Effect of Addition of Antifungal Agents on Physical and Biological Properties of a Tissue Conditioner: An In-Vitro Study

Pragati Rawat*, Swatantra Agarwal, Siddhi Tripathi

Department of Prosthodontics and Crown & Bridge, Kothiwal dental college and research center, Moradabad, UP-244001, India.

Introduction

A removable dental prosthesis is one of the commonest mode of treatment in prosthetic dentistry. However, their long-term use without rest to underlying oral tissues may lead to adverse effect on their integrity. Both rest and tissue conditioners have been advocated to allow the deformed tissues of the residual ridges to return to normal form. The tissue conditioners are soft, resilient temporary liners that have been widely used in dentistry to manage multitude of patient problems and for various clinical applications. Apart from reducing and evenly distributing stresses on the mucosa of the basal seat they can also be used for temporary relining of immediate dentures etc. However, microbial growth results from the adherence of microbial cells promoted by rough surface, and from adhesive interactions between Candida species and oral bacteria, mostly Candida albicans that may lead to Candidiasis. The treatment of Candidiasis includes denture repair or replacement, adoption of prophylactic measures by the patient and the prescription of topical and systemic antifungal drugs. However, the success of topical application of drugs in the oral cavity may be compromised by the copious flow of saliva as well as by the lack of patient compliance. Therefore, antifungal agents can be incorporated in tissue conditioners to simultaneously treat injured peri-prosthetic tissues and infection by Candida. More recently, azole antifungal compounds such as fluconazole, which have excellent efficacy-toxicity profiles, have emerged as the principal drugs used in the treatment of candida infections. However, fluconazole produces few side effects: nausea, vomiting and might lead to the development of drug resistance in Candida albicans. So, to overcome these unpleasant side effects of chemically synthetic antifungals, herbs with antimicrobial property, especially anti-candida effect are of interest. Out of the various natural essential oils such as Oregano oil, Virgin coconut oil, Melaleuca alternifolia oil, etc incorporated by different researchers in tissue conditioner, origanum oil and coconut oil have been found to exhibit superior antifungal activity. However, their use in dental applications is needed to be investigated more.

The most common problems encountered using soft denture liners are water sorption and solubility which are associated with Candida albicans growth and stresses at the liner/denture base interface leading to reduction in the bond strength. However, no data is available to ascertain the effect of incorporation of fluconazole and virgin coconut oil in the tissue conditioner on it’s tensile bond strength to denture base. Also, the viscoelastic properties of soft denture liners are important since they characterize the ability of the material to exert a cushioning effect on the oral tissues and maintain shape during function. The temporary nature of

Abstract

Purpose: Tissue conditioners are used for healing of abused oral tissues. They may harbour microorganisms causing oral diseases such as candidiasis compromising the health of the patient. Also, addition of antifungal agents into tissue conditioner may alter its properties. This study compares the anti-fungal property and mechanical properties of tissue conditioner containing different antifungal agents.

Methods: Three antifungal agents, one synthetic – fluconazole, and two natural - oregano oil and virgin coconut oil were added into the tissue conditioner (Viscogel) in different concentrations. The antifungal property, tensile bond strength and viscoelasticity of Viscogel containing these antifungal agents were assessed after 24 hours, three days and seven days.

Results: While, the highest antifungal activity was shown by Viscogel containing fluconazole, the maximum tensile bond strength was found to be of Viscogel alone (control). Although Viscogel alone and in combination of fluconazole showed deterioration in viscoelasticity, Viscogel in combination of natural agents showed no significant changes over the period of seven days.

Conclusion: Incorporation of the natural agents in the tissue conditioner can be used as an effective alternative to systemic or topical synthetic antifungal agents.
these types of materials is because of the leaching of the alcohol and the plasticizer and adsorption of water and saliva which causes loss of viscoelasticity and therefore, compliance. Apart from few studies, there are no studies to comment upon the effect of incorporation of fluconazole, oregano oil and virgin coconut oil in the tissue conditioner on it’s viscoelastic properties.

Although, Virgin coconut oil and Oregano oil has been found to provide good anti-fungal protection, scarce investigations has been done for their use in dental applications. Moreover, no conclusive data is available for comparison between anti-fungal property, tensile bond strength and viscoelasticity of tissue conditioners when incorporated with oregano oil, virgin coconut oil and fluconazole. Accordingly, this study was designed to compare and evaluate the aforementioned parameters.

**Materials and Methods**

Test materials used for the study were divided into following four groups having 10 samples each :
1. **Group I** - Visco Gel without any antifungal agents (control)
2. **Group II** - Visco Gel + 10 %w/w Fluconazole
3. **Group III** - Visco Gel + 60% v/v Oregano Oil
4. **Group IV** - Visco Gel + 25% v/v Virgin Coconut oil

Following methodology was employed for the study:

**Antifungal property**

Candida albicans strain (ATCC NO. 24433) was taken and inoculated in Sabouraud dextrose agar broth which was incubated at 37°C for 24 hours. Standardization was done by diluting with sterile broth to a density visually equivalent to barium sulphate, standard Mc farland tube no. 5. It was then dispensed into a sterile Sabouraud agar plate and was allowed to dry. When the petri dishes had dried, four wells of diameter 6 mm and depth 1 mm were punched using a tissue punch on each of the ten petri dishes such that the wells lie within the lawn culture. Each of the four wells on each petri dish was filled with different test materials. After the materials had set in the wells, the plates were incubated at 37°C. The plates were then taken out after 24 hours, three days and seven days.

**Results**

**Antifungal property**

Group I showed no antifungal activity throughout the tested time period of seven days. Figure 1 shows intragroup wise graphical representation of zones of inhibition. Table 1 illustrates the intergroup comparison.

![Figure 1. Intragroup wise representation of zones of inhibition](image.png)
Effect of addition of antifungal agents on tissue conditioner

Table 1. Intergroup comparison of Zones of inhibition

| Comparison | T1 MD | T1 SE | T1 ‘p-value’ | T2 MD | T2 SE | T2 ‘p-value’ | T3 MD | T3 SE | T3 ‘p-value’ |
|------------|-------|-------|-------------|-------|-------|-------------|-------|-------|-------------|
| I v/s II   | -18.80| 0.64  | <0.001      | -14.90| 0.57  | <0.001      | -7.60 | 0.80  | <0.001      |
| I v/s III  | -14.30| 0.64  | <0.001      | -11.10| 0.57  | <0.001      | -6.50 | 0.80  | <0.001      |
| I v/s IV   | -10.80| 0.64  | <0.001      | -7.90 | 0.57  | <0.001      | -1.20 | 0.80  | 0.445       |
| II v/s III | 4.50  | 0.64  | <0.001      | 3.80  | 0.57  | <0.001      | 1.10  | 0.80  | 0.520       |
| II v/s IV  | 8.00  | 0.64  | <0.001      | 7.00  | 0.57  | <0.001      | 6.40  | 0.80  | <0.001      |
| III v/s IV | 3.50  | 0.64  | <0.001      | 3.20  | 0.57  | <0.001      | 5.30  | 0.80  | <0.001      |

MD=Mean difference; SE=Standard error; ‘p-value’=level of significance

Tensile Bond Strength

An increasing trend in tensile bond strength was noticed in all the groups at all time intervals. Intragroup comparison of tensile bond strength is depicted graphically in Figure 2. Intergroup comparison of tensile bond strength is given in Table 2.

Viscoelasticity

Viscoelasticity of the tissue conditioner having different antifungal agents in various concentrations was tested through measurement of modulus of elasticity through stress relaxation test. The tested groups showed varying results at different time intervals. Figure 3 depicts intra-group representation of modulus of elasticity graphically. Table 3 illustrates intergroup comparison of modulus of elasticity.

Table 2. Intergroup comparison of tensile bond strength

| Comparison | 24 hours | Day 3 | Day 7 |
|------------|----------|-------|-------|
|            | MD       | SE    | ’p-value’ | MD       | SE    | ’p-value’ | MD       | SE    | ’p-value’ |
| I v/s II   | 0.39     | 0.02  | <0.001    | 0.19     | 0.06  | 0.008     | 0.08     | 0.05  | 0.408     |
| I v/s III  | 0.04     | 0.02  | 0.245     | 0.08     | 0.06  | 0.597     | 0.09     | 0.05  | 0.298     |
| I v/s IV   | 0.22     | 0.02  | <0.001    | 0.17     | 0.06  | 0.047     | 0.15     | 0.05  | 0.038     |
| II v/s III | -0.34    | 0.02  | <0.001    | -0.12    | 0.06  | 0.267     | 0.01     | 0.05  | 0.997     |
| II v/s IV  | -0.16    | 0.02  | <0.001    | -0.02    | 0.06  | 0.980     | 0.06     | 0.05  | 0.606     |
| III v/s IV | 0.18     | 0.02  | <0.001    | 0.09     | 0.06  | 0.469     | 0.05     | 0.05  | 0.735     |

MD=Mean difference; SE=Standard error; ‘p-value’=level of significance

Discussion

Antifungal Property

In the present study, when antifungal property was evaluated, it showed a statistically significant decrease during the period of study. This decrease in zones of inhibition at the end of seven days was due to the regrowth of the fungus. Similar decreasing trends in the antifungal activity have also been seen in the studies conducted by Sharma et al, Chopde et al and Falahatfai et al for fluconazole and Srivastava et al for oregano oil. Although, there are studies confirming the antifungal efficacy of virgin coconut oil as conducted by Ogbolu et al and Kannan et al, studies related to decreasing trend in antifungal activity over a period of time have not been previously reported. Although, the mechanism of action of all the three anti-fungal agents is different from each other and all of them are causing
determination of the fungus cell membrane in one way or the other such as, fluconazole acts by inhibiting the fungal cytochrome P450 enzyme ‘lanosterol 14-demethylase’ causing impairment in ergosterol synthesis leading to a cascade of membrane abnormalities in the fungus;\textsuperscript{6} origanum oil directly inhibits germination and filament formation (the two phases required for tissue invasion) by Candida albicans\textsuperscript{21} and virgin coconut oil acts by causing the cell membrane of the fungus to disintegrate\textsuperscript{13} but still, fluconazole showed highest antifungal activity amongst all. This is because fluconazole is more potent antifungal agent than the oregano oil and virgin coconut oil which could be supported by the fact that the minimum inhibitory concentration of fluconazole against Candida albicans being reported is 0.25-64 µg/ml\textsuperscript{22} for oregano oil it is 2.5 µg/ml\textsuperscript{23} and for virgin coconut oil it is 32 µg/ml.\textsuperscript{12}

Table 3. Intergroup comparison of Modulus of Elasticity

| Comparison            | 24 hours     | Day 3        | Day 7        |
|-----------------------|--------------|--------------|--------------|
|                       | MD        | SE       | 'p-value'    | MD        | SE       | 'p-value'    | MD        | SE       | 'p-value'    |
| I v/s II              | 2.18      | 0.35    | <0.001      | -0.84     | 0.44    | 0.242      | 1.06      | 0.35    | 0.022      |
| I v/s III             | 2.62      | 0.35    | <0.001      | 1.38      | 0.44    | 0.018      | 4.14      | 0.35    | <0.001      |
| I v/s IV              | -1.03     | 0.35    | 0.029       | -1.57     | 0.44    | 0.006      | 0.06      | 0.35    | 0.998      |
| II v/s III            | 0.44      | 0.35    | 0.604       | 2.22      | 0.44    | <0.001     | 3.08      | 0.35    | <0.001      |
| II v/s IV             | -3.21     | 0.35    | <0.001      | -0.72     | 0.44    | 0.374      | -0.99     | 0.35    | 0.034      |
| III v/s IV            | -3.64     | 0.35    | <0.001      | -2.94     | 0.44    | <0.001     | -4.08     | 0.35    | <0.001      |

MD=Mean difference; SE=Standard error; ‘p-value’=level of significance

**Tensile bond strength**

Tensile bond strength showed an increasing trend from day one to day seven in all the groups at all time intervals. These results were in accordance with the study conducted by Huddar et al.\textsuperscript{24} and Yanikoglu et.\textsuperscript{25} The authors were of the opinion that increase in the bond strength could have occurred because of the leaching out of the plasticizer, which resulted in increased stiffness. This in turn resulted in mechanical bonding and chemical adhesion between soft liner material and acrylic resin.\textsuperscript{17,25} According to Srivastava et al,\textsuperscript{11} the bond strength of Viscogel alone and in combination with oregano oil after one day was 3.97 ±0.75 MPa and 3.73±0.65 MPa which increased to 5.40 ±0.49 MPa and 5.11 ±0.66 MPa respectively after one week. These results were contradictory to the results of the studies conducted by Mese et al,\textsuperscript{26} in which the tensile bond strength of the acrylic resin-based liner (Coe-Soft) decreased over the tested time period from 0.45 MPa after 24 hours to 0.39 MPa after one week because of the swelling and stress formation at the bond interface or from a change in the viscoelastic properties of the liner rendering the material stiffer and better able to transmit external loads to the bond site. On intergroup comparison a statistically significant difference in tensile bond strength was seen after day one (p<0.001) and day three (p=0.014), but, no statistically significant difference was noted after day seven (p=0.060). However, in another study conducted by Srivastava et al no significant difference was seen in tensile bond strength after addition of oregano oil after day one and day seven which indicated that the tensile bond strength of Viscogel was not affected by the addition of oregano oil.\textsuperscript{11} Although, antifungal activity of virgin coconut oil has been previously documented by various authors such as Ogbolu et al\textsuperscript{12} and Kannan et al,\textsuperscript{13} its effect on tensile bond strength of tissue conditioner after getting incorporated into it has not been studied as yet.

**Viscoelasticity**

On intragroup comparison, statistically significant difference was found in Group I (p<0.001), Group II (p<0.001) and Group III (p=0.038), while Group IV showed no statistically significant difference (p=0.596). A higher value of modulus of elasticity which was noted at the end of seventh days in all the groups except Group III denoted increased stiffness of material indicating that the viscoelasticity deteriorated with time. This was in accordance with the studies conducted by Saitoh et al,\textsuperscript{27} McCarthy et al,\textsuperscript{19} and Duran et al\textsuperscript{28} in which the authors concluded that an increase in the elastic modulus was noted over the tested time period rendering the material stiffer. This change in the viscoelasticity resulted from leaching out of the components contained in the liquid, especially ethyl alcohol which are replaced by water.\textsuperscript{3,29} On application of Tukey HSD tests, it was seen that statistically significant increase in elastic modulus was seen for Group I for all time intervals except for day one versus day three (p=0.596). This indicated that Viscogel without any antifungal agents got stiffer at the end of seven days and should be replaced after that. For Group II, this increase in elastic modulus was statistically significant for all time intervals except for day three versus day seven (p=0.099) indicating that the viscoelasticity of Viscogel containing 10% w/w fluconazole deteriorated at the end of three days only and so it should not be used beyond that. For Group III and Group IV the change in elastic modulus was not statistically significant for all time intervals (p>0.05). This implicated that tissue conditioner containing 60%v/v oregano oil (Group III) and 25%v/v virgin coconut oil (Group IV) remained viscoelastic even at the
end of seven days and hence, can be used for a longer duration of time. However, studies related to viscoelasticity of the tissue conditioner after addition of fluconazole, oregano oil and virgin coconut oil have not been conducted till now.

The clinical implications of the results of the present study is that the incorporation of various antifungal agents in the tissue conditioner can serve as an alternative to systemic or topical delivery systems of antifungal drug delivery. When mechanical and physical properties were evaluated, oregano oil showed best results amongst all the tested groups. Since, this herbal product have an added advantage of being safe and cost effective, it can be used as an alternative to the synthetic agents that are currently in use.

There are certain limitations in the present study. The present study was aimed at determining the antifungal activity of different antifungal agents incorporated in the tissue conditioner through measurement of zones of inhibition. However, the antifungal potency is also dependent upon the rate of diffusion of these antifungal agents from Viscogel into agar which in turn is affected by it’s concentration, molecular size, viscosity and phase (liquid/solid) of the medium which were not evaluated in the present study. Therefore, studies based on these parameters affecting diffusion rate should be used to evaluate antifungal potency of these antifungal agents. Since, the present study was performed under controlled laboratory conditions therefore, in-vivo studies are suggested for more precise results.

**Conclusion**

Within the limitations of the study, following conclusions were drawn:

- Maximum antifungal activity was shown by Group II followed by Group III and Group IV.
- Group I had the maximum and Group IV had the minimum tensile bond strength at the end of seven days.
- Viscoelasticity deteriorated for Group I and Group II at the end of seven and three days respectively. While, for Group III and Group IV no significant changes were observed for all time intervals.

**Ethical Issues**

Not applicable.

**Conflict of Interest**

Authors declare no conflict of interest in this study.

**References**

1. Zarb GA, Bolender CL, Eckert SE. Prosthodontic treatment for edentulous patients.12th ed. Noida, India: Elsevier India Private Limited; 2004.

2. Jadhav V, Shetty MM, Kalavathy N, Kumar R. Effect of 3 types of antifungal agents on hardness of 2 different commercially available tissue conditioners: An in-vitro study. SRM J Res Dent Sci 2013;4(4):150-3. doi: 10.4103/0976-433X.125590

3. Murata H, Hamada T, Djulaeha E, Nikawa H. Rheology of tissue conditioners. J Prosthet Dent 1998;79(2):188-99. doi: 10.1016/S0022-3913(98)70215-X

4. Nam KY. In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. J Adv Prosthodont 2011;3(1):20-4. doi: 10.4047/jap.2011.3.1.20

5. Mutneja P, Raghavendraswamy K, Gujjar A. Antifungal activity of heat cure acrylic resin after incorporating different percentages of silver zinc zeolite- an in-vitro study. IJCCD 2012;4(3):49-54.

6. 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(5):367-73. doi: 10.1111/jopr.12117

7. Guo N, Liu J, Wu X, Bi X, Meng R, Wang X, et al. Antifungal activity of thymol against clinical isolates of fluconazole-sensitive and -resistant Candida albicans. J Med Microbiol 2009;58( Pt 8):1074-9. doi: 10.1099/jmm.0.008052-0

8. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers, Medical Publishers; 2008.

9. Choonharuengdej S, Amornvit P, Srithavaj T, Alam MK. In Vitro Anti-Candida Effect of Thai Herbs Supplemented in Tissue Conditioner. Int Med J 2014;21(3):331-4.

10. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. J Med Microbiol 2009;58( Pt 11):1454-62. doi: 10.1099/jmm.0.010538-0

11. Srivastava A, Ginjupalli K, Premappalli N, Bhat N, Ballal M. Evaluation of the properties of a tissue conditioner containing organum oil as an antifungal additive. J Prosthet Dent 2013;110(4):313-9. doi: 10.1016/S0022-3913(13)60381-9

12. Ogbulu DO, Oni AA, Daini OA, Olokpo AP. In vitro antimicrobial properties of coconut oil on Candida species in Ibadan, Nigeria. J Med Food 2007;10(2):384-7. doi: 10.1089/jmf.2006.1209

13. Kannan N, Mohammed A. Comparative evaluation of antifungal activity of Cocos nucifera oil against candida albicans. Int J Phytother Res 2014;4(2):27-31.

14. Mertas A, Garbusinska A, Szliska E, Jureczko A, Kowalska M, Król W. The influence of Tea Tree Oil (Melaleuca alternifolia) on fluconazole activity against fluconazole-resistant Candida albicans strains. BioMed Res Int 2015:2015.590470. doi: 10.1155/2015/590470

15. El-Hadary A, Drummond JL. Comparative study of water sorption, solubility, and tensile bond strength of two soft lining materials. J Prostheth Dent 2000;83(3):356-61. doi: 10.1016/S0022-3913(00)70140-5
16. Robinson JG, McCabe JF. Creep and stress relaxation of soft denture liners. *J Prosthet Dent* 1982;48(2):135-40. doi: 10.1016/0022-3913(82)90099-3

17. Safari A, Vojdani M, Mogharrabi S, Iraji Nasrabadini N, Derafshi R. Effect of Beverages on the Hardness and Tensile Bond Strength of Temporary Acrylic Soft Liners to Acrylic Resin Denture Base. *J Dent (Shiraz)* 2013;14(4):178-83.

18. McCarthy JA, Moser JB. Mechanical properties of tissue conditioners. Part I: Theoretical considerations, behavioral characteristics, and tensile properties. *J Prosthet Dent* 1978;40(1):89-97. doi: 10.1016/0022-3913(78)90165-8

19. Chopde N, Pharande A, Khade MN, Khadtare YR, Shah SS, Apratim A. In vitro antifungal activity of two tissue conditioners combined with nystatin, miconazole and fluconazole against candida albicans. *J Contemp Dent Pract* 2012;13(5):695-8. doi: 10.5005/jp-journals-10024-1211

20. Falah-Tafti A, Jafari AA, Lotfi-Kamran MH, Fallahzadeh H, Hayan RS. A comparison of the efficacy of nystatin and fluconazole incorporated into tissue conditioner on the in vitro attachment and colonization of Candida albicans. *Dent Res J (Isfahan)* 2010;7(1):18-22.

21. Manohar V, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, et al. Antifungal activities of Origanum oil against Candida albicans. *Mol Cell Biochem* 2001;228(1-2):111-7. doi: 10.1023/A:1013311632207

22. Clancy CJ, Yu VL, Morris AJ, Snydman DR, Nguyen MH. Flucnazole MIC and the Flucnazole Dose/MIC Ratio Correlate with Therapeutic Response among Patients with Candidemia. *Antimicrob Agents Chemother* 2005;49(8):3171-7. doi: 10.1128/AAC.49.8.3171-3177.2005

23. Cleff MB, Meinerz AR, Xavier M, Schuch LF, Araújo Meireles MC, Alves Rodrigues MR, et al. In Vitro Activity Of Origanum Vulgare Essential Oil Against Candida Species. *Braz J Microbil* 2010;41(1):116-23. doi: 10.1590/S1517-83822010000100018

24. Huddar DA, Hombesh MN, Sandhyarani B, Chandum GS, Nanjannawar GS, Shetty R. Effect of Denture Cleanser on weight, surface roughness and tensile bond strength of two resilient denture liners. *J Contemp Dent Pract* 2012;13(5):607-11.

25. Yanikoglu N, Denizoglu S. The Effect of Different Solutions on the Bond Strength of Soft Lining Materials to Acrylic Resin. *Dent Mater J* 2006;25(1):39-44. doi: 10.4012/dmj.25.39

26. Mese A, Guzel KG. Effect of storage duration on the hardness and tensile bond strength of silicone- and acrylic resin-based resilient denture liners to a processed denture base acrlic resin. *J Prosthet Dent* 2008;99(2):153-9. doi: 10.1016/S0022-3913(08)60032-3

27. Saitoh S, Sasaki K, Nezu T, Taira M. Viscoelastic behavior of commercially available tissue conditioners under compression. *Dent Mater J* 2010;29(4):461-8. doi: 10.4012/dmj.2009-130

28. Duran RL, Powers JM, Craig RG. Viscoelastic and Dynamic Properties of Soft Liners and Tissue Conditioners. *J Dent Res* 1979;58(8):1801-7. doi: 10.1177/002203457905808060

29. Rodrigues S, Shenoy V, Shetty T. Resilient Liners: A Review. *J Indian Prosthodont Soc* 2013;13(3):155-64. doi: 10.1007/s13191-012-0143-8