TO EVALUATE AND COMPARE THE EFFECT OF ADDITION OF DIFFERENT ANTIFUNGAL AGENTS ON TENSILE BOND STRENGTH AND ANTI-FUNGAL EFFICACY OF SOFT TISSUE LINER: AN IN-VITRO STUDY

Ghazi A[1*], Saxena D[2], Bali Y[3], Pandey A[4], Goswami R[5], Aggarwal S[6]

Article History: Received: 20.02.2023 **Revised:** 05.04.2023 **Accepted:** 20.05.2023

Abstract

Purpose: Soft tissue liners 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 soft tissue liner may alter its properties. This study compares tensile bond strength and antifungal properties of soft tissue liner containing different antifungal agents. Methods: 2 Antifungal agents, ketoconazole and voriconazole were added into the soft tissue liner (permasoft). The tensile bond strength of permasoft with poly methyl meth acrylate with and without antifungal agents were assessed. Antifungal efficacy of permasoft containing these antifungal agents were assessed on 1st, 3rd ,7th ,15th and 30th day. Results: Maximum tensile bond strength was found to be of permasoft alone (control). While, the highest antifungal activity was shown by permasoft containing ketoconazole.Conclusion:The ketoconazole in the soft tissue liners can be used as an effective treatment option for c.albicans, then the systemic or topical antifungal agents.

Keywords: Ketoconazole, Tensile Bond Strength, Voriconazole, Antifungal Efficacy.

[1*]PG student, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital, India.
[2]Professor, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital, India.
[3]Associate Professor, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital, India.
[4]Professor & Head, Department of Microbiology, Subharti Medical College & Hospital, India.
[5]Professor & Head, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital, India.
[6]Professor, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital, India.

Email Address: [1*]amreen620.ag@gmail.com

DOI: 10.31838/ecb/2023.12.s3.372

Corresponding Author: Ghazi A[1*]
[1*]PG student, Department of Prosthodontics and Crown & Bridge, Subharti Dental College & Hospital
[1*]amreen620.ag@gmail.com

1. Introduction

Poly methyl methacrylate, a polymer has various applications and is utilized in clinical prosthodontics to fabricate complete dentures, temporary crowns, and artificial teeth. Polymethyl methacrylate is available in the form of a powder-liquid system. This polymer has distinctive properties such as density, aesthetics, cost-effectiveness, ease of manipulation, and tailorable physical and mechanical properties. Polymethyl methacrylate has some concerns associated with water sorption leading to the fracture of dentures, poor impact and flexural strength. Prosthesis manufactured from polymer are prone to microbe adhesion which leads to denture stomatitis.[1]

Denture stomatitis affects denture wearers and is characterized by inflammation and erythema of areas covered by the denture. Options for denture stomatitis treatment are varied and include the use of soft denture liner, topical and systemic antifungal therapy, oral hygiene care, replacement of old dentures, removal of anatomical irregularities, re-establishment of non-traumatic occlusion, and nutritional restitution. In addition to protecting and preserving mucosal integrity patients should sleep without dentures.[2]

Denture soft tissue liners are most commonly used to the intaglio surface of dentures in order to achieve a more equal distribution of the load and reduction of local point pressures.[3]

The available soft tissue liners are either in plasticized acrylic or silicone elastomer form. Accessibility of both types is in the form of auto polymerizing and heat cure. Denture soft tissue liners consist of powder (polymethylmethacrylate and co-polymers) and liquid (methacrylate monomers and plasticizers) and have a tendency to become hard and lose their resiliency due to gradual leaching.[4]Use of high molecular weight acrylic monomers helps to reduce the plasticizer requirement in the acrylic-based soft tissue liner.

Silicone lining material cannot bond chemically which ultimately leads to the potential surface for microbial growth, plaque, and calculus formation at the debonded regions and often cause the functional failure of the prosthesis. Acrylic-based soft tissue liners chemically bond to the denture surface due to similar molecular structure.[5] This eventually help in success of prosthesis.

Topical and/or systemic antifungals are effective in minimizing the signs and symptoms of denture stomatitis. However, these drugs cannot reach a therapeutic antifungal concentration on the denture surfaces, and therefore mucosal reinfection occurs rapidly after treatment completion. Effective treatment of denture stomatitis would ideally require a therapy based on the sustained release of antifungal drugs that may reach sufficient therapeutic concentrations to eliminate the Candida from both the supporting tissues and affected denture surfaces.[6]

Response of topical application of drugs in the oral cavity may be compromised due to copious flow of saliva and diet, as well as lack of patient compliance while systemic administration of drugs causes side effects in effective dose. To subside these limitations, antifungal agents can be incorporated into soft tissue liners to treat injured periprosthetic tissues.[7]

More recently, some azole antifungal compounds emerged as principal drugs in treating candida infection because of their excellent efficacy profile such as Ketoconazole and Voriconazole.

Thus, the present in-vitro study was planned to evaluate and compare the effect of the addition of different anti-fungal agents (ketoconazole and voriconazole) on tensile bond strength and their antifungal efficacy by incorporating these drugs into denture soft tissue liner (Permasoft).

METHODOLOGY

A total of 60 specimens were fabricated:

For tensile bond strength: Consisted of 30 blocks were divided into following 3 groups having 10 samples each.

(1) Group 1-PMMA with Permasoft without any antifungal agents(control).
(2) Group 2-PMMA with Permasoft +ketoconazole
(3) Group 3-PMMA with Permasoft +voriconazole.

For anti-fungal efficacy: Consisted of 30 circular discs were divided into following 3 groups having 10 samples each.

(1) Group 1- Permasoft without any antifungal agents(control).
(2) Group 2- Permasoft +ketoconazole
(3) Group 3-Permasoft +voriconazole.

Following methodology was employed for the study

For tensile bond strength testing

a) Special Metal die fabrication; Stainless steel metal die was prepared which contained a total of three sections upper, middle, and lower. Each section had the same dimensions of 150x100x100 mm. The middle section had four vertical slots with metal separator of dimensions 3 x 10x 10mm (figure1).

b) **Preparation of PMMA blocks;** Middle section of the die was placed on top of the lower section with slidable metal separator between vertical slots in the centre of the die. PMMA powder and liquid mixed until dough stage reached, now homogenous

mixture packed into vertical slots. Polymerization was carried out by following cycle i.e at 74 °C for 2 hours followed by 100 °C for 1 hour. The die was allowed to cool at room temperature.

c) **Addition of soft tissue liner with and without an anti-fungal agent in between the prepared PMMA blocks;** Permasoft was manipulated according to the manufacturer's instructions in a 2:1 P/L ratio and was placed in the 3 mm space between two PMMA blocks. Permasoft with and without incorporating an anti-fungal agent was stirred for 15 seconds and left standing for 4 minutes and a homogenous mixture was packed into the die. After packing die was placed in hot water for 10 minutes at 75 °C, after completing the process, the samples were retrieved from the die. A total of 30 samples were prepared for tensile bond strength testing (figure 2).

Testing of samples for tensile bond strength: Universal testing machine with a digital monitor was used. The samples were gripped vertically and firmly between the upper and lower crosshead jaws (figure 3). Then tensile force was applied gradually (figure 4), at a crosshead speed of 5mm/min until complete debonding occurred (figure 5). All the samples were tested in a similar manner and readings were recorded.

Tensile bond strength = Force at debonding The cross-sectional area of the interface

For Antifungal Efficacy Testing

Following steps were followed:

a) **Special Metal die fabrication:** A stainless steel die was prepared which was divided into three sections: upper, middle, and lower. Each se
b) ction was 80 x 60 x 32 mm in dimension. The middle section had 4 circular slots with a dimension of 10 x 2 mm diameter (figure 6).
c) **Preparation of soft tissue liner circular disc with and without the addition of antifungal agent:** Group 1: (Control) Permasoft powder (2 parts), and liquid (1 part) were mixed according to the manufacturer‘s recommendation without incorporating any anti-fungal agent. Group 2: Permasoft with 10 %w/w Ketoconazole added to Permasoft powder. Group 3: Permasoft with 10 %w/w Voriconazole added to Permasoft powder. The mixture of soft tissue liner with and without incorporation of an antifungal agent was stirred for 15 seconds and left standing for 4 minutes and a homogenous mixture was packed into the die. After packing die was placed in hot water for 10 minutes at 75 °C, after completing the process, the samples were retrieved from the die. A total of 30 samples were prepared for antifungal efficacy testing (figure 7).
d) **Preparation of the C. albicans culture for antifungal efficacy**; Pure culture of the C. albicans with ATCC 90028 strain was prepared. C. albicans strain was inoculated in peptone water and had an optical density of 0.5 McFarland turbidity standards. Muller Hinton agar (MHA) plates were inoculated by swabbing them with a sterile cotton swab. Freshly prepared soft tissue liner samples were placed over the prepared Muller Hinton agar (MHA) plates with sterile forceps (figure 8).

The plates were incubated at 37°C aerobically. The anti-fungal activity was checked on the first day, 3rd day, 7th day, 15th day, and on the 30th day for all the specimens. The absence of growth of C. albicans around the soft tissue liner samples was demonstrated by the zone of low growth/inhibition zone. The increase in the size of the zone of inhibition showed the persistent activity of ketoconazole and voriconazole added to Permasoft.

Statistical Analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical analysis Software in which the obtained data was subjected to one way ANOVA F test and unpaired ‘‘t’’ test.

2. Results

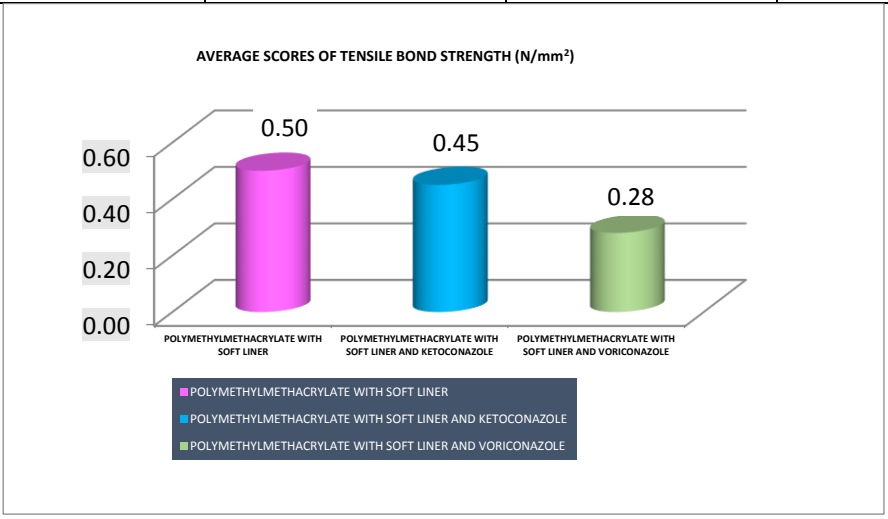
Tensile Bond Strenght

Table 1: illustrates the mean, s.d, maximum, minimum, and c.v scores of tensile bond strength (n/mm^2) for three Groups and Group 1 showed maximum tensile bond strength. Graph 1: shows average tensile bond strength in (n/mm^2) for three different Groups. Table 2: showed inter group comparison for tensile bond strength in (n/mm^2) between different pair of groups (by unpaired ‟t‟ test). Table 3: showed intra group comparison for tensile bond strength (in n/mm^2) between different pair of groups (by one way Anowa-f test).

Table 1. Mean, S.D, maximum, minimum, and C.V scores of tensile bond strength (N/MM^2) for three groups

S.NO	GROUP 1	GROUP 2	GROUP 3:
1	0.53	0.46	0.28
2	0.44	0.48	0.27

3	**0.52**	**0.49**	**0.25**
4	**0.54**	**0.42**	**0.29**
5	**0.51**	**0.44**	**0.29**
6	**0.48**	**0.43**	**0.27**
7	**0.50**	**0.44**	**0.28**
8	**0.53**	**0.46**	**0.28**
9	**0.45**	**0.42**	**0.27**
10	**0.47**	**0.43**	**0.28**
MEAN	**0.50**	**0.45**	**0.28**
STANDARD DEVIATION	**0.035**	**0.025**	**0.012**
MAXIMUM SCORE	**0.54**	**0.49**	**0.29**
MINIMUM SCORE	**0.44**	**0.42**	**0.25**
C.V.	**7.101**	**5.485**	**4.253**



Graph 1- Average Tensile Bond Strength In (N/mm^2) For Three Different Groups

Table:2 Comparison For Tensile Bond Strength (In N/MM^2) Between Different Pair Of Groups (By Unpaired "T" Test)

S.NO.	PAIR OF DIFFERENT SPECIMENS	PROBABLE VALUES OF UN-PAIRED "t" TEST B/W DIFFERENT GROUPS FOR COMPARING THE SIGNIFICANCE IN TENSILE STRENGTH (N/mm2)
1	**GROUP 1**	**P=.0020* P<.05 (SIGNIFICANT)**
2	**GROUP 2**	**P=.0000* P<.05 (SIGNIFICANT)**
3	**GROUP 3**	**P=.0000* P<.05 (SIGNIFICANT)**

*Shows a significant difference b/w different specimens comparing at .05 level of significance. I.e (p<.05)

Table:3 Comparison For Tensile Bond Strength (In N/MM^2) Between Different Pairs Of Groups (By One-Way Anova -F Test)

S.NO.	PAIR OF DIFFERENT SPECIMENS	PROBABLE VALUES OF ONE WAY ANOVA-F TEST FOR COMPARING THE SIGNIFICANT DIFFERENCE IN TENSILE STRENGTH (N/mm^2) AMONG THE GROUPS
1	**AMONG GROUP 1, GROUP 2, AND GROUP 3**	**P=.0000* P<.05 (SIGNIFICANT)**

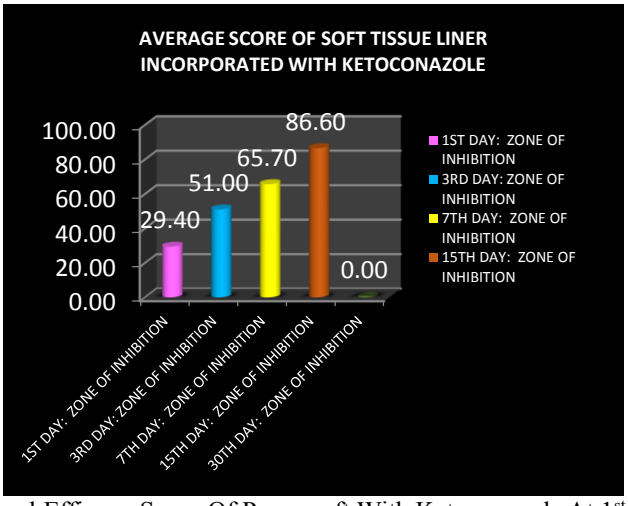
***Shows A Significant Difference B/W Different Specimens Comparing At .05 Level Of Significance. I.E (P<.05)**

Antifungal Efficacy

Table 4: illustrates the mean, s.d, maximum, minimum, and c.v scores of antifungal efficacy in (mm) of Permasoft with ketoconazole. Graph 2: shows average antifungal efficacy in (mm) for Permasoft with ketoconazole. Table 5: illustrates the mean, s.d, maximum, minimum, and c.v scores of antifungal efficacy in (mm) of Permasoft with voriconazole. Graph 3: shows average antifungal efficacy in (mm) for Permasoft with voriconazole.Group 2 and Group 3 showed antifungal efficacy on the 1st day, 3rd day, 7th day, and 15th day. Maximum antifungal efficacy showed by group 2.

Table-4 Mean, S.D, Maximum, Minimum, and C.V Scores of Anti-Fungal Efficacy (Zone Of Inhibition in MM) For Group 2 (Permasoft With Ketoconazole) At Five Different Time Periods

S NO.	1ST DAY: ZONE OF INHIBITION	3RD-DAY: ZONE OF INHIBITION	7TH DAY: ZONE OF INHIBITION	15TH DAY: ZONE OF INHIBITION	30TH DAY: ZONE OF INHIBITION
1	26	56	76	92	0
2	27	66	71	94	0
3	28	56	71	94	0
4	33	47	69	90	0
5	29	43	69	88	0
6	34	56	73	94	0
7	35	51	64	88	0
8	28	46	54	76	0
9	26	46	55	76	0
10	28	43	55	74	0
Mean	**29.40**	**51.00**	**65.70**	**86.60**	**0**
Standard deviation	**3.34**	**7.41**	**8.21**	**8.11**	**0**
Maximum score	**35.00**	**66.00**	**76.00**	**94.00**	**0**
Minimum score	**26.00**	**43.00**	**54.00**	**74.00**	**0**
C.V.	**11.36**	**14.53**	**12.49**	**9.37**	**0**

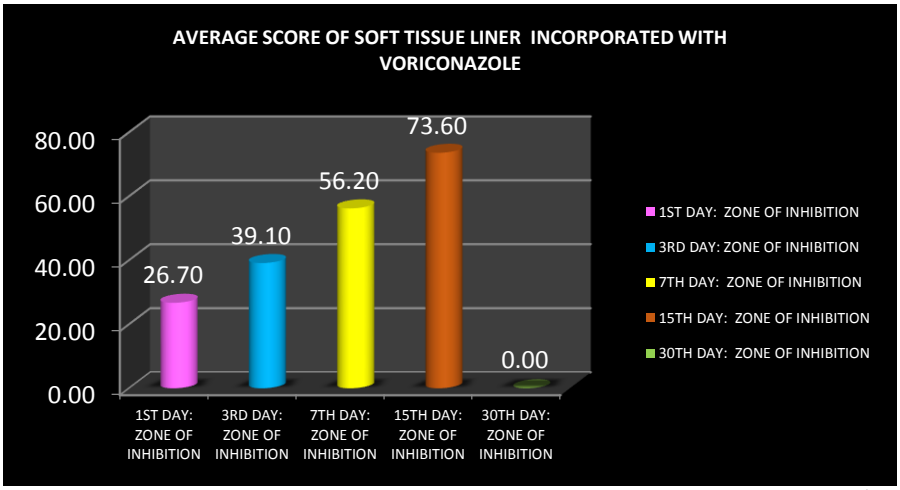


Graph- 2 Average Antifungal Efficacy Score Of Permasoft With Ketoconazole At 1st , 3rd , 7th , 15 Th , And 30th Day (Zone Of Inhibition) In Mm

Table-5 Mean, S.D, Maximum, Minimum, and C.V Scores of Anti-Fungal Efficacy (Zone Of Inhibition in MM) For Group 3 (Permasoft with Voriconazole) In Five Different Time Periods

S NO.	1ST DAY: ZONE OF INHIBITION	3RD-DAY: ZONE OF INHIBITION	7TH DAY: ZONE OF INHIBITION	15TH DAY: ZONE OF INHIBITION	30TH DAY: ZONE OF INHIBITION
1	23	46	54	74	0
2	24	36	52	72	0
3	34	46	57	76	0
4	26	39	58	78	0

5	26	39	59	80	0
6	24	37	65	82	0
7	32	40	60	72	0
8	34	40	55	74	0
9	20	38	53	70	0
10	24	30	49	58	0
Mean	**26.70**	**39.10**	**56.20**	**73.60**	**0**
Standard deviation	**4.90**	**4.65**	**4.59**	**6.65**	**0**
Maximum score	**34.00**	**46.00**	**65.00**	**82.00**	**0**
Minimum score	**20.00**	**30.00**	**49.00**	**58.00**	**0**
C.V.	**18.35**	**11.90**	**8.17**	**9.04**	**0**



Graph 3- Average Antifungal Efficacy Scores Of Group 3 (Permasoft With Voriconazole) At 1st , 3rd , 7th , 15 Th , And 30th Day (Zone Of Inhibition) In Mm

Table:6,6(a),7, and7(a) showed inter and intra group comparison for antifungal efficacy in (mm) between different pair of groups by unpaired "t" test and by one-way anova-f test at five different days. Graph 4 shows average antifungal efficacy scores in three different groups at f day, third day, seventh day, fifteenth day and thirtieth day (zone of inhibition) in mm.

Table -6 Comparison of Zone of Inhibition (In Mm) Between Different Pair of Groups (By Unpaired ''T'' Test) At Five Different Days

S.NO.	PAIR OF DIFFERENT DAYS	PROBABLE VALUES OF PAIRED "t" TEST B/W DIFFERENT DAYS FOR COMPARING THE SIGNIFICANCE OF ANTIFUNGAL EFFICACY IN THREE GROUPS		
		GROUP 1	GROUP 2	GROUP 3
1	B/W 1ST DAY & 3RD DAY	P=.0001* P<.05 (SIGNIFICANT)	P=.0002* P<.05 (SIGNIFICANT)	P=.0003* P<.05 (SIGNIFICANT)
2	B/W 3RD DAY & 7TH DAY	P=.0000* P<.05 (SIGNIFICANT)	P=.0021* P<.05 (SIGNIFICANT	P=.0004* P<.05 (SIGNIFICANT)
3	B/W 1ST DAY & 7TH DAY	P=.0000* P<.05 (SIGNIFICANT)	P=.0001* P<.05 (SIGNIFICANT)	P=.0005* P<.05 (SIGNIFICANT)
4	B/W 1ST DAY & 15TH DAY	P=.0000* P<.05 (SIGNIFICANT)	P=.0001* P<.05 (SIGNIFICANT)	P=.0005* P<.05 (SIGNIFICANT)
5	B/W 1ST DAY & 30TH DAY	P=.0003* P<.05 (SIGNIFICANT)	P=.0021* P<.05 (SIGNIFICANT)	P=.0005* P<.05 (SIGNIFICANT)
6	B/W 3RD DAY & 15TH DAY	P=.0001* P<.05 (SIGNIFICANT)	P=.0001* P<.05 (SIGNIFICANT)	P=.0019* P<.05 (SIGNIFICANT)

7	**B/W 3RD DAY & 30TH DAY**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0001* P<.05 (SIGNIFICANT)**	**P=.0005* P<.05 (SIGNIFICANT)**
8	**B/W 7TH DAY & 15TH DAY**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0011* P<.05 (SIGNIFICANT)**	**P=.0035* P<.05 (SIGNIFICANT)**
9	**B/W 7TH DAY & 30TH DAY**	**P=.0008* P<.05 (SIGNIFICANT)**	**P=.0001* P<.05 (SIGNIFICANT)**	**P=.0005* P<.05 (SIGNIFICANT)**
10	**B/W 15TH DAY & 30TH DAY**	**P=.0004* P<.05 (SIGNIFICANT)**	**P=.0031* P<.05 (SIGNIFICANT)**	**P=.0005* P<.05 (SIGNIFICANT)**

***Shows a significant difference b/w different days for comparing the significance of antifungal efficacy at .05 level f significance.(p<.05)**

Table:6(A)-Comparison For Antifungal Efficacy (Zone Of Inhibition) (In MM) Between Different Pair Of Groups By One-Way Anova-F Test At Five Different Days

S.NO.	**PAIR OF DIFFERENT DAYS**	**PROBABLE VALUES OF ONE WAY ANOVA – F TEST FOR COMPARING THE SIGNIFICANCE OF ANTIFUNGAL EFFICACY IN THREE GROUPS AMONG FIVE TIME-POINTS**		
		GROUP 1	**GROUP 2**	**GROUP 3**
1	**AMONG 1ST, 3RD, 7TH, 15^{TH} & 30^{TH} DAY**	**P=.0011* P<.05 (SIGNIFICANT)**	**P=.0002* P<.05 (SIGNIFICANT**	**P=.0006* P<.05 (SIGNIFICANT**

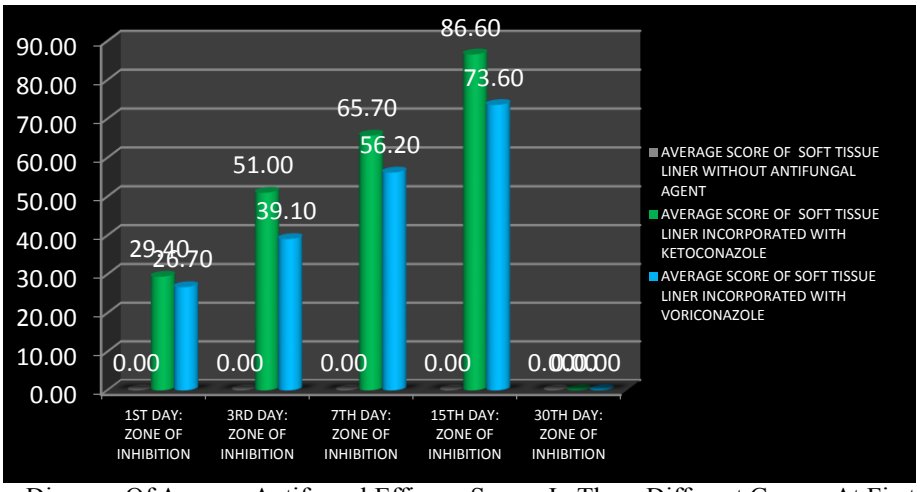
***Shows A Significant Difference B/W Different Days For Comparing The Significance Of Antifungal Efficacy At .05 Level F Significance (P<.05)**

Table: 7 Comparison Of Antifungal Efficacy (Zone Of Inhibition) (In Mm) Between Different Pairs Of Specimens By (Un-Paired / Independent "T" Test) At Each Day / Time-Points

S.NO.	**PAIR OF DIFFERENT DAYS**	**PROBABLE VALUES OF UN-PAIRED / INDEPENDENT "t" TEST B/W DIFFERENT GROUPS FOR COMPARING THE SIGNIFICANCE OF ANTIFUNGAL EFFICACY AT EACH DAY / TIME-POINTS**				
		AT 1ST DAY	**AT 3RD DAY**	**AT 7TH DAY**	**AT 15TH DAY**	**AT 30TH DAY**
1	**GROUP 1 & GROUP 2**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0021* P<.05 (SIGNIFICANT)**	**P=.0003* P<.05 (SIGNIFICANT)**
2	**GROUP 2 & GROUP 3**	**P=.1694** P>.05 (NOT SIGNIFICANT)**	**P=.0006* P<.05 (SIGNIFICANT)**	**P=.0064* P<.05 (SIGNIFICANT)**	**P=.0039* P<.05 (SIGNIFICANT)**	**P=.0049* P<.05 (SIGNIFICANT)**
3	**GROUP 1 & GROUP 3**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0028* P<.05 (SIGNIFICANT)**	**P=.0006* P<.05 (SIGNIFICANT)**

Table:7 (A) Comparison Of Antifungal Efficacy (Zone Of Inhibition) (In Mm) Between Different Pairs Of Specimens By (One-Way Anova-F Test) At Five Time Points

S.NO.	**PAIR OF DIFFERENT DAYS**	**PROBALE VALUES OF ONE WAY ANOVA-F TEST FOR COMPARING THE SIGNIFICANT DIFFERENCE IN ANTIFUNGAL EFFICACY AT THREE TIME POINTS**				
		AT 1ST DAY	**AT 3RD DAY**	**AT 7TH DAY**	**AT 15TH DAY**	**AT 30TH DAY**
1	**AMONG GROUP 1& GROUP 2 & GROUP 3**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0000* P<.05 (SIGNIFICANT)**	**P=.0002* P<.05 (SIGNIFICANT)**	**P=.0015* P<.05 (SIGNIFICANT)**



Graph 4. Bar Diagram Of Average Antifungal Efficacy Scores In Three Different Groups At First Day, Third Day, Seventh Day, Fifteenth Day And Thirtieth Day (Zone Of Inhibition) In MM

3. Discussion

Tensile Bond Strength

In the present study mean, S.D, median, maximum & minimum scores of tensile bond strength (in N/mm^2) for three groups were recorded and tabulated, which reveals the mean score of tensile bond strength was 0.50, 0.45, and 0.28 N/mm^2 respectively. The tensile bond strength was maximum in Group 1 i.e 0.50 N/mm^2, followed by Group 2 i.e 0.45 N/mm^2 & Group 3 i.e 0.28 N/mm^2 shows lowest values.(TABLE -1)

Statistically significant results at a .05 level of significance i.e, ($p<.05$ were obtained, after These results were in accordance with the study conducted by Mese et al., Mese, and Ayse Mese, and Kahraman G Guzel 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 $0.39_{MPa.}$[11,12,13]

On the contrary, Takahashi Jessica mie Ferreira et al stated that soft tissue liners presented higher values for tensile bonds, and the specimens were not affected by the incorporation of antifungal drugs in

and biological assay.[10] The agar punch well method was not appropriate to determine the antifungal efficacy, as it quantifiesthe amount of antimicrobial agent diffused into the tested medium.[11] The Biological assay was a complicated and time taking method, hence, it was not employed in the present study. In the present study agar disk diffusion method was employed as it was more sensitive than the agar punch well diffusion method. It was supported by the study done by Deepika Bainiwal, Kusum Datta, and Pushpa Devi.[25]

To check anti-fungal efficacy, the zones of inhibition [10,14,] method was used In the present study, which was noticed around the circular disc, as incorporating an antifungal agent, for different pairs by the UNPAIRED-‘‘t’’ test and by one-way ANOVA -f test . (TABLE -2,3)

The above results are in support of the study conducted by Grzegorz Chladek, Jaroslaw Zmudzki, and Jacek Kasperski who stated that the tensile bond strength decreases with drug incorporation because of stress build-up at the bond interface, or changing the viscoelastic properties of the soft lining materials, which is in accordance with studies[8,9,10]. This result also indicated that the strengths of soft lining materials were more when used alone, because of the different particle sizes of the used drug.

the samples.[14]

It is also found that poly methyl methacrylate with soft tissue liner (permasoft) incorporated with ketoconazole has a greater tensile bond strength than poly methyl methacrylate with soft tissue liner (permasoft)incorporated with voriconazole.

Anti-Fungal Efficacy

Antifungal properties evaluated by various techniques such as broth dilution method[15], agar disk diffusion[16,17,18], agar punch well[19,20,]

this method was quick and easy.

Results for the control group suggested that the initial value was zero so all the values of mean and standard deviation were zero on the 1st, 3rd, 7th, 15th, and 30th day.

In Group 2 zone of inhibition was noticed around the disc on the1st day, 3rd day, 7th day, and 15th day. On the 1st day mean value was29.40 mm, on 3rd day 51.0 mm, 7th day 65.70 mm, on the 15th day 86.60 mm, and on 30th day mean value was zero respectively. The standard deviation on the 1st day was 3.34 mm, and the maximum on the 7th day i.e 8.21 mm. standard deviation value on the 3rd day was 7.41 and on the 15th day, it was 8.11mm.

The coefficient of variation was maximum on 3rd day observations, which was 14.53 mm, and minimum on the 15th day i.e 9.37 mm.(TABLE-4)
In Group 3, a zone of inhibition was noticed around the disc on 1st day, 3rd day, 7th day, and 15th day. On 1st day mean value was 26.70, on the 3rd day 39.10 mm, 7th day 56.20 mm, on the 15th day 73.60 mm, and on the 30th day mean value was zero Comparison of the zone of inhibition (in mm) between different samples simultaneously at five different time periods by ONE WAY ANOVA-F test and UNPAIRED ‗t'' test. It revealed that a Comparison of antifungal efficacy (zone of inhibition) (in mm) between different pairs of specimens by (UN-PAIRED —t TEST) at each day / time-points and shows significant at different time periods, i.e P=.0000* P<.05 (SIGNIFICANT).(TABLE-7,and 7(a))
The difference in the zone of inhibition at different time periods isbecause the mechanism of action of both anti-fungal agents is different from each other and causes the destruction of the fungus cell membrane in one way or the other such as, ketoconazole acts by inhibiting the fungal cytochrome P450 enzyme 'lanosterol 14-
The increase in the size of the zone of inhibition was noticed till 15days of aerobic incubation at 37°C. Although the MHA plates were further incubated for another 15 days, no increase in the zone of inhibition around the soft tissue liner disc was appreciated. Hence the antifungal efficacy can be determined only for 15 days in vitro. However, the antifungal activity in vivo can be longer than in -vitro activity. The difference in the environmental condition and oral cavity niche can not be nullified, which is responsible for the variation inantifungal efficacy and hence a part of the limitation of this study.

The clinical implications from the results of the present study are that incorporating various antifungal agents in the soft tissue liner can serveas an alternative to systemic or topical delivery systems of antifungal agents. Although ketoconazole showed the highest antifungal activity amongst all the test groups for all the time periods, voriconazole also showed good antifungal activity. When mechanical and physical properties were evaluated, ketoconazole showed the best results among all the tested groups. Since this ketoconazole has the added advantage of being safe and cost-effective, it can be used as an alternative to the systemic delivery of antifungal agents that are currently in use. However, There are certain limitations of the present study. The present study was aimed at determining the

respectively. The standard deviation on the 1st day was 4.90 mm, and the maximum on the 15th day i.e 6.65 mm. standard deviationvalue on the 3rd day was 4.65 mm and on the 7th day, it was 4.59 mm. The coefficient of variation was maximum on 1st day of observations, which was 18.35 mm, and minimum on the 7th day i.e 8.17mm.(TABLE-5)

significant difference was present in the zone of inhibition (in mm) among the three specimens at five different time periods, i.e P=.0000* P<.05 (SIGNIFICANT).(TABLE-6,and 6(a))
demethylase' causing impairment in ergosterol synthesis leading to a cascade of membrane abnormalities in the fungus[26]. It has been proposed that the mode of action of Voriconazole is by inhibiting cytochrome P-450-dependent 14a-demethylase, a key enzyme in ergosterol biosynthesis. Voriconazole completely inhibits the ergosterol synthesis and accumulation of its biosynthetic precursors in Fluconazole-susceptible C. albicans, and Fluconazole-resistant C. albicans but still, Ketoconazole showed the highest antifungal activity.[27,28]

antifungal activity of different antifungal agents incorporated in the soft tissueliners through the measurement of zones of inhibition. However, the antifungal potency is also dependent upon the rate of diffusion of these antifungal agents from permasoft into Muellar- Hinton agar. The rate of diffusion of an antifungal agent is affected by its concentration, molecular size, viscosity, and phase (liquid/solid) of the medium. These factors have not been considered in the present study. Only two properties have been tested in the present study, therefore, other properties such as viscoelastic properties, water sorption, flow etc.should also be tested. Since the present study was performed under controlled laboratory conditions, therefore, in-vivo studies are suggested for more precise results. Apart from that, further studies are required to evaluate the antifungal potency of these antifungal agents based on different parameters affecting the diffusion rate of the drugs from the soft tissue liner.

The surface area of the bonded site tested in this study was very small, in comparison to the entire intaglio surface of thecomplete denture. which is generally greater. Therefore further studies are required to evaluate the bond strength under more closely simulated conditions to understand the nature of the bonding phenomenon.

4. Conclusion

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

1. The tensile bond strength of polymethyl methacrylate with soft tissue liner without antifungal agent was greater than the tensile bond strength of poly methyl methacrylate with soft tissue liner incorporated with ketoconazole and Voriconazole, on statistical analysis, it was found to be significant.
2. Soft tissue liner without any antifungal agent shows no zone of inhibition around the disc at 1st, 3rd, 7th, 15th, and 30th day.
3. Maximum antifungal efficacy was noticed in soft tissue liner incorporated with ketoconazole followed by soft tissue liner incorporated with voriconazole group, respectively. No antifungal efficacy was noticed for soft tissue liner with no antifungal agent.

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To Evaluate And Compare The Effect Of Addition Of Different Anti-Fungal Agents On Tensile Bond Strength And Anti-Fungal Efficacy Of Soft Tissue Liner: An In-Vitro Study

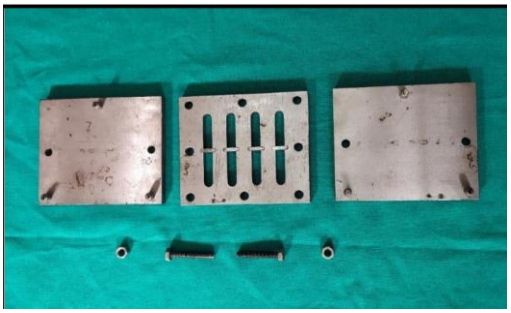
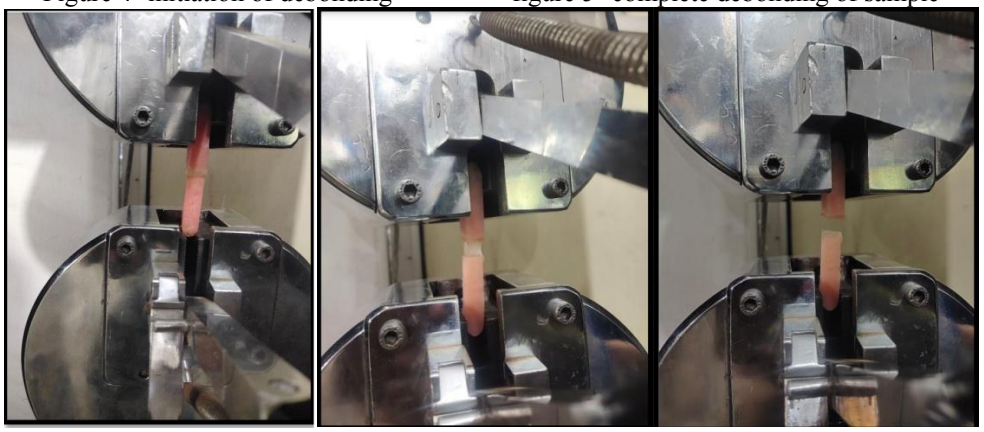


Figure-1 Special Metal Die Used For Tensile Bond Strength Sample Fabrication (Measuring 150 X 100 X 300 MM)

Figure 2- Prepared Samples For Tensile Bond Strength

Figure 3- samples load For tensile bond strength test

Figure 4- initiation of debonding figure 5- complete debonding of sample



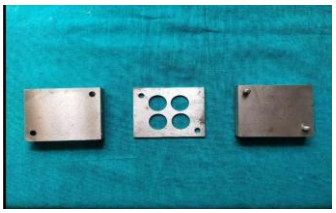


Figure-6 Special Metal Die Used For Anti-Fungal Efficacy Sample Fabrication (Measuring 80 X 60 X 32 MM)

Figure 7- Prepared Sample For Anti-Fungal Efficacy

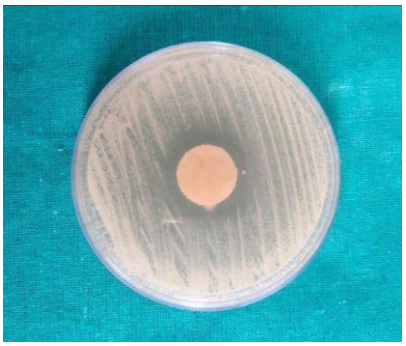


Figure 8- Media With Disc Of Soft Tissue Liner