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EFFICIENCY OF DIFFERENT DENTURE DISINFECTION METHODS

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ABSTRACT
Dentures that are not cleaning and maintained properly may prone to contamination by different microbial pathogens that result in several oral conditions. This study was design to compare the antimicrobial effect of different denture cleansers oxalic, tartaric, citric acids and alkaline peroxide with microwave irradiation on the growth of *Candida albicans* and *Staphylococcus aureus* respectively. Microwave oven used to disinfect specimens of heat-activated acrylic resin and soft linear. Oxalic, citric, tartaric acids, and alkaline peroxide were also used. Microorganisms that tested were *Candida albicans* and *Staphylococcus aureus* separately. Treatment with microwave or tartaric acid could achieve sterilization of both hot cured acrylic resins and soft lining material specimens contaminated with *S. aureus* whereas using of microwave energy, oxalic acid, tartaric acid and Alkaline peroxide can achieve complete sterilization against *C. albicans*. The microwave is the best method to achieve sterilization for both hot cured acrylic resins and soft lining material specimens contaminated with *Staphylococcus aureus* or *Candida albicans*, and tartaric acid ranked secondly.

KEYWORDS: heat-activated acrylic resin, denture cleansers, microwave irradiation, *Candida albicans*, *Staphylococcus aureus*

INTRODUCTION

The presence of a removable prosthesis in the oral cavity may result in accumulations of microbial plaque around and under the denture and this may induce certain pathological mucosal reactions, denture induced stomatitis, and angular cheilitis[1]. Infection of the oral mucosa underlying a removable prosthesis by *Candida albicans* was one of the factors that played a role in the etiology of denture stomatitis[2,3]. Frequently, *Staphylococcus aureus* secondarily infects Angular cheilitis which is mostly associated with the presence of *Candida*- associated stomatitis, and is believed that the infection may initiate under the maxillary denture and spread to the angles of the mouth[4]. Soft denture linears and hot cured acrylic used in the treatment of the completely and partially edentulous patients. The difficultly in the hygienic maintenance of the soft acrylic is one of disadvantages. This is related to their micro-porous surfaces which are support the growth of microorganism such as *Candida albicans* [5-8]. Bal *et al*. (2008) demonstrate that higher numbers of bacteria and *Candida albicans* were adhere to the soft lining materials than acrylic resin materials; As a result, the soft lining materials are more susceptible to microbial adherence than acrylic resin [9]. Mechanical or chemical methods or both were introduced in order to maintain the denture hygiene and prevent denture-related stomatitis. Oxygenating cleansers, alkaline hypochlorite solutions, dilute mineral acids, abrasive powders and pastes, and enzyme-containing materials are the commonly available denture cleansing materials [10]. One of the requirement of the good denture cleansers it should have a bactericidal and fungicidal effects [11,12]. It was found that acrylic resin materials can safely immersed in the prepared denture cleanser solutions such as 4% citric acid, 4% oxalic acid and 4% tartaric acid for 10 minutes without damaging effect to the acrylic resin[13]. However these materials need for daily fresh preparation. Whereas sodium hypochlorite is not preferred due to it's bleaching effect [14-16].

The simplicity and effectiveness of using microwave irradiation makes it alterative to disinfect dentures[17]. For these reasons, this study was conducted to compare the effect of different disinfectant treatments like prepared denture cleansers (oxalic, tartaric, citric acids), alkaline peroxide tablets and microwave disinfection with either immersion in water or not, on the growth of *Candida albicans* and *Staphylococcus aureus* that were contaminated the surfaces of soft lining material and hot cured acrylic respectively. and also to test the hypothesis that said the immersion of hot acrylic resin and soft acrylic resin denture base materials in the water during microwave irradiation may be more effective in the reduction of the growth of *Candida albicans* and *Staphylococcus aureus* than the immersion in denture cleanser (oxalic, tartaric, citric acids and alkaline peroxide tablets) or treatment of specimens with microwave irradiation without immersion in water.

MATERIALS & METHODS

Seventeen specimens (10 x 10 x 2.5 mm) of soft acryl material (Vertex™ Soft, Vertex-Dental, Netherlands) and seventeen specimens of hot cured acrylic (SR Triplex Hot, Ivoclar Vivadent, Liechtenstein) were fabricated according to manufacturer's recommendations.

All specimens were sterilized by using the autoclave for 15 minutes at 121˚C and 15 Psi, and then stored in sterile bags until used.

The microorganisms in this work were Gram positive *Staphylococcus aureus* and yeast *Candida albicans* were obtained from AL-Yarmook Teaching Lab. in Baghdad. These pathogens were choosed as they are considered as indicators pathogens according to the Hand Book of Disinfectants and Antiseptics[18]. On the first day, few colonies (3-5) of *Staphylococcus aureus* and *Candida albicans* were individually inoculated into 10 ml sterile Tryptic Soy Broth (TSB) which incubated aerobically for 24 hours at 37ºc. At the second day, the turbidity of tubes was adjusted to be comparable to the McFarland tube No.5 which corresponds to (10^7) organisms/ml.

100 µl of each previous test tube was inoculated into 10ml of TSB under sterile condition and the sterile specimens were placed individually into the inoculated tubes, vortexed, and incubated at 37ºc for 24 hours under aerobic condition. Then each type of resin materials used in this study was treated with six different disinfectant regimes to test their effect against *Candida albicans* and *Staphylococcus aureus* that contaminated these resin materials respectively. According to the disinfection regime the specimens were immersed in diluted denture cleanser solutions for 10 minutes. A fresh denture cleansers solution is prepared by dissolving four grams for each of the following: oxalic (O), tartaric (T), and citric acids (C) in 100 ml of the isopropanol alcohol[2]. Then, a mixture of equal volumes of each prepared denture cleansers solution and distilled water were prepared freshly before use. While alkaline peroxide solution (Alk) was prepared according to the instructions of manufacturer by addition of one tablet of alkaline peroxide to 100 ml of sterile warm distilled water at 50˚C.

After both positive control and disinfectant solution treatments completed (O, C, T, Alk), the broth was discarded and each specimens was placed into a test tube contained 10 ml sterile saline. Then tubes were exposed to short vortex twice and the number of microorganism was determined in the serial dilution (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}). The procedure includes the addition of 1 ml of normal saline from the first tube in which the specimens were placed to 9 ml of sterile normal saline, the total volume was 10 ml which represent the first dilution (10^{-1}), and other dilutions were prepared sequentially by the same method. Additionally, 100 µl of each dilution were transferred to two types of selective media, Manitol salt agar and Sabouraud dextrose agar for *S. aureus* and *C. albicans* respectively, and the plates incubated aerobically at 37ºC for 48 hours. Then the colony count was determined for each specimen by multiplication the reverse of the dilution factor by the colony number in each plate and the mean of the triplicate were counted for each treatment. Furthermore, only plates with colonies number ranged from 30-300 were depended in this study.

All specimens of group (md) were put in microwave oven at 850 W in dry air (without immersion in water). For group (mw) all specimens were put in microwave oven while immersed in water at 850 W. The exposure times were 5, 10, and 15 min respectively. All specimens of control group were immersed in normal saline for 10 minutes. Then transferred to sterile test tubes containing 10 ml of sterile saline as they were treated as positive control. In addition, Pyrex beaker filled with 150 ml of water was placed in the microwave oven before processing. The microbial colony counts (bacterial and yeast) of each plate were determined after 48 hours under aerobic incubation. The logarithm of colony forming units per milliliter (log cfu/ml) was then calculated. The logarithm effectiveness of microwaved sterilization was tested, by incubation of TSB tubes containing microwaved specimens aerobically at 37ºc for a further 7days.

Six disinfectant regimes were used for each type of resin materials and for each type of microorganisms. Example: HC mw as H, means Hot cured acrylic sample contaminated with C, Candida albicans microorganism disinfected by expose to mw, microwave energy while immerse in water.

Group (mw): 2 specimens were exposed to microwave energy at 850 W for 10 minutes while immersed in distilled water.
Group (md): 2 specimens were exposed to microwave energy at 850 W for 10 minutes in dry air (without immersion in water).
Group (O): 3 specimens were immersed in fresh prepared oxalic acid denture cleansers for 10 min.
Group (C): 3 specimens were immersed in fresh prepared citric acid denture cleansers for 10 min.
Group (T): 3 specimens were immersed in fresh prepared tartaric acid denture cleansers for 10 min.
Group (Alk.): 3 specimens were immersed in fresh prepared alkaline peroxide denture cleansers for 10 min.
Group (control): 1 specimen was immersed in normal saline for 10 min.

Statistical analysis

The multiple comparison tests depending on the Least Significant Difference test (LSD) and also the One-Way Analysis of Variance (ANOVA), was performed for all test groups.

RESULTS

The selection of 15 minutes as the effective time for the microwave irradiation was depended in this work according to a pilot study conducted by the authors. As the experiment showed that treatment time of 10 and 15 minutes for the acrylic specimen that immersed in water during microwave irradiation result in zero colony count whereas the exposure to microwave irradiation in dry condition resulted in 32 colony for 10 minute and zero colony for the 15 minutes.

The colony count of *Candida albicans* or *Staphylococcus aureus* of all specimens was determined before and after each treatment using viable count method.

The colony count of *Candida albicans* of the surface of the hot cured and soft acrylic showed a highly significant reduction in number for the treatment of microwave energy while immersed in water (HC mw and SC mw), Figure (1).

The results of this study revealed that the mean of *Candida albicans* colony count was higher on hot cured acrylic resin samples for the control and alkaline peroxide test groups, while no colony was observed (zero count) for microwave treatment in presence of water, and tartaric acid treatment, when compared with a low colony count in treatment using citric acid, oxalic acid, and exposure to microwave radiation in dry air, Figure (1)

One- way ANOVA and LSD test showed a highly significant difference between all test groups of the hot cured acrylic resin samples, Table (1&2).

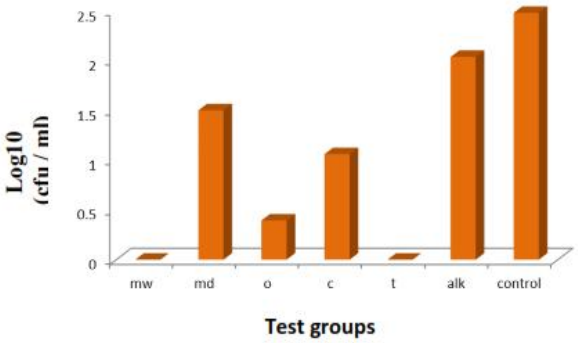

FIGURE 1: Colony count of *Candida albicans* in (log 10) on hot cured acrylic samples for all test groups.

TABLE 1: One-way ANOVA test for all test groups of hot cured acrylic resin samples with *Candida albicans*

	df	Mean Square	F	Sig.
Between Groups	6	3.426	22.055	.000
Within Groups	14	.155		
Total	20			

* The mean difference is significant at the 0.05 level

TABLE 2: LSD for all test groups of hot cured acrylic resin samples with *Candida albicans*

Test groups	Mean Diff.	Std. Error	Sig.
Control-mw	2.47712	.32180	.000
Control- md	2.47712	.32180	.000
Control- O	1.30258	.32180	.001
Control- C	.82124	.32180	.023
Control- T	2.47712	.32180	.000
Control- Alk.	.36608	.32180	.274

* The mean difference is significant at the 0.05 level

Also there was a highly significant reduction in *Staphylococcus aureus* colony count on the hot cured acrylic resin and soft acrylic for the HS mw and SS mw test group, table (3 and 4).

The results show that exposure of sample contaminated with *S. aureus* to microwave energy or tartaric acid will make complete sterilization of sample as no colonies observed while the treatment with citric acid or oxalic acid give a medium antimicrobial activity, whereas the alkaline peroxide treatment as control group show a high number of bacterial colonies. This means it was the less effective disinfectant method being used in this work, Figure (2).

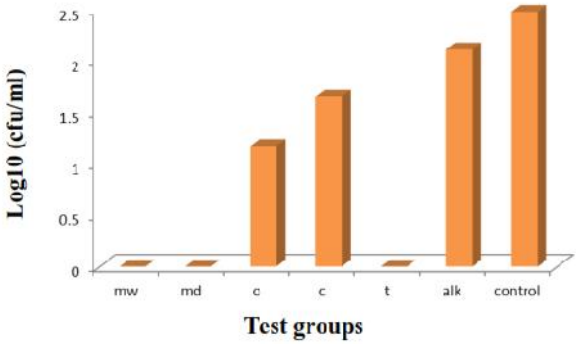

FIGURE 2: Colony count of *Staphylococcus aureus* on hot cured acrylic samples for all test groups.

TABLE 3: One-way ANOVA test for the hot cured acrylic resin samples *Staphylococcus aureus* on the test groups.

	df	Mean Square	F	Sig.
Between Groups	6	3.256	14.365	.000
Within Groups	14	.227		

Total	20

* The mean difference is significant at the 0.05 level

TABLE 4: LSD for all test groups of hot cured acrylic resin samples with *Staphylococcus aureus*.

Test groups	Mean Diff.	Std. Error	Sig.
Control-mw	2.47712	.38869	.000
Control- md	2.47712	.38869	.000
Control- O	.04622	.38869	.907
Control- C	1.00999	.38869	.021
Control- T	1.89439	.38869	.000
Control- Alk.	1.53948	.38869	.001

* The mean difference is significant at the 0.05 level

The higher colony count of *Candida albicans* on soft lining material samples was observed for the control and oxalic acid groups and the lower mean of colonies number (zero colony count) was seen for the treatment with md and, mw, whereas tartaric acid and citric acid treatments showed a medium antimicrobial effect, Figure (3) and tables (5& 6).

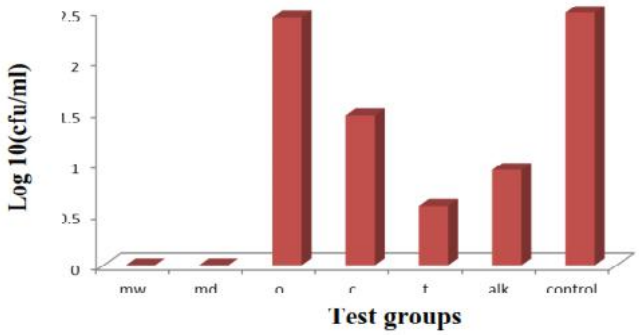

FIGURE 3: Colony count of *Candida albicans* on soft liner samples for all test groups.

TABLE 5: One -way ANOVA test for all test groups of soft liner samples with C*andida albicans*.

	df	Mean Square	F	Sig.
Between Groups	6	3.182	16.520	.000
Within Groups	14	.193		
Total	20			

* The mean difference is significant at the 0.05 level

TABLE 6: LSD for all test groups of soft liner samples with *Candida albicans*.

Test groups	Mean Diff.	Std. Error	Sig.
Control-mw	2.47712	.35837	.000
Control- md	2.47712	.35837	.000
Control- O	.00000	.35837	1.000
Control- C	.78119	.35837	.047
Control- T	1.35781	.35837	.002
Control- Alk.	1.35087	.35837	.002

* The mean difference is significant at the 0.05 level

The higher mean of colony count for *Staphylococcus aureus* on soft lining material samples was observed for control and oxalic acid groups, followed by (citric acid, alkaline peroxide and tartaric acid) test groups which showed a medium number of bacterial colonies. Whereas, the zero colony forming unit was recorded for microwave energy test groups with water and without water (mw &md), figure (4) and tables (7and 8).

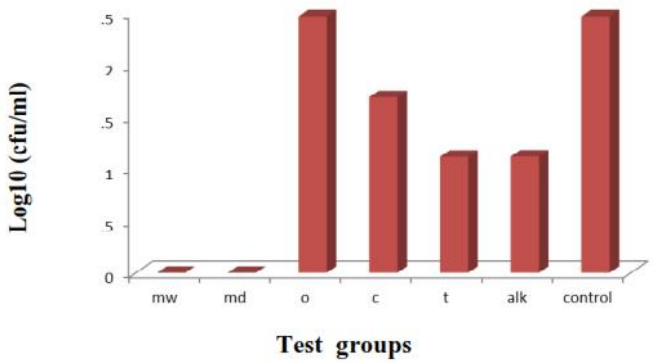

FIGURE 4: Colony count of *Staphylococcus aureus* on soft liner samples after different disinfection methods.

The ANOVA and LSD test, revealed that there were a highly significant differences in colony count of *S.aureus* between all test groups and control group except for oxalic acid test group, table (7and 8).

TABLE 7: One -way ANOVA test for all test groups of soft liner samples with *Staphylococcus aureus*.

	df	Mean Square	F	Sig.
Between Groups	6	3.182	16.520	.000
Within Groups	14	.193		
Total	20			

* The mean difference is significant at the 0.05 level

TABLE 8: LSD analysis for all test groups of soft liner samples with *Staphylococcus aureus*.

Test groups	Mean Diff.	Std. Error	Sig.
Control-mw	2.47712	.35837	.000
Control- md	2.47712	.35837	.000
Control- O	.00000	.35837	1.000
Control- C	.78119	.35837	.047
Control- T	1.35781	.35837	.002
Control- Alk.	1.35087	.35837	.002

* The mean difference is significant at the 0.05 level

DISCUSSION

Dentures could be full of a broad variety of potentially pathogenic microorganisms[20]. These pathogenic microorganisms produce a potential source of contamination from patients to dental and laboratory personnel. The chances of cross-contamination should be reduced by means of disinfection. This work was done to compare between different methods of disinfection in order to decide which type is more effective than the other we compared between the most used methods.

The hypothesis that the immersion in water during microwave irradiation for hot acrylic resin, and soft acrylic resin denture base materials is more effective in the reduction of the growth of *Candida albicans* and *Staphylococcus aureus*, than the immersion in denture cleanser (oxalic, tartaric, citric acids and alkaline peroxide tablets) as well as microwave irradiation without immersion in water was accepted. The results of this study revealed that the immersion in water during microwave irradiation was more effective than the other disinfectant for both hot cure acrylic and soft acrylic with *S. aureus* and *Candida albicans*. A study conducted by Campanha et al. 2007[21] whom evaluated the ability of microwave irradiation in inactivation of *Candida albicans* cells and study the effect of this procedure on cell membrane integrity. They ascertain that irreversible damage and inactivation was occur to *Candida albicans* cells after exposure to microwave irradiation. This may agree with the result of our study and explain the reason for this result. Another research confirmed that dentures contaminated with *Candida* species showed sterilization after microwave irradiation for 3 minutes at 650 watt[22]. The same result was provided by Dixon et al.[23] they afford a promising method to sterilize dentures contaminated with yeast *Candida albicans* by using home microwave oven.

As a conclusion, the treatment with tartaric acid and microwave in presence of water, are useful methods to sterilize hot cured acrylic resins contaminated with *Candida albicans* whereas the using of microwave can sterilize soft lining material contaminated with the same pathogen. Sterilization could also be achieved by using microwave energy or tartaric acid for *S. aureus* contaminated hot cured acrylic resins or using microwave energy for soft lining material contaminated with the same pathogen.

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