

Effect of Incorporation of Clotrimazole on the Zone of Inhibition of Candida Albicans and Surface Roughness in Permanent Soft Liners

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Abstract

Purpose: Permanent soft liners used for the treatment of denture stomatitis have the drawback of increased porosity and surface roughness. This creates a niche for the growth of opportunistic infections. This study aimed to analyze the change in the zone of inhibition and surface roughness with the addition of clotrimazole in soft liner. **Materials and methods:** Long-term permanent soft liners Permasoft (group 1 and 3) and Mollosil (group 2 and 4) were used to fabricate specimen discs (n=48/group) and blocks (n=8/group) with and without clotrimazole in an aseptic environment using a die. Samples were stored in salivary substitute. Discs were placed in petri dishes containing candida albicans culture on Sabouraud dextrose agar medium and incubated for 24 hrs. to measure the zone of inhibition. Surface roughness tester -SJ-210 Mitutoyo Instrument was used on blocks to measure surface roughness. Readings were recorded at time intervals of 1, 7, 14, 21, 35, 42 days. **Results:** One way ANOVA test, post hoc Tukey analysis, and Student t-test were performed. After 42 days decrease in the zone of inhibition was observed ($p < 0.001$) for group 2 and 4 and a constant increase in surface roughness for all groups ($p < 0.001$). Zone of inhibition in control groups was nil. **Conclusion:** The addition of clotrimazole in soft liner inhibits the growth of candida albicans in vitro and can be used for treating candidiasis. Surface roughness values increase within the clinically acceptable limits.

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Introduction-

The oral tissues provide adequate support to the removable dental prosthesis. Adaptation of the prosthesis is compromised due to constant remodeling of alveolar ridges and oral tissues with time. Ill-fitting prosthesis along with predisposing systemic conditions like immunocompromised diseases, diabetes, vitamin deficiency, etc. in geriatric patients leads to the formation of sore spots and tissue trauma¹. For fabrication of a new prosthesis, the abused mucosa needs to be conditioned with soft liners which are applied to the current prosthesis. This helps in regaining adaptation of the prosthesis to the tissues^{1, 2, 3}. The soft liner which can be short-term or long-term and acrylic or silicone-based depending on the duration of use is applied between the impression surface of the prosthesis and oral mucosa. It acts as a shock absorber by providing a cushioning effect. Some known drawbacks of these materials are leaching out of plasticizers over time which makes them more porous in composition. This porosity provides a niche for the growth of opportunistic fungus such as candida albicans causing candidiasis. Treatment of candidiasis includes systemic and topical antifungal agents. The fungi which invade the superficial tissues are better treated using topical agents; but they are less palatable leading to discomfort for the patients and also an uncertainty of application of topical agents by the patient. Based on this, various studies have been conducted by incorporating antifungal drugs into the soft liners it proved to be effective and viable method for reducing biofilm accumulation^{4, 5, 6, 7}.

There are very few studies in literature regarding the incorporation of antifungal agents in soft liners especially long-term liners^{11, 12, 13}. Amongst the properties, the surface roughness of the material

changes because of alcohol and plasticizers which leach out from the liner into the liquid medium. As the surface roughness increases, the biofilm formation increases due to accumulation of oral microorganisms. This poses as a challenge in oral hygiene¹⁴.

This study aimed to examine the change in the zone of inhibition of candida albicans and the surface roughness change by incorporation of clotrimazole into long-term denture soft liners in the in-vitro experimental setting. Based on this a null hypothesis was formulated stating 'There will be no significant change on candida growth and no change in surface roughness between control samples and samples containing clotrimazole.'

Materials and methods-

The permanent long-term soft liners selected for this study were Acrylic based-soft liner (Permasoft DENTSPLY) and Silicone based -soft liner (Mollosil Detax). The material was manipulated according to the manufacturer's guidelines for Group 1- Permasoft control and Group 3-Mollosil control. The antifungal drug Clotrimazole was added at the minimum inhibitory concentration of 5µg/ml. Thus 0.6mg Clotrimazole/60gm of powder was incorporated while manipulating material for Group 2- Permasoft with Clotrimazole and Group 4- Mollosil with Clotrimazole²². The material is transferred into stainless steel dies [Figure 1a, b] in an aseptic environment. Total n=48 discs (radius 2cm, height 0.2cm) and n=8 blocks (2cmx2cmx2cm) were fabricated for each group. [Figure 2] All samples were fabricated by a single operator to maintain uniformity. Samples were stored in 4 separate Petri dishes containing 10 ml salivary substitute (GC Dry Mouth Gel- GC) and incubated at 37°C in an incubator.

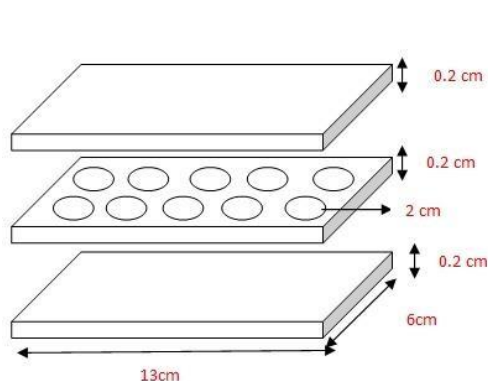


Figure 1a

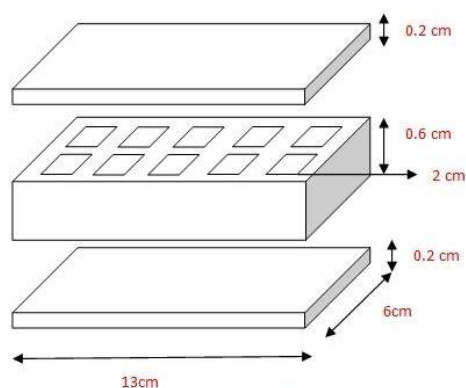


Figure 1b

Figure 1a- Schematic Representation of die for Disc Fabrication. Figure 1b- Schematic Representation of die for Block Fabrication



Figure 2- Sample in Disc and Block

Swabs were obtained from 0.5McFarland concentration candida albicans (ATCC10231) strain [Figure3] and were cultured on petri dishes with Sabaraud dextrose agar medium. Samples were placed in approximate center of each petri dish. For 24 hrs., 8 such samples from each group were incubated at 37°C for 24 to measure the visible zone of inhibition created by the drug was in mm. [Figure 4] Surface roughness tester -SJ-210 Mitutoyo Instrument (JIS1994 Standard) was used to measure surface roughness on the blocks. All 8 samples were

tested from each group. The stylus was passed on the surface and the Ra value displayed on the monitor was noted. [Figure 5]

The procedure was repeated at intervals of first day, first week, second week, third week, fourth week, fifth week, sixth week and results of the zone of inhibition and surface roughness were noted down. The data obtained were evaluated by SPSS software (SPSS 12.0; SPSS, INC, CHICAGO, III) using ANOVA test, Tukey's post hoc

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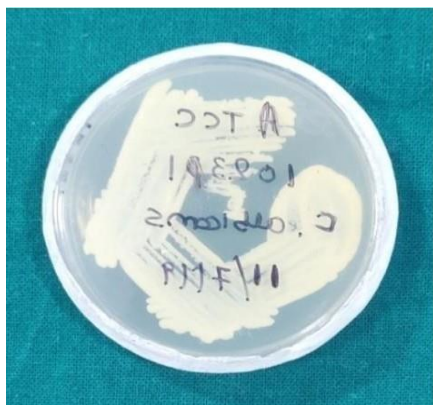


Figure 3

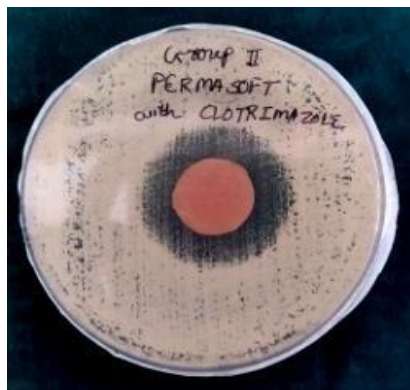


Figure 4



Figure 5

Figure 3- ATCC Strain 10231.

Figure 4- Sample in petri dish showing zone of inhibition.

Figure. 5-Surface Roughness Value tested on roughness tester

Results-

For Groups 1 and 3, no change in values of the zone of inhibition was observed ($p > 0.001$) at intervals of first day, first week, second week, third week, fourth week, fifth week, sixth week. For Group 2 the value of the zone of inhibition decreased on each time interval ($p < 0.001$) except between days 7 and 14 where it remained constant ($p \text{ value} > 0.01$). For Group 4, the value of the zone of inhibition decreased constantly from day 1 to 35 ($p < 0.01$) except between day 35 and 42 ($p > 0.001$).

The maximum zone of inhibition was observed on day 1 for groups 2 and 4. On one way analysis of variance (ANOVA) test for the zone of inhibition, the value decreased over time in between and within groups ($p < 0.001$). [Table-1]. From Tukey post hoc analysis, results for Group 2 and 4 showed significant changes in values at particular and at different time intervals suggesting a change in values of the zone of inhibition ($p < 0.001$). Whereas change in the zone of inhibition was insignificant for Groups 1 and 3.

TABLE 1- Comparison of zone of inhibition (in mm) in between Groups and within Groups by One way ANOVA

		Sum of Squares	df	Mean Square	F	ANOV A P VALUE	Significance
DAY 1	Between Groups	4216.125	3	1405.375	2861.855	<0.0001	S
	Within Groups	13.750	28	0.491			
	Total	4229.875	31				
DAY 7	Between Groups	3044.250	3	1014.750	3071.676	<0.0001	S
	Within Groups	9.250	28	0.330			
	Total	3053.500	31				
	Between Groups	2777.844	3	925.948	1471.009	<0.0001	S
	Within Groups	17.625	28	0.629			

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DAY 14	Total	2795.469	31				
DAY 21	Between Groups	1329.344	3	443.115	848.356	<0.0001	S
	Within Groups	14.625	28	0.522			
	Total	1343.969	31				
DAY 35	Between Groups	774.125	3	258.042	614.908	<0.0001	S
	Within Groups	11.750	28	0.420			
	Total	785.875	31				
DAY 42	Between Groups	760.250	3	253.417	630.726	<0.0001	S
	Within Groups	11.250	28	0.402			
	Total	771.500	31				

TABLE 2- Comparison of surface roughness (Ra value) in between groups and within groups by One way ANOVA

		Sum of Squares	df	Mean Square	F	ANOVA P VALUE	Significance
DAY 1	Between Groups	4.717	3	1.572	1752316.373	<0.0001	S
	Within Groups	.000	28	0.000			
	Total	4.717	31				
DAY 7	Between Groups	10.671	3	3.557	2429162.764	<0.0001	S
	Within Groups	.000	28	0.000			
	Total	10.671	31				
DAY 14	Between Groups	21.946	3	7.315	335367.857	<0.0001	S
	Within Groups	.001	28	0.000			
	Total	21.946	31				
DAY 21	Between Groups	22.813	3	7.604	240.918	<0.0001	S
	Within Groups	.884	28	0.032			
	Total	23.697	31				
DAY 35	Between Groups	69.085	3	23.028	26184528.076	<0.0001	S
	Within Groups	.000	28	0.000			
	Total	69.085	31				
DAY 42	Between Groups	59.120	3	19.707	982042.571	<0.0001	S
	Within Groups	.001	28	0.000			
	Total	59.120	31				

The mean values of surface roughness of 4 groups were measured in Ra value at all-time intervals and noted. (Graph-2). By analysing results in student t-

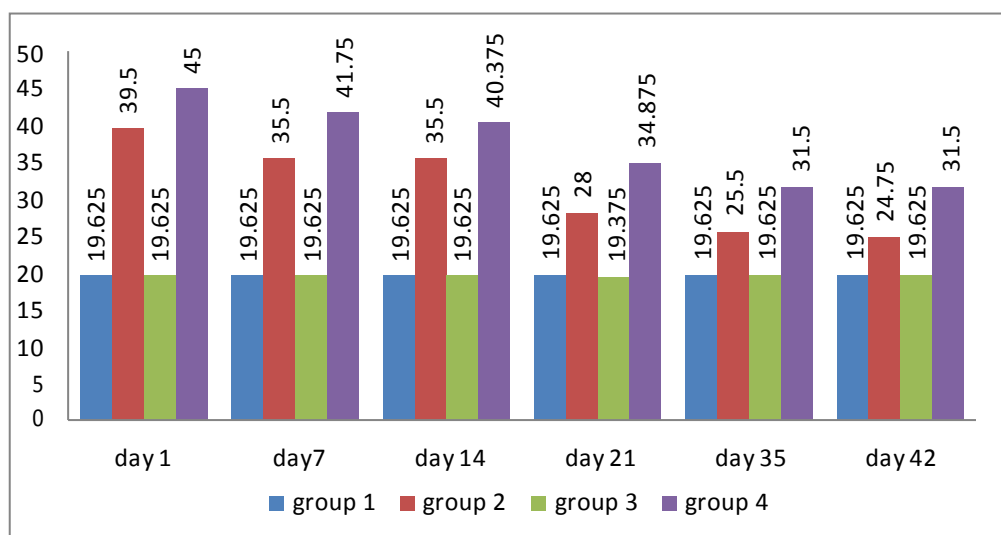
tests for Groups 1, 2, and 4; a linear increase in surface roughness value was recorded from day1 to day 42($p < 0.0001$). Whereas for Group 3, a

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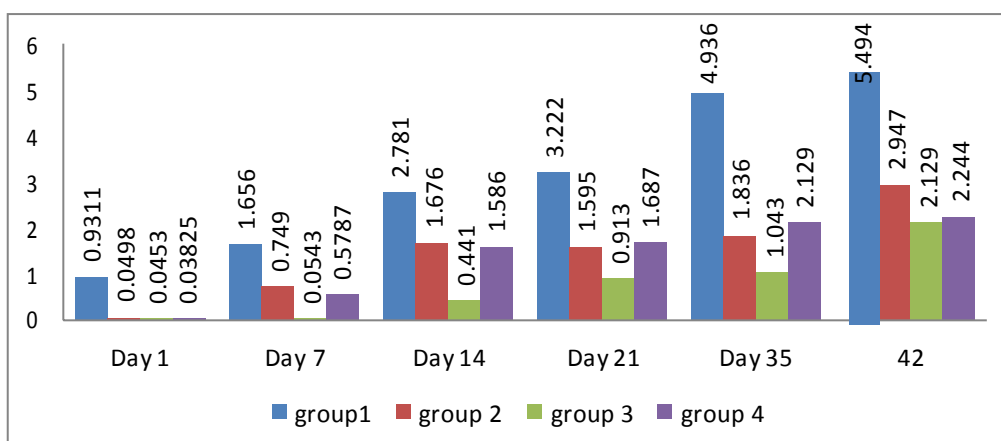
statistically significant increase in surface roughness value was observed from day 1 to 21 and 35 to 42 ($p < 0.0001$). On analyzing a significant increase in roughness was observed over time in between and within all groups from day 1 to 42 ($p < 0.001$) [Table-

2]. On comparing values using the Tukey post hoc analysis test, all groups had statistically significant p-value ($p < 0.0001$) except for groups 2 and 4 on day 21.

GRAPH 1: Shows comparison of zone of inhibition values (in millimeters) for all groups at various time intervals (in days)



GRAPH 2: Shows comparison of surface roughness (Ra) values (in μm) for all groups at various time intervals (in days)



Discussion:

For this study, the addition of clotrimazole lead to the formation of a zone of inhibition in candida growth and also altered the surface roughness of these materials. This proves that the null hypothesis formulated for this study was rejected. There is slow, prolonged release of the drug directly onto the affected tissues which give the benefit of reduced

drug dosage with maximum effect against candidiasis.^{14,15} Topical application of Clotrimazole and Nystatin are most commonly prescribed for treating oral candidiasis¹⁶. Since nystatin is a short-term release drug, its use in permanent soft liners serves lesser importance^{17,18}

Based on statistical findings, groups 1 and 3, it can be proved that long-term soft liners do not possess any in-built antifungal properties. These results are

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similar to Grüber et al. which showed candida growth in silicone and methacrylate soft denture liners⁵. The antifungal and drug releasing effect in group 2 decreases within first week after which the decrease is gradual under the acrylic-based soft liner.^{19,20,21} Acrylic liners are less stable in composition than Silicone soft liners because they do not contain a plasticizer. As there is no leaching out of products when they are stored in salivary substitute, softness and elasticity is not changed over time. Due to this reason, there is constant drug-release from Mollosil with clotrimazole (group 4) along with gradual loss of zone of inhibition that reaches a constant steady level at day 35. These results are similar to the studies done by M. Addy et al.²², Marta Radnai et. Al.²³, Thomas et. Al.⁷ and Vojdani et. Al.²⁴ for various soft liners and antifungal drugs.

There is resistant strain formation occurs due to long time contact of antifungal drugs with mucosa which is not a desirable outcome. The antifungal efficacy of drug after 42 days, this created a lesser chance of formation of resistant strains and a more effective treatment outcome. After 42 days, the soft liner containing clotrimazole have the all properties of liners, But this liners don't show antifungal property.

Permasoft control = Mollosil control < Permasoft with clotrimazole < Mollosil with clotrimazole

There are many factors related to removable dentures linked to surface roughness i.e., stain resistance, retention, oral tissue health and comfort of the patient. In this study, the minimum to maximum values of surface roughness on day one was in the following order; Group 4 < Group 1 = Group 3 < Group 1. While the minimum to maximum values of surface hardness at day forty two was Group 3 < Group 4 < Group 1 < Group 2. There is a constant increase in the value of surface roughness from day one up to day forty two for all the groups, due to leaching out of the plasticizers and low molecular weight antifungal which makes the surface rough by the creation of pores and craters. These results are not similar to the study done on temporary soft liners and tissue conditioners by incorporation of drugs like miconazole, nystatin, and itraconazole by Mirian et. Al.⁵ As the surface roughness increases, the chances of absorption of the

fluids, microbes adhesion and biofilm formation in material increases.^{25,26,27,21} The roughness value of Permasoft with clotrimazole is less than that of Permasoft control because when the bound drug molecules leach out, the polymerization chain reaction continues, making a stronger and uniform matrix. For groups containing mollosil (group 1 and group 3) values of surface roughness is increased due to the release of plasticizers and the release of the drug particles.

Permasoft and Mollosil do not possess antifungal properties of their own. This is the limitation of this study. When clotrimazole is added at the minimum inhibitory concentration in permasoft and mollosil, the zone of inhibition for mollosil with clotrimazole is more than permasoft with clotrimazole and surface roughness of mollosil with clotrimazole is lesser than permasoft with clotrimazole. This makes mollosil with clotrimazole a more effective material for use.

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