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Effect of microwave irradiation on disinfection, dimensional accuracy, and surface porosity of dental casts

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Abstract

To evaluate the effectiveness of different microwave irradiation exposure times on the disinfection of dental stone samples immersed in different solutions, and its affect on the dimensional accuracy and surface porosity.

Dental stone casts were inoculated with an isolate of *Bacillus subtilis* to examine the efficiency of microwave irradiation as a disinfection method while immersed in different solutions; water, 40% sodium chloride, or without immersion for different durations. Dimensional accuracy and surface porosity were also evaluated.

Significant reduction in colony counts of *Bacillus subtilis* were observed after 5 minutes of microwave irradiation of immersed dental casts in water and NaCl solution. No evidence of growth was observed after 10 minutes while immersed in water or NaCl solution. Dimensional accuracy of dental stone was significantly affected by immersion in water for 5 or 10 minutes during exposure to microwave irradiation while it was insignificant affected by immersed in NaCl solution, or in dry air. The surface porosity of dental stone was significantly affected by the immersed in water and NaCl solution for 5 or 10 minutes while it was insignificantly affected by dry conditions during microwave disinfection.

Immersion the dental stone casts improved the effectiveness of microwave irradiation as a disinfection method. The dimensional accuracy and surface porosity was adversely affected but within the clinical limitation.

Key words: microwave, dental, cast, *Bacillus subtilis*.

Introduction

Dental professionals, auxiliaries, and laboratory personnel are at risk of infection with pathogens, including hepatitis B virus, hepatitis C virus, Human Immunodeficiency Virus, herpes simplex and tubercle bacteria which can spread by saliva or blood from a patient as droplets and aerosols.[1] Dental impressions, stone casts…etc transmit these pathogenic microorganisms from the dental office to the dental laboratory.[2,3,4,5] Contamination of the dental stone cast could occur several times in each appointment during fabrication of the prosthesis. If contaminated items were to enter the dental laboratory environment, than the infectious

materials could spread to a prostheses or appliances of another patient.[3,6,7]

The use of effective infection control procedures in the dental office and dental laboratory to prevent cross contamination was recommended by the American Dental Association (ADA). Also, the Centers for Disease Control and Prevention and the American Dental Association (ADA) recommend immersion in or spraying with a disinfectant solution (hypochlorite or iodophor), as methods for disinfection of dental stone casts.[8,9,10] Dental cast disinfection could be either indirectly by disinfecting the impression material or directly by disinfecting the dental stone cast. However, dental impression disinfection was a weak point in the dental hygiene chain since not all impression materials can be disinfected without adversely affecting the properties of the materials.[11,12,13,14]

Several researchers studied the effect of different disinfection procedures on the physical properties, dimensional stability and surface porosity of dental stone. Ivanovski et al.[6] stated that the incorporation of sodium hypochlorite to dental stone resulted in alteration in the physical properties of the set cast. Also they found that glutaraldehyde was the most suited solution for dental stone disinfection, however due to its toxicity there were limitations in using it on a day –to- day basis in the clinic and laboratory.

Taylor et al.[15] worked on disinfection procedures of hydrocolloid impression materials and gypsum casts and found that immersion of the impression or dipping in 1% sodium hypochlorite lead to improved dimensional accuracy of resultant casts but with poor surface quality.

Lucas et al.[16] analyzed the influence of incorporation of different disinfectants into dental stone on dimensional stability and surface quality of dental cast. They found that the addition of sodium hypochlorite negatively altered these properties.

Abdullah[17] studied the surface quality and dimensional accuracy of gypsum casts after repeated immersion in sodium hypochlorite solution. The results of his study demonstrated that repeated immersion caused significant increase in linear dimension and some degree of damage to surface quality of dental stone.

The damaging effects of the immersion technique,[15,17,18] the difficulty in covering the entire surface with the spray disinfecting solution,[18,19] the negative effects of disinfectant additives to the stone mix on the properties of dental casts,[16,20,21,22,23] and the inability to assume that every dental cast presented to the laboratory was disinfected led to the need for an easy, simple, and effective disinfection method which could be used in the dental office and dental laboratory. Microwave irradiation was introduced as a solution for this need by Rohrer & Bulard[24] whom found that microwave radiation could easily kill fungi, viruses, and aerobic and anaerobic bacteria, including spore formers, on the acrylic denture surface with proper modifications. Furthermore, Watanabe et al.[25] found that the presence of NaCl in water accelerated the rate of temperature increase and cell inactivation when disinfecting with microwave irradiation.

This research was designed to investigate the effectiveness of microwave irradiation as a disinfection method for contaminated dental stone casts that were immersed in different solutions and to show the effect at different exposure time on this process. Also, this research was aimed at studying the effect of microwave disinfection on the dimensional

accuracy and surface porosity of type III dental stone.

Materials & Methods

Bactericidal Efficiency

A pilot study was conducted to determine the effective exposure time for microwave disinfection; 1, 2, 3, 4, 5, 10, and 15 minutes respectively, as shown in table (1). The five and ten minute exposure time were selected from the results of this pilot study.

The isolate of *Bacillus subtilis* bacteria used in this research was obtained from the Central Teaching Laboratories of Baghdad Hospital. The bacterium was activated by inoculation of screw capped tubes containing 15 ml nutrient broth and incubation overnight at 35˚C.

This isolate was used to contaminate the dental casts indirectly by contamination of the dental impressions. Irreversible hydrocolloid impression material, alginate (ALGINMAX, Major Prodotti Dentari S.P.A, Italy), was used to make these impressions with a rim lock stock tray for a maxillary master cast. The alginate impressions were individually inoculated with *Bacillus subtilis* broth (1×10^8 cell/ml) one milliliter of bacterial suspension was added to each impression and incubated for 5 minutes at 35˚C under static conditions.[26] The broth was then removed from the impressions and type III dental stone (Zeus sri Loc. Tamburino Roccastrada (GR) Italy) was mixed with water and poured into the impressions according to the manufacturer's instructions. The dental stone casts were removed from the impressions after one hour. Twenty one dental casts were prepared and divided into seven test groups. Each test group included three dental stone casts treated in a different manner, as shown in table (2). This was according to the time of exposure to microwave irradiation and the conditions used simultaneously with the irradiation; immersion in sterile distilled water, 40% NaCl solution or exposed to dry air condition.

The Contaminated dental casts were individually transferred to a Pyrex beaker containing 400ml of sterile distilled water, 400ml 40% NaCl solution, or exposed to a dry air condition adjacent to a beaker of 400ml water. Each beaker was placed in a microwave oven (MB-04344B, LG Electronics Inc.) and irradiated at 850W according to the test groups in table (2).

The dental cast surfaces were vigorously rubbed for 1 minute with a sterile cotton swab then placed in tubes containing 5 ml sterile saline solution. Replicate aliquots (25 microl) of suspensions were plated at dilutions of 10^{-1} to 10^{-6} on plates of nutrient agar. Following the same procedure as above, a first collection of biological material from each dental cast was made before disinfection treatment to insure the presence of bacterial growth at that time. All plates were incubated aerobically at 35˚C for 48 hours, and the presence of *Bacillus subtilis* was investigated by viable count through streaking on nutrient agar plates.[27] The colonies were counted after incubation and the logarithm of colony-forming units per milliliter (log cfu/ml) was then calculated.

Dimensional accuracy

A die was used to make dental stone samples for the evaluation of the dimensional accuracy, according to American Dental Association Specification (ADA) No.19.[28] The die was approximately 30 mm in diameter, on which were engraved 3 parallel lines, X, Y, and Z and two cross lines of (cd) and (c´d´), as seen in figure (1).

One of the objectives of this test was to study the effect of microwave disinfection on dental stone type III, so the effect of the impression material was eliminated by the formation of test stone samples directly onto the test block.

Prior to fabrication of each specimen, the surface of the die was cleaned with cotton gauze soaked in methyl alcohol, rinsed with distilled water, and gently dried with compressed air. Type III dental stone (Zeus sri Loc. Tamburino Roccastrada (GR) Italy) was mixed with water according to manufacturer's instructions and poured into a 30 mm diameter rubber ring of 20 mm height onto the test block. The sample was separated from the die after 1 hour and left for 24 hour at an average room temperature of 17˚C and average relative humidity of 71 % before measurement and during the duration of the testing period.

Thirty samples were subjected to the same microwave disinfection conditions used previously in the bactericidal test, as shown in table (2). Five samples for each test group were used for dimensional accuracy.

Two measurements were recorded for each stone sample; the first was before the disinfection procedure and the second was after one hour of disinfection. The samples were scanned with a scanner to achieve a digital picture from which the measurement of the distance (cd) - (c´d´) was obtained with the computer program Corel Draw X3 version 13.

Surface porosity

Type III dental stone (Zeus sri Loc. Tamburino Roccastrada (GR) Italy) was mixed according to the manufacturer's instructions and poured into a 30 mm diameter rubber ring of 20 mm height with a glass slab as a base. A total of 30 samples were made, five samples for each test group, as in table (2).

Each stone sample was scanned with a scanner twice; before and after one hour of the disinfection procedures. Then, with the use of the program Corel Draw X3 version 13, a circle was drawn to outline the outer border of the sample. In the center of this circle another circle was drawn with a diameter of 4 mm. Surface porosity was assessed by counting the number of pores inside the smaller circle. Surface porosity of each stone sample was read twice and an average of the two attempts was obtained for each of stone samples. Thus an average was obtained before disinfection and one after.

Statistical analysis included descriptive statistics, paired sample t-test, one-way analysis of variance (ANOVA), and multiple comparison tests utilizing the least significant difference test (LSD) and at a significance level of $p<.05$.

Results

The results of the pilot study are summarized in table (1).

Results in table (3, 4, & 5) and fig.(2) showed that growth of *Bacillus subtilis* was eliminated completely when the dental stone casts were exposed to conditions illustrated by groups $M_{10}W$ and $M_{10}Na$. This was highly significantly different ($p<.01$), when compared with the control group. This meant that microwaving immersed dental casts for 10 minute or more may achieve complete sterilization. The growth of *Bacillus subtilis* on the dental casts was reduced when exposed to microwave irradiation for 5 minute in a moistened condition (M_5Na and M_5W) or for 10 minute in a dry condition ($M_{10}D$), although this

was insignificantly different ($p>.05$) from the control group.

The dimensional accuracy of dental stone was significantly ($p<.01$) affected by immersion in water for 5 or 10 minutes during microwave disinfection procedure, while it was insignificantly ($p>.05$) affected by the other microwave disinfection conditions (Na5, Na10, D5, &D10) groups. As shown in table (6 & 7) and figure (3).

The surface porosity of the dental stone was significantly ($p<.05$) increased after microwave disinfection procedures for all test groups, immersed in water or NaCl solution, except for D5 and D10 which were not significantly affected ($p>.05$) when compared with the control group, as seen in table (8 & 9), figure (4).

Discussion

The pilot study was conducted to examine for any microbial growth after microwave irradiation. This differed from the rest of the study which depended on the colony forming unit per milliliter for assessing bactericidal efficiency of microwave irradiation. In the pilot study 10 minutes exposure to microwave irradiation resulted in sterilization of all immersed dental casts (MW and MNa groups). On contrary, the rest of the test groups which were irradiated in dry conditions (MD) were not sterilized even when the exposure time was increased to 15 minutes (table 1). It was clear that increasing the time of exposure to 15 minutes had no additional effect on those groups. According to the obtained results, we depended on the five and ten minutes of exposure in our study.

The *Bacillus subtilis* was chosen in this study because it is easily cultured, safe to use, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Also they were regularly used in testing autoclave efficiency.[29]

The present study evaluated the in vitro effectiveness of microwave irradiation on the disinfection of dental stone casts and showed that a 10 minute microwaving cycle at 850 W was capable of sterilizing all dental stone casts that were immersed in water or 40% NaCl solution, while exposure of the contaminated casts to microwave radiation in a dry condition had less effect on colony count of *Bacillus subtilis*. This could be the result of the lethal effect of the heat generated from microwave irradiations on the microorganisms.[30] Furthermore, microwave irradiation had a direct effect on the DNA, protein, and RNA of microorganisms.[31] The presence of molecules of water around the samples played a major role in absorbing the energy from microwave radiation and the friction of these molecules produced heat that could inactivate microorganisms.[32] The presence of the NaCl ions in the water enhanced microwave irradiation efficiency on inactivation of the microorganisms.[25] This didn't seem to have any effect on the outcome because the NaCl solution and water both gave similar results. The fact that dissolution of the stone cast in water occurred during irradiation and this generated free ions which could have enhance the effect of the microwave irradiation. This was supported by our finding that surface porosity significantly increased after irradiation of the stone samples while immersed in water, as seen from the results of this study.

Berg et al.[33] showed that microwaving gypsum casts had a bactericidal effect at 900W for 5 minute.

Disinfection was observed after 5 minutes in the present investigation,

although some in vitro studies have demonstrated sterilization of specimens after 3 minutes of exposure to microwave irradiation.[34,35] It may be speculated that this was due to the high resistance of the isolate under investigation and 3 minutes of microwaving was not sufficient to cause their inactivation and microbiological growth was observed. Also, one of these studies used *Candida albicans* and they were conducted on acrylic resin while this research was on dental stone type III.

A study by Davis et al.[36] showed that microwave radiation was ineffective in sterilizing dental casts inoculated with *Bacillus subtilis*. The differences in results may due to the differences in resistance among different isolates of *Bacillus subtilis* spp. They used different microwave energy without immersion and at different durations. They also didn't use standardized inoculum on their stone casts and reduction in number of organisms couldn't be measured reliably, although they noticed less microbial growth for 20 minutes of microwave exposure.

The immersion in water for 5 or 10 minutes during microwave irradiation significantly affected the dimensional accuracy of dental stone, contraction was by 0.2131% for 5 minute irradiation and 0.2862% for 10 minutes irradiation, figure(3). The contraction took place because the surface went back to the subhydrate form of $CaSO_4$ and this was due to the prolonged heating of the gypsum product.[37]

The surface porosity of the dental stone samples was significantly higher for W_5, W_{10} Na_5, &Na_{10}. This may be related to the rapid escape of free water from the stone surface during exposure to the microwave radiation or due to the rapid boiling of the free water which may have generated cracks and holes on the surface of the dental stone cast. Our findings were in conformity with the findings of Luebke et al.[38,39] whose photographs of the sample surfaces revealed that the surface of the dental stone had cracks and holes produced from microwave irradiation.

The findings of this investigation concluded that microwaving for 10 minute was an effective method for sterilization of moistened dental casts with least adverse effect on its dimensional accuracy and surface porosity, whereas microwave irradiation for 5 minutes only achieved cast disinfection.

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Table (1). Pilot study of *Bacillus subtilis* growth after different microwave irradiation exposure time

Microwave disinfection treatment	**Exposure time in minutes**						
	1	**2**	**3**	**4**	**5**	**10**	**15**
No treat.	+	+	+	+	+	+	+
MD	+	+	+	+	+	+	+
MW	+	+	+	+	+	-	-
MNa	+	+	+	+	+	-	-

No treat.: dry air exposure (no treatment), MD: microwaved in dry condition, MW: microwaved while immersed in water, MNa: microwaved while immersed in 40%NaCl solution.

Table (2). Test groups and different disinfection procedures.

Test group	**Disinfection procedure**
No treat.	No microwave disinfection & exposed to dry air for 10min. (control group)
M_5D	Five minutes of microwave irradiation in dry air condition.
M_5W	Five minutes of microwave irradiation during immersion in distilled water.
M_5Na	Five minutes of microwave irradiation during immersion in 40% NaCl solution.
$M_{10}D$	Ten minutes of microwave irradiation in dry air condition.
$M_{10}W$	Ten minutes of microwave irradiation during immersion in distilled water.
$M_{10}Na$	Ten minutes of microwave irradiation during immersion in 40%NaCl solution.

Table (3). Mean and standard deviations of viable count of *Bacillus subtilis* cultivated from casts after different disinfection regimens.

Test groups	**Mean**	**S D**
No treat.	2.605	.065
M_5D	3.869	.006
M_5W	1.518	1.319
M_5Na	1.340	1.277
$M_{10}D$	1.651	1.484
$M_{10}W$	.000	.000
$M_{10}Na$	.000	.000

Table (4). One- way analysis of variance test of viable count of *Bacillus subtilis* for all test groups.

	Sum of Squares	F	Sig.
Between Groups	46.475	9.142	.00
Within Groups	11.438		
Total	57.913		

p>.05 (insignificant), p<.05 (significant), and p<.01 (highly significant)

Table (5). LSD analysis of viable count of *Bacillus subtilis* for the test groups under investigation.

	Mean Diff.	Std. Error	Sig.
No treat.-M_5W	1.086	.650	.112
No treat.-M_5Na	1.265	.650	.068
No treat.-M_5D	-1.263	.650	.068
No treat.-$M_{10}W$	2.605	.650	.001
No treat.-$M_{10}D$	.954	.650	.160
No treat.-$M_{10}Na$	2.605	.650	.001

p>.05 (insignificant), p<.05 (significant), and p<.01 (highly significant)

Table (6). Mean and standard deviation statistics for dimensional accuracy

Test groups	**Mean**	**S D**
W_5b	19.800	.015
W_5a	19.758	.020
$W_{10}b$	19.841	.024
$W_{10}a$	19.784	.015
Na_5b	19.757	.029
Na_5a	19.756	.027
$Na_{10}b$	19.801	.044
$Na_{10}a$	19.821	.054
D_5b	19.771	.037
D_5a	19.754	.046
$D_{10}b$	19.823	.029
$D_{10}a$	19.810	.041

a; after, b; before

Table (7). Paired samples t-test for dimensional accuracy

Test groups	**Mean Dif.**	**Std. Err.**	**t**	**Sig.**
W_5b - W_5a	12.346	.003	12.346	.000
$W_{10}b$ - $W_{10}a$	11.140	.005	11.140	.000
Na_5b - Na_5a	.112	.009	.112	.916
$Na_{10}b$-$Na_{10}a$	-2.017	.010	-2.017	.114
D_5b - D_5a	1.300	.013	1.300	.263
$D_{10}b$ - $D_{10}a$	1.006	.012	1.006	.371

p>.05 (insignificant), p<.05 (significant), and p<.01 (highly significant)

Table (8). Mean and standard deviation statistics for surface porosity

Test groups	Mean	S D
W_5b	1.0	1.224
W_5a	13.2	6.220
$W_{10}b$	1.6	1.816
$W_{10}a$	11.2	3.114
Na_5b	.8	.836
Na_5a	13.0	4.582
$Na_{10}b$	.4	.547
$Na_{10}a$	11.8	6.685
D_5b	.2	.447
D_5a	5.2	7.328
$D_{10}b$	.4	.547
$D_{10}a$	4.2	4.324

a; after, b; before

Table (9). Paired samples t-test for surface porosity

Test groups	Mean Dif.	Std. Err.	t	Sig.
W_5b-W_5a	-12.2	2.537	-4.807	.009
$W_{10}b$-$W_{10}a$	-9.6	1.913	-5.018	.007
Na_5b-Na_5a	-12.2	2.332	-5.231	.006
$Na_{10}b$-$Na_{10}a$	-11.4	3.026	-3.767	.020
D_5b-D_5a	-5.0	3.316	-1.508	.206
$D_{10}b$-$D_{10}a$	-3.8	1.984	-1.914	.128

p>.05 (insignificant), p<.05 (significant), and p<.01 (highly significant)

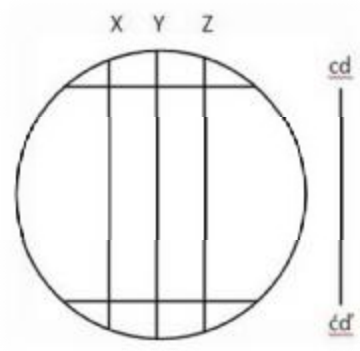

Figure (1). Diagram of die for dimensional accuracy

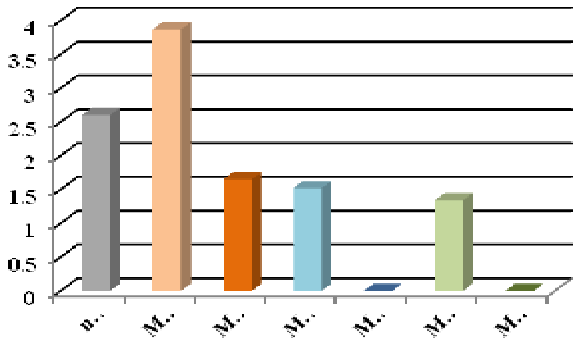

Figure (2). Viable count of *Bacillus subtilis* after different microwave disinfection procedures.

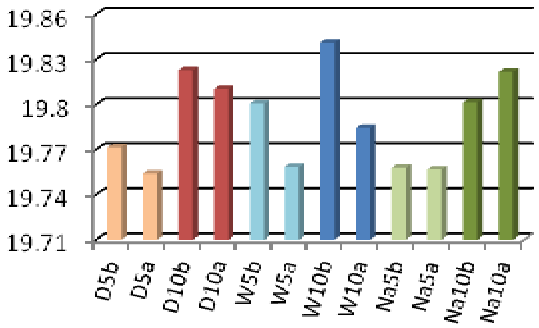

Figure (3). Dimensional accuracy of dental stone

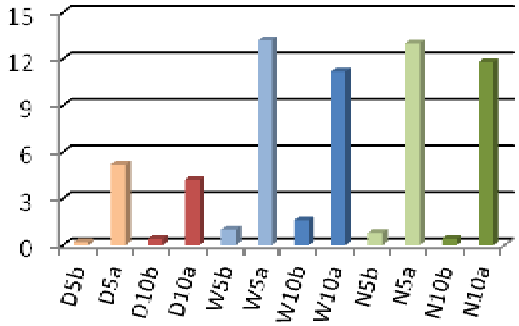

Figure (4) Surface porosity of dental stone