According to the results of present study, soft liner’s containing nystatin or fluconazole used under the denture should be replaced every three days.

**Funding/Supports:**

This work has been financially supported by Dentistry school, Shahid Sadoughi University of Medical Sciences and Health Services as a dentistry student thesis (thesis number 425).

**References:**

2-Bokor-Bratic M, Cankovic M, Dragnic N. Unstimulated whole saliva flow rate and


