



## COMPARATIVE STUDY OF THE ABILITY IN REMOVAL OF CANDIDA ALBICANS MICROORGANISM FROM THE ACRYLIC DENTURE BASE- AN INVITRO STUDY

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### ABSTRACT

Care of any prosthesis is very important and it is mandatory that every patient be informed about the maintenance. But often patients leave the dental clinic without any information of care of denture. As soon as a denture is introduced into the oral cavity, it is surrounded by saliva and the oral environment with its innumerable microorganisms. Due to their porous nature, acrylic resin attracts plaque deposit on its surface. The aim of the study is to Comparing the ability to remove Candida

from surface of denture base acrylic.

**KEYWORDS:** denture is introduced into the oral cavity, it is surrounded by saliva.

### INTRODUCTION

With the introduction of polymers in the early 1930, the dough technique and compression moulding method in mid 1930's made use of acrylic in dentistry possible. In the oral cavity, denture is bathed in protein rich saliva. Some of these proteins are absorbed and altered forming a pellicle. Bacterial colonization of pellicle and food, results in accumulation of plaque, which develops and mature when left undisturbed. This plaque may get colonized extensively by candida albicans in conditions like altered immune response, broad-spectrum antibiotic therapy, ill-fitting dentures etc. Various methods for cleaning like brushing with

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denture brush, soaking in commercial in denture cleanser, use of household products etc. Use of abrasive paste lead to wear of denture, ultrasonic instrument could not be used singly, household products are not reliable. Hence soak type denture cleanser is preferred. There are three major types of denture cleansers namely oxygenating cleanser-Alkaline peroxide, Hypochlorite solution-Dilute sodium hypochlorite, and dilute mineral acids-Dilute hydrochloric acid.

Alkaline peroxide cleanser forms an alkaline solution of hydrogen peroxides, which liberate nascent oxygen in the presence of organic material, and assists in removal of denture deposits. Hypochlorite solution renders mucin and other protein soluble, they also bleach away the stains. Mineral acids dissolve calcareous deposits and denature the proteins.

### **The aim of the study**

To compare the ability to remove candida from surface of denture base acrylic.

### **MATERIALS AND METHODS**

Materials used: Heat cured acrylic (Travelon-Dentsply), 0.75mm spacer wax, Type IV Gypsum(Die-stone), Sabourauds glucose broth(Himedia), Yeast nitrogen base(Himedia), Phosphate buffered saline, Galactose,Candida G.E.Agar,

Denture cleansers- Hydrochloric acid-5% solution

Hypochlorite solution-0.525%

Peroxide Cleanser A-(Fitty dent-Group Pharma)

Peroxide Cleanser B-(Clinsodent-ICPA)

### **Instruments and equipments**

1.5"×2.5"× 1.8 metal dies, Petri dishes, Test-Tubes, Microscope, Reflection spectrometer, Incubator, Centrifuge.

### **Candida removal test**

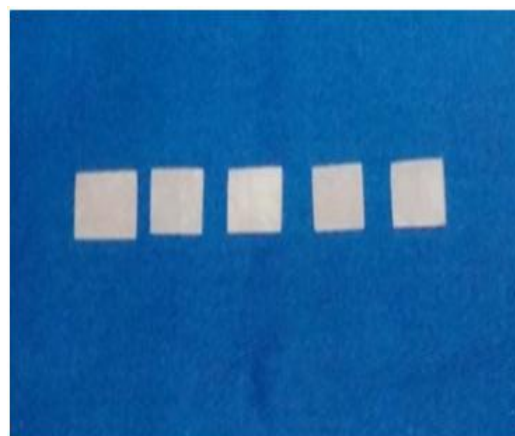
**Preparation of Fungal suspension:** The method advocated by Kaita et al<sup>[1]</sup> was used as more candida adhered to acrylic plates. Candida was grown in 4% glucose 2% peptone and 0.2% yeast extract broth.(Fig 1 ) The preculture was inoculated in yeast nitrogen base with 500 millimoles Galactose and incubated at 37° for 48hrs.Cells were washed, resuspended and diluted to make  $3 \times 10^7$  CFU/ml.

**Preparation of acrylic disks:** They were done according to the method of Nakamoto et al<sup>[2]</sup>, disks measuring 10×10×0.75mm were prepared in heat-cured acrylic. (Fig 2) Then specimens were sterilized by immersion in 5.25% sodium hypochlorite for 5 minutes.

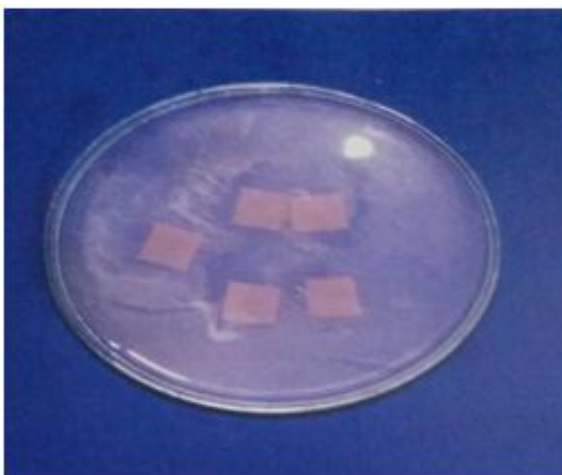
According to the method of Nakamoto et al<sup>2</sup> acrylic plates were placed in a petri dish and covered with 20ml of candida suspension, it was incubated at 37° for 90min. (Fig 3). Then these plates were washed with PBS and these plates were immersed in 50ml of sample denture cleansers (5% hydrochloric acid, 0.525% sodium hypochlorite, two peroxide denture cleansers-Fittydent and Clinsodent) (Fig 4, Fig 5, Fig 6, Fig 7) and distilled water. They were incubated at 37° for 60min and washed with PBS. The cells were fixed with formaldehyde and methanol, stained with crystal violates and dried. Fungal cells that adhered to acrylic were counted under the microscope. (Fig 8) Then high-powered fields (1.1mm square) were counted and totalled. Then the number of cells adherent on the test samples was compared with that of the positive control to get the percentage of adherent cells.



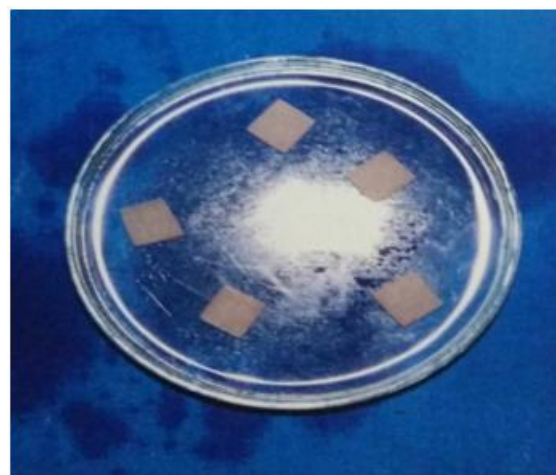
**Fig 1 Candidal Broth**



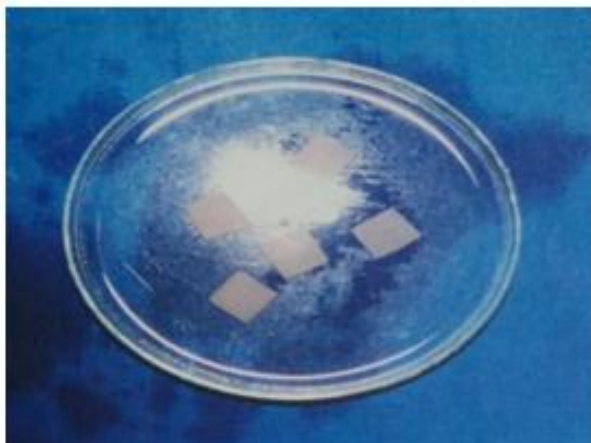
**Fig 2 Samples for Candidal removal test**



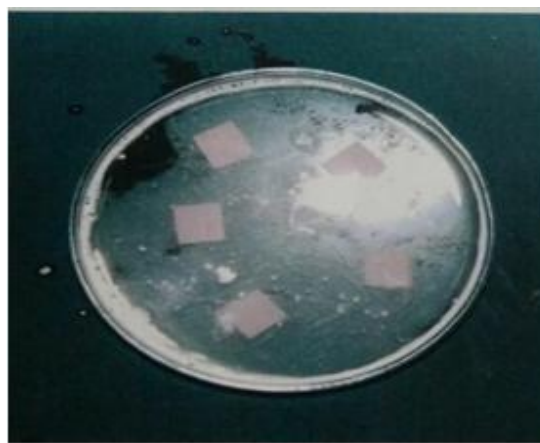
**Fig 3: Samples in Candidal broth**



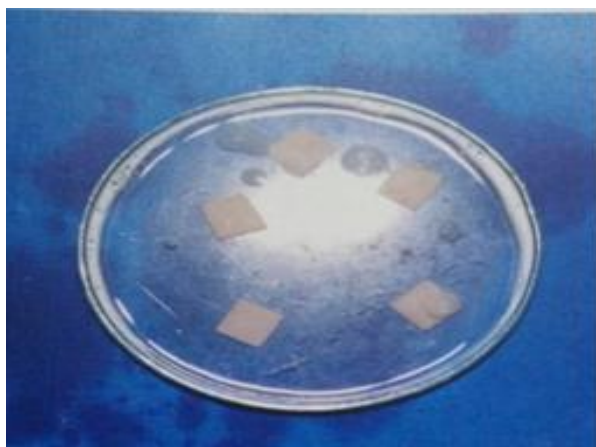
**Fig 4 Samples in 5% Hydrochloric acid**



**Fig 5 Samples in 0.525% Sodium Hypochlorite solution**



**Fig 6 Samples in Fittydent**



**Fig 7: Samples in Clinsodent**



**Fig 8 Samples under the microscope**

## RESULTS

The result of candida removal test, both

(i) The number of adherent cells and

(ii) Percentage adherent cells, done with hydrochloric acid, sodium hypochlorite, Fitty dent & Clinsodent, are shown in table 1, 2, 3&4 respectively. The mean and standard deviation are shown in table 5.

The mean percentage adherent cells after testing with hydrochloric acid; sodium hypochlorite, Fitty dent & Clinsodent are 7.97, 0.49, 4.72 and 5.76 respectively with a standard deviation of 0.8, 0.39, 0.58 & 0.63 respectively.

Table 6 shows the result of student t-test comparing the results of candida removal test done with the hydrochloric acid, sodium hypochlorite, Fitty dent & Clinsodent. It shows that



sodium hypochlorite is the best of the four, followed by Fittydent, Clinsodent and the hydrochloric acid. The comparison values are statistically highly significant.

TABLE - 1

**CANDIDA REMOVAL TEST USING  
HYDROCHLORIC ACID**

SAMPLE	POSITIVE CONTROL AVERAGE	NO. OF CANDIDAL CELLS AFTER TESTING	% ADHERANT CELLS
A1	3840	310	8.07%
A2	3840	330	8.35%
A3	3840	290	7.79%
A4	3840	250	6.56%
A5	3840	360	8.97%
A6	3840	310	8.07%

TABLE - 2

**CANDIDA REMOVAL TEST USING  
SODIUM HYPOCHLORITE**

SAMPLE	POSITIVE CONTROL AVERAGE	NO. OF CANDIDAL CELLS AFTER TESTING	% ADHERANT CELLS
B1	3840	30	0.81%
B2	3840	20	0.53%
B3	3840	40	1.04%
B4	3840	10	0.27%
B5	3840	0	0
B6	3840	10	0.27%

TABLE - 3

**CANDIDA REMOVAL TEST USING FITTYDENT**

SAMPLE	POSITIVE CONTROL AVERAGE	NO. OF CANDIDAL CELLS AFTER TESTING	% ADHERANT CELLS
C1	3840	170	4.42%
C2	3840	190	4.94%
C3	3840	150	3.90%
C4	3840	210	5.46%
C5	3840	200	5.20%
C6	3840	170	4.42%

TABLE - 4

<b>CANDIDA REMOVAL TEST USING CLINSODENT</b>			
<b>SAMPLE</b>	<b>POSITIVE CONTROL AVERAGE</b>	<b>NO. OF CANDIDAL CELLS AFTER TESTING</b>	<b>% ADHERANT CELLS</b>
D1	3840	230	5.98%
D2	3840	210	5.46%
D3	3840	190	4.94%
D4	3840	260	6.77%
D5	3840	230	5.98%
D6	3840	210	5.46%

TABLE - 5

<b>CANDIDA REMOVAL TEST-MEAN&amp;S.D</b>		
<b>GROUP</b>	<b>MEAN</b>	<b>S.D</b>
A	7.97	0.80
B	0.49	0.39
C	4.72	0.58
D	5.76	0.63

TABLE - 6

<b>CANDIDA REMOVAL TEST</b>				
<b>GROUPS COMPARED</b>	<b>MEAN</b>	<b>S.D</b>	<b>t-VALUE</b>	<b>P-VALUE</b>
A	7.97	0.80	20.68	P< 0.001 STATISTICALLY HIGHLY SIGNIFICANT
B	0.49	0.39		
A	7.97	0.80	8.06	P< 0.001 STATISTICALLY HIGHLY SIGNIFICANT
C	4.72	0.58		
A	7.97	0.80	5.32	P< 0.001 STATISTICALLY HIGHLY SIGNIFICANT T
D	5.76	0.63		
B	0.49	0.39	14.91	P< 0.001 STATISTICALLY HIGHLY SIGNIFICANT
C	4.72	0.58		
B	0.49	0.39	17.55	P< 0.001 STATISTICALLY HIGHLY SIGNIFICANT
D	5.76	0.63		
C	4.72	0.58	2.99	P<0.05 STATISTICALLY SIGNIFICANT
D	5.76	0.63		

## DISCUSSION

Though various clinical methods for evaluation of denture cleanser are available as done by Ghalichebaf(1982)<sup>[3]</sup>, Dillis et al(1988)<sup>[4]</sup>, Russell et al(1982)<sup>[5]</sup> and Minagi et al(1987)<sup>[6]</sup>, these studies do not standardize the specimens and only qualitative. Comparison of

effectiveness of denture cleanser is done. In the present study, four different chemical soak type denture cleansers are used. As the efficiency of denture cleanser to remove the plaque from the surface of denture is a primary requisite, it is evaluated by the cleansers ability to remove adherent *Candida albicans* from the surface of acrylic. For the purpose of this study, one mineral acid (5% hydrochloric acid), one hypochlorite solution (0.525% sodium hypochlorite) and two commercially available alkaline peroxide cleansers (Fittydent & Clinsodent) are used. The present study was conducted to evaluate the ability of the denture cleansers to remove candida from the surface of acrylic. According to the present study in the candida removal test, 0.525% sodium hypochlorite removed candida from acrylic surface better than the two peroxide denture cleansers (Fittydent & Clinsodent) & 5% hydrochloric acid, in that order.

### SUMMARY AND CONCLUSION

Due care has to be taken to keep the denture clean. This is important as accumulation of plaque and colonization of candida on the denture leads to denture stomatitis.<sup>[1]</sup> The most common and safe method used to clean a denture is by the use of chemical denture cleanser.

In the present study, four different commonly available chemical denture cleansers (5% hydrochloric acid, 0.525% sodium hypochlorite, and two peroxide denture cleansers, Fittydent & Clinsodent) were compared. The aim of the study was Comparing the ability to remove candida from surface of denture base acrylic. The values were subjected to statistical analysis and following conclusion was drawn. Sodium hypochlorite tested better in the candida removal test followed by Fittydent and Clinsodent and then dilute hydrochloric acid in that order. So on comparing the four denture cleansers, sodium hypochlorite was found to be better than Fittydent, Clinsodent and dilute hydrochloric acid in that order. The above said study was done for a soaking period of 5-15 minutes; further studies with longer time period might be useful to further evaluate the denture cleansers to assess their action on physical properties of materials used to construct denture and their biological effects.

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