Original Article

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Dentistry Section

An In-vitro Evaluation of Retention, Colonization and Penetration of Commonly Used Denture Lining Materials By *Candida albicans*

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ABSTRACT

Introduction: Colonization of the surface by *Candida albicans* and related *Candida* species is one of the major concerns of denture lining materials.

Aim: We evaluated retention, colonization and penetration of the four denture lining materials namely Molloplast B, Permaflex, GC Soft Liner and Ufi Gel Hard C by *Candida albicans*.

Material and Methods: a) Evaluation of retention: Five test discs of each material with smooth surface on one side and rough on the other were prepared and surface roughness (Ra) was measured with profilometer. Retention of *C. albicans* to discs was monitored after one hour of incubation (37°C) with standardized (2.8 x 10^6 cfu/ml) washed cell suspension. Discs were stained with acridine orange and attached cells were counted using inverted microscope; b) Evaluation of colonization and penetration: Eight test discs of each material in sterile artificial saliva, were inoculated with *C. albicans* and incubated for six weeks. Two sections were cut across each test disc to provide three replicate samples. *Candida* cells on cut disc sections were fixed, dehydrated, air dried and viewed via fluorescence microscope; c) Evaluation of antifungal action: Two test discs of each material were placed onto diagnostic sensitivity testing the agar plate. After incubation at 37°C for 24 hours, the zone of inhibition formed around the samples were measured at four places, and the mean calculated.

Results: a) All rough surfaces showed higher retention of *C. albicans* than smooth surfaces. Among the smooth surfaces, Molloplast B and GC Soft Liner showed highest and lowest retention of *C. albicans* respectively (p=0.0090). Among the rough surfaces, the variation in the retention of *C. albicans* was not statistically significant; b) Penetration of *C. albicans* was observed through all three sections of the test discs of each material. There was no statistically significant difference among the test materials; c) Molloplast B and Permaflex produced a mean zone of inhibition of 16.9±4.8mm and 14.80±3.8mm respectively.

Conclusion: a) Smoother surfaces retain fewer cells than rough surfaces; b) Denture lining materials permit infiltration of *Candida* through their structure; c) Denture lining materials have insignificant anti-fungal properties.

Keywords: Adherence, Antifungal, *Candida albicans*, Denture stomatitis, Resilient liner

INTRODUCTION

Recurrent inflammation or erythema and burning sensation of denture bearing tissues are relatively common in denture wearers and are described by the term denture stomatitis. It is frequently found in the maxillary ridge of elderly denture wearers. *C. albicans* is the predominant oral yeast associated with denture stomatitis [1]. The condition is often symptomless, but when symptoms are present they may appear as mucosal bleeding, burning or painful sensations and dryness in the mouth [2].

Resilient denture lining materials are used to limit such injury by providing cushioning effect. They have a therapeutic value in patients with thin atrophic mucosa, sharp alveolar ridge crest, deep anatomic undercuts, bony protuberances, or bruxomania, where the oral mucosa exhibits a reduced tolerance to the load applied by the denture, and in obturators for cleft palate [3]. Studies have reported that the fungi and bacterial species can enter porous spaces within the denture liner and that their colonization may reduce the intra-oral life of the material. The porosity of the denture liner also allows water absorption and the diffusion of nutrient materials that may support growth of oral yeasts [3].

C. albicans, is one of the most frequently isolated and the most virulent *Candida* species of medical importance [4]. Factors such as malignancies, diabetes, immune-deficiency, and malnutrition, which are associated with denture patients promote growth of *C. albicans* [5]. Significantly greater retention of *C. albicans* has been described on denture liners compared with acrylic, and rougher surfaces enhance adhesion and retention [6-10]. The combination of factors such as entrapment of *Candida* species in irregularities of denture base and denture liners, poor oral hygiene and several systemic factors are the most apparent causes for the onset of denture stomatitis [11]. As there are very few reported studies in the literature, the aim of this study was to evaluate retention, colonization and penetration of commonly used denture lining materials by *C. albicans*. The null hypothesis was that there would be no significant differences in the degree of surface roughness, retention, colonization and penetration of *C. albicans*, and anti-fungal action among different denture lining materials.

MATERIAL AND METHODS

This in-vitro study was carried out at the Department of Prosthodontics and Crown and Bridge and Department of Microbiology of Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka, India after approval by the Institutional Ethics Committee. The study consisted of three parts. I) Evaluation of retention of *C. albicans* to denture liners; II) Evaluation of colonization and penetration of denture liners by *C. albicans*; III) Evaluation of inhibition of *C. albicans* by denture liners. Following denture liners were used in the study [Table/Fig-1].

I) Evaluation of Retention

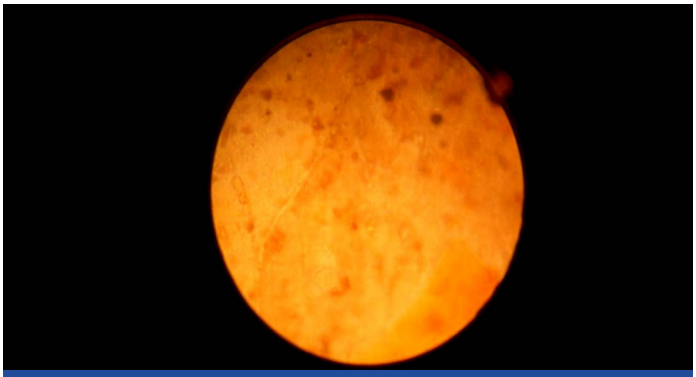
a) Preparation of test discs: Five test discs of 10mm diameter and

1	Molloplast B (Detax, Germany)	Heat cure denture liners
2	Permaflex (Kohler, Germany)	
3	GC Soft Liner (GC Corporation, Tokyo, Japan)	Self cure denture liners
4	Ufi Gel Hard C (Voco, Germany)	

[Table/Fig-1]: Test materials used in the study.

[Table/Fig-2]: Wax discs positioned on glass surface.

[Table/Fig-3]: Test discs of Permaflex, Molloplast B, Ufi gel hard and GC soft liner with smooth surface on one side and rough on the other.



[Table/Fig-4]: Adherent cells as seen under inverted microscope at 40X magnification.

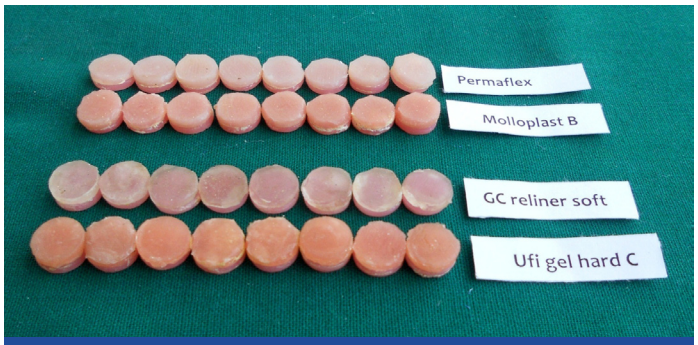
1.5mm thickness prepared from each of the four denture liners were used. Each test disc had smooth surface on one side and rough surface on the other. To prepare this, a glass piece was pressed onto the dental stone mixture (Asian Chemicals, Rajkot, India) in the drag of the flask. After the dental stone had set, wax discs of 10mm diameter and 1.5mm thick were punched from a sheet of modeling wax and placed on top of the glass surface [Table/Fig-2]. The cope of the flask was positioned and the dental stone poured over the wax discs. The voids produced after de-waxing were filled by denture liners. The discs thus obtained had one glass-smooth surface and one dental stone-rough surface [Table/Fig-3]. Curing of test discs of heat cure and cold cure denture liners were carried out according to standard procedures.

b) Surface roughness measurements: Surface roughness (Ra) was measured at three areas of each surface (i.e., smooth and rough) of each specimen, yielding six measurements for each disc, using profilometer (Surtronic 3+, Taylor Hobson Ltd., UK) and the mean value was calculated. Subsequently, all discs were cleaned ultrasonically in distilled water for 15 seconds, sterilized and stored in sterile distilled water at 37°C for 24 hours prior to *C. albicans* contamination, and for the evaluation of retention of *C. albicans* to denture liners.

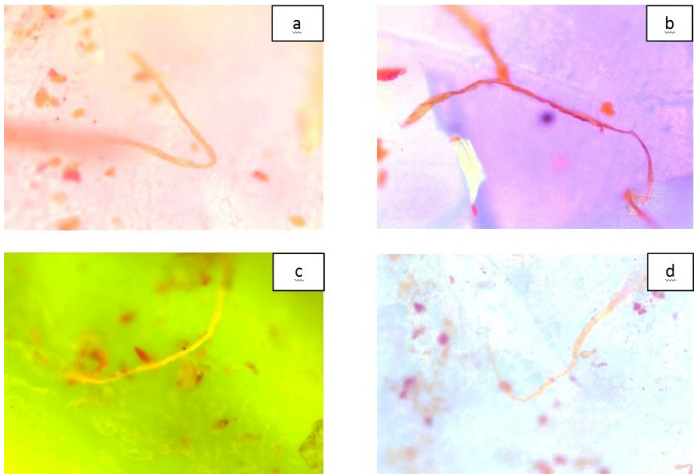
c) Microbiological procedure: About (4–6) colonies of *C. albicans* (ATCC 2091) from a Sabouraud's plate were inoculated into 100ml artificial saliva (Saleva, Global Dent Aids Pvt., Ltd., India) and incubated at 37°C for 24 hours. Cells were collected by centrifugation, and re-suspended in Phosphate Buffered Saline (PBS). Each sterile test disc was placed in a sterile 5ml glass bottle in a vertical position and 2ml of the standardized cell suspension (2.8×10^6 cfu/ml) were added to each bottle, the apparatus was incubated, without agitation at 37°C for one hour. Test discs were then removed from the suspension, rinsed by dipping gently three times in sterile PBS to remove loosely attached cells and left to air dry. Attached cells remaining on the discs were fixed by immersion in 100% methanol (Himedia Pvt., Ltd., India) for one minute, stained by immersing in 0.03% acridine orange (Himedia Pvt., Ltd., India) for 2 minute, followed by washing in distilled water and air drying. Test discs were viewed using inverted microscope at 40X magnification (Magnus Analytics, Italy). The number of adherent cells in 10 random fields was counted for each test disc and the mean number was obtained [Table/Fig-4].

II) Evaluation of Colonization and Penetration.

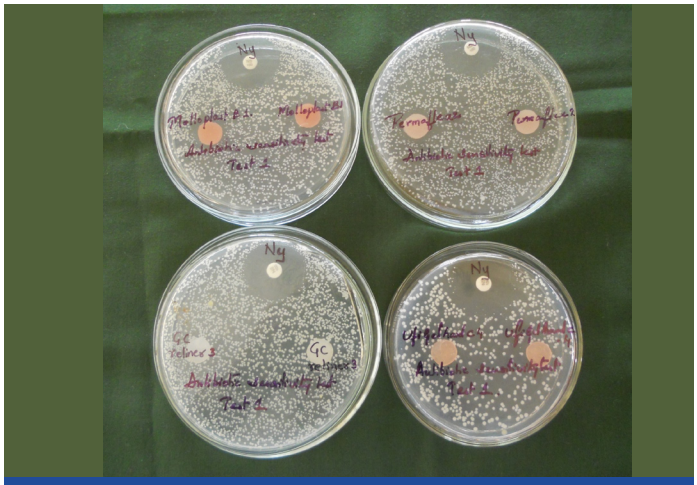
a) Preparation of test discs: Eight test discs of each of the four materials were tested. Disc shaped voids were prepared as described above. Acrylic resin powder and liquid (Trevalon, Dentsply. India) were mixed and the dough was filled into the voids in the flasks. After polymerization, acrylic discs were removed. Discs of modeling wax were anchored to each acrylic disc and flasked. This procedure produced acrylic discs surmounted by a void which was filled by the denture liners [Table/Fig-5].



[Table/Fig-5]: Acrylic discs surmounted by liner.



[Table/Fig-6]: Penetration of *Candida albicans* into section 3 of the test discs of denture lining materials (fluorescence microscope 25000X): (a) Permaflex; (b) Molloplast; (c) GC soft liner and (d) Ufi gel hard C.



[Table/Fig-7]: Agar diffusion test for Molloplast B and Permaflex, GC soft liner and Ufi gel hard C.

b) Microbiological procedure: Test acrylic/liner discs were placed into 15 ml glass bottles containing 10 ml artificial saliva and sterilized. Each of the bottles was inoculated with 0.1 ml of an 18 hours culture of *C. albicans* in artificial saliva and incubated at 37^{0}C for 6 weeks.

After this, test discs were removed from the bottles and placed in 4% glutaraldehyde in PBS for at least 2 hour to fix the cells and dehydrated in alcohol. Two sections were cut across each test disc with hard tissue microtome (Leica SP 1600, Leica Microsystems Germany) to provide three replicate samples. Sections were immersed in 0.03% acridine orange for one minute, rinsed in distilled water, air dried and viewed via fluorescence microscope 25000X (Magus Analytics, Italy) [Table/Fig-6]. The penetration of *C. albicans* into sections of denture liners was evaluated by counting the number of blastospores/hyphae visible within each microscopic field of each section of test disc, as described below [8].

Class I: Little hyphal presence, where by 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: Large amount of hyphal presence, whereby most of the microscopic field was covered by mycelial forms.

III) Evaluation of antifungal action.

a) Preparation of test discs: Four test discs of each of the four denture liners were tested. For this, discs of modeling wax were punched out from a sheet, dental stone was poured into the shallow part of the dental flask, and the punched wax discs were placed on the surface of the stone. After setting, separating medium was applied, the upper part of the flask was then placed into position and dental stone was poured into it. After de-waxing, denture lining materials were packed and polymerized. Subsequently, all specimens were cleaned ultrasonically in distilled water for 15 seconds, sterilized and used for the evaluation of zone of inhibition.

b) Microbiological procedure: An overnight culture of *C. albicans* diluted with 1:1000 in sterile PBS, was used to inoculate diagnostic sensitivity testing agar (Himedia laboratories Pvt., Ltd., India). The plates were dried, and two test discs of each denture liner were placed in each agar plate and the experiment was repeated twice. Nystatin susceptibility test discs (100 units/disc) (Himedia Laboratories Pvt., Ltd., India) were used as controls [Table/Fig-7]. After incubation at 37°C for 24 hours, the zone of inhibition formed around the samples were measured using a transparent ruler at four places around each test disc and the mean determined.

STATISTICAL ANALYSIS

The summary data was tabulated as mean values and standard deviation. One-way ANOVA and was used for comparing multiple unmatched groups for parametric data. Kruskal Wallis test was used for comparing multiple unmatched groups for non-parametric data. Pair wise comparison of groups was done by Mann-Whitney U test. Chi square test was used for analyzing categorical data. The confidence level of the study was set at 95%.

RESULTS

As indicated in [Table/Fig-8,9], there was a significant variation in the surface roughness (Ra) values of smooth surfaces, with Permaflex and Ufi Gel Hard C showing higher values and GC Soft Liner showing the least value. The variation in the surface roughness values was not statistically significant for rough surfaces.

All rough surfaces showed higher retention of *C. albicans* than smooth surfaces. Among the smooth surfaces, Molloplast B and GC Soft Liner showed highest and lowest retention of *C. albicans* respectively. Among the rough surfaces, the variation in the retention of *C. albicans* was not statistically significant [Table/Fig-10].

Materials	Mean± SD	Anova F*		Difference between materials†			
		F-value	p-value	Mollo-plast B	Perm-aflex	GC Soft Liner	Ufi Gel Hard C
Molloplast B	0.33±0.09	8.9258	0.0010 S	1.0000 NS	--	--	--
Permaflex	0.69±0.29			0.0279 S	1.0000 NS	--	--
GC Soft Liner	0.18±0.10			0.5638 NS	0.0020* S	1.0000 NS	--
Ufi Gel Hard C	0.62±0.17			0.0918 NS	0.9224 NS	0.0070 S	1.0000 NS

[Table/Fig-8]: Comparison of smooth surface values of test discs.
† Tukeys multiple post hoc procedure

Materials	Mean± SD	Anova F*		Difference between materials†			
		F-value	p-value	Mollo-plast B	Perm-aflex	GC Soft Liner	Ufi Gel Hard C
Molloplast B	1.47±0.18	2.8526	0.0701	p=1.0000	--	--	--
Permaflex	1.88±0.31			p=0.3005	p=1.0000		--
GC Soft Liner	1.70±0.48			p=0.7466	p=0.8485	p=1.0000	--
Ufi Gel Hard C	2.10±0.37			p=0.0500*	p=0.7566	p=0.3084	p=1.0000

[Table/Fig-9]: Comparison of rough surface values of test discs.
† Tukeys multiple post hoc procedure

There was no statistically significant difference among the test materials with respect to penetration of *C. albicans*. Third (deepest) section of all the discs had *candida* penetration [Table/Fig-11].

Molloplast B and Permaflex produced a mean zone of inhibition of 16.9±4.8mm and 14.80±3.8mm respectively, GC Soft Liner and Ufi Gel Hard C did not show any zone of inhibition when compared with the positive control (Nystatin susceptibility test discs), which showed a mean zone of inhibition of 30.37±3.33mm [Table/Fig-12].

DISCUSSION

There is a rise in the number of elderly people using complete dentures which has resulted in consequent increase in the use of denture liners. The purpose of this study was to comprehensively evaluate significant aspects related to performance of commonly used denture liners such as retention, colonization and penetration and anti-fungal action.

Retention of *C. albicans* to denture lining materials: The adherence of *C. albicans* to denture liners is the first step in

Materials	Smooth Surface*		Rough Surface*	
	Mean± SD	Median	Mean± SD	Median
Molloplast B	11.20±1.82	11.00	12.80±3.01	13.50
Permaflex	8.70±1.99	9.00	14.80±2.05	13.50
GC Soft Liner	3.50±1.22	3.50	12.30±3.23	11.00
Ufi Gel Hard C	9.40±3.31	9.00	14.00±3.30	13.50
H-value	11.9846		2.4384	
p-value	0.0074 S		0.4865 NS	
Pair wise comparison of test discs†				
Molloplast B vs. Permaflex	p=0.0758		p=0.3472	
Molloplast B vs. GC Soft Liner	p=0.0090*		p=0.6761	
Molloplast B vs.Ufi Gel Hard C	p=0.3472		p=0.5309	
Permaflex vs. GC Soft Liner	p=0.0122*		p=0.1172	
Permaflex vs. Ufi Gel Hard C	p=0.6761		p=0.9168	
Ufi Gel Hard C vs. GC Soft Liner	p=0.0163*		p=0.4034	

[Table/Fig-10]: Comparison of test discs with respect to colonies of *C. albicans* per field on smooth and rough surfaces.
* Kruskal Wallis ANOVA, † Mann-Whitney U test, S-significant, NS-not significant

Materials	Section I			Section II				Section III		
	Class I	Class II	Class III	Class I	Class II	Class III	None	Class I	Class II	Class III
Molloplast B	--	4	4	7	1	--	5	3	--	--
Permaflex	--	3	5	8	0	--	5	3	--	--
GC Soft Liner	--	6	2	7	1	--	4	4	--	--
Ufi Gel Hard C	--	5	3	8	0	--	3	5	--	--
Total	--	18	14	30	2	--	18	14	--	--
p*	0.4681			0.5452			0.4681			

[Table/Fig-11]: Comparison of test discs with respect to penetration of *C. albicans* into different sections of test discs.
*Chi square test

Material	Mean(mm)
Molloplast B	16.93±4.8
Permaflex	14.8±3.8
GC Soft Liner	--
Ufi Gel Hard C	--
Nystatin	30.37 ±3.33

[Table/Fig-12]: Mean values of zone of inhibition (mm) of test discs.

Authors	Materials tested	Parameters evaluated	Conclusion
Gedik H et al., [6]	Ufi Gel P * Ufi Gel C* Mollosil* Soft-Liner* Moloplast B** Luci Soft** *Room Temperature Polymerised Denture Liner **High Temperature Polymerised Denture Liner	In-vitro evaluation of surface roughness and adherence of *C. albicans*	High temperature polymerised denture liners showed lower surface roughness value and lower adhesion of *C. albicans* than room temperature polymerised denture liners
Nevzatoğlu EU et al., [7]	Denture Base Acrylic Resins and Silicone-Based Resilient Liners	Adherence of *C. albicans* to denture base acrylics and silicone-based resilient liner materials with different surface finishes	In all types of surface finishes, *C. albicans* adhesion on denture base acrylics was significantly less than those of silicone liners
Bulad K et al., [8]	Molloplast B* Flexor * Permaflex * Luci-Soft * Eversoft ** Ufi Gel Hard C*** *Heat Curing Silicone ** Chair-Side Curing Denture Liner ***Cold Curing Hard Liner	• Adhesion • Colonization and penetration • Anti-fungal action	• There was more adhesion on rough surfaces. More cells were attached to Molloplast-B (silicone material) than to Ufi-Gel Hard C • Penetration was greatest into Ufi Gel Hard C and least into Eversoft. • None of the materials produced a zone of inhibition
Radford DR et al., [9]	Molloplast B Novus	In-vitro adherence of *C. albicans* to heat-cured hard and soft denture-base materials with varying surface roughness	Rough surfaces on denture-base materials promote the adhesion of *C. albicans*
Verran J, et al., [10]	Acrylic resin and silicone surfaces	Retention of *C. albicans* on smooth and rough surfaces	Significantly higher numbers of cells were observed on roughened surfaces (silicone > acrylic resin) than on smooth surfaces

[Table/Fig-13]: Summary of studies done to evaluate adhesion and penetration of *C. albicans* into denture liners.

colonization, followed by initiation of pathogenesis and eventually causing infection [11,12]. *C. albicans* seen on the surfaces were mainly blastospores, as the incubation of the cells was only for one hour, though, a few hyphae were also seen. The results are in agreement with earlier studies on short-term attachment, which showed pseudo hyphae and hyphae and not yeast growth or surface colonization [8,13].

Surface roughness is a major factor in the attachment and retention of microorganisms on surfaces, with an increase in roughness causing increased retention of cells. It has been known as a factor for the entrapment of microorganisms and subsequent protection from shear forces. *C. albicans* were retained on rough surfaces in higher numbers than on smooth surfaces. Similar effects have been reported in the past [6-10] [Table/Fig-13]. There are clear implications for these findings in terms of surface cleanability, denture hygiene and materials fabrication. Overall, surface structure, properties and composition of biomaterials, hydrophobicity and roughness affect the adhesion of microorganisms on biomaterials [14].

Colonization and penetration of denture lining materials by *C. albicans*: Penetration of *C. albicans* within denture liners was evaluated by observing all three sections of test discs of each material. Colonization was visible with the naked eye on the surface, when materials were removed from the saliva culture. Cells were observed on the outer surface of all three sections of each test disc, indicating that penetration of the lining materials had occurred. Both blastospores and hyphal forms were visible. *C. albicans* is a microscopic, oval shaped, thin walled fungus usually measuring 2 to 4µm; however, filamentous shapes of variable length have also been identified having 3 to 5µm rounded ends in infected tissues [14]. *C. albicans* colonize not only the surface of the denture liners, but they also penetrate inside the materials.

Penetration of *C. albicans* (blastospores and hyphae) through Molloplast B and Permaflex was least and was relatively comparable. This is probably because both are similar in composition and are heat cure denture liners. The most significant penetration of *Candida* blastospores was observed in Ufi Gel Hard C, a hard denture liner. This might be because of the presence of porosities inside the matrix of the cured material which facilitate the penetration of blastospores [15]. Significant hyphal formation was seen all through the other soft denture liners. The penetration of *C. albicans* at the acrylic-liner junction point was observed in a few samples, particularly GC Soft Liner. This can be attributed to the failure of bonding between the liner and the acrylic base

material, which may contribute to deterioration of the prosthesis lining and function.

Inhibition of *C. albicans* by denture lining materials: In the current study, Molloplast B and Permaflex exhibited zone of inhibition against *C. albicans*, but the effect was small. Of the denture lining materials tested, only Molloplast B has been studied previously for any inhibitory effect on the growth of *C. albicans*, with differing success, one study investigated inhibitory effect of Permaflex and Ufi Gel Hard C and there are no reported studies regarding inhibitory effect of GC Soft Liner. Uncured Molloplast B material has been reported to cause a definite inhibition of *Candida* growth in-vitro, with the cured material showing no growth inhibition. The active constituent, methacryloloxy-propyl-trimethoxy-silane, would become inactive during the cross-linking curing process [16]. If a small excess remained, the cured material might still exhibit minimal inhibition of growth. The curing of all the test materials used in this study was done using standard procedures. One study reported that Molloplast B demonstrated such a small degree of inhibition that the zone area was not measurable [17]. Another study found that Molloplast-B had no inhibitory influence on growth of *C. albicans* [16]. One more study reported no inhibitory effect of Molloplast B and Permaflex. Clearly, the method of preparation of the material and its storage, and the experimental protocol appear to have some effect on findings [8].

One of the most serious disadvantages of these materials is their reported lack of antimicrobial activity. A denture liner material with anti-fungal property is yet to be developed. To overcome these problems, attempts have been made to incorporate different anti-fungal agents to the resilient liners such as Propolis, Zeolite, Chlorhexidine, Fluconazole, Punica granatum, Nystatin, Itraconazole, Miconazole, Ketoconazole, Clotrimazole [18-25] in the resilient liners with varying degree of success.

Ideal properties of soft lining material include superior biocompatibility, enduring resilience, low water sorption, high bond strength to denture base, high dimensional stability, good color stability and resistance to microbial growth. It appears that currently available denture liners are deficient in few areas. Selection of denture liner in a given situation should be based on the knowledge about how the adherence and bio-film formation takes place. This will be helpful to diminish *Candida* colonization in clinical practice.

LIMITATION

The outcome obtained and the conclusions drawn are based on in-vitro studies, correlation to clinical practice requires further in-vivo research.

CONCLUSION

a) All rough surfaces showed higher retention of *C. albicans* than smooth surfaces. Among the smooth surfaces, Molloplast B and GC Soft Liner showed highest and lowest retention of *C. albicans* respectively. Among the rough surfaces, the variation in the retention of *C. albicans* was not statistically significant.

b) All four materials showed penetration of *C. albicans* into the deepest section of discs.

c) Molloplast B and Permaflex exhibited zone of inhibition against *C. albicans*, but the effect was small.

REFERENCES

[1] 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;19:252-57.

[2] Samaranayake LP, Keung Leung W, Jin JL. Oral mucosal fungal infections. *Periodontology* 2000. 2009;49:39-59.

[3] Wright PS, Young KA, Parker S, Kalchandra S. Evaluating the effect of soft lining materials on the growth of yeast. *J Prosthet Dent.* 1998;79:404-09.

[4] 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.

[5] Reichart PA, Samaranayake LP, Philipsen HP. Pathology and clinical correlates in oral candidiasis and its variants: A review. *Oral Diseases*. 2000;6:85-91.

[6] Gedik H, Ozkan YK. The effect of surface roughness of silicone-based resilient liner materials on the adherence of *Candida albicans* and inhibition of *Candida albicans* with different disinfectants. *Oral Health Prev Dent*. 2009;7:347-53.

[7] Nevzatoğlu EU, Ozcan M, Kulak-Ozkan Y, Kadir T. Adherence of *Candida albicans* to denture base acrylics and silicone-based resilient liner materials with different surface finishes. *Clin Oral Investig*. 2007;11:231-36.

[8] Bulad K, Taylor RL, Verran J, Fraser J. Colonization and penetration of denture soft lining materials by *Candida albicans*. *Dental Materials*. 2004;20:167-75.

[9] Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent*. 1998;26:577-83.

[10] Verran J, Maryan CJ. Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *J Prosthet Dent*. 1997;77:535-39.

[11] Vural C, Ozdemir G, Kurtulmus H, Kumbuloglu O, Ozcan M. Comparative effects of two different artificial body fluids on *Candida albicans* adhesion to soft lining materials. *Dental Materials Journal.* 2010;29:206–12.

[12] Waters MGJ, Williams DW, Jagger RG, Lewis MAO. Adherence of *Candida albicans* to experimental denture soft lining materials. *J Prosthet Dent*. 1997;77:306-12.

[13] Verran J, Lees GC, Shakespeare AP. The effect of surface roughness on the adhesion of *C. albicans* to acrylic. *Biofouling.*1991;3:183-92.

[14] Emira N, Mejdi S, Dorra K, Amina B, Eulogio V. Comparison of the adhesion ability of *Candida albicans* strains to biotic and abiotic surfaces. *African Journal of Biotechnology.* 2011;10:977-85.

[15] Kawano F, Dootz ER, Koran A. Sorption and solubility of 12 soft denture liners. *J Prosthet Dent*. 1994;72:393-98.

[16] Wright PS. The effect of soft lining materials on the growth of *Candida albicans*. *J Dent.* 1980;8:144-51.

[17] Burns DR, Bruns DA, Dipietro GJ, Gregory RL. Response of processed resilient denture liners to *Candida albicans*. *J Prosthet Dent.* 1987;57:507-12.

[18] 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;22:1544-47.

[19] Nikawa H, Yamamoto T, Hamada T, Rahardjo MB, Murata H, Nakanoda S. Antifungal effect of zeolite incorporated tissue conditioner against *Candida albicans* growth and/or acid production. *J Oral Rehabil*. 1997;24:350-57.

[20] Jang KS. Inhibitory effect of antifungal agents incorporated in denture lining materials against *Candida albicans*. *J Korean Acad Prosthodont.* 1999;37:293-300.

[21] Amin WM, Al – Ali MH, Salim NA, Al – Tarawneh SK. A new form of intra oral delivery of antifungal drugs for the treatment of denture induced oral candidosis. *European J Dent*. 2009;3:257-66.

[22] Vasconcelos LC, Sampaio MC, Sampaio FC, Higino JS. Use of Punica granatum as an antifungal agent against candidosis associated with denture stomatitis. *Mycoses*. 2003;46:192-96.

[23] 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.

[24] 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.

[25] Vojdani M, Zibaei M, Khaledi AAR, Zomorodian K, Ranjbar MA, Boshehri S. In-vitro study of the effect of clotrimazole incorporation into silicone denture liner on fungal colonization. *Shiraz Univ Dent J*. 2009;9(Suppl. 1):19-23.

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