Original Article

Effect of *Melaleuca alternifolia* Mixed with Tissue Conditioners in Varying Doses on Colonization and Inhibition of *Candida albicans*: An *In Vitro* Study

Siva Kumar Vankadara, Rajendra Basavaraj Hallikerimath[1], Viraj Patil[1], Kishore Bhat[2], Mallikarjun Hanumantappa Doddamani[3]

Department of Prosthodontics and Crown and Bridge, SJM Dental College and Hospital, Chitradurga, Departments of [1]Prosthodontics and Crown and Bridge and [2]Microbiology and Immunology, Maratha Mandal's N. G. Halgekar Institute of Dental Sciences and Research Centre, [3]Department of Prosthodontics and Crown and Bridge, KLE VK Institute of Dental Sciences, Belgaum, Karnataka, India

Abstract

Aims and Objectives: The study was conducted to evaluate the Colonization & Inhibition of Candida albicans in selected commercially available denture lining materials material by mixing them with varying concentrations and doses of tea tree oil. **Materials and Methods:** Five test discs of 10mm diameter and 1.5mm thickness were prepared using commercially available soft denture lining materials (Viscogel and GC-soft). Tea tree oil of varying concentrations (10%, 20%, 30%, and 40%) and doses (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml) were added during manipulation. Test discs kept in sterile artificial saliva were inoculated with Candida albicans (ATCC-2091 strain) and incubated for 6 weeks. These discs were fixed, dehydrated air dried and stained using 0.03% acridine orange stain and observed under Fluorescent microscope to count the colonies on the surface of each disc to evaluate the colonization. To evaluate inhibition, test discs were placed on the top of Sabouraud's dextrose agar inoculated with Candida albicans (ATCC-2091 strain). After incubation at 370C for 48 hours, the zone of Inhibition formed around the samples was measured. **Results:** The GC soft liner had higher mean colonization and lesser zone of inhibition of C.albicans when compared to Viscogel soft liner ($P < 0.001$) and highest zone of inhibition observed with 2 ml volume and 40% vol/vol concentration of melaleuca alternifolia ($P < 0.05$). **Interpretation and Conclusion:** By the addition of Tea Tree oil, Viscogel had good acquired good antifungal properties than GC-soft lining materials.

Keywords: *Candida albicans denture soft-liners, colonization, denture stomatitis, inhibition, nystatin, tea tree oil*

Introduction

Resilient denture soft liners have been available in dental practice for many years and have a good therapeutic purpose. They mainly reduce the traumatic effect that the denture may have on patients with thin atrophic mucosa or with normal mucosa exhibiting a reduced tolerance to the load applied by the denture. However, it has also been suggested that the porosity of soft liner also allows water absorption and diffusion of nutrient materials that may support the growth of oral yeasts[1] predominantly by *Candida albicans* and related *Candida* species.[2,3]

Denture stomatitis more commonly known as "denture sore mouth" is a term used to describe certain pathologic changes such as recurrent inflammation or erythema and burning sensation of denture-bearing tissues under complete or partial dentures. *C. albicans* is the predominant oral yeast associated with denture stomatitis.[4]

Effective therapy must focus on the multifactorial etiology of the disease.[5] Treatment plan should include substitution of old prosthesis, elimination of anatomical irregularities, establishment of a nontraumatic occlusion, nutritional reconstitution, oral hygiene instructions, antifungal treatment, and systemic evaluation.[6] However, the success of topical application of drugs in the oral cavity may be compromised by some factors such as discomfort caused by the infection, unpleasant taste,[7] and frequency of dosage.[5]

These limitations have led to the development of other methods of drug elution such as incorporation of antifungal and antimicrobial agents with denture soft liners. Several attempts have been made to incorporate different antifungal agents such as propolis,[8] zeolite,[9,10] chlorhexidine,[11] fluconazole,[11] *Punica granatum*,[12] nystatin,[13,14] itraconazole,[13] miconazole,[15]

How to cite this article: Vankadara SK, Hallikerimath RB, Patil V, Bhat K, Doddamani MH. Effect of *Melaleuca alternifolia* mixed with tissue conditioners in varying doses on colonization and inhibition of *Candida albicans*: An *in vitro* study. Contemp Clin Dent 2017;8:446-50.

Address for correspondence:
Dr. Siva Vankadara Kumar, Department of Prosthodontics and Crown and Bridge, SJM Dental College and Hospital, B D Road, Chitradurga - 577 501, Karnataka, India.
E-mail: drvankadara@gmail.com

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_542_17

Quick Response Code:

ketoconazole,[15] and clotrimazole[16] with varying degree of success.

However, in the light of increased awareness and demand for natural health, commercially available naturopathic remedies are being used in the treatment of denture stomatitis. The essential oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO) is a multipurpose medicinal herb that can be obtained from its leaves by steam distillation.[17] It has shown promising effect as a topical antifungal agent in the treatment of dandruff, acne, and oral candidiasis.[18]

This *in vitro* study is undertaken with the aim to test the efficacy of TTO mixed with two different denture soft-lining materials against colonization and inhibition of *C. albicans* under varying concentrations and doses.

Materials and Methods

The study was conducted in the Department of Prosthodontics and Crown and Bridge and Department of Microbiology, Maratha Mandal's N. G Halgekar Institute of Dental Sciences and Research Center, Belgaum, Karnataka, India. The denture soft liners selected were Visco-Gel (Dentsply, India) and GC Soft-Liner (GC Corporation India). TTO was brought from A. G. Aromatics, Noida, India.

Specimen preparation

The study consisted of four groups and four subgroups of soft liner specimens to which TTO was added in varying concentrations (10, 20, 30, and 40% vol/vol) and varying doses (0.5, 1, 1.5, and 2 ml) with a sample size (n = 5). Wax discs of 10 mm diameter and 1.5 mm thick were punched from a sheet of modeling wax using sectioned gutta-percha box and flasked in a way similar to processing the complete dentures. Doses of *M. alternifolia* (0.5, 1.0, 1.5, and 2.0 ml) of varying concentrations were added to the conditioner liquids and homogenized in a sterile glass beaker for 30 s. Immediately afterward, the conditioning powder was added and mixed according to the manufacturer's instructions and then filled into the voids left by the wax discs. These discs were taken out of the denture flask after the setting time of the denture soft liners as recommended by the manufacturer. All the above-mentioned procedures were done in laminar air flow chamber under ultraviolet light and were used immediately for the microbiological procedure discussed below to prevent any microbial contamination.

Evaluation of candidal colonization on the test specimens

Standard ATCC (2091) approved *C. albicans* strains were collected. Test discs of soft liners were placed into glass test tubes containing 10 ml of artificial saliva. Each of these bottles was inoculated with 0.1 ml of an 18 h culture of *C. albicans* in artificial saliva and incubated in an incubator at 37°C for 6 weeks. Test denture liner discs were transferred aseptically to fresh sterile artificial saliva on a weekly basis. Five replicates were used for each material type. After 6 weeks of incubation, test pieces were removed from the bottles and placed in 4% glutaraldehyde in phosphate-buffered saline for at least 2 h to fix the cells. Substrata plus attached cells were then dehydrated in alcohol (30%, 50%, 70%, 90%, and 100%) for 10 min for each concentration in universal jars. These discs were immersed in 0.03% Acridine orange for 1 min, rinsed in distilled water, then air dried. Replicates were mounted on microscopic slide for examination and viewed through fluorescent microscope (×40 objective, Phase contrast condenser-PS-1, No. 3-WB filter used). The objective was focused on one end of the section and racked down so that the entire length of the sample was examined. Colonization of *C. albicans* into the denture lining material was measured by counting the number of blastospores visible within the each field,[19] as described below.

- Class I: Little hyphal presence, whereby mycelia forms cover up to a quarter of the microscopic field
- Class II: Moderate amount of hyphal presence, where half of the field of view was covered by mycelial forms
- Class III: A large amount of hyphal presence, whereby most of the microscopic field was covered by mycelial forms.

Evaluation of inhibition of growth of *Candida albicans* around test specimens by disc diffusion method

Five test discs for each concentration and dose of TTO mixed with of the test materials (GC-soft liner and Visco-Gel soft liner) were tested. Kirby–Bauer disc diffusion susceptibility test protocol was followed for assessing the zones of inhibition. Sabouraud's agar medium was poured into the agar plates, and they were brought to room temperature before use. A loop was used to transfer the colonies to the broth. Visually turbidity of the broth was adjusted equal to that of a 0.5 McFarland turbidity standard. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of agar plate was swabbed thrice, rotating plates approximately 60° between streaking to ensure even distribution. Inoculated plates were allowed to stand for at least 3 min but no longer than 15 min. Four discs of test lining materials mixed with varying volumes and concentrations of TTO were placed in each agar plate, and the experiment was repeated five times. Nystatin susceptibility test discs (100 units/disc) were used as controls and placed in the center of agar plate. Within 15 min of compound application, plates were incubated for 48 h at 37ºC in incubator. Plates were read only if the lawn of growth was confluent or nearly confluent. Diameter of inhibition zones was measured to the nearest whole millimeter by holding the measuring device.

Results

Pair-wise comparison of two groups, four concentrations, and five volumes by three-way ANOVA and *post hoc* Bonferroni test gives the following results [Figures 1 and 2]:

- The GC soft liner had higher mean colonization and lower zone of inhibition when compared to Visco-Gel soft liner, and the comparison was statistically significant ($P < 0.001$)
- All comparisons showed significant reduction in colony-forming units (CFUs) with each increasing volume with highest reduction observed in 2 ml volume ($P < 0.05$)
- All comparisons showed a significant reduction in CFUs with each increasing concentration with highest reduction observed in 40% vol/vol concentration ($P < 0.05$).

Discussion

Human body as a host has many kinds of resident microorganisms that could be classified as both domestic and opportunistic pathogens.[20] One such residential human microflora is *C. albicans* which is a eukaryotic and opportunistic pathogen. This organism being mainly found in the oral cavity causes stomatitis while its presence on the fitting surface of the denture causes denture stomatitis or denture sore mouth.[4] Even though many organisms are associated with this condition, most of the studies show that *C. albicans* and ill-fitting dentures are commonly associated with this condition. Adhesion of Candida to the denture base materials, particularly when worn continuously in conditions of trauma and poor hygiene can usually cause denture stomatitis.[21-23] One of the initial steps in the prevention of denture stomatitis is improving denture adaptation and providing the suitable conditions for the denture bearing areas to recover. As evident from many studies, even though tissue conditioners can improve the adaptation of the dentures,[23] their susceptibility to attachment and colonization of the oral microorganisms, especially candida would result in the irritation of the tissues, leading to denture stomatitis in the oral cavity of the denture wearers.[24,25]

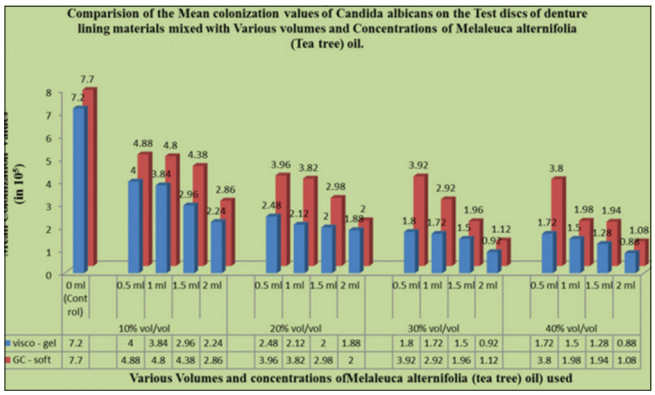

Figure 1: Graph comparing the mean colonization values of *Candida albicans* on the test discs of the denture lining materials mixed with various volumes and concentrations of *Melaleuca alternifolia*

Most of the tissue conditioners apart from not having any antifungal activity also showed their supporting role in adhesion and growth of candida. Hence, a tissue conditioner with antifungal activity could be of great advantage for patients with high risk of denture stomatitis.[23] Several attempts have been made to incorporate additives and antifungal agents to tissue conditioners as a drug delivery method for controlling microbial colonization and inhibit the biofilm formation.[8-16] However, the widespread use of these antibiotics is often expensive, toxic, and can create host resistance. Hence, the focus has now shifted to the safer natural alternatives as it was scientifically proven that some of the plant extracts have antimicrobial activity. *M. alternifolia* (TTO) is one of those plant extracts which have gained popularity over the past few decades. TTO, the volatile essential oil from Australian native plant *M. alternifolia*, has been largely employed primarily for its antimicrobial and anti-inflammatory properties and shows promise as a topical antifungal agent. This reputation is due to its safe antiseptic effect that is incorporated in many pharmaceutical and cosmetic products.[18]

Results of the current study suggested that the discs treated with TTO showed significantly lesser colonization and maximum zone of inhibition of *C. albicans* than the discs which were not treated with TTO, showing the lack of intrinsic antifungal activity of the soft denture-lining test materials (Visco-Gel and GC-soft liners). This was in accordance with the study conducted by Thomas and Nutt (1978) which states that there was no inhibitory effect for tissue conditioning material (Visco-Gel) on *C. albicans* for periods of time varying from 3 days to 1 week. The later results are also in accordance with the studies conducted by Gruber *et al*.[26] and Wright's[27] findings. Even in these studies, tissue conditioners appeared to have a supporting effect of fungal growth.

Comparisons between the mean CFUs and maximum zones of inhibitions of the test denture liner discs mixed with TTO of various concentrations and various volumes showed that there was a significant reduction in the colonization and maximum zone of inhibition of *C. albicans* was observed when 2 ml, 40% vol/vol of TTO was used with Visco-Gel soft liner and Gc-soft liner but Visco-Gel showed

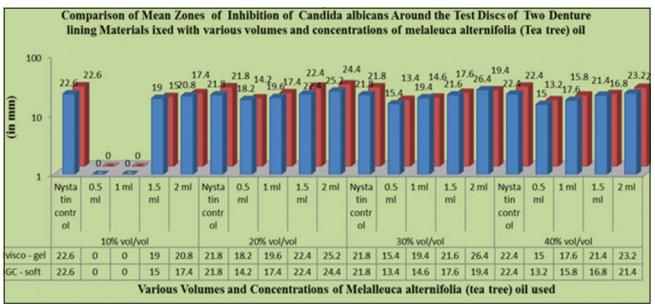

Figure 2: Graph comparing the mean of zones of inhibition around the test discs of the two denture-lining materials mixed with various volumes and concentrations of *Melaleuca alternifolia*

significantly less colonization. This difference might be attributed to some interaction between both the materials. This observation is also in agreement with the study done by Truhlar *et al*., who observed that combination of Visco-Gel and Nystatin had a greater efficacy in denture stomatitis treatment than that of Lynal and Nystatin.[7] These results were also in agreement with the study conducted by Hammer *et al*.,[18] which showed that the treatment *C. albicans* with TTO exert antifungal action by altering membrane properties of fungal cells which may alter their permeability and effect the membranes ability to osmoregulate the cells adequately or to exclude the toxic materials.[28]

Discs treated with 40% *M. alternifolia* (TTO) showed lesser colonization; this finding is in contrast to the findings of Catalan *et al*., (2008)[29] which states that Coe-comfort and Fitt conditioners mixed with 1 ml, 20% vol/vol of melaleuca oil exhibited a total inhibition of *C. albicans* growth. While a study conducted by Sharma and Hegde[30] revealed that melaleuca oil does not show significant antifungal activity until its concentration in Visco-Gel is increased beyond 27.5% concentration. A concentration of 30% showed statistically significant antifungal activity based on minimum inhibitory dose (MID) values. Increasing the concentration to 35% was not found to increase its MID value significantly. However, in contrast, our study showed the maximum inhibition and least colonization of *C. albicans* at 2 ml and 40% vol/vol in both Visco-Gel and GC-soft; this might be attributed to the variation in the strain of *C. albicans* used for the study or due to the difference in the procedure followed.

Comparison of the inhibitory effect of *M. alternifolia* (tea tree) oil and 5% Nystatin susceptibility discs showed that nystatin had a great inhibitory effect than melaleuca oil. This is also in accordance with the study conducted by Sharma and Hegde[30] and Chow *et al*., (1999)[13] which suggest that 5% fluconazole in Visco-Gel showed maximum antifungal activity. However, in that study after 72 h, there was a significant decrease in the antifungal activity, and it had become totally ineffective on day 7 as shown by the regrowth of *C. albicans*. Similar results were also elicited by Truhlar *et al*.,[7] where Visco-Gel incorporating nystatin had a significant loss of antifungal activity by day 2.

Conclusion

Resilient soft liners combined with TTO have shown *in vitro* antifungal efficacy at 40% vol/vol concentration at a dose of 2 ml suggesting the possibility of this essential oil for the therapeutic use against denture stomatitis and possibly other oral infections.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. ten Cate JM, Klis FM, Pereira-Cenci T, Crielaard W, de Groot PW. Molecular and cellular mechanisms that lead to candida biofilm formation. J Dent Res 2009;88:105-15.
2. McCullough MJ, Ross BC, Reade PC. *Candida albicans*: A review of its history, taxonomy, epidemiology, virulence attributes, and methods of strain differentiation. Int J Oral Maxillofac Surg 1996;25:136-44.
3. Reichart PA, Samaranayake LP, Philipsen HP. Pathology and clinical correlates in oral candidiasis and its variants: A review. Oral Dis 2000;6:85-91.
4. Jose A, Coco BJ, Milligan S, Young B, Lappin DF, Bagg J, *et al.* Reducing the incidence of denture stomatitis: Are denture cleansers sufficient? J Prosthodont 2010;19:252-7.
5. Schneid TR. An *in vitro* analysis of a sustained release system for the treatment of denture stomatitis. Spec Care Dentist 1992;12:245-50.
6. Aldana L, Marker VA, Kolstad R, Iacopino AM. Effects of candida treatment regimens on the physical properties of denture resins. Int J Prosthodont 1994;7:473-8.
7. Truhlar MR, Shay K, Sohnle P. Use of a new assay technique for quantification of antifungal activity of nystatin incorporated in denture liners. J Prosthet Dent 1994;71:517-24.
8. Santos VR, Gomes RT, de Mesquita RA, de Moura MD, França EC, de Aguiar EG, *et al.* Efficacy of Brazilian propolis gel for the management of denture stomatitis: A pilot study. Phytother Res 2008;22:1544-7.
9. Nikawa H, Yamamoto T, Hamada T, Rahardjo MB, Murata H, Nakanoda S, *et al.* Antifungal effect of zeolite-incorporated tissue conditioner against *Candida albicans* growth and/or acid production. J Oral Rehabil 1997;24:350-7.
10. Jang KS. Inhibitory effect of antifungal agents incorporated in denture lining materials against *Candida albicans*. J Korean Acad Prosthodont 1999;37:293-300.
11. Amin WM, Al-Ali MH, Salim NA, Al-Tarawneh SK. A new form of intraoral delivery of antifungal drugs for the treatment of denture-induced oral candidosis. Eur J Dent 2009;3:257-66.
12. Vasconcelos LC, Sampaio MC, Sampaio FC, Higino JS. Use of *Punica granatum* as an antifungal agent against candidosis associated with denture stomatitis. Mycoses 2003;46:192-6.
13. Chow CK, Matear DW, Lawrence HP. Efficacy of antifungal agents in tissue conditioners in treating candidiasis. Gerodontology 1999;16:110-8.
14. Thomas CJ, Nutt GM. The *in vitro* fungicidal properties of Visco-gel, alone and combined with nystatin and amphotericin B. J Oral Rehabil 1978;5:167-72.
15. Quinn DM. The effectiveness, *in vitro*, of miconazole and ketoconazole combined with tissue conditioners in inhibiting the growth of *Candida albicans*. J Oral Rehabil 1985;12:177-82.
16. Vojdani M, Zibaei M, Khaledi AAR, Zomorodian K, Ranjbar MA, Boshehri S. *In vitro* study of the effect of clotrimazole incorporation into silicone soft liner on fungal colonization. Shiraz Univ Dent J 2009;9 Suppl 1:19-23.
17. Al-Mashhadane FA. Tea tree oil: A new antifungal agents against *Candida albicans* cells on heat cured acrylic resin denture base material. An *in vitro* study. Al Rafiadain Dent J 2007;7:54-7s.
18. Hammer KA, Carson CF, Riley TV. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces*

cerevisiae. J Antimicrob Chemother 2004;53:1081-5.

19. Bulad K, Taylor RL, Verran J, McCord JF. Colonization and penetration of denture soft lining materials by *Candida albicans*. Dent Mater 2004;20:167-75.
20. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279-94.
21. Moura JS, da Silva WJ, Pereira T, Del Bel Cury AA, Rodrigues Garcia RC. Influence of acrylic resin polymerization methods and saliva on the adherence of four candida species. J Prosthet Dent 2006;96:205-11.
22. Fanello S, Bouchara JP, Sauteron M, Delbos V, Parot E, Marot-Leblond A, *et al*. Predictive value of oral colonization by candida yeasts for the onset of a nosocomial infection in elderly hospitalized patients. J Med Microbiol 2006;55:223-8.
23. Chander S, Hill M, Moore D, Morrow L. Tissue conditioning materials as functional impression materials. Eur J Prosthodont Restor Dent 2007;15:67-71.
24. Nikawa H, Hamada T, Yamamoto T. Denture plaque – Past and recent concerns. J Dent 1998;26:299-304.
25. Jandourek A, Vaishampayan JK, Vazquez JA. Efficacy of melaleuca oral solution for the treatment of fluconazole refractory oral candidiasis in AIDS patients. AIDS 1998;12:1033-7.
26. Gruber RG, Lucatorto FM, Molnar EJ. Fungus growth on tissue conditioners and soft liners. J Am Dent Assoc 1966;73:641.
27. Wright PS. The effect of soft lining materials on the growth of *Candida albicans*. J Dent 1980;8:144-51.
28. Carson CF, Mee BJ, Riley TV. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother 2002;46:1914-20.
29. Catalan A, Pacheco JG, Martinez A, Mondaca MA. Invitro and Invivo activity of Melaleuca alternifolia mixes with tissue conditioner on Candida albicans. Oral Surg Oral Med Oral Pathol Oral RAdiol Endod 2008;105:327-32.
30. Sharma S, Hegde V. Comparative evaluation of antifungal activity of melaleuca oil and fluconazole when incorporated in tissue conditioner: An *in vitro* study. J Prosthodont 2014;23:367-73.