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Comparative Evaluation of Adherence of *Candida Albicans* on Heat Cure Acrylic Resin, Cobalt Chromium Alloy and Flexible Denture Base Material: An In Vitro Study

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ABSTRACT

Background: Human oral cavity harbors a multitude of microorganisms. Among them Candida albicans has become a cause of great concern to dental profession. Candida albicans has been widely associated with the etiology of denture related stomatitis. These lesions are very frequent complications in removable denture patients, especially the geriatric patients. A vast number of materials are used in the fabrication of denture bases, including acrylics, metals &more recently flexible denture base materials. Hence the objective of this study is to evaluate the adherence of Candida albicans on heat cure acrylic resin, cobalt-chromium alloy and flexible denture base materials.

Methodology: Total 90 samples were made, 30 from each type of denture base material i.e. Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy. All disc samples were sterilized with ultraviolet rays. For Candida albicans adherence, all group samples were placed in standardized cell suspension (ATCC 26555) of Candida albicans for 1 hour at room temperature. After the samples were removed, drained and placed in 1ml phosphate buffer saline (PBS), and vortexed for 1h. A loop full of the content were transferred from phosphate buffer saline (PBS) on to Yeast Peptone Dextrose (YPD) Agar plate using 4mm diameter standard wire loop. This material were spread on the plate in a lawn culture and incubated at 37°c for 48 hr. Viable counts of the microorganism were determined by colony count. Results were expressed in cfu/mm^2.

Results: It showed The mean of CFU/mm^2 of C. albicans on heat cure acrylic resin, flexible acrylic resin and cobalt-chromium alloy were 99.83 cfu/mm^2, 80.21 cfu/mm^2 and 69.90 cfu/mm^2 respectively.

Interpretation & Conclusion: The high candidal retention was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy.

Keywords: Denture Stomatitis, Denture biofilm, C.albicans, denture base material.

INTRODUCTION

Dental plaque has been identified as the main etiological factor in denture stomatitis, dental caries and periodontal diseases. Adherence mechanisms of oral bacteria are essential to bacterial colonization of the oral cavity. Dentures , like teeth, are hard, non shedding surfaces and

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accumulate plaque and calculus in a similar way. Surface roughness and surface free energy may contribute to the positively correlated rate of microbial colonization and plaque maturation on surfaces. The behavior of oral mucous membrane in contact with dentures has been the subject of considerable interest. The oral epithelium protects the underlying tissues mainly against physical, chemical, and bacterial irritants. The denture base rests on mucous membrane which serves as a cushion between the base and the supporting bone. Basically as the oral mucosa is functioning in a new environment, it is difficult to predict the nature of tissues changes even if stress which is created is within the biologic limits of tissue tolerance. Injury of the mucosa by way of traumatic/inflammatory causes, results in inflammatory response and denture stomatitis. Human oral cavity harbours a multitude of microorganisms. Among them candida albicans has become a cause of great concern to dental profession. Candida albicans has been widely associated with the etiology of denture related stomatitis. These lesions are very frequent complications in removable denture patients, especially the geriatric patients.[1]

METHODOLOGY

This study was conducted at (1) the dept of Prosthodontics KVG Dental College and Hospital, Sullia, Karnataka, India. (2) the dept of Microbiology, Maratha Mandal Dental College, Belgaum Karnataka, India. The proposed study was undertaken to evaluate adherence of Candida albicans on heat cure acrylic resin, cobalt chromium alloy and flexible denture base material.

Various materials studied are

1. Denture base materials
a. Heat cure acrylic resin material (group 1)
b. Flexible denture base material (group 2)
c. Cobalt – chromium alloy material (group 3)
2. Laboratory isolates of candida albicans (ATCC-26555) were used.

Total 90 samples were made, 30 from each type of denture base material i.e. Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy. All disc samples were sterilized with ultraviolet rays. For Candida albicans adherence, all group samples were placed in standardized cell suspension (ATCC 26555) of Candida albicans for 1hour at room temperature. After the samples were removed, drained and placed in 1ml phosphate buffer saline (PBS), and vortexed for 1h. A loop full of the contents were transferred from phosphate buffer saline (PBS) on to Yeast Peptone Dextrose (YPD) Agar plate using 4mm diameter standard wire loop. This material were spread on the plate in a lawn culture and incubated at 37°c for 48 hr. Viable count of the microorganism were determined by colony count.

RESULTS

In this present study adherence of Candida albicans on heat cure acrylic resin; cobalt-chromium alloy and flexible denture base material were measured. Results were expressed in cfu/mm^2.

Data Analysis: The C. albicans colonies were calculated in cfu/mm^2 on three different denture base materials are shown in Annexure I.The dimensions shown were the average of three readings. The mean value and standard deviation were calculated. The data were then subjected to detailed statistical analys\

Table and Graph interpretations

Table 1 and Graph 1

It shows the mean values, standard deviation and one way ANOVA test for number of C. albicans colony attachment on heat cure resin, flexible resin and Co-Cr Alloy i.e. 99.83 cfu/mm^2, 80.21 cfu/mm^2 and 69.90 cfu/mm^2 respectively. The $p<0.001$ indicate that there are significant difference between groups so further multiple comparison test was carried out.

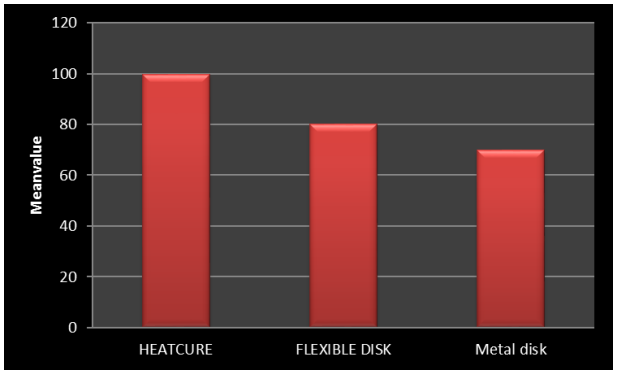
Table 1: Mean, standard deviation, and one way anova test for various denture base materials.

Parameter	n	Min	Max	Mean	SD	SE	ANOVA F value	P value
Group 1	30	94	107	99.83	3.602	0.658	485.652	<0.001 HS
Group 2	30	73	86	80.21	3.931	0.730		
Group 3	30	61	79	69.90	3.867	0.695		

Table 2: Tukeys HSD multiple comparison test shows that there were significant difference between group1, group2, and group 3.

Table 2: Multiple comparison test (Tukeys HSD test) for group 1, group 2, and group 3.

(I) Parameter	(J) Parameter	MD (I-J)	SD	p	
Group 1	Group 2	19.626	0.990	0.001	HS
	Group 3	29.930	0.974	0.001	HS
Group 2	Group 3	10.304	0.982	0.001	HS



Graph 1: Mean values of C. albicans colony attachment on heat cure resin, flexible resin and Co-Cr Alloy (metal disc).

DISCUSSION

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 a great concern, the difficulties that arise may not be attributed to denture construction but also to associated problems with continuous denture wearing. The use of a dental prosthesis is indispensable for functional and esthetic rehabilitation of edentulous patients improving their oral health related quality of life.[2]

A commonly occurring condition observed frequently is denture stomatitis secondary to candidal infection. The material most commonly used for fabricating removable partial and complete dentures is heat cure acrylic resin.[3] However; this material presents limitations, particularly in terms of fungal adhesion. Candida among all fungal infection acts as main culprit for oral mycosis and plays an important role in denture stomatitis.[4] it is a well known fact that removable denture bases fabricated from heat cure acrylic resin act as a reservoir for microorganism and contribute to re-infection to denture wearers.[5]

The ability to form biofilm is intimately associated with the ability to cause infection and as such should be considered an important virulence determinant during denture stomatitis.[6] From the literature the picture emerges that many factors determine Candida harboring-biofilms. These factors include surface properties, micro-organisms interactions, biofilm architecture, and saliva. Despite therapeutic progress, opportunistic oral fungal infectious diseases have increased in prevalence, especially in denture wearers.

Candida albicans existence presents a high significance in the etiology of denture stomatitis; its incidence has been reported to occur among 11-67 % of the denture wearers.[7] and is found on surfaces of hard and resilient acrylic resin materials in vivo. Among many studies concerning the adhesion mechanisms of C. albicans to denture base materials and factors affecting their mechanisms, surface roughness[8] and type of materials[9] are known to be two major factors for the adherence mechanism directly. The yeasts, being a part of the denture plaque, adhere and accumulate on the surface of the prosthesis that plays a storing role for them. [10, 11, 12]

A lot of studies have been done on attachment of candida albicans on acrylic resin material. But a very few studies was done on flexible and metallic denture base material. cobalt chromium alloy is most commonly used metallic denture base and flexible denture base material is newer material in dentistry. So flexible and cobalt chromium alloy denture base material along with heat cure resin material was used for this study.

This study was carried out to evaluate the adherence of Candida albicans to three type of denture base materials (heat cure denture base material, flexible denture base material and Cobalt Chromium alloy denture base material) 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.[13] These features alter the degree of adherence of C.albicans to acrylics, which may be contrasting to the metals. Among the metals, gold is been shown to have better surface topography than acrylics. However, the use of gold is impracticable, Co-Cr alloy is commonly used metallic denture base material, is been used in this study.

In this study the adherence of *C*. albicans strains ATCC 26555 *was* studied on heat cure acrylic resin denture-base material, flexible denture base material and a Co-Cr alloy denture-base material. It was found that the adherence of *C.* albicans ATCC 26555 was more on heat cure acrylic resin material compare to flexible denture base material and Co-Cr alloy denture-base material. Minimum candidal attachment was found on Co-Cr alloy denture-base material. These results demonstrated that the type of denture-base material could play an important role at the attachment of microorganisms.

A study was done by Celik GY et al, to evaluate the *In vitro* attachment on acrylic resin and a Co-Cr alloy denture-base materials using the two type strains of *C. albicans(*ATCC 26555 and Serotype B Netherland CBS 5983) *and* S. mutans (NCTC 8177). The results of assays showed that there was more attachment of two type strains of *C.* albicans to acrylic resin base material than to Co-Cr alloy base material, while there was more attachment of S. mutans to Co-Cr alloy than to acrylic resin. The results obtained in our study were relatively similiar to the results obtained from Celik GY et al study.[14] Some researchers had reported that the attachment of microorganisms to different materials was depend on the surface finishes.[15]Cell-surface hydrophobicity has been associated with bacterial attachment to variety surfaces.[16] The relationship between hydrophobicity and attachment has changed according to used materials. While the greater hydrophobicity of the cells result in the greater attachment to some surfaces.

CONCLUSION

The highest C. albicans attachment was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy denture base material.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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