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Effectiveness of Sterilization by Microwave Irradiation on Polyvinyl Siloxane Contaminated with *Candida Albicans*: An Invitro Study

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Abstract

Aims: This in-vitro study aimed to evaluate the effect of microwave irradiation on polyvinyl siloxane impression material contaminated with candida Albicans. **Materials and methods:** 30 samples were fabricated from the acrylic mold with disk shape 5*2 (diameter*thickness). Samples incubated with Brain Heart Infusion Broth (BHI) media containing Candida albicans. Then divided 30 samples into 6 groups: C (positive control); 5 non-irradiated specimens, 3MWD (samples irradiated for 3minute in dry condition), 6MWD (samples irradiated for 6minute in dry condition), 3MWW (samples irradiated for 3minute in wet condition), 6MWW (samples irradiated for 6minute in wet condition), CHX (samples immersed in 0.5% chlorhexidine) After incubation of all samples for 24 hours at 37°C, the samples were got vortex and then serial dilution carried out of suspensions then cultured on Sabouraude Dextrose Agar after incubation for 24 hours at 37°C bacteria were counted. A further 7 days of incubation for microwaved samples was done to verify the effectiveness of both dry and wet microwave sterilization for two periods of time. **Results**: There was a significant reduction in cfu /ml of candida albicans at 24 hours of incubation. No growth of C. albicans was recorded after 7 days of incubation. **Conclusions**: Microwave irradiation at 640W for both wet and dry conditions for 3 and 6 min was proved to be effective in the disinfection of polyvinyl siloxane specimens contaminated with. *Candida. albicans.*

تأثير التعقيم بالموجات الدقيقة على مادة البولى فينال سيليكون الملوثة بالمبيضات البيضاء (دراسة مختبرية)

الملخص

الأهداف: تهدف هذه الدراسة المختبرية الى تقييم تأثير التعقيم بالتشعيع بالموجات الدقيقة على مادة الطبعة السنية (بولي فينال سيليكون) السيليكون من النوع الإضافي الملوثة بالمبيضات البيضاء. **المواد وطرائق العمل:** تم تحضير ثلاثون عينة باستخدام قالب رغوي، كانت العينات بشكل أقراص بابعاد 5*2 (القطر * السمك) وعقمت بجهاز الموصدة بعد ذلك تم تحصين جميع العينات بالمبيضات البيضاء لمدة 24 ساعة، قسمت العينات الى ست مجاميع تحتوي كل مجموعة على خمس عينات وتشمل: مجموعة السيطرة واربع مجاميع للتعقيم بالموجات الدقيقة 640 وات (جاف ورطب) ومجموعة الكلورهكسيدين بنسبة 0.5% النقع لمدة ساعة. **النتائج:** أظهرت الدراسة ان هناك تأثير مطهر واضح على فطريات المبيضات البيضاء بعد 24 ساعة تحصين و وتأثير التعقيم بعد سبعة أيام تحضين. **الاستنتاجات:** اثبت أن التعقيم بالموجات الدقيقة 640 وات، بنوعيه (الجاف والرطب) لمدة 3 و6 دقائق لمادة الطبعة السنية السيليكونية (بولي فينال سيليكون) والملوثة بالمبيضات البيضاء، هو الأكثر فعالية.

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INTRODUCTION

Dental impressions are used to make replicas (models or casts) of oral tissues and teeth. In dentistry daily works, we take impressions of teeth and their supporting structures. The replicas are used to construct restorations and other prosthesis. (1)

Dental impressions, which are required during the laboratory stages of restorations, can contribute to the spread of infectious diseases between patients and dental workers. Pathogens such as E. coli Cytomegalovirus, hepatitis B and C viruses, and herpes simplex virus Types 1 and 2 viruses, as well as the human immunodeficiency virus, are all present. transmitted, endangering the health of dental laboratory personnel, mainly as a result of patient saliva and blood contact. (2)

Candida albicans is the most common etiological factor of opportunistic affected humans and causes fungal infections. (3)

Because the impression must be transmitted to the laboratory, it is fundamental to disinfect it. The majority of manufacturers advise using a specific disinfectant. Iodophor, bleach, or glutaraldehyde are examples of possible agents. If the necessary immersion period is followed and the impression is poured quickly, there appears to be little distortion. (4)

Microwave disinfection of polyvinyl siloxane impressions resulted in a disinfecting effect with no change in the physical properties of the impressions. Therefore, a microwave oven can serve as an effective disinfection tool for dental impression materials. (5)

MATERIALS AND METHODS

Approval of the present study was from the Scientific Research Committee / Department of Prosthodontics / College of Dentistry (UoM.Dent / DM. L.22/22)

Thirty specimens of Addition silicone impression material (light body, normal set: hydrophilic; elite HD + Zhermak) were prepared as circular (disk-shaped) 2 millimetres in thickness and 5 millimetres in diameter. (6)

Preparation is done by using a computerized program (corel DRAW, 2020 Corel Corporation) to design the Mold for specimens of the microbiological test. Image design was transferred to a computer-controlled Laser cutting machine (Boye Laser Application Technology Co., Ltd, China) used for cutting the hole that represented the sample dimensions in hardened acrylic mold, two layers of same hardened acrylic plates used as upper and lower covers. (7,8)

The impression materials were injected into the space of the mold by the tip of the syringe which was always immersed in the impression materials to prevent void formation duplication at the setting time, specimens were isolated and trimmed by a sharp scalpel Figure (1). (9,10)

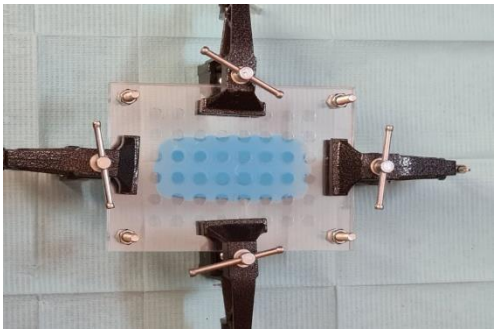

Figure (1): Acrylic Mold for microbiological samples with four pins and four G-clamps

All specimens were sterilized by autoclave (RAYPA steam sterilizer) at 121ºc for 15 minutes at 15 pounds/inch^2 /21ºC., then transmitted to sterile vials containing 10 ml brain heart infusion broth. (11)

The procedure needed four days to complete except the day of sample preparation, brain heart infusion, and Sabouraude Dextrose Agar preparation. Here listed the steps according to the days of the procedure:

1. *Candida albicans* (12) was inoculated to the turbidity of 0.5 ml of McFarland standard which corresponds to 10^8 organism/ml in 10ml of distal water, then incubated in 37ºc in an incubator (BINDER Gmbh).
2. Transferred 50µl of incubated media to 10 ml of brain heart infusion broth which contained a sterile sample for each group, closed test tube, and incubated for 24 hours at 37º C. (13)
3. Samples and then further divided into subgroups:

- Control groups: five samples for each microorganism without any sterilization methods neither by microwave nor chlorhexidine
- Dry microwave: 680W further divided into Three-minute groups: had five samples were placed in a dry sterile tube in the center of the rotational plate of the microwave in the same manner as 3min group, the procedure took place Six minutes
- Wet microwave also sub-divided into Three minute transferred samples to a sterile test tube with 10 ml of sterile distal water for Six minutes as the above procedure done with a 6-minute microwave
- Chlorhexidine group five samples immersed in 0.5 % Chlorhexidine (GLUCO-CHeX2%) for one hour in 10ml of CHX. (14)

After completing the grouping and sterilization method, each specimen except the control group was transferred to a tube containing 10 ml of sterile brain heart infusion broth were got vortex vigorously (DRAGON lab MG-X) for one minute and stood off for nine minutes then short vortex undergone to resuspend all microorganism. Then serial dilution started. (13,14)

4. Reading the results by colony forming unit (CFU)/ml using this equation:
CFU/ml=numbers of microorganisms in each quadrant *10*4*inverted mitigation.

All samples treated in microwave dry and wet groups were incubated for 7 days at 37ºc then cultures were recorded as positive or negative results (Figures 2 and 3).

Figure (2): Sample with brain heart broth in an incubator after seven days of microwave sterilization.

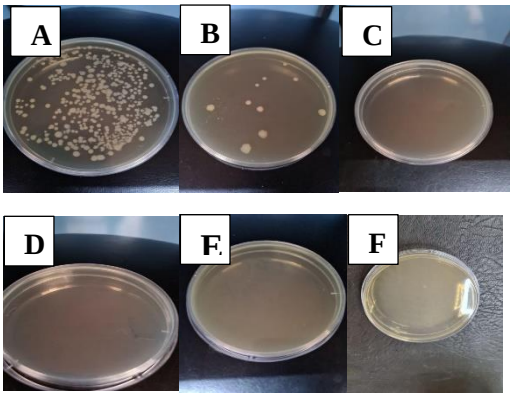

Figure (3): A: Control, B: Three-minute dry microwave, C: Six-minute dry microwave, D: Three-minute wet microwave, E: Six-minute wet microwave, F: Chlourhixidine 0.5%.

RESULTS

The mean range and standard deviation of colony forming unit per millimetres (CFU/ml) of antifungal effect of microwave on polyvinyl siloxane showed in Table (1) Figure (1) by using SPSS statistic software version 23 (IMB, USA) to analyses the results. Kruskal–Wallis analysis of variance was used to compare the control group and tested groups (microwaved groups and CHX group). Table (1), showed there was a statistically significant difference between control and microwaved groups. Microwave decreased the number of colonies forming unit to zero in both dry and wet conditions. Three minutes in dry microwave condition had less effect on CFU/ml counting of *Candida albicans.*

Table (1): Means, standard deviation, and Kruskal– Wallis test of microwave antifungal effect against *Candida albicans*.

Fungi	Group	Mean	SD	K-W	Sig
Candida albicans	C	6.751e+11	1.070e+11	26.26	0.000**
	3 MWD	28000	34387.4977		
	3 MWW	0	0		
	6 MWD	0	0		
	6 MWW	0	0		
	CHX	0	0		

Table (2): showed the effect of microwave after seven days of disinfection of microwave groups. *Candida albicans* appeared more sensitive to sterilization in wet condition either three or six minutes. But reappeared to growth in 6minute dry condition at 60% resistance, more resistance group was 3minute dry microwave with 100% resistance.

Table (2): Fungal growth present after one week.

Fungi	Group	Growth	Present %	Significant
Candida	3 MWD	+++++	100	-
	6 MWD	+++--	60	-
	3 MWW	-----	0	-
	6 MWW	-----	0	-

DISCUSSION

The previous Tables' results, concluded that the lethal effect of microwave against *Candida albicans* decreased in colony-forming units of *Candida albicans* in all wet conditions of microwave. the differences were in dry conditions when 3MWD was not enough for *Candida*

albicans to disappear, it needed six minutes in dry conditions to decrease to zero

Microwave ovens increase the temperature of water-containing materials by vibrating the molecules 2 to 3 billion times per second. causing friction, which causes water to heat up After that, the water began to boil after about 2 minutes, and this supplied heating the object or sample uniformly.

Microwave irradiation has many directions of action one of them is the direct effect on the biological state of bacteria and end lethal effect, other effects due to electrical field generation that influenced the charge distribution later affect the permeability of cell membrane that alter the function of Na-K pump and disabled cell membrane. Even the growth of bacteria can be affected by irradiation when destroys or alters the normal metabolism of bacteria which leads to death. [15,16]

CONCLUSION

Within the limitation of this study, colony-forming units of *Candida albicans* were decreased after sterilization with 640W microwave irradiation. Resistance of Candida albicans was observed after 7 days of incubation in dry conditions.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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