Original Article

The influence of surface roughness on the retention of candida albicans to denture base acrylic resins – an in vitro study

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ABSTRACT

Background: The adherence of Candida albicans to host cells or polymers such as denture acrylic resin is an essential and necessary first step in successful colonization and the development of pathogenesis and infection. A denture may then function as a reservoir of infection and surface irregularities can increase the adherence of microorganisms even after adequate hygiene measures.

Aim and Objectives: To compare and evaluate the adhesion of Candida albicans on heat polymerized polymethyl methacrylate (PMMA) denture base resin with three different surface finishes.

Materials and method: To evaluate and compare adherence of Candida albicans on three different surface finishes of PMMA. Two commercial brands namely, Trevalon (Dentsply; Gurgaon, India), DPI (Dental Products of India; Mumbai, India) and a reference strain of Candida albicans (ATCC 60193) was used for this experimental purpose. Viable and adherence assay methods were used to evaluate this adherence.

Results: The results of this study confirm earlier work which demonstrated that increased surface roughness increased retention of yeast on PMMA surfaces. The results obtained by adherence assay revealed results similar to the viable assay with higher adherence of Candida albicans to rough surface as compared to the polished surface.
Conclusion: Surface finished with tungsten carbide bur had the maximum roughness and adherence of Candida albicans to its surface was the highest. DPI acrylic denture base material had less number of adherent Candida albicans cells to its surface with similar finish than that of Trevalon.

Keywords: *Candida albicans*, denture, polymethyl methacrylate

INTRODUCTION

The base of complete denture is largely responsible for providing the prosthesis with retention, stability, and support by being closely adapted to the oral mucosa. The material most commonly used for fabricating denture base is heat polymerized acrylic resin[1] but on occasions, soft lining materials are used to provide a cushion between the hard denture base and supporting tissue.[2]

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The advantages of heat polymerized acrylic resin include good esthetic quality, ease of processing and repairs, favourable physical and mechanical properties, material availability and economics.[3] The disadvantages, Substratum roughness of materials like acrylic resins used in prosthodontics and restorative dentistry significantly influence bacterial retention. Surface roughness provides niches in which microorganisms are protected from the shear forces and oral hygiene measures, thus allowing the entrapped microbial cells to attach irreversibly to a surface.[4] The denture may function as a reservoir of infection and surface irregularities would increase the likelihood of microorganisms remaining on the surface after the prosthesis has been cleaned.[5] The adherence of *Candida albicans* to host cells or polymers such as, denture acrylic resin and soft lining materials is an essential and necessary first step in successful colonization and the development of pathogenesis and infection.[6]

Candida albicans is a dimorphic fungus that is commensal in the gastrointestinal and reproductive tracts of healthy individuals. Under certain predisposing conditions, Candida albicans can convert into a pathogen capable of causing a variety of oral infections including pseudomembranous candidiasis, erythematous candidiasis and hyperplastic candidiasis, as well as Candida-associated denture stomatitis, Candida associated angular cheilitis, rhomboid glossitis and chronic mucocutaneous candidiasis.7 It is estimated that 60% of denture wearers are affected with Candida-associated denture stomatitis.8

The first step implicated in denture stomatitis is adherence of Candida to acrylic or to salivary pellicles adsorbed on the surface of dental prosthesis. This is considered the most important event in the ability of Candida albicans to colonize dentures in the mouth.7.

Aim:

The aim of this study was to investigate, compare and evaluate the adhesion of Candida albicans on two commonly used brands of heat polymerized polymethyl methacrylate (PMMA) denture base resin with three different surface finishes:

1. Acrylic resin surfaces finished with tungsten carbide bur.
2. Acrylic resin surfaces finished with emery paper (P 600) following the use of tungsten carbide bur.
3. Acrylic resin surface finished with pumice buffing following the first two steps.

MATERIALS USED

This study was conducted in the Department of Prosthodontics, of this institution for in vitro evaluation and comparison of adherence of Candida albicans on three different surface finishes of two commonly used heat polymerized polymethyl methacrylate denture base resin.

Two commercially available brands of heat polymerized polymethyl methacrylate (PMMA) denture base resin were used in the study and are listed in Table 1.

Table 1: Commercially available brands used in the study

Brand of material	Trade name	Manufacturer
(PMMA) denture base resin	Trevalon	Dentsply, Gurgaon, India.
PMMA) denture base resin	DPI	Mumbai

METHOD EMPLOYED

Specimen preparation:

A total of 120 wax patterns were made using custom made brass mould (Fig 1) having two compartments, each of dimension (20 × 20 × 2) mm. The specimens were invested in type III dental stone. The flasks were dewaxed, packed and processed according to the manufacturer's instructions, to obtain an equal number of specimens from the two brands of heat polymerised acrylic resin (Fig 2).

Grouping of specimens:

60 specimens of each of the two groups were divided into three subgroups (A1, A2, A3 & B1, B2, B3) each of 20 samples.

A1 & B1 specimens were finished with tungsten carbide bur (No: RL 40/13) at the speed of 15000 rpm.
A2 & B2 specimens were finished with emery paper (P 600) following step 1
A3 & B3 specimens were finished with wet pumice on cotton buff at slow speed following usage of tungsten carbide bur and emery paper.

Obtaining *Candida Albicans*

Candida albicans strain ATCC (60193) was obtained as a stock culture (Pathological Analysis Department of the Kasturba Medical College, Mangalore, India), and 100 ml stationary phase culture of candida was incubated on Sabouraud dextrose broth. The culture was then centrifuged (Function Line, Labofuge 400 R, Hereaus Instruments, Germany) at 750 rpm for 10 min and the resultant cell pellet was washed thrice with phosphate-buffered saline (PBS) solution (0.15 M, pH 7.2) to obtain a cell concentration of 2 MacFarland equivalent to $1.5\pm0.3\times10^{-7}$ cells/ml.

Viable Assay

50 ml of standardized cell suspension was added to a petridish that contains test samples and then incubated without agitation for 1 hour at room temperature (24°C), following which the samples with adherent cells were removed and washed by gently dipping 10 times in 100 ml sterile PBS . This was done 3 times to remove loosely adherent cells.

All the samples were submerged in Sabouraud dextrose and incubated aerobically at 37°C for 18 – 20 hours. The Sabouraud dextrose broth undiluted and dilutions 10^{-2} were prepared. For each dilution, the SDB were plated in a duplicate Sabouraud dextrose agar with Chloramphenicol (SDA) and the inoculated plates were incubated aerobically at 37°C for 24 hours. Figure 3 demonstrates the microbiological armamentarium used in the study. (Fig 3) After incubation of the SDA plates, the median value for each sample was calculated as CFU/ml. (Fig 4)

Adherence assay

The square samples of denture base resin materials were deposited in 20 ml *Candida albicans* suspension containing 1.5±0.3×10-7 cells/ml. in sterile petridishes. The samples were incubated for 1 hour at room temperature. The samples were washed twice by gentle agitation of the samples in phosphate buffered saline solution for 1 minute. After the samples were dried, adherent candida cells were fixed in methanol and stained for 60 seconds with crystal violet. All samples were washed in phosphate buffered saline solution for 30 seconds and examined by light microscopy under x10, x40 and x1000 magnification.

Adherent *candida* cells in 30 fields of view ($0.25mm^2$ per field) were enumerated and the results were expressed as yeast cells/$mm^2$of material.(Fig 5,6 &7)

RESULTS

The statistical data obtained was analysed using SPSS (USA version 16).
Mann – Whitney U test was used for pair wise comparisons of viable assay. Adherence assay results were presented as mean ± SD. Multiple group comparisons were made by one way ANOVA test. A p-value of ≤ 0.05 was considered for statistical significance. The results are presented in three tables and two graphs.

Table 2: Represents the specimens in groups of different surface finishes allotted to the different Microbial retention Assay methods.

Table 2: Grouping of tested materials prior to Viable and Adherent assay

Specimen	**Viable assay**	**Adherent assay**	**Total**
A1	**10**	**10**	**20**
A2	**10**	**10**	**20**
A3	**10**	**10**	**20**
B1	**10**	**10**	**20**
B2	**10**	**10**	**20**
B3	**10**	**10**	**20**
Total		**120**	

Table 3: Represents the median value of microbial retention of the tested materials by Viable assay.

Table 3: Microbial retention of the tested materials of different surface finishes by use of viable assay method in colony forming units per millilitre (CFU/ml)

Material	**Samples**	**Median**	**Z value**	**P value**
Trevalon	A1	10^4	- 4.700	<0.05
	A2	10^3		
	A3	10^3		
DPI	B1	10^3		
	B2	10^2		
	B3	10^2		

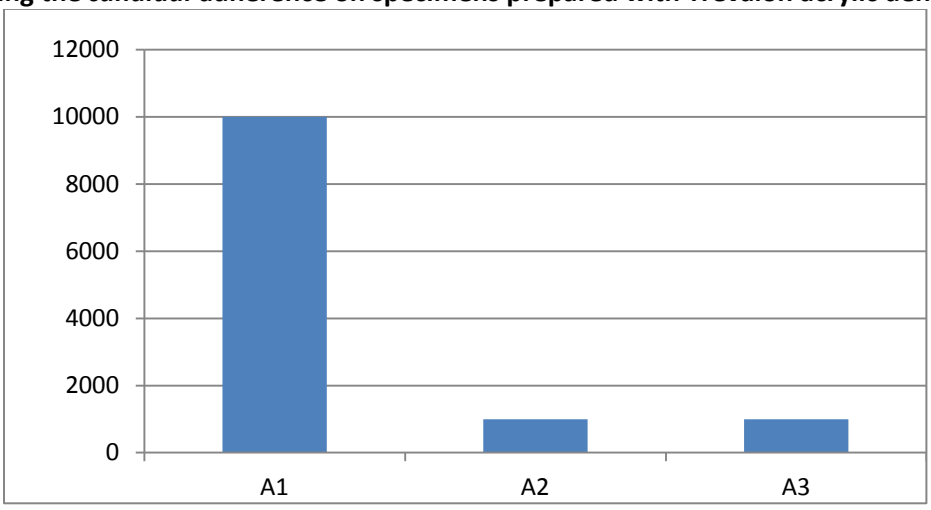
Mann-Whitney U Test

Table 4: Represents the mean and standard deviations value of adherence of Candida albicans for all tested materials by adherence assay.

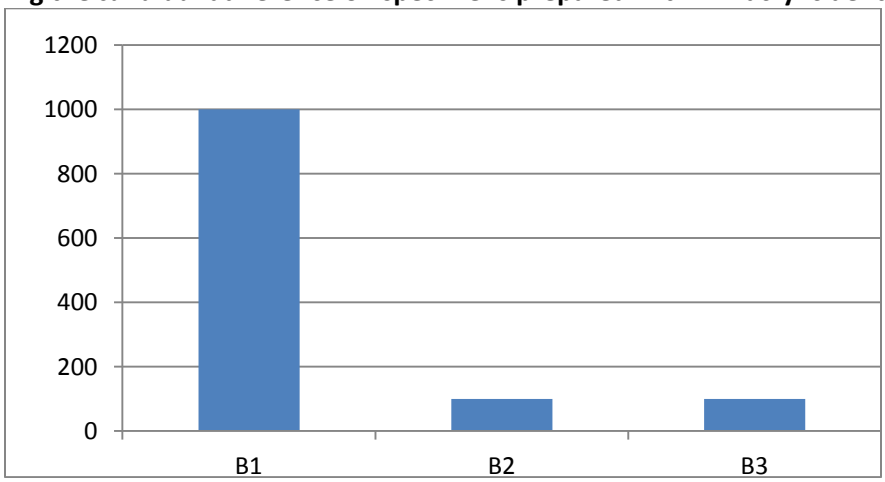
Table 4: Microbial retention of the tested materials of different surface finishes by the use of Adherence assay in *Candida albicans* cells per square millimetre.

Material	Samples	Mean	SD	F value	P value	Tukey's posthoc test
Trevalon	A1	479.50	19.63	1.186	<0.05	**<**0.05
	A2	288.10	15.13			
	A3	267.70	19.31			
DPI	B1	350.30	17.90			
	B2	262.00	17.02			
	B3	205.00	13.12			

Bar diagram showing the *candidal* adherence on specimens prepared with Trevalon acrylic denture base material



Bar diagram showing the *candidal* adherence on specimens prepared with DPI acrylic denture base material



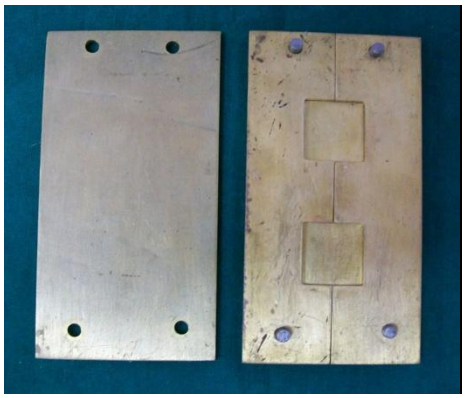


Figure 1: Custom made brass split mould

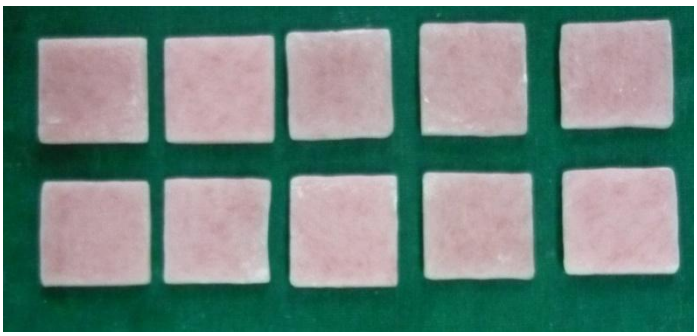


Figure 2: Specimens used in the study

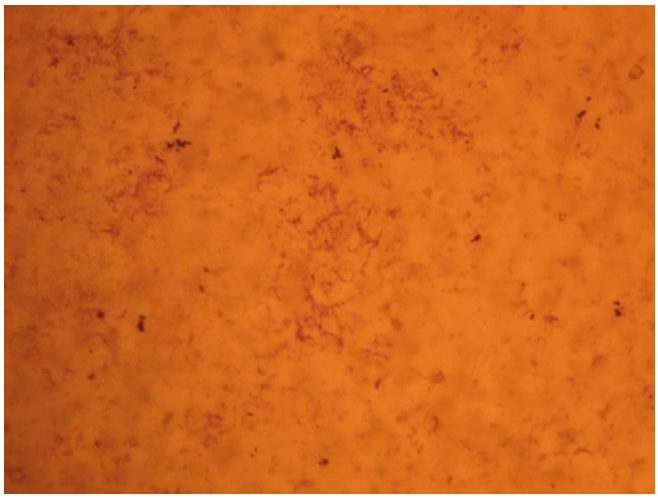


Figure 3: Microbiology armamentarium

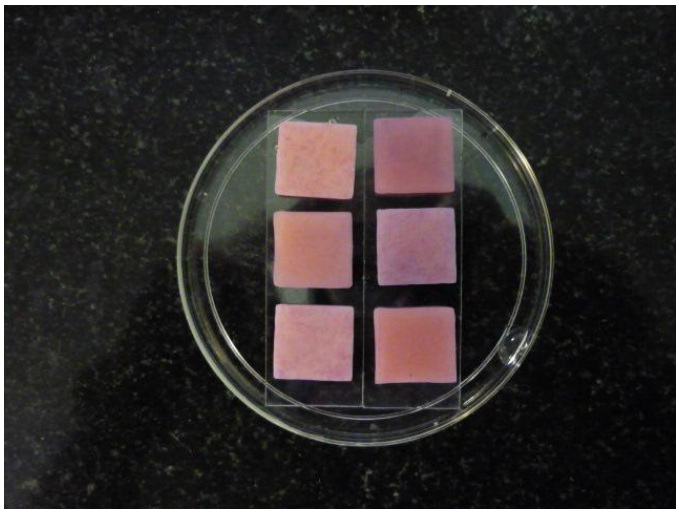


Figure 4: Viable assay: Growth of Candida albicans in CFU

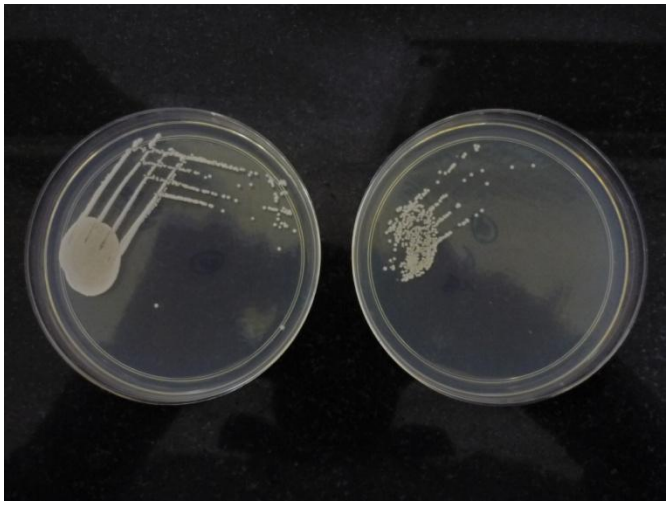


Figure 5: Adherence assay: Samples after staining

Figure 6: Microphotograph showing *Candida* by adherence assay, sample A

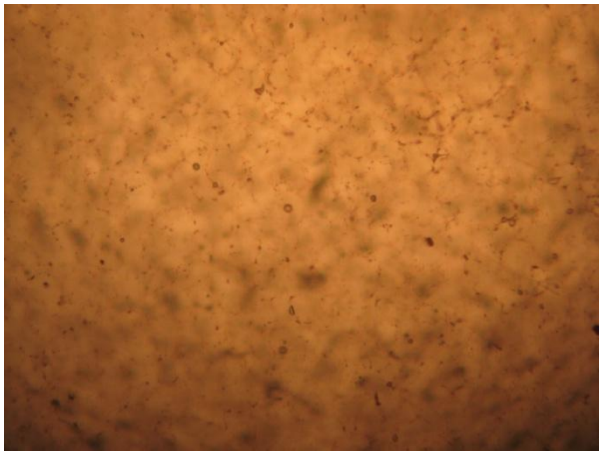


Figure 7: Microphotograph showing Candida by adherence assay, sample B

Candida albican adhesion

The median value of microbial retention of the tested materials by Viable assay is presented in Table 3. The highest value of retention was observed in A1 (10^4 CFU/ ml) & B1 (10^3 CFU/ ml).The lowest value of retention was observed in B2 & B3 (10^2 CFU/ ml) .The mean and standard deviations value of adherence of *Candida albicans* for all tested materials by adherence assay is presented in Table 4. The highest value of adhesion was observed in A1 (479.50± 19.63) & B1 (350.30 ±17.90); whereas the lowest in A3 (267.70±19.31) and B3 (205 ±13.12). The results also indicated that DPI acrylic denture base material had less number of adherent *Candida albicans* cells to its surface with similar finish than that of Trevalon. Multiple group comparisons were made by one way ANOVA test. There was a statistically significant difference between the groups ($P<0.05$), and this indicates that, the surface finish of the tested material influences the microbial retention of *Candida albicans.*

DISCUSSION

The prevalence of denture stomatitis, which has a multifactorial aetiology, is high with 60% of denture wearers being affected with *Candida*-associated denture stomatitis.[8]

Candida albicans is an important oral fungal pathogen, which has the ability to proliferate on both hard and soft tissues forming complex biofilm structures. This proliferation would depend on the physical properties of the material itself, such as porosity, roughness, surface free energy and hydrophobicity. Among other factors, denture base material is one contributing factor associated with denture stomatitis. There are many studies on the adhesion of *Candida albicans* to denture acrylic resin, caused by the association of the commensal opportunistic pathogen yeast with denture stomatitis.[2,4,5] Surfaces used in these studies tend to be smooth and transparent and not representative of the in vivo environment.[5] Other studies have been done using denture soft lining materials.[9] The aim of this study is to determine the influence of surface roughness on the retention of *Candida albicans* to heat polymerized polymethyl methacrylate denture base acrylic resins with three different surface finishes.

The surface finish of a material used for a removable prosthesis influences the retention, staining resistance, plaque accumulation, as well as oral tissue health and patient's comfort.[10] In both the tested materials, with the three different surface finishes, there was an adhesion of *Candida albicans* on rough surfaces in greater number than that on smooth surfaces and cells were observed in surface irregularities after the washing process. The results obtained by staining technique revealed results similar to the viable assay with higher number of *Candida albicans* adherence to rough surface as compared to polished surface. The Trevalon (A1) finished with tungsten carbide bur was reported to have highest number of adherent cells of all tested groups. The surface finish with pumice buffing compared to tungsten carbide finishing significantly decreased the adherence of Candida albicans to denture base acrylic resins. There was also significant difference between surface finish with emery paper and pumice buffing. The results of this study confirm earlier work which demonstrated that increased surface roughness increased retention of yeast on PMMA surfaces. [2,4,5,10-15] One possible explanation for the differences in surface topography to influence the attachment of microorganisms is that the roughness observed in the surface of tested materials processed against dental stone are presumably caused by the crystalline structure of the surface of the stone since these pits were often filled with yeast cells.[5] In vivo, such surface defects would provide an ideal protective area for microorganisms and the potential for a focus from which outgrowth and infection might proceed.[5]Materials with rough surfaces make the cleaning of the prosthesis and mechanical removal of the microorganisms difficult, as well as cause discoloration of the denture base materials.[10] In addition during microbial colonization, cells produce acidic substances as an outcome of their natural metabolisms that affects the pH of the surface they interact with. One more interesting fact that was noticed was that DPI acrylic denture base material had less number of adherent *Candida albicans* cells to its surface as compared to that of Trevalon in all three types of surface finish. This suggests that the physical and chemical composition of the denture base material is important in terms of the ability to promote or prevent adhesion of yeast cells to its surface.

Consideration must be given to studies investigating modification and development of improved denture base materials that may provide a more biocompatible

material to the oral environment. Further, results obtained from an invitro experiment might differ from similar in vivo experiments since saliva reduces adhesion of *C. albicans* and thus diminishes the effect of surface roughness and free surface energy differences between materials. In addition to surface characteristics of the denture base materials, surface properties of the microorganisms and the surface tension of the suspending medium, role of salivary proteins and use of antimicrobial agents are all likely to affect the retention of *Candida albicans* to denture base acrylic resins and need to be considered in further investigations. Lastly, use of confocal microscopy complimentary to Scanning Electron microscopy and profilometer are required to produce quantitative data on surface roughness, to permit a level of investigation well above that of "standard" investigation.

CONCLUSION

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

Surface finished with tungsten carbide bur had the maximum roughness and higher number of *Candida albicans* adherent to its surface.

There was statistically less significant difference in number of *Candida albicans* adherent to surface finished with emery paper following tungsten carbide bur and surface finished with pumice buffing following tungsten carbide and emery paper.

DPI acrylic denture base material had less number of adherent *Candida albicans* cells to its surface with similar finish than that of Trevalon.

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