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Antifungal Effect: Comparison of Commercial Denture Cleansers and Microwave Energy

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ABSTRACT

Aims: To evaluate the ability of commercial denture cleanser on decreasing *Candida albicans* biofilm and comparing this with microwave sterilization technique on heat cured acrylic resin denture base material which was cured by two different techniques. **Materials and Methods:** thirty two samples of heat cured acrylic resin denture base material had been used, half of them was cured by conventional water bath technique, and the other half by microwave technique. A simple method to measure Candida biofilm activity using pH change of Stomastat. Modified Sabouraud broth developed and used to evaluate the efficacy of the following denture cleansers: Chlorhexidine 0.2% and sodium hypochlorite 0.5%, and microwave sterilization on *Candida albicans* biofilm. The initial number of yeasts inoculated was correlated with pH value of Stomastat after 24 hours incubation period. **Results:** The acrylic resin samples that cured by conventional water bath technique were most effectively disinfected by chlorhexidine 0.2%. This demonstrated by increasing the pH value of Stomastat significantly than the control group. The same result was obtained with those samples which were cured by microwave oven. The initial number of *Candida albicans* cells in Stomastat was significantly decreased than the control group after treatment of acrylic samples with denture cleansers and microwave sterilization technique, while for those samples that cured by microwave technique the least number was obtained after treatment with chlorhexidine 0.2%. **Conclusion:** Both of chlorhexidine 0.2% and sodium hypochlorite 0.5% and microwave technique had the ability to disinfect acrylic samples that cured by water bath and microwave techniques effectively. However, chlorhexidine 0.2% was the most effective one.

Key Words: Denture cleansers, microwave, antifungal agent.

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INTRODUCTION

Removable prosthesis may be potential source of infection[1–3]. Several studies have focused on the materials and methods necessary to ensure proper disinfection control of dental prosthesis[4–6]. When selecting a disinfection procedure, the effect of the disinfectant on denture must be carefully considered. Although chemical disinfection has been largely recommended [5, 6], it is possible that certain components of the disinfectant solutions may penetrate the material and not be completely eliminated by rinsing. In addition, the use of disinfectants has been considered time consuming or inappropriate[4, 6].

Various methods of chemically disinfecting dental prosthesis have been evaluated; those include sodium hypochlorite, glutaraldehyde and chlorine dioxide [5, 6]. Although most of these solutions have shown no deleterious effects on acrylic resins [7], they may be introduced to the oral cavity.

If the denture is heavily contaminated, it probably would not be completely disinfected by immersion only. Recently, the microwave disinfection method is claimed to be a useful alternative to immersion disinfection [8].

Microwave irradiation may be used for decontamination of food, microbiologic laboratory materials, dental instruments, nitrous oxide nasal hoods, contact lenses,

household sponges, Candida contaminated underwear, clinical waste and material used in clinical laboratory and home health care [9, 10]. On the other hand, little information is available concerning the effect of microwave disinfection upon acrylic resins [11–13].

A study showed that microwave sterilization would be effective for sterilization of hard chairside reline resins that contaminated with pathogenic microorganisms and fungi (Candida albicans) [14, 15].

The aim of this study was to evaluate the effect of microwave radiation on Candida albicans biofilm on acrylic resin denture base surface compared with commercial denture cleanser.

MATERIALS AND METHODS

Thirty two specimens were prepared with dimensions of 1 cm × 1 cm × 1 mm using heat cured acrylic resin (Major Base 2, Major Prodotti Dentari, Italy) that had been processed against acetate sheet and glass slab [16]. Half of them were cured by conventional water bath technique according to manufacturers' instruction, and the other half were cured by microwave curing technique using fiber–reinforced plastic–flask [17] at curing cycle (30 minutes) at low setting (80 watts), 15 minutes per side, followed by 1 ½ minutes at high setting (500 watts)[18] and by using domestic microwave oven (Samsung, Model RE–570 D, 0.6 cu–ft, Korea).

All specimens were conditioned by storing them in distilled water for 48 + 2 hours at 37 + 1 ºC before the experiment [15–17].

Candida albicans from inner surface of upper denture was obtained according to Kassab,[16] and examined the ability of denture cleaning agents to reduce or remove the colonized yeast and fungal biofilm by quantifying the number of vital cell of colonized Candida albicans (biofilm activety) [19, 20].

Thirty two samples of acrylic resin were sterilized by autoclave at 121 ºC for 15 minutes and were tested in this study; each one had been placed in the bottom of sterile beaker, 0.5 ml of serum was added, then incubated for 1 hour at 37 ºC. After that serum was aspirated and 0.025 ml of yeast suspension 106 cell/ml was inoculated (yeast suspension was determined by total cell count using haemocytometer, and incubated for 2 hours at 37ºC. After that 2ml of Sabouraud broth was added to each beaker, and incubated for 6 days at 37ºC) [21].

To assess the ability of different solutions to remove Candidal biofilm, the colonized yeast specimens were immersed into 50 ml of the following tested solutions:

Fifty ml of chlorhexidine 0.2% as acontrol positive.

Fifty ml sodium hypochlorite 0.5%.

Fifty ml sterile distilled water as a control negative.

In addition, microwave was used to sterilize some of specimens. The acrylic resin samples were divided into two main groups in this study:

The first group represents acrylic resin samples that cured by conventional water bath technique and the total number of specimens were 16; those divided into:

Four samples were treated with 0.2% chlorhexidine.

Four samples were treated with 0.5% sodium hypochlorite.

Four samples were treated with distilled water.

Four samples were sterilized by using microwave oven.

The second group represents acrylic resin samples that cured by microwave technique and the total number of specimens were 16 and divided as in group A.

For all the specimens in groups A and B that treated with solutions, each beaker was incubated for 2 hours at room temperature, washed with sterile distilled water and then 2 ml of Stomastat (modified Sabouraud broth containing 0.01% wt/vol chloramphenicol),(22, 23) were added to each beaker. Then, the initial number of yeasts inoculated was determined by total cell count using haemocytometer. After 24 hours of incubation, the pH of medium with each specimen was measured using a pH meter (HNNA, CE, Italy).

Specimens that sterilized by microwave oven were 8: 4 cured by water bath technique and 4 cured by microwave technique, were placed in distilled water during microwave exposure and irradiated with microwave energy for 6 minutes at

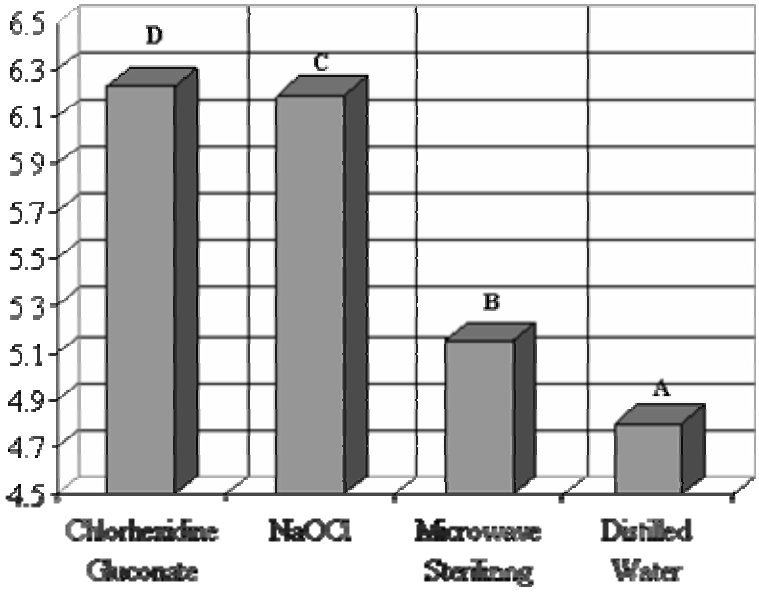
650 w (12, 14).

RESULTS

Results showed that the pH value of Stomastat after treatment of specimens of acrylic that cured by water bath technique with chlorhexidine 0.2% and sodium hypochlorite 0.5% were increased significantly than control samples in distilled water and there was significant difference (Table 1 and Figure 1) and the most effective one is chlorhexidine 0.2%. This indicated the ability of those solutions in disinfection of acrylic specimens. Also this Table showed the significant effect of microwave in disinfection of acrylic samples that cured by water bath. However, its effect was less than chlorhexidine and sodium hypochlorite.

Table (1): Analysis of variance for the pH value of Stomastat in the samples cured by water bath technique

	d.f	SS	MS	F–value	*p*–value
Between Groups	3	6.336	2.112	4546.274	0.000 Significant
Within Groups	12	5.575E–03	4.646E–04		
Total	15	6.342			



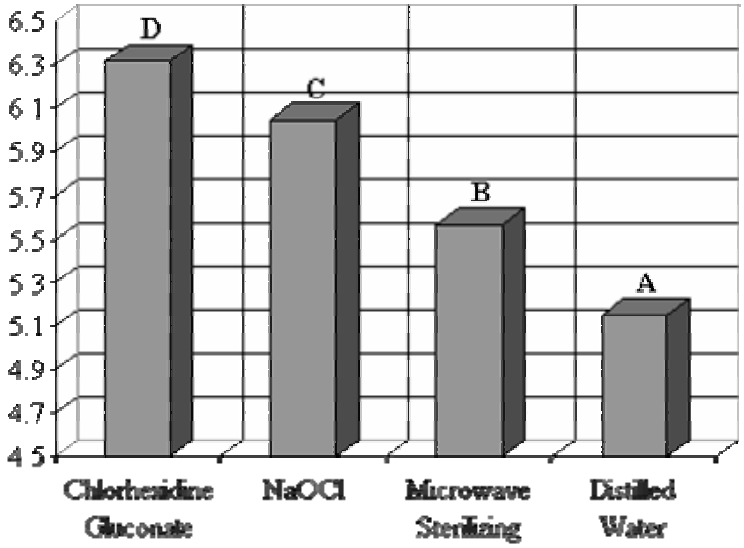
Means with the same letter were statistically not significant ($p > 0.05$).

Figure (1): Duncan's Multiple Range test for the pH value of Stomastat in the samples cured by water bath technique.

Table (2) and Figure (2) demonstrated the similar results that mentioned in previous one. The only difference in that the acrylic samples were cured by microwave technique. The increase in pH value than the control group indicated that the media of Stomastat become less acidic which caused by reduction in the growth of Candida albicans.

Table (2): Analysis of variance for the pH value of Stomastat in the samples cured by microwave technique

	d.f	SS	MS	F–value	*p*–value
Between Groups	3	3.217	1.072	535.620	0.000 Significant
Within Groups	12	2.403 E–02	2.002 E–03		
Total	15	3.241			



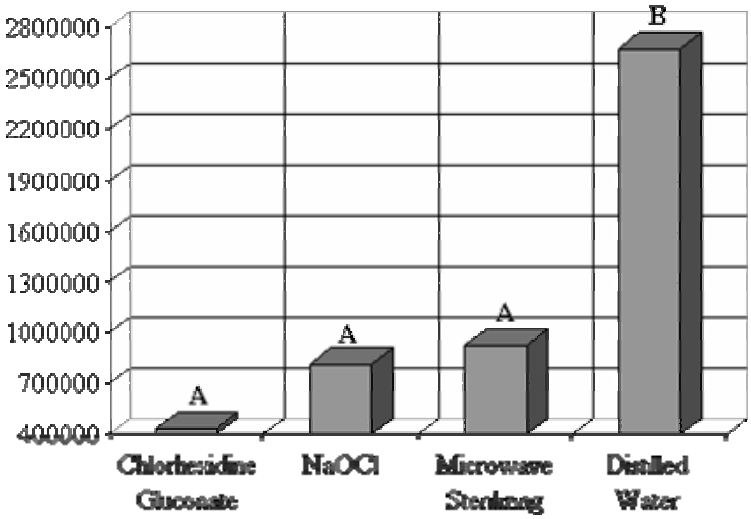
Means with the same letter were statistically not significant ($p > 0.05$).

Figure (2): Duncan's Multiple Range test for the pH value of Stomastat in the samples cured by microwave technique

Table (3) and Figure (3) demonstrated that the initial number of Candida albicans cells inoculated in Stomastat after treatment of acrylic samples that cured by water bath with chlorhexidine, sodium hypochlorite and microwave were significantly decreased than the control group in distilled water. However, there were no differences among them. This indicated that the pH of Stomastat in the control group was more acidic than others which cause by acid production of Candida albicans.

Table (3): Analysis of variance for the initial number of *Candida albicans* in the samples cured by water bath technique

	d.f	**SS**	**MS**	**F–value**	***p*–value**
Between Groups	3	1.19 E+13	3.98 E+12	16.884	0.000 Significant
Within Groups	12	2.83 E+12	2.35 E+11		
Total	15	1.48 E+13			



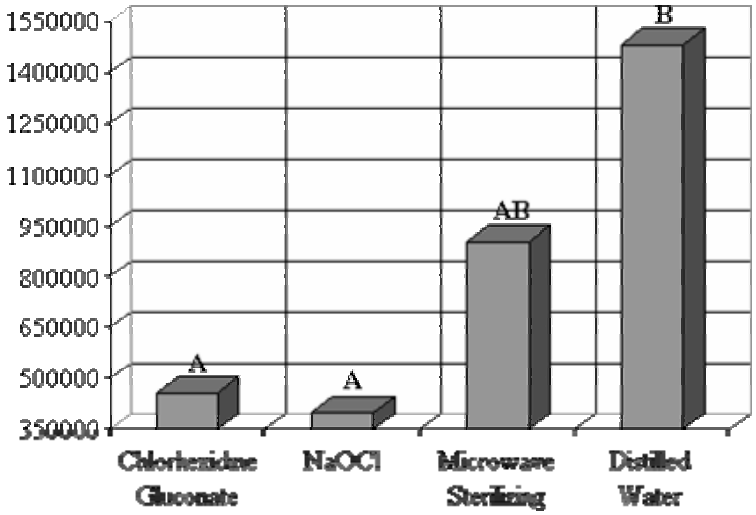
Means with the same letter were statistically not significant ($p > 0.05$).

Figure (3): Duncan's Multiple Range test for the initial number of *Candida albicans* in the samples cured by water bath technique

In Table (4) and Figure (4), the acrylic samples were cured by microwave. The initial number of Candida albicans in Stomastat after treatment with chlorhexidine, sodium hypochlorite and microwave were significantly decreased than the control group (distilled water). However, both of chlorhexidine and sodium hypochlorite had the least number with no difference between them but significantly different from microwave. Also, microwave had the similar effect but in less degree than chlorhexidine and sodium hypochlorite.

Table (4): Analysis of variance for the initial number of *Candida albicans* in the samples cured by microwave technique

	d.f	SS	MS	F–value	*p*–value
Between Groups	3	3.01 E+12	1.00 E+12	3.262	0.049
Within Groups	12	3.70 E+12	3.08 E+11		Significant
Total	15	6.71 E+12			



Means with the same letter were statistically not significant ($p > 0.05$).

Figure (4): Duncan's Multiple Range test for the initial number of *Candida albicans* in the samples cured by microwave technique.

DISCUSSION

It has been reported that contaminated prosthesis can transfer microorganisms to prosthesis, materials, equipment, personnel and patients[8,24], and cross contamination of the prosthesis may occur when infected units are pumiced in dental laboratories[25]. Therefore, dental office, personnel and laboratory technicians should protect themselves against cross contamination[4, 12] and prevent denture stomatitis by incorporating relatively simple sterilization–disinfection procedures into their daily routines[13]. Also, the formation of Candidal biofilms on prosthetic appliance is significant medical problem and often necessitates removal of the appliance[26].

In the present study, 6–day Candidal biofilm on serum coated acrylic samples was used to evaluate antifungal efficacy of cleansers. Drake et al[27]. used one day bacteria–yeast colonization model and showed that Candida albicans appeared to become resistant to cleansers than bacteria. It is generally accepted that denture plaque matures in vivo within one week (demonstrating a large amount of Candida albicans) [19]. Also, they observed that the activity of Candida albicans biofilm which composed of large amount of blastospore and hyphae formed on saliva or serum–coated acrylic resins increased during 4 or 5 days of incubation in contrast to uncoated specimens. Furthermore, the activity of fungal biofilm on serum–coated sample was approximately 100 times

higher than the activity on an uncoated sample and about 10 times than that on saliva–coated sample. Thus, in the present study, we used 6 days Candidal biofilm on serum–coated acrylic resin samples.

This study showed that NaOCl was effective antifungal and disinfectant agent on acrylic resin denture base material. Webb et al [28]. showed that NaOCl was effective denturee disinfectant agent and it reduced the adhesion of Candida albicans cells on denture acrylic. The present study also demonstrated the effectiveness of microwave energy in disinfection of acrylic resin denture base material whether cured by microwave or water bath technique.

In this study, it could be concluded that NaOCl was more effective disinfectant than microwave energy on acrylic resin in concentration of 0.5%. Webb et al [11]. demonstrated that microwave was more effective in denture sterilization than NaOCl in concentrations of 0.02% and 0.0125%. The difference may be due to the difference in the concentration of NaOCl and microwave oven watts used. However, despite the ability of NaOCl at 0.5% as a disinfectant on denture acrylic, it is not well recommended to be used for this purpose because of its possibility of bleaching effect on acrylic denture base [10, 11, 29].

In an in vitro study, NaOCl was found to be more effective disinfecting procedure than microwave for 5 minutes, while in other studies microwave produced superior effect [11, 12]. The results of Silva et al [15]. agreed with the results of the present study on sterilization of complete denture contaminated by Candida albicans by microwave energy.

In the present study, the effectiveness of chlorhexidine as disinfectant was concluded. Banting and Hill [13] showed the effectiveness of chlorhexidine in eradication of invasive form of Candida albicans organism. Kassab[16] showed the effectiveness of chlorhexidine as antifungal agent against adherent Candida albicana cells on acrylic resin denture base material that cured by water bath technique.

The present study demonstrated the effectiveness of chlorhexidine as disinfectant and more powerful than microwave, which disagreed with the results of Banting and Hill[13]. In spite of that microwave energy is considered more practical procedure for disinfection of complete denture as adjunct to treatment of oral candidiasis because of it is less expensive, more convenient and requires considerably less effort on the part, without affecting other properties of acrylic[11, 12].

The specimens that had been sterilized by microwave oven were placed in water during microwave exposure to produce a uniform heating of specimens which was considered to be adequate to kill organisms even within pores of the materials [12, 14]. Others suggested that wetting of materials before microwave irradiation to obtain more effective disinfection[30].

CONCLUSION

Both of chlorhexidine 0.2% and sodium hypochlorite 0.5% and microwave technique had the ability to disinfect acrylic samples that cured by water bath and microwave techniques effectively. However, chlorhexidine 0.2% was the most effective one.

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