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Effectiveness of Microwave Sterilization on Soft Lining Material

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الخلاصة

الأهداف :تقييم فاعلية التعقيم بالتشعيع بالموجات المايكروية لمادة التبطين الطرية لطقم الأسنان. **طريقة العمل**: تم تحضير ستون عينة من مادة التبطين الطرية لطقم الأسنان تبعا للطريقة القياسية و عقمت بجهاز التعقيم بالموصدة. قسمت العينات إلى أربعة مجاميع وتم تحضين عينات كل مجموعة مع أحد أنواع الأحياء المجهرية المختبرة التالية:المكورات العنقودية،عصيات سبتلس، زوائف ايروجينوزا،والمبيضات البيضاء. عوملت عينات كل مجموعة بالتشعيع بالموجات المايكروية 540 وات لستة دقائق وقورنت مع مجموعة السيطرة . **النتائج:** أظهرت الدراسة أن هناك تأثير مطهر واضح على جميع الأحياء المجهرية المختبرة بعد 48 ساعة تحضين ، وتأثير معقم على المبيضات البيضاء بعد 48 ساعة تحضين و 7 أيام تحضين.**الكلمات المفتاحية**مادة التبطين الطرية لطقم الأسنان، الموجات المايكروية.

ABSTRACT

Aims: The aim of this study was to evaluate the effectiveness of microwave irradiation sterilization on Molloplast –B soft denture liner. **Materials and Methods:** Sixty specimens of Molloplast –B soft denture liner were fabricated in a standardize procedure and autoclaved. The total 60 specimens were divided into 4 groups. Each group has 15 specimens inculated with Brain Heart Infusion Broth (BHI) media containing one of the tested microorganisms (Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa and Candida albicans). Then the 15 specimens in each group was further divided into 3 subgroups: group C (positive control); 5 none irradiated specimens, group D (dry microwave treatment); 5 specimens placed in a dry beaker and microwave irradiated at 540W for 6 minutes, group W (wet microwave treatment; 5 specimens immersed in distalled water and irradiated in the same manner as group D. After incubation of all specimens for 24 hours at 37°C, the specimens were got vortex and then the replicated specimens (100µL) of suspensions were plated on 4 selective media appropriate for each organism. All plates were incubated at 37°C for 48 hours. After incubation, colonies were counted. Further 7 days incubation for microwaved specimens was done to verify the effectiveness of dry and wet microwave sterilization. **Results:** Significant reduction in cfu/ml of all microorganisms was observed at 48 hours. No growth of C. albicans was recorded at 48 hours and after 7 days incubation. **Conclusions:** Microwave irradiation at 540W for 6 min in dry and wet conditions was proved to be effective in the disinfection of soft lining material specimens contaminated with Staph. aureus, Ps. aeruginosa and B. subtilis. Wet treatment was more effective than dry one. Dry and wet microwave treatment sterilized specimens contaminated with C. albicans.

Key Words: Soft lining material, microwave sterilization and disinfection..

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INTRODUCTION

Soft lining materials are often used when treating patients unable to tolerate conventional heat – polymerized acrylic resin prostheses.[1] These materials are widely used to restore the health of inflamed and distorted denture – supporting tissues, to make dynamic impressions, as tissue conditioners, to maintain the proper fit of the denture, to prevent trauma and for trail evaluation of border extension.[2-4]

Additional uses of soft lining materials have emerged in the past few years, such as using for oral cancer patients with post-operative defects requiring obturation and to modify transitional prostheses after

stage I and stage II implant surgery.[5]

Soft lining materials have some disadvantage related to their physical properties and their response to microorganisms. They are easily colonized and infected by microorganisms. Investigations showed that these materials are more susceptible to the microbial adhesion than the acrylic resin.[6] This is because these materials have more porous surfaces than the conventional acrylic resins.[7] Studies have reported that yeast and bacterial species can enter porous spaces within the soft lining materials and that their colonization may reduce the intraoral life of the material[8] and vice versa, i.e., the aging of these materials can promote the colonization of microorganisms[9] and this may affect the underlying tissues causing denture – related stomatitis which may affect as many as one half of an elderly population of denture wearers[10-15]. Therefore, simple and effective denture disinfection procedures should be incorporated into the daily routines of dental office personnel and denture wearers to avoid a cycle of cross – contamination and prevent denture – related stomatitis.[16-19] However, maintaining cleanness of soft lining materials is difficult because of porosity, incompatibility with some types of denture cleansers and low abrasion resistance. This makes the use of disinfectant solutions as daily prostheses hygiene is not the best choice.[7,20] Therefore, due to the variable degradation of soft lining materials and the potential harmful consequences that occur, there is a need for improvement of these materials. Accordingly, many investigators were searching about more valid and convenient method of disinfecting the prostheses.

The microwave disinfection method is claimed to be a useful alternative to immersion disinfection. Microwave irradiation may be used for decontamination of food, microbiologic laboratory materials, dental instruments, underwear and clinical waste.[21]

Microwave irradiation has been suggested as a simple and effective method for denture disinfection, and different regimens have been tested.[22-25] More recently, studies have demonstrated that the effectiveness of microwave irradiation is improved when the specimens are irradiated while immersed in water.[7,21,25] Dixon *et al*.[7] found that 5 – minute wet microwave irradiation at full power effectively sterilized all the specimens contaminated with C. albicans. Neppelenbroek *et al*.[21] reported the same results after microwave irradiation of three hard chairside reline resins immersed in water. The specimens were contaminated with 3 types of bacteria in addition to C. albicans and microwaved in wet condition for 6 minutes at 650W. The same contamination and wet microwave regimn were tested by Silva et al[25] on simulated complete dentures. They differentiated between two terms, denture sterilization or disinfection, as sterilization is the process by which all forms of microorganisms, including viruses, bacteria, fungi and spores, are destroyed, while disinfection is the destruction of most but not necessarily all microorganisms; particularly the highly resistant microbial spores may survive. Accordingly, they observed sterilization of dentures contaminated with Staph. aureus and C. albicans and disinfection of the dentures contaminated with Ps. aeruginosa and B. subtilis.

The aim of this study was to determine the effectiveness of microwave sterilization (540 W for 6 min), in dry and wet conditions, on Molloplast – B soft lining material contaminated with Staph. aureus, Ps. aeroginosa, B. subtilis and C. albicans.

MATERIALS AND METHODS

1. Specimen Preparation:

Sixty specimens of the soft lining material were prepared. The selected material was Molloplast – B (DETAX GmbH and Co. K G, Germany). First, 60 circular specimens of 12 mm diameter and 3 mm thickness were prepared from silicone material. These silicone specimens were molded in dental die stone in metal dental flask. After the stone was set, the flasks were opened and the silicone specimens were removed carefully. Two coats of sodium alginate were used as a mold separator. Then Molloplast – B liner was packed, presses and cured according to manufacturer instruction. After polymerization, the flasks were bench cooled and then the soft

lining material specimens were removed carefully. The excess materials were trimmed by sharp scalpel gently.

2. Sterilization of Specimens:

All specimens were sterilized by autoclave at 121.5°C for 15 minutes[26]. To confirm the effectiveness of this procedure, specimens were added individually to 10 ml of Brain Heart Infusion Broth (Oxoid) in sterile test tubes, which were then incubated at 37°C for 7 days. At 48 hours and 7 day, the broths were evaluated for microbial growth (turbidity). No turbidity in the broth tubes was observed at 48 hours and 7 days (Figure 1).

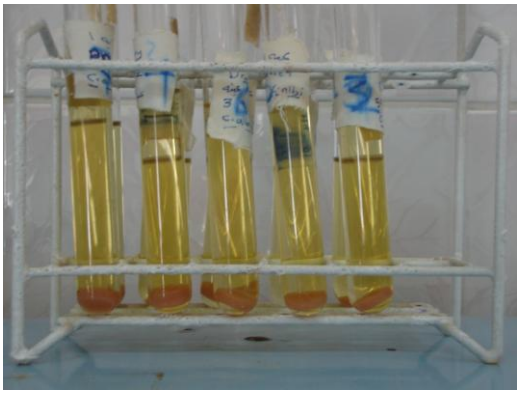

Figure (1): Test tubes containing specimens in (BHI) broth after 48 hours incubation to verify sterility; no growth was observed.

3. **Contamination and Microwave Disinfection Pocedures:**

The recently published Handbook of Disinfection and Antiseptics[27] recommended that gram – positive Staph. aureus, gram– negative Ps. aeruginosa, resistant spore B. subtilis and fungus C. albicans be used as indicators of this study were obtained from Department of Biology, Science College, University of Mosul. For more confidence, each of isolated microorganisms was recultured and then the suitable biochemical tests were done.[28]

On day 1, bacterial (Staph. aureus, Ps. Aeruginosa, B. subtilis) and yeast (C. albicans) isolates were individually inoculated to a turbidity of 0.5 of the McFarland standard[29], corresponding to 10^8 organisms/ml in 10 ml of BHI broth and incubated for 24 hours at 37°C. The following day 50 µl of inoculated BHI broth were transferred to each test tube containing 10 ml of sterile BHI broth. Each sterile specimen to be tested was especially placed into the test tube, sealed with foil and incubated for 24 hours at 37°C. The distribution of the specimens was done according to the type of microorganisms used for contamination and the microwave treatment.

The total sixty specimens were divided into 4 groups. Each group has 15 specimens contaminated with one type of involved microorganisms. Then the 15 specimens in each group was further divided into 3 subgroups:

1. Group C (Control) : The positive control group, 5 specimens were not treated by microwave after their contamination.
2. Group D (Dry): Dry microwave treatment, 5 specimens were contained in a dry beaker and placed on the rotational plate of the microwave and irradiated at 540W for 6 minutes after their contamination. The used microwave was Multiwave cooking/ 5 power level/ LG MODEL No. MS - 305A/Serial No. 305 KM 00157; Korea).
3. Group W (Wet): Wet microwave treatment, 5 specimens were contained in a beaker filled with 200 ml of distilled water[21] and irradiated in the same manner as group D (Figure 2).

Figure (2): A beaker containing group W specimens immersed in 200 ml of sterile distilled water to be microwaved.

Accordingly, after incubation of all the soft lining material specimens for 24 hours at 37°C, 40 specimens were selected for microwaving (20 specimens were undergone dry irradiation and 20 specimens were undergone wet irradiation) and the last 20 specimens were not microwaved (positive controls). The tubes containing positive control specimens (group C) were got vortex vigorously (Tucker instruments LTD/ England) for 1 minute and allowed to stand for 9 minutes, followed by a short vortex to resuspend and organisms present.[21] To determine the number of microorganisms in the 10^{-5} and 10^{-6} dilutions replicate specimens (100 µl) of the suspension were transferred to plates of 3 selective media, Nutrient agar media for Staph. aureus and B. subtilis, Mueller Hinton for Ps. aeruginosa and sabouraud agar containing 5µg/ml gentamicin for C. albicans. The plates were incubated at 37°C for 48 hours.

The microwaved specimens (group D and group W) were individually placed in sterile glass test tubes containing 10 ml of sterile BHI broth and treated identically to positive control specimens.

After incubation for 48 hours, bacteria and yeast colony counts of each plated specimen were quantified (Figures 3 and 4). The colony – forming units per milliliter (cfu/ml) were then calculated[30]. To verify the long – term effectiveness of dry and wet microwave sterilization, the BHI broth tubes with the microwaved cimens were incubated at 37°C for a ther 7 days. Cultures were interpreted by a single microbiologist as positive or tive growth.

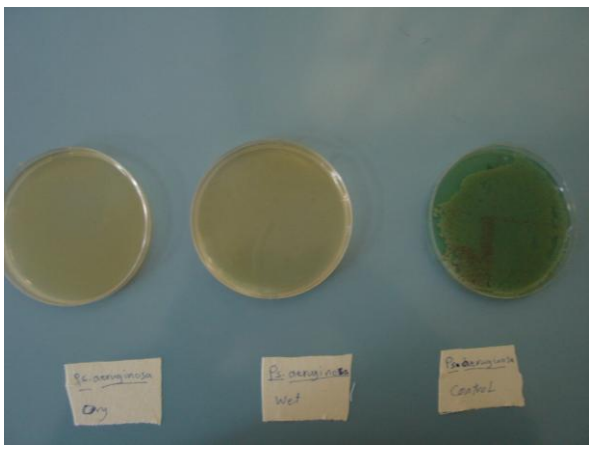

Figure (3): Mueller Hinton Agar plates exhibit Ps. aeruginosa growth from group C mens and no growth from group D and group W.

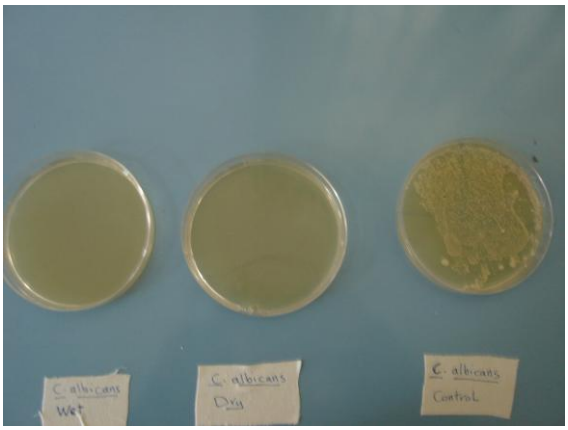

Figure (4): Sabouraud agar plates exhibit C. albicans growth from group C specimens and no growth from group D and group W.

Since the cfu/ml values among the positive control specimens had an inhomogenity distribution, a Post Hoc test one – way analysis of variance (ANOVA) at a 95% confidence level, on ranks was used. If significant differences in the cfu/ml numbers, Duncan method was performed to analyze the data.

RESULTS

Microwave irradiation at 540 W for 6 min. of Molloplast – B soft lining material specimens in dry and wet groups resulted in a significant reduction (at $p< 0.05$) of the cfu/ml of microorganisms on all specimens contaminated with individual suspension of Staph. aureus, Ps. aeruginosa, B. subtilis and C. albicans when compared with control group (Table 1). This reduction is equal in dry and wet groups of each tested organisms except in those contaminated with Staph. aureus which had significant difference in sterilization between dry and wet conditions.

Table (1) and Figure (5) explained no growth of Staph. aureus, Ps. aeruginosa and C. albicans exist after 6 min wet microwaving. Only B. subtilis showed some resistance.

Table (1): The mean and standard deviation of cfu/ml for each microorganisms in control (C) group and dry (D) and wet (W) microwave disinfected groups.

Microorganisms	Group	Mean	SD
Staph. aureus	**C**	63×10^8	3.6
	D	24×10^3*#	2.3
	W	0.00*#	0.0
Ps. aeruginosa	**C**	11×10^9	5.6
	D	0.00*	0.0
	W	0.00*	0.0
B. subtilis	**C**	72.4×10^8	5.2
	D	4×10^3*	0.6
	W	4×10^3*	0.6
C. albicans	**C**	48.2×10^6	0.0
	D	0.00*	0.0
	W	0.00*	0.0

*A significant difference exists at $p< 0.05$ between groups D and C,or groups W and C.; # A significant difference exists at $P< 0.05$ between group D and W.

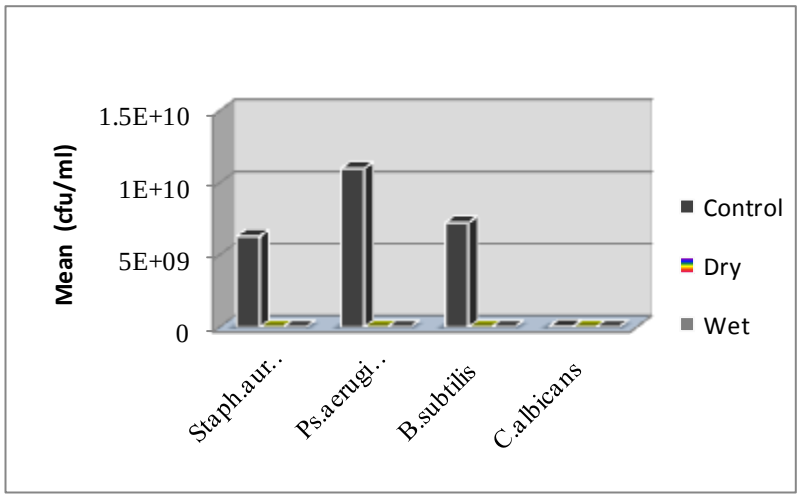

Figure (5): The mean of growth of each microorganisms on specimens of control (C) group, dry (D) and wet (W) microwave disinfected groups after 48 hours incubation.

Table (2) displayed the long – term effectiveness of dry and wet microwave sterilization. Results obtained after 7 days incubation of group D recorded surviving of organisms in all microwaved specimens (100% resistance) except those specimens contaminated with C. albicans.

Table (2): The growth of microorganisms on microwaved specimens of dry (D) and wet (W) microwave disinfected groups after 7 days incubation.

Microorganisms	Group	growth	Percentage (%) of resistance	Significany
Staph. Aureus	**D**	+ + + + +	100	Sig.
	W	+ + - - -	40	
Ps. Aeruginosa	**D**	+ + + + +	100	Sig.
	W	+ - - - -	20	
B. subtilis	**D**	+ + + + +	100	
	W	+ + + + +	100	————
C. albicans	**D**	- - - - -	0	————
	W	- - - - -	0	

+ Positive growth on culture media; - Negative growth on culture media

The results of wet irradiation were clearly variable B. subtilis showed 100% resistance, followed by Staph. aureus and Ps. aeruginosa (40% and 20% resistance respectively) which were significantly lower than those in dry condition. C. albicans appeared to be the most sensitive one among the tested microorganisms as it displayed 0% resistance in dry and wet microwaving (Table 2).

Positive group contaminated with individual suspensions showed substantial microbial growth on plates at 48 hours of incubation. There was no significant difference ($p > 0.05$) in cfu/ml mean values between Staph. aureus, Ps. aeruginosa and B. subtilis in positive control group. The mean numbers of cfu/ml for Staph. aureus, Ps. aeruginosa and B. subtilis were significantly ($p < 0.05$) higher than those observed for C. albicans (Table 1 and Figure 5).

DISCUSSION

This study was arranged as a crossover trail to reveal the real influence of microwave sterilization on microbe counts. We did not begin with in vivo study because patients often tend to improve the level of oral hygiene during this kind of study, which can lead to misinterpretation.

The soft lining material chosen for the present study was Molloplast – B. It is a methacryloxy propyl trimethoxy silane heat – polymerized silicone rubber. Its chemical properties account for its great compatibility with oral tissues and its dimensional stability, resiliency and compliance. Molloplast – B has shown long – term serviceability and stability, which to a great extent depend on proper manipulation during processing and good home care practices afterward.[31]

The selection of the microorganisms used in the present study was based on peer – reviewed scientific data regarding concepts of indicator and surrogate pathogen organisms, as well as, their intrinsic microbial resistance to validate the effectiveness of sterilization procedures.[27]

Rohrer and Bulard[22] demonstrated that the consistent sterilization could only be accomplished if the dentures were rotated in a three – dimensional manner within the microwave oven to avoid "cold spots or areas" where no bacteriocidal effect is achieved. Such a modified oven is not commercially available or practical for use by a person or healthy care facility. However, in this study a domestic microwave oven with a rotating table was used and this is commonly available.Procedures Similar to those carried out by Neppelenbroek *et al*.[21] and Silva *et al*.[25] were followed, as they also used a household oven.

The present study showed that dry microwave irradiation at 540W for 6 minutes of Molloplast – B soft lining material specimens contaminated with Staph. aureus, Ps. aeruginosa and B. subtilis resulted in an effective disinfection , but not sterilization . This treatment significantly restricted the growth but did not kill all the viable organisms as this was very clear after 7 –day incubation period when the microorganisms still survived in all the shared specimens.

Wet microwaving is better than dry one in that it produced significant disinfection of the growth of all tested bacteria and this disinfection still significant after 7 – day incubation in two of them; Staph. aureus and Ps. Aeruginosa .

B. subtilis showed some resistance after dry and wet microwaving and maximum growth among other bacteria after 7 –days incubation. Sporulated bacteria are more resistant than non sporulated bacteria because the spore is a resting cell, highly resistant to desiccation , heat and chemical agents.[32]

Consistent sterilization was proved only against C. albicans (cfu/ml = 0.0) (Table 1), which failed to grow even after 7 – days incubation. This effective sterilization was produced in dry and wet microwaving.

This investigation demonstrated that the wet microwave irradiation of Molloplast – B soft lining material for 6 min. at 540W setting produces effective disinfection against Staph. aureus and Ps. aeruginosa and effective sterilization against C. albicans which is believed to be the most important factor in the etiology of denture stomatitis; a pathogenic condition observed in more than half of healthy denture wearers[10-15] Therefore, this disinfection protocol may be a reliable alternative for the disinfection of the prostheses lined with this material.

The results of the present study confirmed Baysan *et al*.[23] findings that microwave exposure of soft lining material contaminated with Staph. aureus and C. albicans led to a greater reduction in the microorganisms counts than leaving the lining material dry overnight. Webb *et al*.[24] found that the microwaving of dentures for 6 min at medium setting (350W) does not remove non – viable C. albicans. While at high microwave setting (604 ± 92), C. albicans were be undetectable beginning from 2 min exposure time. These findings confirm our results regarding the long exposure time and high microwave setting be used in the present study. Also, the results supported Dixon *et al*.[7] investigation which recorded effective sterilization of all C. albicans contaminated Molloplast – B specimens with 6 min of wet irradiation.

The present study is in agreement with the findings of Neppelenbroek *et al*.[21] concerning wet microwave sterilization against C. albicans but it is in disagreement concerning sterilization against Staph. aureus, Ps. aeruginosa and B. subtilis. These differences could be attributed to the distinct processing of the specimens.

In Neppelenbroek *et al*.[21] study , the specimens were processed against acetate sheet and glass slab. This procedure resulted in a mirror – like finish of the specimens, which is less likely to facilitate microbial entrapment and retention than a surface with a higher roughness such as unpolished surfaces of specimens molded in die stone used in the present study. Therefore, a more clinically relevant in vitro approach is necessary to predicting the effectiveness of microwave sterilization. This explains why the results of this study were the nearest to Silva *et al*[25] results in that wet microwave irradiation of simulated complete dentures resulted in sterilization against C. albicans and Staph. aureus and disinfection against Ps. aeruginosa and B. subtilis. These differences may be due to the use of higher microwave setting (650W) in Silva *et al*[25] than that used in our study (540W).

On positive control specimens, the cfu/ml of C. albicans was significantly lower than those of Staph. aureus , Ps. aeruginosa and

B. subtilis. Larger yeast cells (5 to 10 μm) required larger surface defects to enhance their retention compared with small bacteria (0.5 to 3.0 μm), i.e., yeast cells are more easily dislodged from rough surfaces compared with smaller bacteria.[25]

Although the lethal action of microwaves on various microorganisms is well established, the mechanism of destruction is not completely understood. However, destruction of microorganisms, by microwave irradiation at temperatures lower than the thermal destruction point has been observed, which suggested that the destruction of the electromagnetic field with the molecules of the cells and the surrounding liquid medium, creating effects that could be caused by thermal action alone. Microwave ovens heat materials containing water by making the molecules vibrate 2 to 3 billion times a second, thus producing friction that results in the heating of water. The water started to boil after approximately 2 min., and this provided uniform heating of the specimens. The high temperature associated with the movements of molecules probably cause the water molecules to diffuse more rapidly into the material. Since cells contain water molecules, it can be assumed that they are vulnerable to microwave irradiation. Apparently, this was adequate to kill organisms even within the pores of the materials. Moreover, microorganisms generally contain high intracellular concentrations of ionizable compounds which may absorb microwave thermal heat at a much greater rate than a surrounding liquid medium such as distilled water. In addition, mechanical disruption would occur if the oscillations of the cells in the electromagnetic field were rapid enough and of sufficient displacement to exceed the elastic limitations of the cell wall.[25] Whether the nature of the lethality of the microwave irradiation for microorganisms in the present study molecular, mechanical, or selective heating, remains to be investigated.

Further investigations were needed to check if the microwave exposure time (6 min.) and setting (540W) can be increased to improve disinfection. Although no apparent deformation or color changes was observed on the microwaved specimens, the effect of microwave irradiation on the physical and mechanical properties of the soft lining materials also need further investigations.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions were drawn: Microwave irradiation at 540W for 6 min. in dry condition resulted in significant disinfection of soft lining material specimens contaminated with Staph. aureus, Ps. aeruginosa and B. subtilis. The same microwave treatment was used in wet condition produced more effective disinfection against Staph. aureus, Ps. aeruginosa and B. subtilis. Both dry and wet microwave treatment resulted in consistent sterilization against C. albicans.

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