

An Evaluation of the Adherence of *Candida albicans* on the Surface of Heat Cure Denture Base Material Subjected to Different Stages of Polishing

ASHISH SATPATHY¹, DHAKSHAINI M.R.², ANIL KUMAR GUJJARI³

ABSTRACT

Background: Candidiasis is common in denture wearers. Rough surfaces of dentures promote denture plaque formation and maturation, thus justifying the need for smooth surfaces. Proper finishing and polishing of dentures provide smooth surfaces.

Thus, this study was done to assess the adherence of *Candida* to heat polymerized acrylic resin surfaces which were subjected to different stipulated polishing methods.

Material and Methods: A total of 48 acrylic resin specimens of dimension 10x10x3 mm³ were fabricated and divided into four groups, A, B,C and D, with 12 specimens in each group, according to method of polishing step which was employed. Subsequently, they were subjected to an adherence assay. All the data was statistically analyzed by using One-way ANOVA and differences within the groups were analyzed by using independent sample t-test.

Results: Results revealed that group A which was unpolished had a mean adherence of 2.92 and it was finished by using sand papers. Group B had a mean adherence of 0.5 and it was finished by using sand papers, followed by polishing with pumice. Group C had a mean adherence of 2.92 and finishing was done by using sandpaper which was followed by polishing with the use of pumice and rouge. Group D had a mean adherence of 37.1667.

Conclusion: Finishing specimens of heat cure denture base resin with sand paper of progressive grits alone exhibited minimum Candidal adherence. Specimens which were finished by using sand papers of progressive grits, followed by polishing with pumice reduced Candidal adherence marginally as compared to that in control group. Finishing specimens of heat cure denture base resin by using above steps, followed by rouge, showed maximum Candidal adherence.

Key words: *Candida albicans*, Polishing, Adherence, Yeast, Candidiasis, *Candida*

INTRODUCTION

In normal practice, emphasis is given to the polishing of dentures for aesthetic reasons only. Polishing is one of the methods that aides in maintenance of prosthesis hygiene, by enhancing the reduction of Candidal colonization. Polishing of denture surfaces results in reduction in surface free energy, which can directly affect the adherence of microorganisms [1]. Surface characteristics such as microporosities and roughness increase surface free energy, that may cause the dentures to harbour microorganisms. There is only sparse literature on the effect of polishing steps on the Candidal adherence on denture base material.

Hence, this study was conducted to assess different finishing and polishing procedures which reduced surface irregularity to produce a smooth surface and their effect on Candidal adherence.

MATERIAL AND METHODS

This in vitro study was carried out in following sequence:

1. Specimen fabrication.
2. Finishing and polishing.
3. Adherence assay.

1. Specimen Fabrication

A stainless steel mould was used to fabricate wax specimens of dimension 10x10x3 mm³ [2,3]. The mould had an upper plate, a spacer plate and lower plates with aligning pins. The spacer plate which was of 3mm thickness had 8 square perforations. A total of 48 specimens were fabricated [Table/Fig-1 and 2].

Grouping of Specimens:

- Specimens were divided into four groups which had 12 specimens each.
- Each of these groups was grouped as per the exposure to different finishing and polishing procedures, except control Group A which had unpolished specimens.

2. Polishing Procedure which was Employed to Polish the Specimen

A wooden jig was fabricated, where specimens were fixed and mechanically polished, as the specimens were very small to be held in the hands. The speed of abrasion was standardized to 1500 rpm³, load under which specimens were abraded was standardized to 400 gram [2] and time for which the specimens were abraded was standardized to 90 seconds [2].

First, the specimens which had to be polished were placed over a glass surface, with the surface which had to be polished facing towards glass. This was done to make sure that all surfaces of specimens were at the same level. Then, all the specimens were attached to a wooden jig at once by using cyanoacrylate glue, making sure that all surfaces were touching the glass surface. Now, the surfaces of the attached specimens which had to be polished faced upwards. This was done to make sure that the surfaces of all the specimens which had to be polished were at the same level, which could help abrasive agents to equally finish and polish specimens at the same time [Table/Fig-3].

The finishing protocol which was used in the study was: [1,4].

- Demarcated specimens which were finished by using sand papers of (320/400/600) grits which were used progressively.

- Demarcated specimens which were finished by using sand papers of (320/400/600) grits, followed by polishing with pumice slurry.
- Demarcated specimens which were finished by using sand papers of (320/400/600) grits which were used progressively, followed by polishing with pumice slurry and rouge.
- Then, the specimens were numbered before the adherence assay was done, to check for the adherence of *Candida albicans*.

3. Adherence Assay [3]

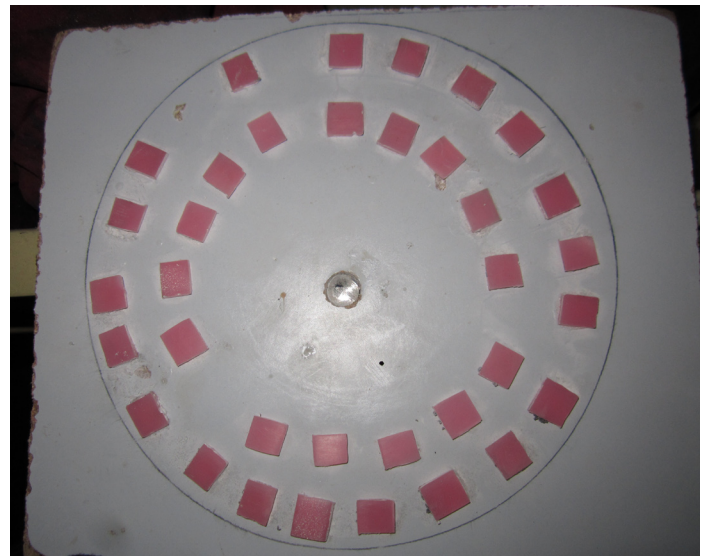
Before beginning with the adherence assay, an yeast suspension was prepared.

Preparation of Yeast Suspension

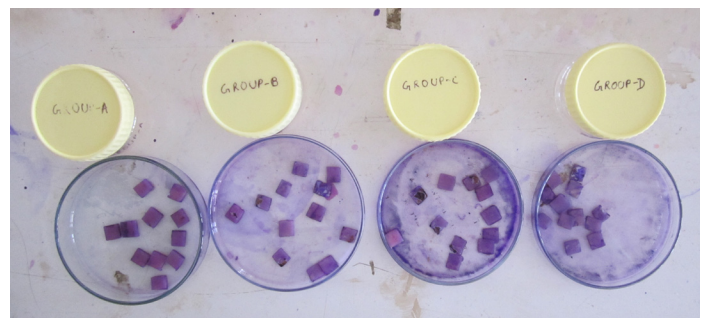
Candida albicans strains were incubated in Sabouraud’s broth which was supplemented with sucrose at 37°C for 24 hours. Candidal growth was harvested after 24 hours by centrifugation. The pellet which resulted from centrifugation was washed twice in phosphate buffered saline solution. The yeast cells were enumerated with McFarland’s unit which was diluted in phosphate buffered saline solution to 10⁷ cells/ml.

Adherence Assay

All the specimens were inoculated with 20 ml of yeast suspension and they were placed in a sterile petri dish and were incubated for 1 hour. Then, the specimens were washed twice by gentle agitation in phosphate buffered saline for 1 minute and they were air dried. Adherent yeast cells were fixed and stained. All the stained specimens were examined under a microscope [5]. Adherent yeast cells in 30 fields of view (0.25 mm² per field) were expressed as yeast cells/mm² of specimen [Table/Fig-4 and 5].



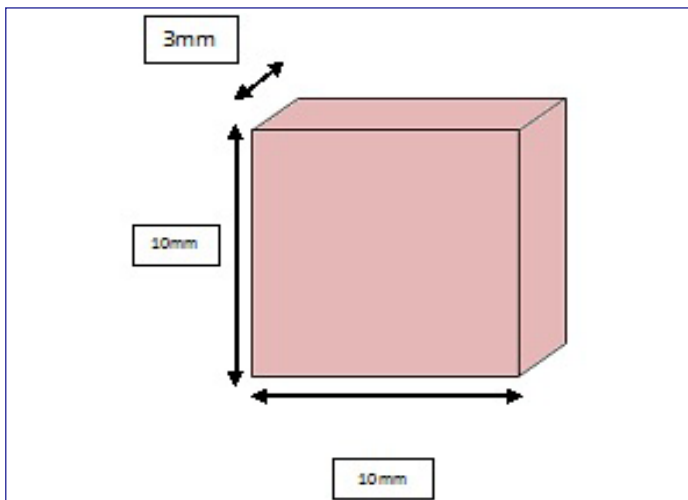
[Table/Fig-3]: Specimen after polishing attached to wooden jig



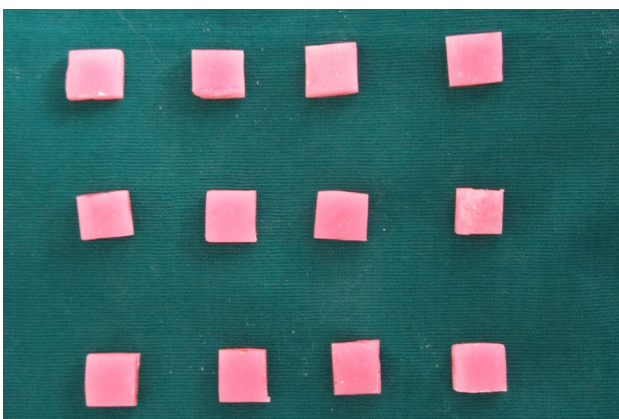
[Table/Fig-4]: Stained specimens



[Table/Fig-5]: Candida under microscope (40x magnification)



[Table/Fig-1]: Acrylic specimens



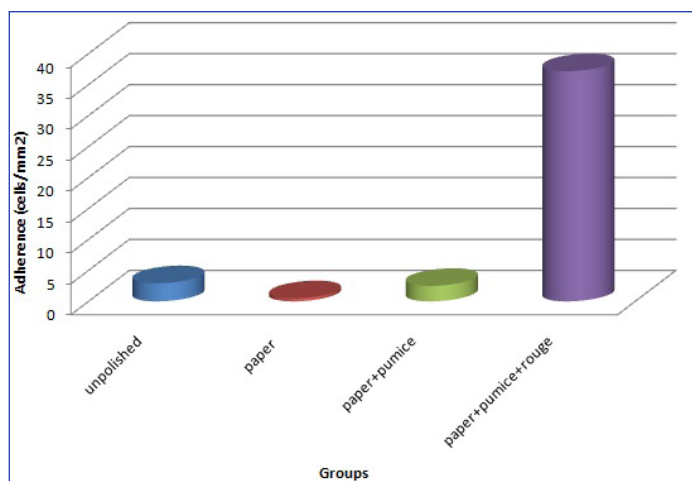
[Table/Fig-2]: Specimens before polishing

RESULTS AND STATISTICS

[Table/Fig-6] shows descriptive analysis: Dependent variable – adherence of *Candida*. [Table/Fig-7] depicts mean adherence values of all the four groups. The highest mean adherence value was seen with the Group D and the lowest mean adherence value was seen with the Group B. Group A and Group C showed intermediate adherence values, with Group A showing greater values than the Group C.

Groups	n	Mean	Std.deviation
A	12	2.9167	.79296
B	12	.5000	.52223
C	12	2.5000	.67420
D	12	37.1667	9.16350
Total	48	10.7708	16.06270

[Table/Fig-6]: Descriptive statistics showing candidal adherence in control Group A and other test groups B,C.,D



[Table/Fig-7]: Comparison of adherence of *Candida albicans* in cells/mm² over the surface of acrylic specimens between different groups

Inter Group Comparison	Level of Significance (p- value)
A-B	.000
A-C	.179
A-D	.000
B-C	.000
B-D	.000
C-D	.000

[Table/Fig-8]:

*Inter-group comparison of significance between Groups A,B,C and D

DISCUSSION

Rough surfaces of dentures allow food and other debris to cling to them. Irregular surfaces are likely to occur unavoidably during the construction of an appliance. Irregularity can be avoided by proper finishing of dentures, which involves both abrading and polishing.

The most commonly used materials for finishing and polishing of dentures in day to day practice are: emery papers, rouge and pumice. So, the polishing agents which were used for polishing specimens in the present study were emery papers of grits 320,400,600 respectively, pumice and rouge [1,4].

An important prerequisite for a successful Candidal colonization and infection is the ability of the yeast cells to adhere to the superficial epithelial cells as well as fitting surfaces of dentures [6]. As per authors, both free energy and the roughness of intra-oral hard surfaces have a major impact on the initial adhesion and retention of oral micro organisms [7]. So, the present study was aimed to find out whether polishing, which altered surface roughness could affect adherence of *Candida albicans*. In the present study, the efficacies of various polishing steps of a polishing protocol for inhibiting the adherence of *Candida* on surface of heat cure denture base resins were tested. It was found that control group specimens, Group A, which were unpolished, showed a significantly higher adherence of *Candida* than the Group B and Group C [Table/Fig-8].

The results which were obtained were in accordance to findings of Donnel et al, who compared inter-procedural variations by using three polishing systems i.e., conventional, acrylic polishing system and silicone polishing system to polish heat cure acrylic specimens. It was found that conventional polishing with the use of carbide bur, sand paper, pumice slurry and Tripoli produced most superior surfaces [8]. Group C showed a marginally lower adherence than control Group A, that was not statistically significant, but adherence in group C was higher than that in Group B [Table/Fig-8]. Where the specimens were polished only by using sand papers. This result was in accordance to the findings of Srividya et al., who showed that surface roughness of acrylic specimens which were

polished with pumice slurry produced significantly rough surfaces as compared to metallic polishing liquid and polishing paste, due to loose and larger particle sizes of the pumice. But the polishing agent which was used in this study was only pumice and not sandpaper, followed by pumice [9]. In the present study, adherence of *Candida* was found to be maximum in test group, where polishing was done by using sandpaper, followed by pumice and rouge (Group D). Moreover, the adherence of *Candida* increased more than that of the control group (Group A). These results may be due to- interaction between the rouge and *Candida* such as to have such a significant Candidal adhesion. *Candida albicans* requires iron for metabolism. Iron is also present in rouge, which is present over the surface of acrylic after polishing, that needs to be removed. This rouge could have increased the adherence of *Candida albicans* on denture base [9,10,11]. *Candida albicans* expresses very similar systems of iron uptake as *Sacharomyces cerevisiae*. *C. albicans* efficiently uses haem as a nutritional source of iron by secreting haemolytic factors to break down haemoglobin. It is broken down by haem oxygenase [11]. Thus, iron is needed for metabolism of *Candida albicans*. Iron is also present in rouge, which is present over the surface of acrylic after polishing. As it contains wax or grease as a binder [12], it leaves deposits on the work, that after buffing must be cleaned off [10]. Thus, iron from rouge may be used by *Candida* for its metabolism, which may cause increased cell counts.

LIMITATIONS OF THE STUDY

Though the Candidal adherence is on the tissue surface, it was not considered here, as the polishing was not done on tissue surface, and as saliva was not used [13,14]. Further, excessive dependence on finishing and polishing procedures for obtaining smoothness of dentures results in reduction of thickness of dentures, that affects the strengths of dentures. So, other ways such as proper wax up, flasking and processing should be emphasized, that produce smooth surfaces without affecting thickness of dentures.

Other limitations such as metal denture bases and other type of denture base materials were not used in this study. Research needs be done, to describe in detail the effect of rouge on Candidal adherence.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that: Finishing specimens of heat cure denture base resin with sand paper of progressive grits alone exhibited minimum Candidal adherence. It was followed by finishing specimens of heat cure denture base resin by using sand paper of progressive grits and polishing with pumice, which reduced Candidal adherence marginally as compared to that in control group. Finishing specimens of heat cure denture base resin by using sandpaper of progressive grits, followed by polishing with pumice and rouge showed maximum Candidal adherence. Therefore, it may be suggested that the specimens which are polished with rouge must be cleaned with solvent to remove residual rouge which remains on the denture surfaces.

ACKNOWLEDGEMENT

Department of prosthodontics, JSS dental college for preparation of specimens and polishing of the specimens Department of Microbiology, JSS Medical College, Mysore, India for helping to carry out the adherence assay.

REFERENCES

- [1] Moura JS, Silva WJD, Pereria T, Cury AADB, Garcia R. Influence of acrylic resin polymerisation methods and saliva on the adherence of four *Candida* species. *J Prosthet Dent*. 2006;96:205-11.

- [2] Kuhar M, Funduk N. Effect of polishing techniques on the surface roughness of acrylic denture base resins. *J Prosthet Dent.* 2005;93:76-85.
- [3] Walters MGJ, Williams DW, Jagger RG, Lewis MAO. Adherence of *Candida albicans* to experimental denture soft lining materials. *J Prosthet Dent.* 1997;77:306-12.
- [4] Morrow RM, Rudd KD, Rhoads JE. Dental laboratory procedures complete dentures (volume one). C.V. Mosby Company. 2nd edition 1986;334-7.
- [5] Kalla R, Rao H, MV Sunil Kumar. Surface adherence of *Candida albicans* to different Polymethylmethacrylate denture base resins: An in vitro study. *Int J Prosthet Dent.* 2011;2(1)2-7.
- [6] Heylings JF Cutting and abrasion. The alliance professional development programme.
- [7] Quirynen M, Bollen CML. The influence of surface roughness and surface free energy on supra- and subgingival plaque formation in man. a review of literature. *J Clin Periodontol.* 1995;22:1-14.
- [8] Donnel E FO, Radford DR, Sinclair GF, Clark RKF. Chairside polishing of heat cure acrylic resin: an SEM and EDA study. *Int J Prosthodont.* 2003;16:233-38.
- [9] Srividya S, Nair C.K., Shetty J. Effect of different polishing agents on surface finish and hardness of denture base acrylic resins: a comparative study. *IJOPRD.* 2011;1(11);7-11.
- [10] Popular Mechanics September. 1969; 191.
- [11] Crichton R. Iron Metabolism: From Molecular Mechanisms to Clinical Consequences, 3rd edition, Wiley, 2008; 130.
- [12] Wikipedia search Fe III Oxide.
- [13] Edgerton M, Scannapieco FA, Reddy MS, Levine MJ. Human sub-mandibular-sublingual saliva promotes adhesion of *Candida albicans* to polymethyl methacrylate. *Infect and Immunity.* 1993;2644-52.
- [14] Busscher HJ, Evans LV. Oral biofilms and plaque control. *Harwood Academic Publishers.* 1998. 1st edition:175-88.

PARTICULARS OF CONTRIBUTORS:

1. Post Graduate Student, Department of Prosthodontics, JSS Dental College and Hospital, Mysore, India.
2. Professor, Department of Prosthodontics, JSS Dental College and Hospital, Mysore, India.
3. Professor and Head, Department of Prosthodontics, JSS Dental College and Hospital, Mysore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Anil Kumar Gujjari,
Room No. 10, Department of Prosthodontics, JSS Dental College and Hospital, SS Nagar, Mysore-570015, India.
Phone: 9448390982, E-mail: anilgujjari@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 22, 2013**
Date of Peer Review: **May 09, 2013**
Date of Acceptance: **May 25, 2013**
Date of Publishing: **Oct 05, 2013**