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Effect of 2% Chlorhexidine and 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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ABSTRACT

Aim: Hence the objective of this study is to evaluate the effectiveness of 2% chlorhexidine and 2% glutaraldehyde on 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. To test the antifungal efficacy of glutaraldehyde and chlorhexidine, the procedure were repeated for all the disc samples of the all group where in the disc samples were treated with both the disinfectants separately for 12 hrs and tested for Candidal adherence.

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. The mean of CFU/mm^2 of C. albicans after treated with 2% chlorhexidine and 2% Glutaraldehyde were zero. The mean of CFU/mm^2 of C. albicans after treated with 0.2% chlorhexidine on heat cure acrylic resin and flexible acrylic resin were 0.87 cfu/mm^2 and 1.33 cfu/mm^2 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 and 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. At 2% chlorhexidine and 2% Glutaraldehyde there were no growth of c. albicans on all three types of denture base material. Hence 2%

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chlorhexidine and 2% Glutaraldehyde can be used to soak the denture in denture wearing patients to avoid denture stomatitis.

Keywords: Denture Stomatitis, Denture biofilm, Chlorhexidine, 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. 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 way. Surface roughness and surface free energy may contribute to the positively correlated rate of microbial colonization and plaque maturation on surfaces.

The behaviour 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]

MATERIALS AND METHODS

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.
3. Disinfectant
a.Chlorhexidine
b.Glutaraldehyde

RESULTS

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

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.

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

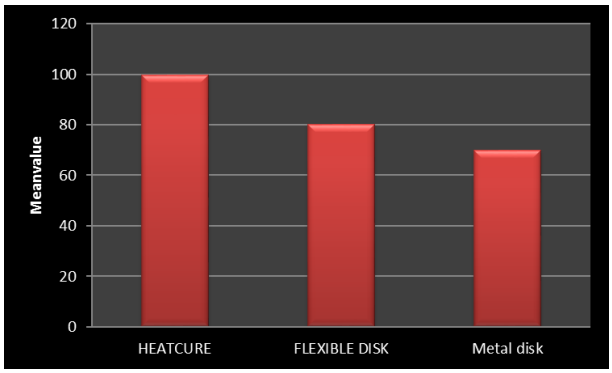
parameter	parameter	n	Min	Max	Mean	SD	SE	H	Pvalue
Chlorhexidine 0.2%	Group 1	15	0	2	0.87	0.516	0.133	25.870	<0.001 HS
	Group 2	15	0	2	1.33	0.724	0.187		
	Group 3	15	0	0	0.00	0.000	0.000		
Glutaraldehyde 0.2%	Group 1	15	0	1	0.47	0.516	0.133	17.117	<0.001 HS
	Group 2	15	0	2	0.87	0.640	0.165		
	Group 3	15	0	0	0.00	0.000	0.000		

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

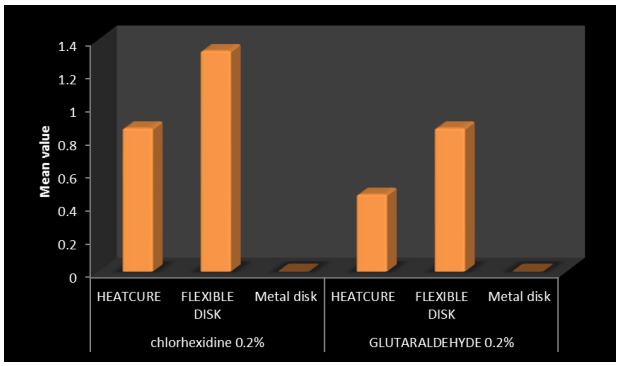
parameter	parameter	parameter	MD	P value	
Chlorhexidine 0.2%	Group 1	Group 2	-0.467	0.046	Sig
		Group 3	0.867	0.000	HS
	Group 2	Group 3	1.333	0.000	HS
Glutaraldehyde 0.2%	Group 1	Group 2	-0.400	0.083	NS
		Group 3	0.467	0.003	HS
	Group 2	Group 3	0.867	0.000	HS

Table 5: Mann-Whitney U test (Z) for inter comparison of Chlorhexidine and Glutaraldehyde on individual groups.

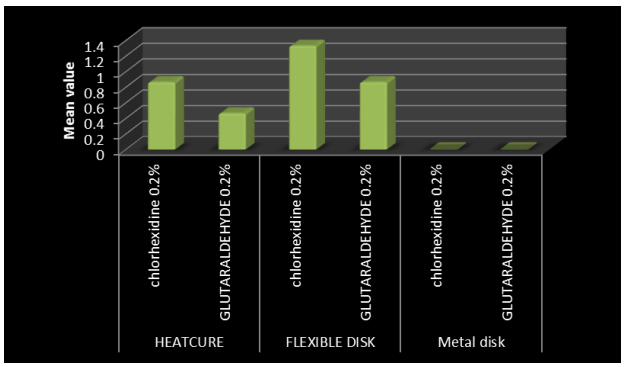
parameter	parameter	n	Min	Max	Mean	SD	SE	Z	P value
Group 1	Chlorhexidine 0.2%	15	0	2	0.87	0.516	0.133	1.983	0.047 Sig
	Glutaraldehyde 0.2%	15	0	1	0.47	0.516	0.133		
Group 2	Chlorhexidine 0.2%	15	0	2	1.33	0.724	0.187	1.832	0.067
	Glutaraldehyde 0.2%	15	0	2	0.87	0.640	0.165		NS
Group 3	Chlorhexidine 0.2%	15	0	0	0.00	0.000	0.000	0.000	1.000 NS
	Glutaraldehyde 0.2%	15	0	0	0.00	0.000	0.000		



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 Chlorhexidine and Glutaraldehyde.



Graph 3: Mean values of C. albicans colony attachment on individual groups after treating with 0.2% chlorhexidine and 0.2% glutaraldehyde.

To test the antifungal efficacy of glutaraldehyde and chlorhexidine, the procedure were repeated for all the disc samples of the all group where in the disc samples were treated with both the disinfectants separately for 12 hrs and tested for Candidal adherence. There were no growth of candida albicans on these three groups after treating with 2% Chlorhexidine and 2% glutaraldehyde. So an additional study was done to evaluate the disinfectant efficacy of 0.2% chlorhexidine and 0.2% glutaraldehyde on the adherence of Candida albicans on heat cure acrylic resin; cobalt-chromium alloy and flexible denture base material.

Data Analysis

The C. albicans colonies were calculated in cfu/mm^2 on three different denture base materials are shown in Annexure I, II, and III .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/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 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 no significant difference

for group 1 and group 2, after treating with 0.2% glutaraldehyde. There were significant difference in number of colony forming units among the remaining group 1, 2, and 3 for 0.2% chlorhexidine and 0.2% glutaraldehyde.

Table 5 and graph 3

It shows the intra comparison of mean number of colony formed on individual group after treating with 0.2% chlorhexidine and 0.2% glutaraldehyde. Here the probability 'P' and the 'Z' values were given. (Where 'Z' indicates Mann-Whitney U test). Test concluded that there are significant difference when heat cure resin treated with 0.2% Chlorhexidine and 0.2% glutaraldehyde but no significant difference on flexible and metal disc (Co-Cr alloy) when treated with 0.2% Chlorhexidine and 0.2% Glutaraldehyde.

DISCUSSION

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 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.[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 two types of disinfectants. 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. Heat sterilization of acrylic materials is not viable, mainly due to the low ebullition temperature of the monomer that composes the resin. Therefore, disinfection based on chemical substances is essential. 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]

Budtz jorgenson et al[16] in his studies on clinical aspect of candida infection in denture wearers hypothesized that the Candida induced denture stomatitis usually does not reflect on any deep seated systemic abnormality, but the dentures are primary predisposing factors. Therefore preventive measures must be taken against colonization of Candida on palatal mucosa and dentures. Various drugs have been employed to treat Candida induced denture stomatitis. Chlorhexidine is commonly used by dental patient as antiseptics [17]and the Glutaraldehyde-based disinfectant are often used in dentistry. [15]

In this study an attempt has been made to evaluate and compare the growth of candidal species in three type of denture base material (heat cure denture base material, flexible denture base material and Cobalt Chromium alloy denture base material) & to evaluate and compare the disinfectant efficacy of 2% Chlorhexidine and 2% glutaraldehyde on these three type of denture base material.

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) and evaluate the effect of two type disinfectant (2%Chlorhexidine and 2%Glutaraldehyde) on adherence of Candida albicans to these three type of denture base materials was studied. 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.[18] 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.[6] 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. [19]The results obtained in our study were relatively similiar to the results obtained from Celik GY et al study. Some researchers had reported that the attachment of microorganisms to different materials was depend on the surface finishes.[20] Cell-surface hydrophobicity has been associated with bacterial attachment to variety surfaces.[21] 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.

This study was carried out to evaluate the effectiveness of two type disinfectant (2%Chlorhexidine and 2%Glutaraldehyde) 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 these two type of disinfectant (2% glutaraldehyde, and 2%chlorhexidine). The results showed that 2% glutaraldehyde, and 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 (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]

This study was also carried out to evaluate the effectiveness of two type disinfectant (0.2%Chlorhexidine and 0.2%Glutaraldehyde) 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 these two types of disinfectant (0.2%Chlorhexidine and 0.2%Glutaraldehyde). But little growth was found on flexible denture base material and heat cure resin material (by using these two types of disinfectant(0.2%Chlorhexidine and 0.2%Glutaraldehyde). The candidal growth was more in flexible base material compared to heat cure resin material by using these two types of disinfectant (0.2%Chlorhexidine and 0.2%Glutaraldehyde). And less candidal colonies were found that flexible material disc and heat cure resin disc was treated with 0.2%glutaraldehyde compared to 0.2%chlorhexidine. The results showed that 0.2% glutaraldehyde, and 0.2%chlorhexidine were effective against the candida albicans on cobalt chromium alloy material. But results demonstrated that 0.2 glutaraldehyde was more effective compared to 0.2 chlorhexidine against the candida albicans on heat cure acrylic material and flexible denture base material. And result also showed that 0.2% glutaraldehyde, and 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 and 2%Glutaraldehyde is highly effective against C. *albicans.* Result of present studied showed that 2%Chlorhexidine and 2%Glutaraldehyde is highly effective against C. *albicans adherence* on three type of denture base material.(heat cure denture base material, flexible denture base material and Cobalt Chromium alloy denture base material). 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.

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 2% glutaraldehyde on all three type of denture base material.

4. C. albicans showed no growth when treated with 0.2% chlorhexidine on Co-Cr alloy denture base material.

5. C. albicans showed no growth when treated with 0.2% glutaraldehyde on Co-Cr alloy denture base material.

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

7. Minimal growth was found on heat cure acrylic resin, compared to flexible resin when treated with 0.2% glutaraldehyde but overall growth was less with 0.2% glutaraldehyde compared to 0.2% chlorhexidine.

8. Adherence of candida albicans was less in flexible denture base material compared to heat cure resin material. But after treated with 0.2%Chlorhexidine and 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.

CONFLICT OF INTEREST

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

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