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Antimicrobial efficacy and surface roughness of type III & type IV gypsum products when subjected to microwave radiation for effective disinfection

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**Conflicts of Interest:** Nil

### Abstract

**Introduction:** A dental practitioner is exposed to a wide range of bacteria as they mainly deal with the oral environment which harbours mainly harmful bacteria. The present study is to know the efficacy of microwave radiation on dental cast and its effect on surface roughness. **Materials and method:** Type III and Type IV gypsum products were used to make the samples and were exposed to two different micro-organisms i.e Staphylococcus aureus and Candida albicans. All the samples were subjected to microwave radiation at 3, 5 and 7mins time intervals respectively at 900watts.The control and test group were evaluated to know the effect of microwave radiation and its effect on surface details of the samples which was evaluated with profilometer.

**Results:** Statistical analysis was done by Repeated ANOVA and P value was found significant (p<0.01) indicating decrease in microbial load after irradiation and change in surface roughness at 3 different time intervals.

**Conclusion:** From this study we infer that there was markable changes in surface roughness as the irradiation time increased and the least time 3mins had effective elimination of all the microorganisms.

**Keywords:** Microwave irradiation, surface roughness, Type III & Type IV gypsum products, microorganisms.

#### Introduction

Gypsum products are widely used as materials for the preparation of models in dentistry. Though it is recommended to disinfect the impression, due to inherent with various disinfectants. Still there might be scope for transfer of microorganisms on to the casts. Disinfection of casts that are poured after impression making is important to prevent cross contamination and spread of infection<sup>(1)</sup>. The potential for cross-contamination with dental casts is especially prevalent in prosthodontics because of multiple opportunities for the transfer of infectious agents from saliva to dental casts. Hence disinfection of these cast should be done after each clinical and laboratory procedures. Spraying the disinfecting solution on the casts, usage of die stone that contains disinfectant, immersing the casts in disinfecting solution or incorporating chemicals into gypsum at the time of mixing, microwave radiation are various methods recommended for disinfection of dental casts <sup>(2)</sup>. Some of these procedures resulted in adverse effect on strength, hardness and roughness of gypsum materials. It is important that disinfectant solutions be effective as antimicrobial agents, but not depreciate the physical property of gypsum cast or significantly alter the accuracy of the casts. Gas sterilization is costly and time consuming. Microwave sterilization may be an alternative that might prove to be a more viable option for sterilizing dental stone casts <sup>(3)</sup>.

#### Materials and methods

A total of 160 samples were prepared with Type III and Type IV gypsum products with dimension of 40\*10\*2mm (Figure 1) under aseptic conditions. Specimens were further divided into 2 groups (n=80) based on the tests performed for Type III and Type IV gypsum products i.e antimicrobial efficacy and Surface roughness. They were again subdivided into four subgroups of 10 samples each based on the time interval of microwave radiation i.e, control, 3mins, 5min and 7mins respectively.

#### Microbiological method

Micro-organisms used in this study are Staphylococcus aureus and Candida albicans. Standard cultures of bacteria (S. aureus, and yeast (C. albicans) were individually inoculated in blood agar for bacteria and Sabouraud broth (Figure 2) for fungus. The turbidity of the broths was adjusted using a 0.5 Mcfarland standard turbidity tube corresponding to 105 organism/ml in 10 ml of broth. The tubes were incubated at 37 degrees for 24hrs. Using sterile forceps, one specimen was transferred in each test tube. 1 ml of appropriate broth and standard inoculums of organism was added in respective test samples using micropippete (1 ml) and the tubes were incubated. The specimens incubated were collected in containers filled with distilled water for microwave exposure. The samples were individually exposed to microwaves in a domestic microwave according to the different exposure parameters i.e 900 watt and for three intervals (3mins, 5mins and 7mins). Plates of selective media, Blood agar for bacteria and Sabouraud agar for yeast were prepared in Petri dishes and were cultured in plates by loop inoculation (Figure 3). Broth from the tubes were transferred on the plate in using

 $25 \ \mu$ l micropippete. The Petri dishes were incubated at  $37 \ C$  for 48 hr for fungi and 24 hr for bacteria. Colonies on each labeled section of the plates were counted by using a magnifying lens (Figure 4). Each count was multiplied with the dilution factor (100) and expressed as CFU/ml.

### Surface roughness

The prepared samples were subjected to microwave radiation for 3mins, 5mins and 7mins. The control samples were remained unexposed. Evaluation of surface roughness was done by using profilometer (Figure 5). The diamond styli of the profilometer moves about 3mm to and fro and evaluates the roughness of the samples and the values are displayed.

#### Results

Mean and standard deviation were employed to describe the data. The obtained antimicrobial efficiency, and surface roughness values were tabulated and statistically analysed between the study groups by using repeated ANOVA test. The mean microbial load of candida on type 3 gypsum product at 3 mins is 56 whereas the sample unexposed to radiation(control) is 111.5. The mean microbial load of candida on type 4 at 3mins is 46.50 and the control group has 108.70. There was a statistically significant difference seen in all the time intervals (p<0.01) (Table-1, Graph-1). The mean microbial load of staphylococcus on type 3 gypsum product at 3 mins is 2.45 and the sample unexposed to radiation(control) is 26.80. The mean microbial load of Staphylococcus on type 4 at 3mins is 2.40 and the control group has 25.60. There was a statistically significant difference seen in all the time intervals (p<0.01) (Table-2, Graph-2). The mean surface roughness of type 3 gypsum product at 3 mins is 1.62, at 5mins is 1.68 and at 7mins is 2.10 whereas the sample unexposed to radiation(control) is 1.54. The mean surface roughness of type 4 at 3mins is 1.00, at 5mins is 1.53, at 7mins is 1.64 and the control group has 0.94 (Table-3, Graph-3). There was a statistically significant difference seen in all the time intervals (p<0.01).

#### Discussion

The dentists and health workers are exposed to wide variety of potentially dangerous microorganisms. The general routes for transmission of microbial agents in dental clinics are as follows: Direct contact with infectious lesions or infected saliva or blood. Indirect transmission via transfer of micro-organisms from contaminated objects including impressions, impression trays and gypsum casts. Cast poured against contaminated impression have shown micro-organisms therefore disinfection of cast is an important measure for the control of cross-contamination <sup>(4)</sup>. Two modes of action of microwaves for sterilization is described. This include thermal effect which is conversion of microwave energy into heat by prolonged kinetic motion of polar molecules and non-thermal effect by direct interaction of electromagnetic field with the biologic molecule, creating effects that cannot be caused by thermal action alone. The suggested possible mechanism include: (1) Denaturation of proteins. (2)

Cell wall membrane phenomenon involving loss in selective permeability and molecular resonance resulting in cleavage. (3) Certain intracellular changes e.g., orientation of cell organelles.

A viable cell count is a measure of number of living cells capable of multiplying and producing a visible colony of cells in a sample. It is commonly estimated by spreading a known volume of cell suspension onto an agar plate and counting the number of colonies that arise after a period of incubation. A viable cell count is a measure of number of living cells capable of multiplying and producing a visible colony of cells in a sample. It is commonly estimated by spreading a known volume of cell suspension onto an agar plate and counting the number of colonies that arise after a period of incubation. Precaution was taken to avoid

## Dr. Y. Ravi Shankar, et al. International Journal of Dental Science and Innovative Research (IJDSIR)

contamination of the specimens and experimental setup. Microbial inoculation was carried out in laminar air flow chamber, which is considered to be a completely sterile chamber.

In the present study, S. aureus and C. albicans were chosen as a test indicator to check the disinfecting efficacy of microwave oven irradiation based on association of analytical chemist guidelines for bactericidal testing of hospital based disinfectant and as they are the prototype for gram +ve, gram –ve organisms. These tested organisms are basically opportunistic pathogens, which are transiently found in the oral cavity. They are important human pathogens that can cause a broad spectrum of infections, from the trivial to the life threatening.

The results showed that complete sterilization of specimens, inoculated with individual suspensions of micro-organisms was achieved at 5 min and subsequent exposure for C. albicans and S. aureus, as negative colonies were obtained on respective plate cultures which were consistent with the studies conducted by K. Meghasri,Deepthi kalahasti and Abhilasha Bhasin <sup>(5,6,7,9)</sup>. The loss of surface details at can be explained on the basis of porosity or microcracks formed by the steam during microwave irradiation which was in accordance with the study conducted by Neha Malaviya and Kishore Ginjupalli <sup>(1)</sup>.

#### Conclusion

- Based on the results of the above study, we can infer that 5mins of exposure was effective in disinfection of the casts.
- Microwave disinfection can be performed quickly, without the use of toxic, pungent, or allergenic chemicals.
- 3. Surface roughness of the casts was increased as the radiation time increased.

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### Legend Table and Figures

Table 1: Antimicrobial efficacy after microwave radiation inoculated with Candida

Parameter		Type III		Type IV	
	Time	Mean	SD	Mean	SD
candida	3 min	56.00	4.11	46.50	3.47
	5 min	0.00	0.00	0.00	0.00
	7 min	0.00	0.00	0.00	0.00
	Control	111.50	6.08	108.70	6.41
	P-value	<0.01*		<0.01*	

Table2: Antimicrobial efficacy after microwave radiation inoculated with Staphylocoocus.

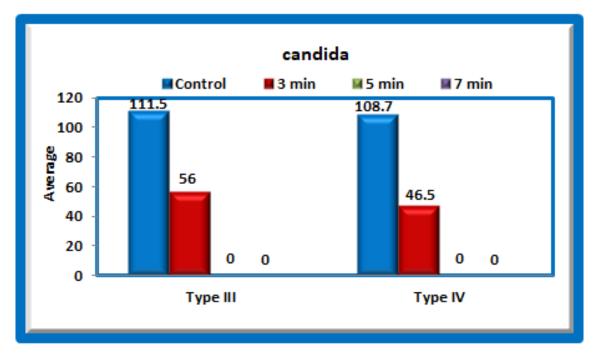
Parameter		Type III		Type IV	
	Time	Mean	SD	Mean	SD
Staph	3 min	2.45	0.18	2.40	0.28
	5 min	0.00	0.00	0.00	0.00
	7 min	0.00	0.00	0.00	0.00

Table 3: Surface roughness of samples subjected to microwave radiation.

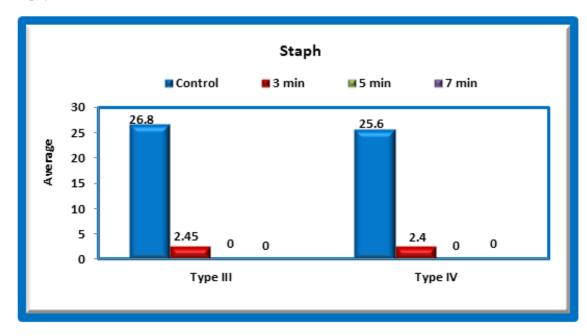
Parameter		Type III		T ype IV	
	Time	Mean	SD	Mean	SD
Surface rough ness	3 min	1.62	0.42	1.00	0.17
	5 min	1.68	0.46	1.53	0.80
	7 min	2.10	0.45	1.64	0.87
	Control	1.54	0.37	0.94	0.30
	P-value	<0.01*		<0.01*	

## Dr. Y. Ravi Shankar, et al. International Journal of Dental Science and Innovative Research (IJDSIR)

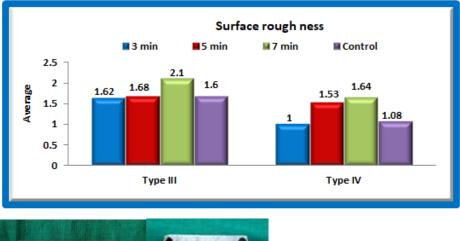
Graph 1: Schematic representation of Anti-microbial efficacy of Type 3 and type 4 gypsum products inoculated with Candida after 3,5 and 7 mins of microwave radiation.



Graph 2: Schematic representation of Anti-microbial efficacy of Type 3 and type 4 gypsum products inoculated with Staphylococcus after 3, 5 and 7 mins of microwave radiation.



Graph 3: Schematic representation of Surface roughness of Type 3 and type 4 gypsum products for 3 mins, 5mins and 7mins of microwave radiation.



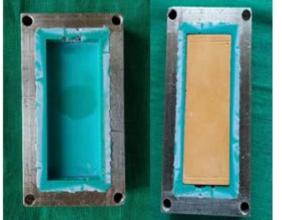


Figure 1: master die and samples prepared with dimension of 40\*10\*2mm

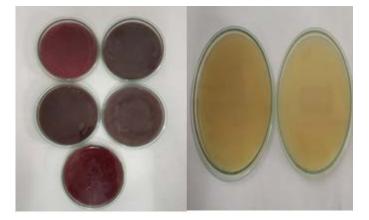


Figure 2: blood agar for Staphylococcus aureus and sabouraud broth for Candida albicans



Figure 3: loop inoculation of bacteria for culturing



Figure 4: Colonies of Staphylococcus formed after incubation



Figure 5: Surface roughness measured using Profilometer