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ORIGINAL RESEARCH

Surface Adherence of *Candida albicans* to Different Polymethyl Methacrylate Resin Denture Base Materials

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ABSTRACT

Over the years researchers have reported on the frequency and distribution of yeast in the oropharynx of apparently normal individuals and those with systemic or mycotic diseases wearing dentures. Few workers have taken into consideration the role played by the legion of denture base materials on the occurrence and progression of denture stomatitis. Hence this study was undertaken with the objective of evaluating the *in vitro* adherence of *Candida albicans* to different denture base materials namely reinforced polymethyl methacrylate, Non-reinforced polymethyl methacrylate and Cobalt chrome alloy of which chrome cobalt alloy showed less adherence to candidal cells compared to the two polymethyl methacrylate resins.

Keywords: *Candida albicans*, Candidiasis, Oral thrush, Nonreinforced polymethyl methacrylate, Poly methyl methacrylate, Chrome cobalt alloy.

INTRODUCTION

The human oral cavity is known to harbor a multitude of organisms. Among them, *Candida albicans* has lately become a cause of great concern to the dental profession. Coexistence of Candida species, either as a commensal and/or as a pathogen has attracted the attention of many investigators. *Candida albicans* has been termed as a notorious "opportunistic pathogen"[1] amongst similar species.

Among the edentulous individuals most particularly the elderly, this pathogen has been observed to harbor the oral cavity.[2-4] Surveys on randomly selected patients in dental clinics have revealed high incidence of palatal inflammation among denture wearers.[2]

An essential prerequisite for successful candidial colonization and infection as the ability of the yeast to adhere to the superficial epithelial cells as well as to the fitting surface of the denture,[5] considering the latter as the reservoir of infection.[2,3]

The purpose of this study is, therefore, to conduct an *in vitro* experiment to evaluate the amount of surface adherence of *Candida albicans* to nonreinforced PMMA, reinforced PMMA and cobalt chrome base metal denture base materials and to compare the adherence of the yeast among these materials.

AIMS AND OBJECTIVES

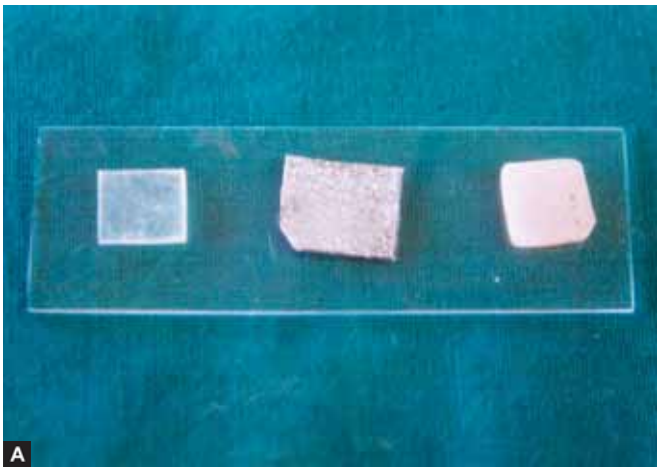
The present study was done to study the adherence of *Candida albicans* to cross linked and fiber reinforced acrylic denture base materials at periodic intervals.

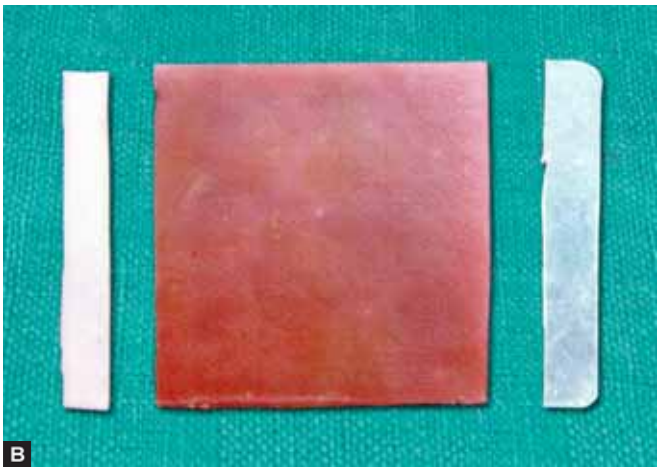
MATERIALS AND METHODS

The materials used in this study were polymethyl methacrylate nonreinforced, polymethyl methacrylate reinforced, and a base metal alloy (Fig. 1).

Test samples were fabricated in the following way. A casting wax sheet with tissue topography simulated on one surface was used. The thickness of the sheet was adjusted to 3 mm. These sheets were cut into smaller squares of 7 × 7 cm squares, so as to fit into the mould of the dental flask (Figs 2A and B). These were invested using Type III dental stone (Rapidur, Dentaurum) to have good reproduction of the tissue surface. Two types of poly-methyl methacrylate were used, reinforced (Lucitone 199) and non-reinforced (Stellon), which were packed in separate

Fig. 1: Materials used





Figs 2A and B: Test samples

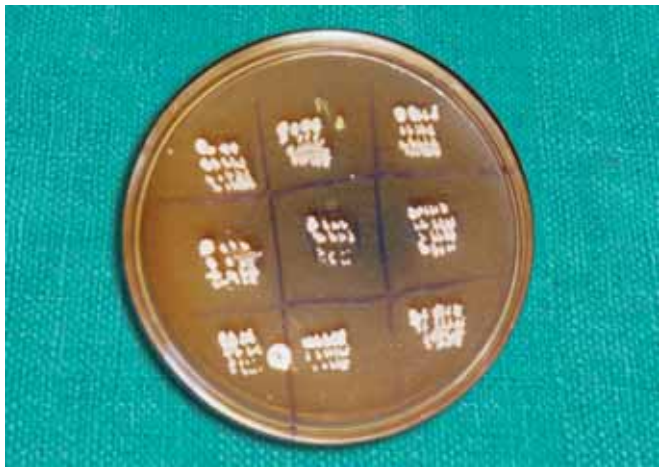


Fig. 4: Culture preparation

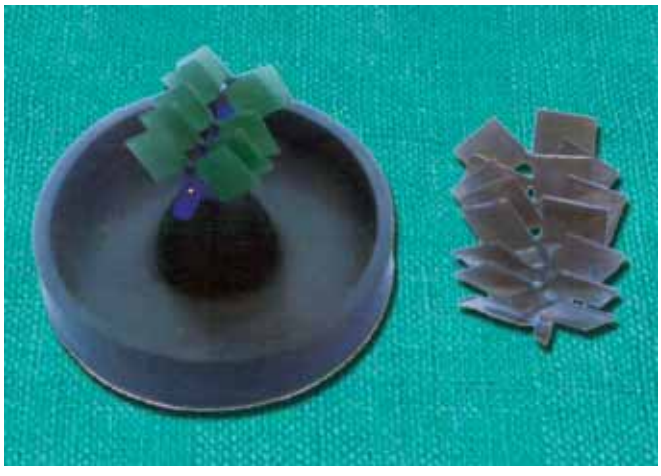


Fig. 3: Fabrication of test specimens

flasks and excess removed following a trial closure under a hydraulic bench press. Then the flasks were secured in a dental clamp and polymerized in an acrylizer. A long curing cycle (70 degree for 9 hours) was employed to decrease the residual monomer content and also to obtain good transparency in the material.

These acrylized sheets were cut into squares of 1×1 cm. An identification mark was made on the nontissues surface to distinguish between fiber reinforced and nonfiber reinforced acrylics. They were then subjected to standard finishing and polishing technique.

Wax patterns with similar tissue topography on one surface were prepared in casting wax (Dentaurum, Germany). They were then invested in a 9x casting ring with phosphate bonded investment material (Hi-temp, Whipmix, Germany) (Fig. 4). The investment was proportioned as per the manufacturer's specifications and then vaccum mixed and invested. The casting rings were subjected to burnout procedure and cast in an induction casting machine (Degussa, Germany). The metal specimens were then retrieved and subjected to sand blasting after cutting the sprues. They were then finished, polished, dried, and cleaned with an ultrasonic cleaner (Sonorex, Italy). Finally, they were electropolished. All the samples were washed, dried, and sterilized by ultraviolet rays.

CULTURE PREPARATION

Laboratory isolates of *Candida albicans* were used for the study. Ten milliliters of sabouraud's broth was incubated with *Candida albicans* and incubated at 37°C for 24 hrs (Fig. 5).

INOCULATION

The 48 hrs broth culture with *Candida albicans* (10 ml) was dispensed in 20 sterile containers. One sample of each type of

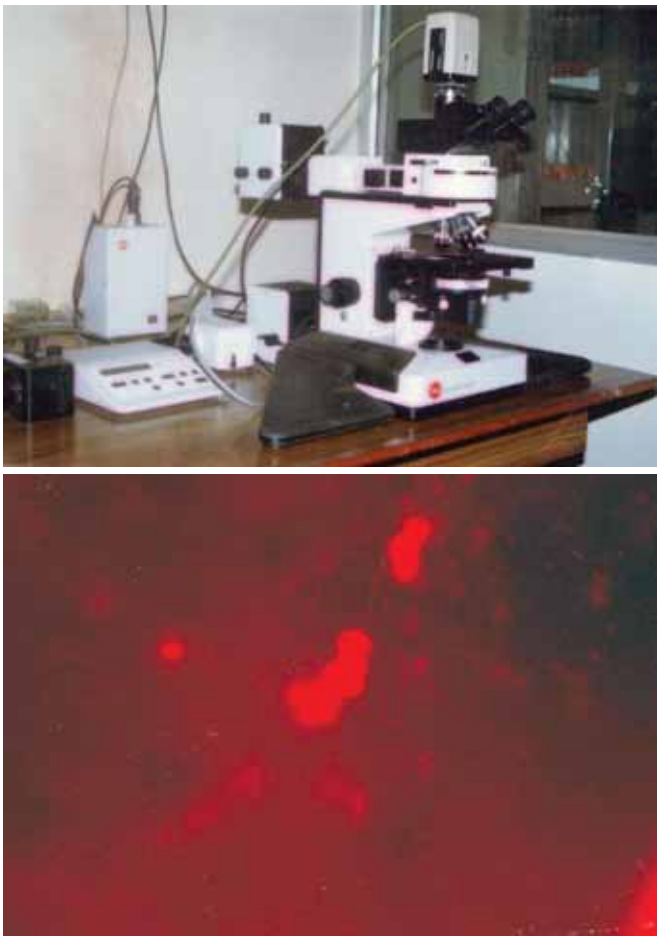


Fig. 5: Fluorescent study

denture base study material was introduced into each broth culture container. These were then incubated at 37°C for ten different intervals of time (1/2 to 1 hour, 2, 4, 6, 8, 18, 20, 24 and 48 hours) and labeled accordingly.

FLUORESCENT STUDY

One set of samples was then air dried and fixed in methyl alcohol for one minute. They were then stained by 0.01% acrydine orange (Himedia), a fluorescent dye for 2 minutes. They were washed and air dried in a slanted position. The samples were observed under oil immersion objective (1000x), using fluorescent microscope (Leitez, Germany) (Fig. 5).

RESULTS

Three types of materials *viz* reinforced polymethyl methacrylate, nonreinforced polymethyl methacrylate, and cobalt chrome metal were the materials used. These were evaluated at various intervals of 30 mins, 1, 2, 4, 6, 8, 16, 20, 24 and 48 hours. Both microscopic as well as macroscopic estimation of adherence were carried out.

The samples were examined under fluorescent microscopes to count the adherent yeast. This was a part of microscopic study and the numbers of adherent cells were counted for different material samples at varying intervals of time. A particular sample was focussed under the microscope and the numbers of cells adherent were counted at 10 random fields. So, three hundred folds were seen for thirty samples.

Fluorescent Study

The study showed that there was significant difference in the number of cells adhered among the three test materials ($p < 0.05$). There was also a significant increase in the degree of adherence with the increase in the incubation periods ($p < 0.05$). It was observed that cobalt chrome alloy showed least adherence in comparison to reinforced and nonreinforced acrylic resins (Table 1). This was statistically analyzed and found to be significant.

Among the two acrylic resins, reinforced acrylic resin showed more adherence than nonreinforced acrylic resin. However, there was no significant difference between the amount of candidal cells between the two resins ($p < 0.05$). The increase in the number of adherent candidal cells with the increase in incubation periods was not uniform for any tested specimen (Table 1).

Culture Study

The observations of the culture study were similar to that of fluorescent study. Thus, verifying the results of the fluorescent study. Cobalt chrome alloy showed the least number of colony forming units compared with reinforce and nonreinforced acrylic resins with the increase in the incubation periods (Table 2).

Table 1: The adherent cells in fluorescent study

Incubation period	*Reinforced acrylic*	*Nonreinforced acrylic*	*Cobalt chrome alloy (metal)*
30 minutes	0.4	0.4	0.2
1 hour	0.8	0.6	0.4
2 hours	1.1	0.9	0.5
4 hours	1.1	0.8	0.4
6 hours	1.2	1.1	0.7
8 hours	1.3	1.1	0.6
16 hours	1.3	1.2	0.7
20 hours	1.5	1.3	1.0
24 hours	1.7	1.5	0.8
48 hours	2.1	1.5	1.0

Table 2: The colony forming units in culture study

Incubation period	Reinforced acrylic	Nonreinforced acrylic	Cobalt chrome alloy (metal)
30 minutes	40	40	20
1 hour	80	60	40
2 hours	100	90	50
4 hours	100	80	40
6 hours	100	100	70
8 hours	100	100	60
16 hours	100	100	70
20 hours	100	100	100
24 hours	100	100	80
48 hours	100	100	100

This was found statistically significant ($p < 0.05$). However, between the two acrylic resins, there was no significant difference ($p < 0.05$).

DISCUSSION

Edentulousness is not a disease entity in itself, but rather a consequence of pathology. Increasing incidence of edentulousness over the recent years has questioned the adequacy of dental treatment. Yet, the mainstay for the management of the edentulous state till date remains to be an acrylic complete denture. Treatment of these individuals with complete dentures not only rehabilitates them functionally but also esthetically and psychologically. However, prosthetic rehabilitation of the aged has been of great concern. The difficulties that arise may not only be attributed to denture construction but also to associated problems with continuous denture wearing. A commonly occurring condition observed frequently is denture stomatitis secondary to candidial infection.

Denture stomatitis is a term applied to an inflammation of the denture bearing mucosa which may affect as many as two-third of denture wearers.[6] Its incidence has been reported to occur among 11 to 67% of the denture wearers.[7] The incidence is further exaggerated in the elderly due to a compromised oral and general health. Certain disease conditions, like diabetes mellitus, corticosteroid therapy, antibiotic therapy, xerostomia and radiation therapy,[3,8] have shown to predispose to denture stomatitis.

The occurrence of an infection is directly related to two major criteria—the virulence of the organism and the number of organisms colonizing the area. These two factors though independent are related to each other. Any reason that would cause a fall in the number of organism colonizing would decrease the occurrence of infection. *Candida albicans* has been demonstrated on the fitting surface of the dentures.[9,6] This was attributed to the acidic pH prevalent under the fitting surface of the dentures which aided the proliferation of the fungi (LP Samaranayake 1982) opined that denture stomatitis is associated with the proliferation of *Candida albicans* on the fitting surface of the denture than on the mucosal surface of the palate. Olson (1974) considered the fitting surface of the denture as a reservoir of infection.

This study was carried out to evaluate the surface adherence of *Candida albicans* to three commonly used denture base materials. The three denture base materials used for this study were reinforced polymethyl methacrylate, nonreinforced polymethyl methacrylate, and base metal alloy (see Fig. 1). Acrylics and metals have been used in the fabrication of denture bases; however, acrylics have been more widely used. Acrylics, though economical and easy to manipulate, show some features such as water sorption and permissive surface in contrast to metallic denture bases.[10] These features may alter the degree of adherence of *Candida albicans* to acrylics, which may be contrasting to the metals. Among the metals, gold has been shown to have better surface topography than acrylics.[11] However, the use of gold is impracticable. Therefore, cobalt chrome, a commonly used metallic denture base material, is been used in this study.

It has been reported that other factors such as surface-free energy, hydrostatic forces, hydrophilic or hydrophobic nature of the material, pervious nature of the material, and water sorption affect the nature of adherence of the yeast to the denture base materials.[10,12,13] Therefore, two types of polymethyl methacrylate were used inferring some differences in the above factors.

The methodology chosen was based on a study by Verran and Maryan (1997)[14] with following modifications. Different periods of incubation were considered to evaluate whether the amount of adherence varied with time lapse. The surface topography of the specimen was altered to simulate tissue topography similar to the fitting surface of the denture. The specimens were observed under incident beam fluorescent microscopy was used because the specimens tested were either translucent or opaque and reflected beam microscopy was not useful.

The specimens were observed for the adherent cells and some interesting results were obtained. Cobalt chrome, the base metal alloy used, showed the least adherence to the candidial cells when compared with the two types of polymethyl methacrylates used (Table 1). This when statistically evaluated was found to be significant ($p < 0.05$). The number of adherent cells and the periods of incubation when compared showed that there was a definite increase in the number of adherent cells

with increase in the incubation time. However, this increase in the number of adherent cells was not regular when compared between different methods of incubation (Table 1). The number of fields and the number of adherent candidal cells were also compared. It was observed that there is no correlation between the presence of adherent cells and the fields observed. Some of the fields showed adherent candidial cells and some did not show any cells. This can be attributed to the irregular surface due to the simulation of tissue topography. Thus, after washing the specimens, the adherent cells on the elevated areas would have been removed easily compared with the cells in the depressed areas. This can be attributed to the fields without cells and fields with cells, respectively.

Polymethyl methacrylate when examined for adherence showed that both the polymethyl methacrylates specimens exhibited more adherences when compared with the cobalt chrome alloy specimens. This can be related to the relatively even surface of the cobalt chrome alloy specimens than the polymethyl methacrylate specimen which show a rough porous surface with most intricate and diverse surface contours.[11] The decreased adherence to cobalt chrome alloy specimens can also be attributed to the surface finish of the material. However, in practice, the fitting surface of acrylic dentures with metallic denture bases, the surface of which can be electropolished. This gives the metals an even surface as compared with acrylic resins thereby decreasing the adherence of *Candida albicans* cells of them.

It has been reported that acrylic resins are hydrophilic, pervious, and exhibit water sorption in contrast to metals, which are hydrophobic, nonpervious, and show no water sorption property. The water sorption may help the candidial cells to adhere or to even penetrate the surface of the acrylic resin. However, this is postulated and yet to be proved. Further, indentations measuring 1 to 2 um on the surface of the acrylic resins have been recorded. These indentations are of greater width and depth and, thus, yeast with diameter of 5 um[15] cannot be dislodged easily. Hydrostatic forces and surface-free energy have also been shown to play an important role in the adherence of *Candida albicans* to substrate surfaces.[12,13] *Candida albicans* possess surface-free energy closer to that of polymethyl methacrylate than cobalt chrome alloy.[13] This may be the reason for increased adherence of *Candida albicans* to polymethyl methacrylate when compared with cobalt chrome.

Among the two types of polymethyl methacrylate resins, it was observed that reinforced resin shows more adherence as compared to nonreinforced resin. However, this difference in adherence between the two resins was not statistically significant (Table 1). Furthermore, this difference in the adherence was thought to be due to the difference in factors like surface-free energy and contact angle of the two materials. This can be further attributed to the reinforcing fibers and veined fibers incorporated in the reinforced resins which may be difficult to finish on the surface, thus, making the surface more irregular compared to the nonreinforced resins.

The imprint culture study was conducted to verify the results of the microscopic study. The number of colony forming units (CFU) was counted following the incubation period. The number of colony forming units was expressed as 100CFU wherever confluent growth was encountered. The study indicated an increase in the adherence of candidial cells with increase in the periods of incubation with respect to all the test specimens (Table 2). Among all the specimens checked, cobalt chrome showed the least adherence compared with the polymethyl methacrylates. Statistically, comparison of adherence between cobalt chrome and polymethyl methacrylate was seen to be significant ($p < 0.05$), but between the two polymethyl methacrylates it was found to be nonsignificant ($p > 0.05$). Thus, the data obtained from the imprint culture study were found similar to that from fluorescent microscopy study verifying the results of the latter study. It has been shown that frequent cleaning of denture reduces the occurrence of candidial infections.[15]

CONCLUSION

Our study indicated that brushing the test specimens with the help of denture brush, decreased the number of adherent candidial cells. Furthermore, it was evident that specimens that were cleaned after 24 hours showed less adherent candidial cells than that were cleaned after 48 hours. However, brushing did not completely eliminate the adherent candidial cells. Above findings suggest that the metallic denture bases are far better than acrylic denture bases in terms of incidence of denture stomatitis due to candidial infection. Further, they are seemed to be more biocompatible with the oral tissues with minimal tissue reactions if any.[15] However, usage of these base metal alloys as denture bases may not be practicable in regard to their cost, difficulty in relining the necessary set up for their fabrication. Thus, acrylic resins still remain the materials of choice answering the requisites of economy and ease of fabrication. However, patient's oral hygiene should be given prime importance irrespective of the denture base materials used.

REFERENCES

1. Samaranayake Lakshman P, Mc Farlane Wallace T. Oral Candidosis. Cambridge, Butterworth and Co Ltd 265.
2. Davenport JC. The oral distribution of Candida in denture stomatitis. Brit Dent J 1970;129:151.
3. Jorgenson Budtz Ejvind. Clinical aspects of Candida infection in denture wearers. JADA March 1978;96:474.
4. Olsen Ingar. Denture stomatitis; Occurrence and distribution of fungi. Acta Odont Scand 1974;32:329-33.
5. Samaranayake LP, Mc Farlane TW. An in vitro study of adherence of Candida albicans to acrylic surfaces. Arch Oral Biol 1980;25:603-09.
6. Jorgenson Budtz E. Denture stomatitis V Candida Agglutinins in Human Sera. Acta Odont Scand 1972;30:313-25.
7. Arendorf TM, Walker DM. Denture stomatitis: A review. J Oral Rehab 1987;14:217-27.
8. Knight Lawrence, Fletcher John. Growth of Candida albicans in saliva: Stimulation by glucose associated with antibiotics corticosteroids and diabetes mellitus. J Infect Dis 1971;123: 371-77.

9. Cawson RA. Role of Candida. Dent Practit 1965;16:138-42.
10. Braden M. The absorption of water by acrylic resins and other materials. J Prosthet Dent 1964;14:307-16.
11. Miner John F. The nature of denture base: A key factor in denture base mouth sore mouth. J Prosthet Dent 1973;29(3):250-55.
12. Klotz A Stephen, Drutz David J, Zaije James E. Factors governing adherence Candida species to plastic surfaces. Infect Immune 1985;50(1):97-101.
13. Minagi Shogo, Miyake Yochiro, Inagaki Kumiko, Tsuru Hiromichi, Suginaka Hidekazu. Hydrophonic interaction in Candida albicans and Candida tropicalis adherence to various denture base resin materials. Infect Immune 1985;47:11-14.
14. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resins and silicone of different surface topography. J Prosthet Dent 1997;77:535-39.
15. Smith Dennis C, Williams David F. Biocompatibility of dental materials. Florida, CRC press, Inc, 4:273.