

Evaluation of Bacteriocidal Efficacy of Microwave Oven Irradiation and UV Light in Disinfection of Dental Gypsum Casts-An in Vitro Study

Dr. Bidyabati Huyam¹, Dr. Trupti Dahane², Dr. AJPakhan³,
Dr. Surekha Godbole⁴

^{1,2,3,4}(Department of prosthodontics, Sharad Pawar Dental college/Datta Meghe Institute of Medical Sciences, India)

Abstract: The aim of this study was to evaluate and compare microwave disinfection with ultraviolet light disinfection of dental gypsum casts. **Materials and methods :** A total of 60 blocks were prepared from an L- key mold using Type III dental stone. Of the 60 blocks, 30 blocks were contaminated with 1 ml suspension of *Staphylococcus aureus* and 30 blocks were contaminated with 1 ml suspension of *Pseudomonas aeruginosa*. Then, the blocks were disinfected with microwave irradiation using the microwave oven and UV light disinfection. Bacteriologic procedures were performed; the cfu/ml for each block was calculated using colony counter. The results were analyzed using Chi-square test. **Results:** Multiple comparisons revealed that there is a significant difference between microwave irradiation and UV light disinfection on observing the microbial load of *Staphylococcus aureus* ($p < 0.05$) and *Pseudomonas aeruginosa* ($p < 0.05$). **Conclusion:** On the basis of observations made for the antimicrobial assessment the microwave irradiation of gypsum blocks has proved to be a better disinfection method when compared with ultraviolet light disinfection.

Keywords: Cast, disinfection, gypsum, microwave, uv light

I. Introduction

Infection control is an important concept in the present day practice of dentistry because the dental health care professionals are at high risk of cross infection and emergence of new communicable diseases like hepatitis, HIV and prevailing diseases like tuberculosis makes it all the more important to control the transmission.

Gypsum products are widely used as materials for the preparation of models and casts in dentistry. Dental casts are transferred several times between the dental laboratory and the dental office. It has been established that bacteria and viruses can be transmitted from patients to the gypsum models during the fabrication of the prosthesis,¹ if the plaster is poured into contaminated impressions or through contamination of bite blocks and trial bases.² The usual solution to this problem has been to rinse the impressions under running water and to place them in an appropriate disinfection solution.³ This should be done upon removal of the impression from the patient's mouth or in the dental laboratory prior to casting the model. However, two problems may arise. One is the risk that infectious organisms may still contaminate the gypsum models during the subsequent dental procedures such as jaw registration and the try-in procedures. The second is the

Conventional autoclaving of the cast could easily damage the surface of the dental stone, and gas sterilization is expensive and time consuming. Immersion of the cast in chemical disinfectant could lead to dissolution of sufficient amount of gypsum to cause measurable reduction in dimensions of the cast and decrease in the compressive strength of the dental stone, so microwave oven disinfection and UV light disinfection might provide a convenient solution.⁴ Recently, several investigations have been carried out as regards the use of ultraviolet and microwave energy as an alternative method to other sterilization methods.⁵ Microwave irradiation is thought to be effective in eliminating micro-organisms⁶, it is also practical and can be repeated many times without affecting the dental casts.⁷ Ultraviolet radiation is that part of the electromagnetic spectrum lying between the softest ionizing radiation on the one side and visible radiation on the other. UV lights can kill microorganisms, or inactivate them by damaging their DNA. Ultraviolet radiation has emerged as an effective approach for inactivation of micro organisms in the last few decades as compared to other methods of disinfection like spraying and immersion method.⁸ The present study was aimed to determine bacteriocidal activity of microwave and ultraviolet on bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

II. Materials And Methods

2.1 Materials

- 1.L-key mold
- 2.Type -3 dental stone (kalstone Kalabhai pvt ltd)
- 3.Brain heart infusion broth(Hi Media)
- 4.Mc Conkey(Hi Media) and blood agar plates

2.2 Equipments

- 1.Incubator
2. Microwave oven 700 watt and 2450 MHz(LG intellowave) Fig.2
- 3.UV light disinfection chamber Fig.3

2.3 Method:

The study was conducted in the Department Of Prosthodontics & Department of Oral Pathology at Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences Wardha.

To conduct this study 60 blocks were prepared from a standard L-key mold using Type III dental stone(Fig.1), namely (Kalstone, Kalabhai Pvt. Ltd.,) mixed in accordance with the manufacturers recommendations (w/p ratio of 0.23). The samples were grouped as:

- Control group - untreated group (20 blocks),
Group I - microwave disinfection group (20 blocks),
Group II – UV light disinfection group (20 blocks).
Criteria for selection of samples

2.4 Inclusion criteria

- 1) Non-spore forming bacteria which are commonly found in the oral cavity and in environment
- 2) 24-48 hour dried cast with adequate strength and surface hardness.

2.5 Exclusion criteria

- 1) Spore-forming bacteria
- 2) Casts dried <24-48 h/wet casts with inadequate strength and surface hardness.

2.6 Aseptic procedures

Throughout the study, all the laboratory and bacteriological procedures were carried out under aseptic conditions. A standard barrier technique was used with sterile gloves and facemask. The study mold, spatulas, mixing bowls, forceps, and tweezers were disinfected with 70% ethanol before each use. All bacteriological procedures were carried out in a flow bench except plating, which was carried out in the laminar flow.

Bacterial strains was carried out in peptone water suspension and adjusted according to 0.5 Mc Farland turbidity. Prepared suspension contained 10^6 bacteria/ml..

Control group(-untreated group) .10 blocks were contaminated with 1 ml suspension of Staphylococcus aureus and another 10 blocks were contaminated with 1 ml suspension of Pseudomonas aeruginosa

Group I –(Microwave irradiated group.) 10 blocks were contaminated with 1 ml suspension of Staphylococcus aureus and another 10 blocks were contaminated with 1 ml suspension of Pseudomonas aeruginosa. After 10 min, bacterial suspension was gently shaken off the blocks to remove excess liquid and was disinfected in a microwave oven.

The microwave oven irradiation/disinfection was performed in LG intellowave microwave oven set at 700 watt and 2450 MHz frequency, for a total of 5 min.

Group II – (UV light disinfected group). 10 blocks were contaminated with 1 ml suspension of Staphylococcus aureus and another 10 blocks contaminated with 1 ml suspension of Pseudomonas aeruginosa. The blocks were disinfected in the shoebox shaped UVdisinfection chamber for 5 min. which consists of outer body and inner body. Outer box is made up of galvanized tin sheets of dimensions 455 mm x 200 mm x160mm. Inner box is made up of copper and then silver plated and is meant for keeping gypsum casts for disinfection of dimensions 125mm x 325mm x 125 mm. Two UV germicidal lamps (Sankyo Denki Co Ltd,Japan) of 8 watt,254 nm wavelength are fitted. U.V. output is 2.5 watt, rated life -6000hrs , length of tube is 1 feet , indicator on top centre of lid indicates lamp is glowing. This unit is designed to reflect the UV light emitted so that the cast within the unit is exposed to UV radiations from many directions. It consists of mains to control the supply of electricity to whole system, timer to start the apparatus after setting of time. Time was set by rotating the knob between 1-60 min. Voltmeter indicates the volt. Distance between sample and tube was 10 cms.

2.7 Bacteriological procedures

All disinfected blocks by microwave irradiation and UV light and non disinfected blocks from control group were submerged in brain heart infusion (BHI) broth (Fig 4) and incubated aerobically at 37° C for 6 hrs .Then, BHI was plated in McConkey for pseudomonas aeruginosa and blood agar plates for staphylococcus aureus(Fig.5). The inoculated plates were incubated aerobically at 37°C for 18 hrs. After incubation of the plates, the cfu/ml for each cast was calculated using colony counter.

III. Results

The data thus obtained was tabulated and analyzed by using Statistical analysis was done by using descriptive and inferential statistics using Chi square test and software used in the analysis was Graph Pad Prism 5.0 version and EPI INFO 7.0 version, $p < 0.05$ is considered as level of significance.

Multiple comparisons revealed that there is a significant difference between all the three combinations on observing the microbial load of Staphylococcus aureus ($p < 0.05$) [Table 1 and Table 2]. On assessing the presence of microbial load i.e. Pseudomonas aeruginosa colony a statistically significant difference was observed ($p < 0.05$). Multiple comparisons revealed a statistically significant ($p = 0.0001$) difference for all the three combinations on assessing the microbial load of Pseudomonas aeruginosa (Table 3 and Table 4). On the basis of observations made for the microbial assessment the order of efficacy obtained was Microwave > UV light > Control for both the organisms.

IV. Discussion

A cast from a properly disinfected impression may subsequently become contaminated by a technician or clinician. In addition, the prosthesis that is contaminated by the patient after trial and adjustment in the mouth will recontaminate the cast after repositioning

In practice, contaminated gypsum casts are not possible to disinfect chemically. If elimination of possible cross contamination is considered a requirement, the disinfection measures should be applied throughout all phases of treatment to both the casts and the prosthesis.

Concerning this, the present investigation was undertaken to evaluate the effectiveness of microwave disinfection and UV light disinfection .Microwaves are electromagnetic waves produced by a generator called as magnetron. The principle of heating by microwaves is that they cause polar molecules to oscillate because the molecules are electrically unbalanced. Molecular vibration produces heat, a rise in temperature, and possibly some degradation of the effected molecule. Microorganisms contain polar molecules which when excited at high frequency, might cause disaggregation of the microbial structure. The process is fast and does not affect nonpolar molecules.⁹

An in vitro and in vivo study conducted on high-level microwave disinfection of dental gypsum cast revealed that there was a striking reduction of bacteria on the casts after 5 min of microwave oven irradiation in an ordinary household microwave oven set at 900 Watt.⁶

An investigation of bacteriocidal activity of a microwave set at 2450 MHz, 324 W, 650 W and 1400 W on suspensions of various non-sporogenic bacteria including *S. aureus* and *P. aeruginosa*, and sporogenic medically important bacteria showed that the vegetative bacteria were promptly killed in 5 min or less. Bacterial spores, on the other hand, were only killed in aqueous suspension when a 1400 W setting was used for 10-20

An investigation undertaken to evaluate the effectiveness of microwave irradiation in disinfection of complete dentures and long term soft lining materials concluded that MW energy could be considered as effective and safe alternative.^{11,12}

The results of the present study showed striking reduction of bacteria on the casts after 5 min of microwave irradiation in a microwave oven set at 700 W and 2450 MHz.

Research data on UV light disinfection is scarce but Singh S et al., and Wakefield CW¹³ has reported the clinical relevance of disinfection by UV rays by revealing the application of "germicidal" UV rays for disinfecting drinking water, culture media, titanium implants , impression materials, dental hand pieces etc. Boylan RJ⁸ evaluated the disinfectant properties of the buffalo ultraviolet disinfection unit (BDU), an instrument that emitted UV rays in an enclosed area which was designed to disinfect materials routinely used in dental operator .In the BDU ,UV light emitted from a single lamp is reflected off of the walls of the unit and bombards the item being disinfected from a

variety of directions .UV light kills the microorganisms by damaging their DNA by the formation of thymine-containing photoproducts in the DNA of affected cells. Also, UV lights cause DNA mutations. The longer that cells are exposed to UV light the greater are the chances that lethal mutations will arise as a result of the formation of more of these photoproducts in the cells 'DNA. The results of this study showed that BDU reduced the number of microorganism remaining on the surface of impression ,dentures and removable partial dentures.

Aksen et al in their study have found that 3 minutes of UV exposure was enough for the destruction of *Staphylococcus aureus* and *pseudomonas aeruginosa* using UV source (Steristom 2537A⁰).¹⁴

Concerning clinical practice, provided these procedures does not harm the gypsum casts, disinfection can be performed quickly, repeatedly, without the use of toxic, pungent, or allergenic chemicals. However, in regard to the above provision, MW irradiation of the gypsum casts has been tested previously as to its effect on the strength and hardness of the cast. The results indicated an improvement in these qualities, although there was some concern that cracks or porosities in the surface might occur when Type IV gypsum casts were exposed to irradiation with a very high wattage of 1450W.^{15,16}

Figures and Tables

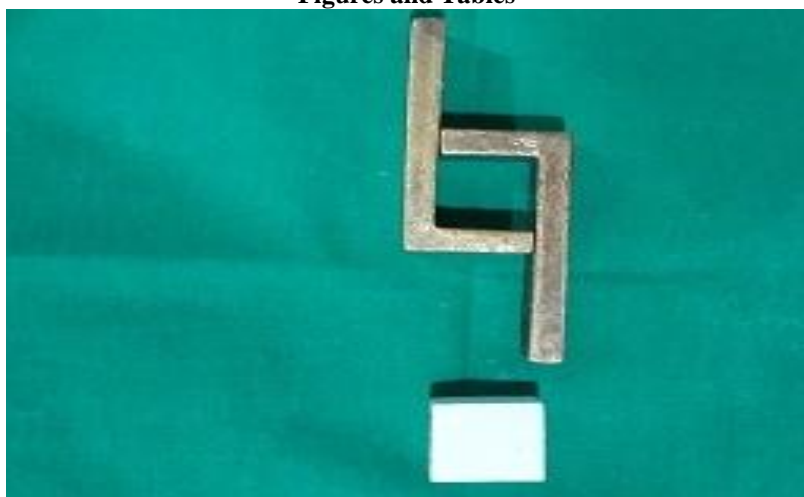


Fig.1 L-key and the gypsum blocks



Fig.2- microwave oven



Fig.3 U V light chamber



Fig.4 gypsum blocks immersed in BHI broth

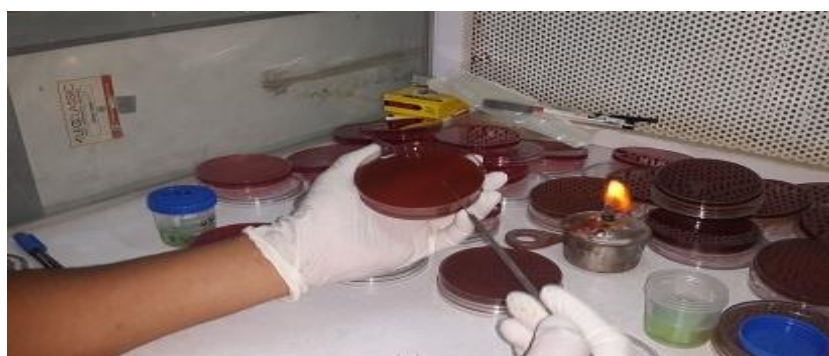


Fig.5 plating of BHI on culture plates

Table 1: To assess the presence of microbial load i.e Staphylococcus aureus colony in control group and after disinfection via microwave irradiation and ultraviolet light

SN	Level of microbial load	Number of specimens					
		Control		Microwave irradiated		UV Light	
		N	%	N	%	N	%
1	10 ⁰	0	0	18	90	0	0
2	10 ¹	0	0	2	10	9	45
3	10 ²	0	0	0	0	10	50
4	10 ³	0	0	0	0	1	5
5	10 ⁴	0	0	0	0	0	0
6	10 ⁵	0	0	0	0	0	0
7	10 ⁶	20	100	0	0	0	0
Total		20	100	20	100	20	100

Table 2: Multiple comparisons of three groups control, microwave and ultraviolet light on disinfection of Staphylococcus aureus

SN	Comparison	z-value	p-value
1	Control Vs Microwave	40.00	0.0001, S
2	Control Vs. UV Light	120.0	0.0001, S
3	MicrowavVs UV Light	33.45	0.0001, S

Table 3: To assess the presence of microbial load i.e pseudomonas aeruginosa colony in control group and after disinfection via microwave irradiation and ultraviolet light

SN	Level of microbial load	Number of specimens					
		Control		Microwave irradiated		UV Light	
		N	%	N	%	N	%
1	10 ⁰	0	0	19	95	0	0
2	10 ¹	0	0	1	5	5	25
3	10 ²	0	0	0	0	14	70
4	10 ³	0	0	0	0	1	5
5	10 ⁴	0	0	0	0	0	0

6	10 ⁵	0	0	0	0	0	0
7	10 ⁶	20	100	0	0	0	0
Total		20	100	20	100	20	100

Table 4: Multiple comparisons of three groups control, microwave and ultraviolet light on disinfection of pseudomonas aeruginosa

SN	Comparison	z-value	p-value
1	Control Vs Microwave	40.00	0.0001, S
2	Control Vs. UV Light	40.00	0.0001, S
3	Microwave Vs UV Light	26.67	0.0001, S

VI. Conclusion

Within the limitation of this in vitro study, the following conclusions can be drawn:

On the basis of observations made for the antimicrobial assessment, the microwave irradiation proved to be a better disinfection method for gypsum cast as compared with Ultraviolet light disinfection. UV light kills, within seconds, microorganisms that are not shadowed from its emissions. Thus, microbes on the surfaces of items or, if located below the surface, positioned so that UV light will shine on them, will be killed. UV radiation is a powerful bacteriocidal agent. Nevertheless, because of the shadowing effect that allows for the survival of unexposed microorganisms, the reliance on UV light as the sole means of disinfection of gypsum casts cannot be recommended. Additionally, residual blood and organic materials on the cast as well as dust particles in the environment would reduce the already poor penetrability of UV radiation. Another problem is the loss of output of UV radiation by the bulbs with time, a process that is impossible to detect without the use of sophisticated measuring devices. Frequent changes of the bulb would seem to be imperative. Thus, the major use for the UV light in the dental operator may be as an instrument to sanitize dental cast and prostheses that have already been thoroughly rinsed and dried.

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