Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Dentistry

Effect Of 0.2% Glutaraldehyde 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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Original Research Article

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Article History
Received: 01.12.2017
Accepted: 11.12.2017
Published: 30.12.2017

DOI:
10.36347/sjams.2017.v05i12.014

Abstract: Objective of this study is to evaluate the effectiveness of 0.2% glutaraldehyde on the adherence of Candida albicans on heat cure acrylic resin, cobalt-chromium alloy and flexible denture base materials. 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/mm^2.To test the antifungal efficacy of Glutaraldehyde, 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. 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. The mean of CFU/mm2 of C. albicans after treated with 0.2% Glutaraldehyde on heat cure acrylic resin and flexible acrylic resin were 0.47 cfu/mm2 and 0.87 cfu/mm2 respectively.The high candidal retention was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy. At 0.2% Glutaraldehyde there were slight growth of c. Albicans on Heat cure and flexible acrylic resin but no growth on Co-Cr alloy material.
Keywords: Denture Stomatitis, Denture biofilm, Glutaraldehyde, C. albicans, Denture base material.

INTRODUCTION

Dental plaque has been identified as the main etiological factor in denture stomatitis, dental caries and periodontal diseases. 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. 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]. Glutaraldehyde-based disinfectants are often used in dentistry since they are non-corrosive; do not degrade plastic or rubber materials & which are also not inactivated when in contact with organic materials.

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

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)

- Laboratory isolates of candida albicans (ATCC-26555) were used.
- Disinfectant
    i. Glutaraldehyde

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 was 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 Glutaraldehyde the procedure were repeated for all the disc samples of the all group where in the disc samples were treated with the Glutaraldehyde disinfectants separately for 12 hrs and tested for Candidal adherence. There was no growth of candida albicans on these three groups after treating with 2% Glutaraldehyde. So an additional study was done to evaluate the disinfectant efficacy of 0.2% Glutaraldehyde 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/mm^2 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 (Figures) interpretations

Table 1 and Fig-1 show 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
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		

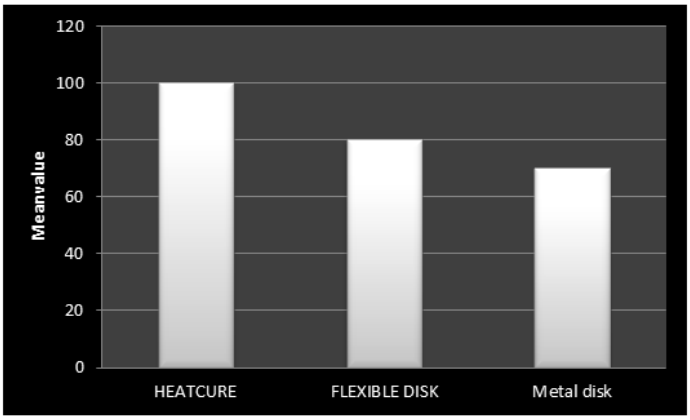

Fig-1: Mean values of C. albicans colony attachment on Heat cure resin, Flexible resin and Co-Cr Alloy (metal disc)

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

Table-2: **multiple comparison tests (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

Table 3 and Fig-2 show 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-3: **Kruskal wallis test (H) for inter comparison of groups**

parameter	parameter	n	Min	Max	Mean	SD	SE	H	P value
Glutaraldehyde 0.2%	Group 1	15	0	1	0.47	0.516	0.133	17.117	<0.001
	Group 2	15	0	2	0.87	0.640	0.165		HS
	Group 3	15	0	0	0.00	0.000	0.000		

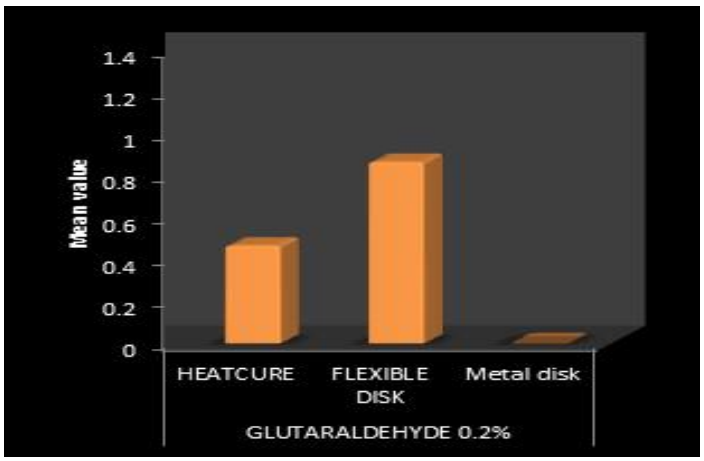

Fig-2: Mean values of C. albicans colony attachment on Heat cure resin, Flexible resin and Co-Cr Alloy (metal disc) after treating with 0.2% Glutareldehyde

Table 4 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% Glutaraldehyde.

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

parameter	parameter	parameter	MD	P value	
Glutaraldehyde 0.2%	Group 1	Group 2	-0.400	0.083	NS
		Group 3	0.467	0.003	HS
	Group 2	Group 3	0.867	0.000	HS

DISCUSSIONS

Edentulousness is not a disease entity itself, but rather a consequence of pathology. Increasing incidence of edentulousness over recent years has questioned the adequacy of dental treatment. Yet the mainstay for the management of 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 aesthetically and psychologically. The use of a dental prosthesis is indispensable for functional and aesthetic rehabilitation of edentulous patients improving their oral health related quality of life [2].

A commonly occurring condition observed frequently is denture stomatitis secondary to candida 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 [11-13].

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 Glutaraldehyde 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. Glutaraldehyde-based solutions are commonly indicated for the disinfection of prostheses. Because they are non-corrosive; do not degrade plastic or rubber materials & are also not inactivated when in contact with organic materials [15].

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 candida 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% Glutaraldehyde 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 types of denture base materials after treating the three type of material with 2% Glutaraldehyde disinfectant. The results showed that 2% Glutaraldehyde 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 (l % 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 similiar 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].

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. mutants to Co-Cr

alloy than to acrylic resin [1]. The results obtained in our study were relatively similar to the results obtained from Celik GY et al study.

This study was also carried out to evaluate the effectiveness of 0.2% Glutaraldehyde disinfectant on adherence of Candida albinos 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 albinos found on Cobalt Chromium alloy denture base materials by using 0.2% Glutaraldehyde disinfectant. But little growth was found on flexible denture base material and heat cure resin material by using 0.2% Glutaraldehyde disinfectant. The candida growth was more in flexible base material compared to heat cure resin material by using 0.2% Glutaraldehyde disinfectant. The results showed that 0.2% Glutaraldehyde were effective against the candida albicans on cobalt chromium alloy material. And result also showed that 0.2% Glutaraldehyde was less effective in removing candida colonies on flexible material disc and heat cure resin disc compare to cobalt chromium alloy disc.

A study was done by I.A. Orsi *et al.* to evaluate the antimicrobial efficacy of disinfectants (1% and 2% sodium hypochlorite and 2% Glutaraldehyde) on full metal crowns contaminated with microorganisms (S. aureus, P. aeruginosa, S. mutans, E. faecalis and C. albicans). Result of this study showed Microbial growth was observed only in the control group. In the experimental group, the 3 chemical disinfectants destroyed all microbial strains at the 3 periods of disinfection evaluated (5, 10 or 15 min), as no turbidity of the media was detected [16].

Previous studies and the results of the present study prove 2% Glutaraldehyde 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.

CONCLUSION

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

- The highest C. albicans attachment was observed on Heat cure acrylic resin followed by flexible acrylic resin and cobalt chromium alloy denture base material.
- C. albicans showed no growth when treated with 2% Glutaraldehyde on all three type of denture base material.
- C. albicans showed no growth when treated with 0.2% Glutaraldehyde on Co-Cr alloy denture base material.
- Minimal growth was found on heat cure acrylic resin, compared to flexible resin when treated with 0.2% Glutaraldehyde.
- Adherence of candida albicans was less in flexible denture base material compared to heat cure resin material. But after treated with 0.2% Glutaraldehyde, 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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