

Evaluation and Comparison of High-Level Microwave Oven Disinfection with Chemical Disinfection of Dental Gypsum Casts

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Abstract:

Background: The aim of this study was to evaluate and compare microwave disinfection with chemical disinfection of dental gypsum casts.

Materials and Methods: A total of 120 casts were prepared from a silicone mold using Type III dental stone. Of the 120 casts, 60 casts were contaminated with 1 ml suspension of *Staphylococcus aureus* and 60 casts were contaminated with 1 ml suspension of *Pseudomonas aeruginosa*. Then, the casts were disinfected with microwave irradiation and chemical disinfection using the microwave oven and 0.5% sodium hypochlorite. Bacteriologic procedures were performed; the cfu/ml for each cast was calculated as a weighted mean. The results were analyzed using Kruskal-Wallis test and Mann-Whitney test.

Results: The untreated casts showed Brain heart infusion broth counts of 10^6 log cfu/ml compared to irradiated and chemically disinfected casts, in which 10^5 log reduction of cfu/ml was seen. These results satisfied the requirements of current infection control guidelines for the dental laboratory. The results obtained for chemical disinfection were in equivalence with microwave disinfection.

Conclusions: Within the limitation of this *in vitro* study, it was found that microwave disinfection of casts for 5 min at 900 W gives high-level disinfection that complies with the current infection control guidelines for the dental laboratory and microwave disinfection method is an effective and validated method as chemical disinfection.

Key Words: Chemical disinfection, dental gypsum cast, microwave disinfection

Introduction

In clinical practice, objects potentially contaminated with pathogenic microorganisms are transported between dental clinic and the dental laboratory during various treatment modalities. It has been claimed that to avoid cross-contamination, specific disinfection measures should be followed.

The centers for disease control¹ and the American Dental Association (ADA)² have recommended the use of infection control procedures in dental practices. Compared to sterilization, disinfection is normally a less lethal process for eliminating virtually all recognized pathogenic microorganisms, but not necessarily all microbial agents particularly the highly resistant microbial spores may survive.³

Microorganisms have been recovered from dental casts under various experimental and environmental conditions.⁴ Although the ADA council on infection control provides recommendations for the disinfection of sources of cross-contamination, no recommendations have been outlined for casts, a common vector for cross-contamination.

The potential for cross-contamination with stone casts is especially prevalent in restorative dentistry because of multiple opportunities for the transfer of infectious agents from saliva to casts. Therefore, these casts should be disinfected after each clinical and laboratory procedures.

Sterilization and disinfection of impressions will probably sacrifice the dimensional accuracy of the impression due to heat and or time lapse.⁴ More practical approach would be, to clean the impression of visible contaminants, such as saliva, make the stone cast and then disinfect the cast.

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, microwave oven disinfection might provide a convenient solution.⁵

Therefore, the present laboratory investigation was undertaken with the following objectives: (1) To test whether microwave

oven irradiation can disinfect gypsum casts effectively. (2) To compare the effectiveness of microwave oven disinfection with that of chemical disinfection of dental gypsum cast.

Materials and Methods

To conduct this study 120 casts were prepared from a standard silicone mold (Nissin molds) using Type III dental stone, namely (Kalstone, Kalabhai Pvt. Ltd.,) mixed in accordance with the manufacturers recommendations (w/p ratio of 0.23). The materials and methods were selected in an attempt to simulate the conditions under which the materials are used clinically and in the laboratory. One hundred and twenty dental gypsum casts were grouped as: Control group - untreated group (40 casts), Group I - microwave disinfection group (40 casts), Group II - chemical disinfection group (40 casts).

Criteria for selection of samples

Inclusion criteria

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

Exclusion criteria

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

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.

Control group-untreated group

Out of 40 casts in the control group, 20 casts were contaminated with 1 ml suspension of *Staphylococcus aureus* and 20 casts were contaminated with 1 ml suspension of *Pseudomonas aeruginosa*. Both the organisms were selected and suspensions prepared to the European standard EN 1040 with turbidity corresponding to McFarland standard 1 (3×10^6 colony forming units/ml).

Group I - Microwave irradiated group

Out of 40 casts in this group, 20 casts were contaminated with 1 ml suspension of *S. aureus* and 20 casts were contaminated with 1 ml suspension of *P. aeruginosa*. After 10 min, bacterial suspension was gently shaken off the casts to remove excess liquid and was disinfected in a microwave oven.

The microwave oven irradiation/disinfection was performed in Godrej microwave oven type, model no: GMS 19A, set at 900 watt and 2450 MHz frequency, for a total of 5 min. To ensure

that the casts were adequately irradiated on all surfaces, they were first exposed for 2.5 min and subsequently turned upside down and irradiated again for the same amount of time.

Group II - Chemically disinfected group

Out of 40 casts in this group, 20 casts were contaminated with 1 ml suspension of *S. aureus* and 20 casts were contaminated with 1 ml suspension of *P. aeruginosa*. After 10 min, bacterial suspension was gently shaken off the casts to remove excess liquid and was disinfected chemically with 0.5% sodium hypochlorite (NaOCl), pH adjusted to 10 by immersion method for 10 min.

Bacteriological procedures

Bacteriologic procedures were performed in compliance with European standard EN 1040. All casts were submerged in brain heart infusion (BHI) broth and incubated aerobically at 37°C for 6 h. BHI aliquots, undiluted and diluted to 10^{-6} were then prepared from the cultures of each of infected (control group) and disinfected casts (Group I, Group II) using micro titer pipettes and spinx centrifuge. All dilutions were made with normal saline solution. For each dilution, BHI was plated in McConkey and blood agar plates. The inoculated plates were incubated aerobically at 37°C for 18 h. After incubation of the plates, the cfu/ml for each cast was calculated using colony counter. The data thus obtained was tabulated and analyzed by using Kruskal-Wallis test and Mann-Witney test.

Results

The results of the study showed very high significance existing between control and test groups with the *P* value being <0.0005 (Table 1). Comparison between the control group and microwave disinfection; and between the control group and chemical disinfection group using Mann-Whitney test indicated a very high significance existing between the groups with the *P* value being obtained <0.0005 (Tables 2 and 3). Results comparing microwave disinfection and chemical disinfection showed no statistical significance existing between the groups with the *P* value being not <0.05. This indicates that the efficacy of microwave and chemical disinfection were equivalent (Table 4).

Table 1: Mean and standard deviation of control and test groups.

Bacteria type	Group	N	Mean	Standard deviation	Kruskal-Wallis test	P
<i>S. aureus</i>	Untreated group	20	2.915	1.0297	46.747	0.0005
	Microwave disinfected	20	0.185	0.5806		
	Chemically disinfected	20	0.145	0.4605		
	Total	60				
<i>P. aeruginosa</i>	Untreated group	20	4.505	2.2444	41.68	0.0005
	Microwave disinfected	20	0.645	1.3839		
	Chemically disinfected	20	0.145	0.6485		
	Total	60				

S. aureus: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 2: Comparison between the control group and microwave disinfection group for *S. aureus* and *P. aeruginosa*.

Bacteria type	Group	N	Mann-Whitney test	P
<i>S. aureus</i>	Untreated group	20	5.4	<0.0005
	Microwave disinfected	20		
	Total	40		
<i>P. aeruginosa</i>	Untreated group	20	4.7	<0.0005
	Microwave disinfected	20		
	Total	40		

S. aureus: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 3: Comparison between the control group and chemical disinfection group for *S. aureus* and *P. aeruginosa*.

Bacteria type	Group	N	Mann-Whitney test	P
<i>S. aureus</i>	Untreated group	20	5.5	<0.0005
	Chemically disinfected	20		
	Total	40		
<i>P. aeruginosa</i>	Untreated group	20	5.6	<0.0005
	Chemically disinfected	20		
	Total	40		

S. aureus: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 4: Comparison between microwave disinfection group and chemical disinfection group for *S. aureus* and *P. aeruginosa*.

Bacteria type	Group	N	Mann-Whitney test	P
<i>S. aureus</i>	Microwave disinfected	20	-0.052	0.989
	Chemically disinfected	20		
	Total	40		
<i>P. aeruginosa</i>	Microwave disinfected	20	-1.412	0.429
	Chemically disinfected	20		
	Total	40		

S. aureus: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*

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. 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.⁶

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.⁷

An investigation of bactericidal activity of a microwave set at 2450 MHz, 324 W, 650 W and 1400 W on suspensions of various non-sporogenous bacteria including *S. aureus* and *P. aeruginosa*, and sporogenous 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 min.⁸

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 900 W and 2450 MHz. The untreated casts showed BHI counts of 10^6 log cfu/ml compared with irradiated ones, in which 10^5 log reduction of cfu/ml was seen (Tables 1 and 2). This result satisfied the requirements of European standard EN 1040⁹ and current infection control guidelines for the dental laboratory.¹

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.^{10,11}

Concerning clinical practice, provided this procedure does not harm the gypsum casts, disinfection can be performed quickly, repeatedly, without the use of toxic, pungent, or allergenic chemicals. 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.^{12,13}

Chemical disinfectants are used as usual solutions to break the chain of cross-infection and efficacy of such disinfectants has been the subject of several studies. In the present study, an investigation was also carried out to compare the level of disinfection provided by MW irradiation with chemical disinfection. For this purpose 0.5% NaOCl, at pH adjusted to 10, 10 min immersion method was chosen.

Sodium hypochlorite is a halogen. They are oxidizing agents and act by releasing halide ions. They behave as protoplasmic poisons by oxidizing the cellular constituents of microorganisms.

A study recommended that NaOCl solution be used in concentrations ranging from 0.05% to 0.5% (500-5000 ppm) diluted with water. In addition, it should be used at least 30 min for thorough disinfection of heavily contaminated objects.¹⁴ An another study conducted to evaluate the antimicrobial properties of NaOCl, 0.525%, at pH levels adjusted from 6 to 12 on experimentally contaminated irreversible hydrocolloid impressions concluded that the pH of 10 was the only level that was consistently effective at decreased immersion times. It was effective in times of 3 min or greater.^{15,16}

The findings in the present study with regards to chemical disinfection showed that casts immersed in 0.5% NaOCl, pH 10 for 10 min resulted in 10^5 log reduction of cfu/ml when compared to untreated casts that showed BHI counts of 10^6 log cfu/ml (Tables 1 and 3). These results were in equivalence with MW disinfection (Table 4).

However, chemical disinfection of cast lead to dissolution of the minor amount of gypsum, which may affect the dimensions of the cast. On the contrary, the results of neither previous studies nor the present study furnished sufficient information as to all possible effects of MW irradiation on gypsum cast. Macroscopically, the surfaces of the casts appeared unaffected by the MW irradiation and no obvious cracks or porosities were observed.

In the present *in vitro* study, the results showed that there was no statistical significance between MW disinfection and chemical disinfection. Their efficacy levels were equivalent. Contrary to these results, a study on high level MW disinfection of dental gypsum cast found that efficacy MW disinfection was better than chemical disinfection.⁷ NaOCl was used as a chemical disinfectant at a concentration of 0.07%, at pH 10 for 3 min; in the present study 0.5% NaOCl pH adjusted to 10 for 10 min was used. This could be the fact in variation of results in that study and the present study.

This indicates that the concentration of chemical disinfectant, exposure time, pH levels are crucial factors to be considered in evaluating the efficacy levels of chemical disinfection.

In the present study, single irradiation was used on non sporogenic microorganisms, however further investigations are required to evaluate the effect of single and multiple irradiations on the casts physical properties; weight of the casts irradiated at one time and multiple times; efficacy of MW disinfection with respect to non sporogenic as well sporogenic.

Conclusion

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

1. Microwave disinfection of casts for 5 min at 900 W gives high-level disinfection that complies with European standard EN 1040 and the current infection control guidelines for the dental laboratory
2. Microwave disinfection method is an effective and validated method as chemical disinfection
3. Chemical disinfection with 0.5% NaOCl, pH adjusted to 10 for 10 min was as effective as microwave disinfection
4. Microwave disinfection can be performed quickly, without the use of toxic, pungent, or allergenic chemicals.

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