

## Evaluation of Antimicrobial Activity of Disinfectants on Acrylic Resins

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### **Abstract:**

**Purpose:** The aim of this study is to evaluate effectiveness of regularly used disinfectant solution in the disinfection of acrylic resin specimens contaminated with *Candida albicans*.

**Materials and methods:** Thirty six acrylic resin specimens contaminated in vitro with  $1.5 \times 10^8$  cfu/ml suspensions of microorganisms were immersed in disinfectants for 10 minutes, the control group was not submitted to any disinfection process. Final counts of microorganisms per ml were performed by plating method for evaluation of microbial level reduction. Results were compare statistically by wilcoxon test ( $p < 0.05$ )

**Results:** The results showed that 1% sodium hypochlorite and 0.2% w/v chlorhexidine gluconate were most effective against *Candida albicans*, followed by 3.8% sodium perborate.

**Conclusion:** within the limits of this study, it could be concluded that 1% sodium hypochlorite, 0.2% chlorhexidine gluconate and 3.8% sodium perborate are valid alternatives for disinfection of acrylic resins.

**Key words:** Acrylic resins, antimicrobial activity, *Candida albicans*, disinfectants.

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### **I. Introduction**

Polymethyl methacrylate was introduced in 1937 by Dr. Walter Wright and since then it has been successfully used for denture bases, artificial teeth, denture repair, impression trays and many other applications in dentistry<sup>1</sup>.

As dental personnel become more aware of modes of transmission of numerous infections microorganisms during dental procedures, infection control in dental practice has received increasing attention<sup>1</sup>. For the disinfection of acrylic resin several disinfectants have been suggested. They are home use like bleach, white vinegar, baking soda based, peroxides etc and in office use like chlorhexidine gluconate, chlorine, iodophors, sodium perborate, aldehyde components, sodium hypochloride, hydrogen peroxide etc<sup>2,5</sup>.

*Candida albicans* is suggested as major causative organism for denture stomatitis, which is most common amongst complete denture wearers. However, various other bacteria were suggested to play role in denture stomatitis, of all *Candida albicans* has high capacity to adhere to denture base resins and form structured biofilm<sup>2</sup>. There are other factors that favour the develop of oral candidiasis, such as denture base fit, metabolic disorder, patient's age, mucosa conditions, epithelial changes, poor diet, appropriate denture hygiene, xerostomia and salivary flow<sup>2</sup>.

Oral candidiasis is clinically characterized by different degree of inflammation of palatal mucosa under tissue surface of upper denture, ranging from petechiae to generalized inflammation with papillary hyperplasia<sup>3</sup>. Though it is the responsibility of denture users' dentist should inform patients about various disinfectants have been suggested<sup>4</sup>.

In this study, our aim is to evaluate the effectiveness of disinfectants on acrylic resin surfaces.

### **II. Materials and method**

Thirty six heat cured acrylic resin cubes of polymethyl methacrylate resin (DPI company) of size  $10 \times 10 \times 2$  mm according to American society for testing and material standard were made. These are prepared according to manufacturer's instructions, by mixing 3 parts of polymer to 1 part of monomer. After curing using short curing cycle they were polished with pumice and kept in water until use<sup>1</sup> (fig.1). *Candida albicans* is included for antimicrobial activity test.

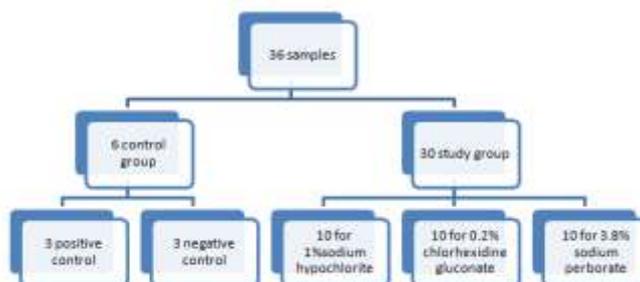


Fig.1 Acrylic resin specimens



Fig.2 Disinfectant solutions

The following disinfectants were included in study are 1% sodium hypochlorite, 0.2% w/v chlorhexidine gluconate and 3.8% sodium perborate (fig.2). The specimens were distributed into ten groups for each disinfectant <sup>1,2</sup>. The control group of six samples in which three are positive control and three are negative control i.e, positive control are contaminated whereas negative control are not contaminated and are sterile <sup>1,2</sup>.



### III. Microbiology lab procedures

#### Preparation of yeast suspension

For any susceptibility testing, standard inoculums must be employed. The standard inoculums were prepared according to 0.5 McFarland (0.05ml 1% barium chloride added to 9.95ml 1% sulphuric acid) a reference to adjust turbidity of yeast suspensions so that number of yeasts will be within a given range i.e,  $1.5 \times 10^8$  cfu/ml by transferring 1-2 colonies of 48 hours culture to BHI broth and incubated them at 35°C until the turbidity of media was equal to 0.5 McFarland <sup>2,6</sup>.(fig.3)



Fig.3 Inoculum preparation

#### Contamination of samples :

The acrylic resin specimens were first sterilized under autoclave and then separately put in sterile test tubes with 1cc inoculums and then they were incubated at 35°C for 1 hour <sup>2</sup>(fig.4)



Fig.4 contaminated with inoculum

Fig.5 samples in disinfectants

#### Disinfection of samples:

After contamination, all samples were rinsed with sterile distilled water for 30 sec. except the control group, remaining samples were immersed in 10 ml of each disinfectant to be tested for 10 minutes <sup>1,2</sup>(Fig.5). After 10 minutes, again the disinfected specimens were immersed in sterile distilled water for 2 sec to remove excess disinfectant solution.

#### IV. Microbiological survey

After excess disinfectant removal, the samples were transferred to 10 ml of normal saline solution and were agitated for 5 min to remove the adhered cells(fig.6). From this suspension, 100 microliters were plated on sabaroud's dextrose agar media and were spread uniformly <sup>2,5</sup>(fig.7). After drying, the petridishes were placed in biological oxygen demand incubator at 25°C for 48 hours. After incubation , the grown fungal colonies of *Candida albicans* on SDA were counted before and after disinfection <sup>1,2</sup>(fig.8,9)



Fig.6 Samples in normal saline

Fig.7 Microbial suspension after disinfection



Fig.8 Candida before disinfection



Fig.9 Candida after disinfection

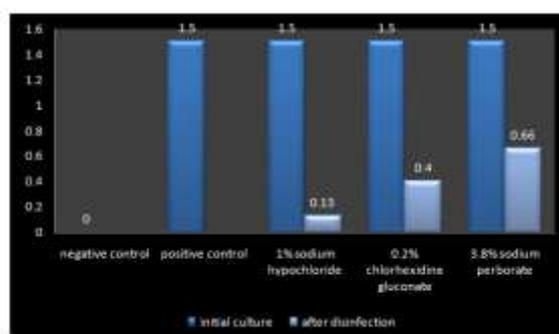
The data was submitted to statistical package for social sciences (SPSS) software and wilcoxon test was run to analyze the data <sup>5</sup>.

#### V. Results

The results obtained for the antimicrobial effectiveness are shown in table 1 .The number of Colony forming units obtained during initial cultures is constant i.e,  $1.5 \times 10^8$  cells/ml (P - 0.05). Confluent growth (100% growth) of CFU was observed. Among all the specimens of the control group, the positive control group showed viable bacteria at all experimental times, which indicated the efficiency of method. In contrast, the negative control group showed no viable bacteria at all experimental times. Of the 10 specimens disinfected with 1% sodium hypochlorite showed statistically significant difference (P - 0.016) among final counts of

*Candida albicans* after disinfection which was found to be  $0.13 \times 10^8$  cells/ml. Of the 10 specimens disinfected with 0.2% chlorhexidine gluconate showed statistically significant difference (P - 0.025) among final counts which was found to be  $0.40 \times 10^8$  cells/ml and remaining 10 specimens disinfected with 3.8% sodium perborate showed no statistically significant difference (P - 0.045) among the final counts which was found to be  $0.66 \times 10^8$  cells/ml. 1% sodium hypochlorite and 0.2% w/v chlorhexidine gluconate showed similar effectiveness among the tested disinfectants against *Candida albicans*. 3.8% sodium perborate showed least effect against *Candida albicans* compared with initial growth or cultures, all of the immersion solutions were found to reduce the growth of *Candida albicans*.

Disinfectant solution	n	Initial culture (Mean)	After disinfection (Mean)	P-value
1% Sodium hypochlorite	10	$1.5 \times 10^8$ cells/ml	$0.13 \times 10^8$ cells/ml	0.016
0.2% Chlorhexidine gluconate	10	$1.5 \times 10^8$ cells/ml	$0.40 \times 10^8$ cells/ml	0.025
3.8% Sodium perborate	10	$1.5 \times 10^8$ cells/ml	$0.66 \times 10^8$ cells/ml	0.045



## VI. Discussion

Oral environment temperature and acquired pellicle formed over dentures promote *Candida* adhesion to resin materials, indicating need of an adequate plaque control for maintaining oral health<sup>3,4</sup>. Chandra et al showed that development of this yeast on acrylic resins happens in three distinct stages. They are initial stage upto 11 hours from colonization when some microcolonies begin to be formed. Intermediate stage from 12-30 hours after colonization when some extracellular material begins to accumulate over colonies<sup>3,8</sup>.

Maturation stage from 38-72 hours after colonization when *Candida albicans* colonies become totally involved by extracellular matrix forming a biofilm. They also concluded that antifungal resistance increases during biofilm development, as extracellular matrix acts as barrier to action of antifungal agents<sup>3,8</sup>.

Sodium hypochlorite has the capacity to dissolve mucins and other organic substances in the biofilm matrix, inhibiting its formation and microorganism recolonization, yet promoting the degradation of the acrylic resin, depending on its concentration and immersion time. It can be a bactericide and fungicide, because it acts directly on the organic matrix of the plaque, resulting in the dissolution of the polymer's structure, probably because of oxidation of the protean component or significantly reducing the adhesion of most *Candida* sp. to the oral epithelial cells. These characteristics allow the hypochlorite to reduce *Candida* sp. adhesive ability, but it does not work as an antiinvasive barrier, as it is not able to prevent the production of proteinases by the *Candida* sp.

Chlorhexidine has a broad spectrum of activity against a variety of organisms, including *C. albicans*. It has been pointed out that the resistance of biofilms originates typically from the recalcitrance of a small subpopulation<sup>22</sup>. Chlorhexidine is a cationic agent, which is adsorbed by oral surfaces and negatively charged microbes, interfering with osmotic equilibrium, the formation of acquired films and the microbial adsorption of oral surfaces. Therefore, it has the capacity to reduce biofilm formation and inhibit the synthesis of insoluble polysaccharides in the microbial matrix of the dental biofilm. The rate of propidium iodide (PI) penetration into the cytoplasm of cells as plasma membrane integrity is compromised by the action of chlorhexidine<sup>22</sup>. The kinetics of appearance of PI fluorescence reflects the rate, or at least the extent, of membrane permeabilization that is a consequence of membrane disruption by chlorhexidine. However, continued use may cause staining and alterations of color in the acrylic

When sodium peroxide is dissolved in water, they become alkaline hydrogen peroxide, which decomposes when it comes into contact with certain substances and releases small oxygen bubbles with the mechanical action of detaching the biofilm from the denture surface. The oxidant agents help to remove stains and have some antibacterial action. This type of solution can be used alone or in combination with a mechanical method. In the literature, Budtz – Jorgensen and Bertram, 1970 et al found that *Candida albicans* were found to

be most prevalent of all candidal species both in healthy and diseased oral cavity<sup>5</sup>. Arendof and walker,1987 et al reported that *Candida albicans* to be most pathogenic and capable of adhering to epithelial cells and acrylic resin surfaces<sup>4,5</sup>. The results of present study demonstrated that 1% sodium hypochlorite showed best antimicrobial effectiveness against tested microorganisms. These data are in accordance with previous studies analyzing disinfection with this solution. Despite of its high antimicrobial activity, this disinfectant presents serious limitations such as corrosive activity on metal surfaces<sup>4</sup>.

Iacopino et al reported that chlorhexidine is highly effective against candidiasis<sup>7,9</sup>. In this study 0.2% w/v chlorhexidine mouthwash is used which is effective on *Candida albicans* for 10 min immersion. Pavarina et al recommended the use of 4% chlorhexidine for 10 minutes to disinfect complete dentures. In this study of all disinfectants 3.8% sodium perborate is least effective and showed no statistical significant difference between final counts after disinfection and control group<sup>21</sup>.

## VII. Conclusion

Within the limits of this study 1% sodium hypochloride is most effective than 0.2% w/v chloride and 3.8% sodium perborate is least effective on *Candida albicans*. Sodium hypochlorite and chlorhexidine are cost effective or economical, non toxic and easily accessible hence are appropriate for household use in disinfection of complete dentures.

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