Comparative Evaluation of Adhesion of *Candida albicans* to Heat Cure Polymethyl Methacrylate, Self-Cure Polymethyl Methacrylate and Vacuum formed Thermoplastic Resin - An In Vitro Study

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ABSTRACT

BACKGROUND

Polymethyl methacrylates are the most common materials for rehabilitation of complete or partial edentulous patients, Rehabilitation of such patients with tissue / mucosa supported dentures with lack of hygiene, surface roughness of prosthesis act as possible agents of tissue damage inducing denture stomatitis. Among the *Candida* species, *Candida albicans* are notable "opportunistic pathogens" which harbour the oral cavity and further its adherence of yeast to superficial epithelial cells and denture surfaces are important for successful *Candida* colonization and infection. This study aims to evaluate and compare *Candida* adherence to surface of thermoplastic resin sheets, heat cure PMMA (Poly-Methyl Metha-Crylates) and self-cure PMMA. We wanted to evaluate and compare the adhesion of *Candida albicans* to three different denture base materials - heat cure polymethyl methacrylates, self-cure polymethyl methacrylates and vacuum formed thermoplastic resin sheets.

METHODS

A 10 samples of each heat cure PMMA, self-cure PMMA and thermoplastic resin sheet were prepared of 10X 10X 3 size. All samples were immersed in distilled water for 24 hrs. *C. albicans* GDH 2346 (NCYC 1467) was incubated at 37º C for 24 hours in 500 mL Sabouraud's broth and the specimens were independently deposited in 20 mL yeast suspension in sterile Petri dishes and incubated for 24 hours at room temperature. Adherent yeast cells in 90 fields of view (0.25 mm 2 per field) in the 30 specimens of materials (3 fields from each specimen) were enumerated.

RESULTS

The adhesion of *Candida albicans* to a surface was least for thermoplastic resin sheet compared to self-cure PMMA and heat cure PMMA.

CONCLUSIONS

Adhered candidal count to thermoplastic resin sheet was lesser compared heat cure PMMA and self-cure PMMA.

KEY WORDS

Candida albicans, Heat Cure Polymethyl Methacrylates (PMMA), Self-Cure Polymethyl Methacrylates (PMMA), Thermoplastic Resin Sheet

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BACKGROUND

Most widely used materials for removable prosthesis in dentistry are polymethyl methacrylates, either self-cure or heat cure acrylic resins as denture base materials for complete or partial dental prosthesis, surgical obturators, TMJ (Temporo-Mandibular Joint) splints and others. With advancements in dentistry there is development of various new materials and modified older materials that are being used to rehabilitate complete or partial edentulous patients. Rehabilitation of such patients with tissue / mucosa supported dentures with lack of hygiene, surface roughness of prosthesis act as possible agents of tissue damage and denture stomatitis present in 11 % to 67 % of denture wearers.[1] Denture induced pathosis also known as sore mouth under plates was 1st recorded in 1885 by Black.[2] Later was reported in 1936 by Cahn that the acidogenic microorganisms which were responsible "denture sore mouth" were *Monilia albicans*.[3]

The ability of these microorganisms to adhere to oral and denture surfaces is crucial in pathogenesis and such adherence enables the microorganism to withstand the mechanical washing action of saliva and it is a prerequisite for successful colonization.[4] Initial adhesion phase of micro- organisms to surfaces is influenced by surface free energy and adhesion phase is affected by surface roughness,[5] Hence, surface of prosthesis are finished and polished well to reduce surface free energy and surface roughness to avoid microbial adhesion and colonization. Advancements with nylon like materials in 1950s,[6] thermoplastic resins have gained the interest for its usage in removable flexible partial dentures, preformed partial denture clasps, fiber-reinforced fixed partial dentures, temporary crowns and bridges, provisional crowns and bridge, speech therapy appliances, orthodontic retainers and brackets, impression tray and border molding materials, sleep apnea appliances, splints for TMJ disorders, immediate obturators, feeding plates in palatal defects patients with other denture base materials due to their ease of adaptation, flexibility, abrasion resistant, smooth surface and unbreakable material which gives comfort to patient.[7] Hence, this study aims to evaluate and compare *Candida* adherence to surface of thermoplastic resin sheets, heat cure PMMA and self-cure PMMA.

METHODS

Sample Fabrication

10 blocks of each heat cure polymethyl methacrylate (DPI Company India), self-cure polymethyl methacrylate (DPI Company India) and thermoplastic resin sheets were prepared of 10 * 10 * 13 mm in size respectively. A wax block of 10*10*13 mm dimension was made using modelling wax (Hindustan modelling wax no. 2, India) which was then duplicated in putty elastomeric rubber base material (Aquasil, Dentsply, India) further required number of wax samples were prepared by pouring modelling wax into the rubber base index. Later test samples were fabricated by investing wax models in type III dental stone (KALABHAI, Kalstone) for good surface reproduction. After dental stone setting and wax elimination, the resulted stone moulds were used for the fabrication of samples.

(Group A) Heat cure PMMA samples considering sample size and number the samples were fabricated by mixing polymer powder and monomer liquid in ratio of 3:1 by volume which provides sufficient monomer to thoroughly wet polymer particles. Following the manufacturer instructions flasks were bench cured for 30 minutes and curing / polymerization was done, after completion of polymerization flask is cooled slowly to the room temperature and later samples were retrieved from the flask.

(Group B) Self-cure PMMA samples-monomer and polymer are mixed following the manufacturer's instructions to achieve dough like consistency and then packed into dental stone mould and to ensure complete polymerization the flask was held under pressure.

After complete polymerization samples were retrieved from moulds only excess material was trimmed without any fine finishing and polishing.

(Group C) Thermoplastic resin sheet samples of 2 mm thickness were achieved with positive pressure moulding (Bioacryl) by directly pressing thermoplastic sheets over the acrylic samples. Excess sheet was trimmed using scissors.

Candida albicans Preparation

One standard strain organism was used (*C. albicans*). *Candida albicans* was incubated at 37º C for 24 hours in 500 mL Sabouraud's broth for growth and was harvested after 24 hours by cold centrifugation. The growth obtained was washed using phosphate-buffered saline (0.15 mol / L, pH 7.2) twice. Haemocytometer was used for enumeration of yeast cells which were then diluted to 10^7 yeast cells / mL by using phosphate buffered saline.

Adherence Assay

The adherence assay specimens / samples were independently deposited in 20 mL yeast suspension in sterile Petri dishes and incubated for 24 hours at room temperature to attain stationary phase. After the incubation period samples were removed using sterile forceps to avoid any contamination and were washed twice in phosphate buffered saline for 1 minute, dried, fixed in methanol 80 % and stained for 30 seconds with crystal violet.[8]

Candida Adherence Counts

Adherent yeast cells in 90 fields of view (0.25 mm 2 per field) in the 30 specimens of materials (3 fields from each specimen) were enumerated and the mean was calculated and the results were expressed as yeast cells per mm^2 of materials**.** The specimens were washed twice in phosphate buffered saline for 1 minute, dried, fixed in methanol 80 % and stained for 30 seconds with crystal violet.

Statistical Analysis

Data were presented as mean and Standard Deviation (SD) values. Kruskal Wallis test was used for comparison between mean values and comparison between three groups using Dunn's Post hoc test.

RESULTS

Data obtained was subjected to statistical analysis using SPSS software version 19 to draw conclusion from experimental data. Candidal count with mean and standard deviation of all the samples is shown in Table 1.

Materials	N	Mean	Standard Deviation
Group A	10	344.2	144.7
Group B	10	510.2	240.8
Group C	10	241.2	186.4

Table 1. Mean and Standard Deviation of all the Samples

P = 0.012

Materials	Comparison	P Value
Group A	Group B	0.156
	Group C	0.473
Group B	Group A	0.156
	Group C	0.012
Group C	Group B	0.012
	Group A	0.473

Table 2. Comparison of the Three Groups Using Post Hoc Test

Candidal count of all the samples is shown in Table 1. Results showed that highest surface adhesion was recorded in self cure acrylic resin followed by heat cure acrylic resin and thermoplastic resin sheet.

DISCUSSION

Important issue of the denture base materials which compromises its service and efficacy is the adhesion of microorganisms, especially yeasts.[3] Microbial colonization, biofilm formation and its adhesion is highest onto rougher surface of denture base materials.[9] In present study *Candida albicans* species were chosen because it is the most related *Candida* species to oral fungal infection[10] and all the surfaces were prepared in a type III stone mould to have a good reproduction of surfaces and were kept without finishing and polishing to simulate the usual denture fit surfaces which act as a primary reservoir of pathogenesis and it explains why *Candida* adherence counts on self-cure acrylic resin specimens (Group B) was more when compared to thermoplastic resin sheet specimens which was smooth (Group C). Surface roughness is of prime importance in the early stages of adhesion of *Candida albicans* according to Quirynen et al[11] and also affirmed that surface roughness is important in plaque development intra-orally.

Aggregation of *Candida* species on the acrylic material as examined by microscope is seen during drying of the material mainly due to surface tension forces according to D W Williams et al[12], hence, in this methodology, yeast aggregation was minimised by keeping all the samples immersed in buffer solution and prevent drying all over the experiment. The technique therefore offers an improved method of measuring adherence of *Candida* to acrylics. *Candida* adherence on heat cure and self-cure (Group A and B) PMMA samples showed greater adhesion than thermoplastic material (Group C). Positive correlation of surface roughness and surface free energy may contribute to increased rate of microbial colonization and plaque maturation on surfaces hence, rougher the surface more the Candidal count.[13] The thermoplastic resin sheet (Group C) show lower surface roughness value have a smaller number of Candidal cells attachment according to microbial study conducted. Surface irregularities which imparts roughness to surface may serve as reservoir and increase microorganism retention and protect them from shear forces. Other variables such as yeast concentration and viability and culture condition were kept constant in study, hence, can be explained that materials with the superficial defects such as voids and micro cracks on surface may serve as reservoir and were possible sites for Candidal adhesion. Thermoplastic resin sheets which is used in various clinical scenarios due to its ease of adaptation and flexibility showed minimal adhesion of *Candida albicans* compared to self-cure acrylic and heat cure acrylic.

Further investigations are needed to establish the useful relations of other affecting factors like material surface hydrophobicity and surface free energy on the degree of *Candida* adherence.

CONCLUSIONS

Within the limitations of this in vitro study, we conclude that thermoplastic resin sheet has lesser *Candida* adherence onto surfaces compared to heat cure and self-cure PMMA resins.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
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REFERENCES

[1] Nevalainen MJ, Narhi TO, Ainamo A. Oral mucosal lesions and oral hygiene habits in the home-living elderly. J Oral Rehabil 1997;24(5):332-7.
[2] Black GV. Sore mouth under plates. Dent Items Interest 1885;7:492.
[3] Cahn LR. The denture sore mouth. Ann Dent 1936;3:33-6.
[4] Marcotte H, Lavoie MC. Oral microbial ecology and the role of salivary immunoglobulin A. Microbiol Mol Biol Rev 1998;62(1):71-109.
[5] Rizzatti-Barbosa CM, Gabriotti MN, Silva-Concilio LR, et al. Surface roughness of acrylic resins processed by microwave energy and polished by mechanical and chemical process. Brazilian Journal of Oral Sciences 2015;5(16):977-81.
[6] Tannamala PK, Pulagam M, Pottem SR, et al. Flexible resins in the rehabilitation of maxillectomy patient. Indian J Dent Res 2012;23(1):97-100.
[7] Pusz A, Szymiczek M, Michalik K. Ageing process influence on mechanical properties of polyamide - glass composites applied in dentistry. Journal of Achievements in Materials and Manufacturing Engineering 2010;38(1):49-55.
[8] Agrawal H, Shah R, Agrawal N. The adherence of *Candida albicans* on surface of different denture base materials (an in-vitro study). Indian J Basic Appl Med Res 2013;2(6):576-81.

[9] Gharechahi M, Moosavi H, Forghani M. Effect of surface roughness and materials composition on biofilm formation. Journal of Biomaterials and Nanobiotechnology 2012;(3):541-6.
[10] Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence 2013;4(2):119-28.
[11] Quirynen M, Marechal M, Busscher HJ, et al. The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. J Clin Periodontol 1990;17(3):138-44.
[12] Williams DW, Waters MG, Potts AJ, et al. A novel technique for assessment of adherence of *Candida albicans* to solid surfaces. J Clin Pathol 1998 390-91.
[13] Radford DR, Sweet SP, Challacombe SJ, et al. Adherence of *Candida albicans* to denture-base materials with different surface finishes. J Dent 1998;26(7):577-83.