



EFFECT OF 0.2% CHLORHEXIDINE DISINFECTANTS ON 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 and Objective: 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. Its properties like Candidal attachment on it need to be evaluated. Chlorhexidine is commonly used by dental patient as antiseptic and the glutaraldehyde-based disinfectant are often used in dentistry. Hence the objective of this study is to evaluate the effectiveness of 0.2% chlorhexidine on the adherence of *Candida albicans* on heat cure acrylic resin, cobalt-chromium alloy and flexible denture base materials.

Methodology: Total 45 samples were made, 15 from each type of denture base material i.e. Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy. For *Candida albicans* adherence, all group samples were placed in standardized cell suspension (ATCC 26555) of *Candida albicans*. Viable counts of the microorganism were determined by colony count. Results were expressed in cfu/mm². To test the antifungal efficacy of Chlorhexidine, the procedure were repeated for all the disc samples of the all group where in the disc samples were treated with the disinfectants separately for 12 hrs and tested for Candidal adherence.

Results: The mean of CFU/mm² of *C. albicans* on heat cure acrylic resin, flexible acrylic resin and cobalt-chromium alloy were 99.83 cfu/mm², 80.21 cfu/mm² and 69.90 cfu/mm² respectively. The mean of CFU/mm² of *C. albicans* after treated with 0.2% chlorhexidine on heat cure acrylic resin and flexible acrylic resin were 0.87 cfu/mm² and 1.33 cfu/mm² respectively.

Conclusion: The high candidal retention was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy. At 0.2% Chlorhexidine there were slight growth of *C. albicans* on Heat cure and flexible acrylic resin but no growth on Co-Cr alloy material. Hence Chlorhexidine can be used to soak the denture in denture wearing patients to avoid denture stomatitis.

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 accumulate plaque and calculus in a similar

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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) Department of Prosthodontics, KVG Dental College and Hospital, Sullia, Karnataka, India. (2) Department of Microbiology, Maratha Mandal Dental College, Belgaum Karnataka, India.

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.
3. Disinfectant
 - a. Chlorhexidine.

Total 45 samples were made, 15 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.

To test the antifungal efficacy of Chlorhexidine, the procedure were repeated for all the disc samples of the all group where in the disc samples were treated with the

Chlorhexidine disinfectants separately for 12 hrs and tested for *Candida albicans* adherence. There were no growth of *Candida albicans* on these three groups after treating with 2% Chlorhexidine. So an additional study was done to evaluate the disinfectant efficacy of 0.2% Chlorhexidine on the adherence of *Candida albicans* on heat cure acrylic resin; cobalt-chromium alloy and flexible denture base material

RESULTS

Data Analysis

The *C. albicans* colonies were calculated in cfu/mm² on three different denture base materials. 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 analysis.

The statistical analysis was carried out using Kruskal-Wallis test (H) for group comparison and Mann-Whitney U test (Z) for inter comparison of groups, with statistical package for social science (SPSS) version 16 for windows.

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/mm², 80.21 cfu/mm² and 69.90 cfu/mm² respectively. The p<0.001 indicate that there are significant difference between groups so further multiple comparison test was carried out.

Table 2

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

Table 3 and graph 2

It shows the comparison of the mean and standard deviation (SD) along with probability 'p' and 'H' values were given. (Where 'H' indicates Kruskal Wallis test).

Table 4

It shows the comparisons of number of colony forming units on group 1, 2, and 3. it concluded that There were significant difference in number of colony forming units among the group 1, 2, and 3 for 0.2% Chlorhexidine.

DISCUSSION

Prosthetic rehabilitation of the edentulous 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].

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 [8]. Among many studies concerning the adhesion mechanisms of *C. albicans* to denture base materials and factors affecting their mechanisms, surface roughness [9] and type of materials [10] 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 [11, 12, 13].

Based on the clinical need for alternative disinfectants that might be used for this purpose, this study was designed to evaluate the antimicrobial effect of Chlorhexidine disinfectant. The selection of microorganisms was based on the Pathogenic potential or representative importance for antimicrobial effectiveness evaluation studies. *C. albicans*, in association with other factors (i.e., traumatizing prosthesis, unsatisfactory hygiene conditions, systemic factors), is related to the occurrence of denture stomatitis. Therefore, studies in dentistry focus on this microorganism not only as a cross infection problem, but also as a stomatitis-related factor [14].

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.

The possibility of cross-infection between the dental office and laboratory is high. Therefore, the disinfection of prostheses before sending them to and after receiving them from the laboratory is an important step for cross-infection control.

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. 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.

This study was carried out to evaluate the effectiveness of disinfectant 0.2% Chlorhexidine disinfectant on 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) was studied. The results showed there was no growth of *Candida albicans* found on three type of denture base materials after treating the these three type of material with 2% chlorhexidine disinfectant. The results showed that 2% chlorhexidine were most effective against the growth of *Candida albicans* on these three types of denture base material.

A study was done by Cristina da Silva F et al [15] to evaluate the effectiveness of disinfectant solutions (1 % sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, tabs of sodium perborate-based denture cleanser, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens contaminated in vitro by *Candida albicans*, *Streptococcus mutans*, *S. aureus*, *Escherichia coli*, or *Bacillus subtilis* as measured by residual colony-forming unit (CFU). The results showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms followed by 100% vinegar, 3.8% sodium perborate, and tabs of sodium perborate-based denture cleanser. The results obtained in our study were relatively similar to the results obtained from Cristina da Silva F et al. According to Cristina da Silva F et al, Sodium hypochlorite, *Chlorhexidine* and *glutaraldehyde* exhibited the most pronounced inhibitory effect against *C. albicans* ATCC 26555 [15].

This study was also carried out to evaluate the effectiveness of 0.2% Chlorhexidine disinfectant on 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) was studied. The results showed there was no growth of candida albicans found on Cobalt Chromium alloy denture base materials by using 0.2%Chlorhexidine disinfectant But little growth was found on flexible denture base material and heat cure resin material by using 0.2% Chlorhexidine disinfectant. The candidal growth was more in flexible base material compared to heat cure resin material by using 0.2% Chlorhexidine disinfectant. The results showed that 0.2% chlorhexidine were effective against the candida albicans on cobalt chromium alloy material. And result also showed that 0.2%chlorhexidine

were less effective in removing candidal colonies on flexible material disc and heat cure resin disc compare to cobalt chromium alloy disc.

Previous studies and the results of the present study prove 2% Chlorhexidine is highly effective against *C. albicans*. Further investigations should be made to overcome the before mentioned problems associated with microbiologic study to determine the candidal count in denture biofilm. However exclusive electron microscopic analysis gives better information regarding biofilm forms but rarely gives quantitative information of Candida in a biofilm condition.

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
Group1	30	94	107	99.83	3.602	0.658	485.652	<0.001 HS
Group2	30	73	86	80.21	3.931	0.730		
Group3	30	61	79	69.90	3.867	0.695		

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

Parameter	Parameter	n	Min	Max	Mean	SD	SE	H	Pvalue
Chlorhexidine 0.2%	Group 1	15	0	2	0.87	0.516	0.133	25.870	<0.001 HS
	Group 2	15	0	2	1.33	0.724	0.187		
	Group 3	15	0	0	0.00	0.000	0.000		

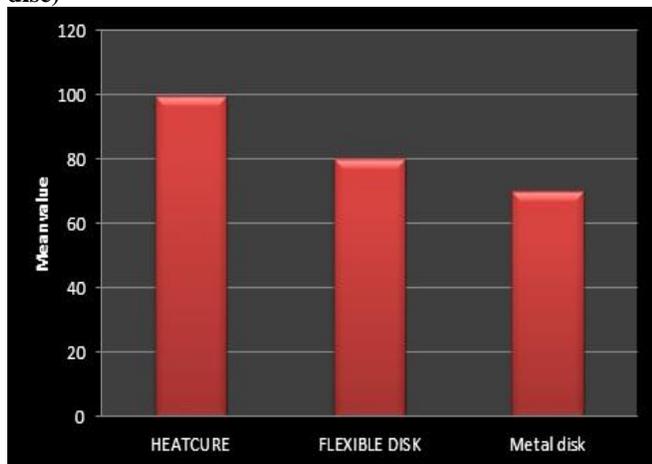
Table 3. Kruskal wallis test (H) for inter comparison of groups

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

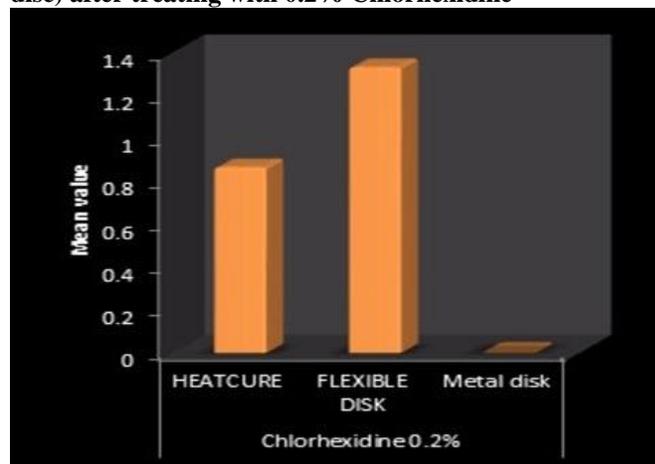
Table 4. Mann-Whitney U test (Z) for inter comparison of groups

Parameter	Parameter	Parameter	MD	P value	
Chlorhexidine 0.2%	Group 1	Group 2	-0.467	0.046	Sig
		Group 3	0.867	0.000	HS
	Group 2	Group 3	1.333	0.000	HS

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



Graph 2. Mean values of C. albicans colony attachment on Heat cure resin, Flexible resin and Co-Cr Alloy (metal disc) after treating with 0.2% Chlorhexidine



CONCLUSION

Within the limitations of this study, the following conclusions were drawn.

1. The highest *C. albicans* attachment was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy denture base material.
2. *C. albicans* showed no growth when treated with 2% Chlorhexidine on all three type of denture base material.
3. *C. albicans* showed no growth when treated with 0.2% Chlorhexidine on Co-Cr alloy denture base material.

4. Minimal growth was found on heat cure acrylic resin, compared to flexible resin when treated with 0.2% Chlorhexidine.

5. Adherence of candida albicans was less in flexible denture base material compared to heat cure resin material. But after treated with 0.2% Chlorhexidine *C. albicans* adherence was more in flexible denture base material compared to heat cure resin material. Because *C. albicans* colony was difficult to remove in flexible denture base material compared to heat cure resin material.

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