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ORIGINAL ARTICLE

Evaluation of Efficacy of Microwave Irradiation in Disinfecting Dental Gypsum Casts: An Ex Vivo Study

Deepthi Kalahasti · Veena Hegde · Kranti Kosaraju · Srikala Baliga · N. Kulasekhar Reddy · BK Sujatha

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Abstract The aim of this study is to assess the efficacy of microwave irradiation in disinfecting gypsum casts and also to compare its efficacy with validated method of chemical disinfection. The present study is an ex vivo study conducted on a sample of five irreversible hydrocolloid impressions in vitro and on ten patients gypsum casts in vivo following standard impression techniques to check the efficacy of microwave oven irradiation and compare its efficacy with standard chemical method of disinfection. Results were analysed using Mann–Whitney test and Wilcoxon signed rank test. Untreated gypsum casts showed cfu/ml counts with a median log value of 6, while microwave-irradiated ones had median cfu/ml counts of 0. Casts poured from chemically disinfected impressions demonstrated cfu/ml counts with a median log value of 5. Microwave irradiation was found to be effective in disinfecting gypsum casts when compared to chemical disinfectant in disinfecting dental impressions.

Keywords Cross contamination · Dental gypsum casts · *Staphylococcus* · *Pseudomonas* · Disinfection methods · Microwave radiation

Introduction

Increased awareness of the importance of infectious diseases and recognition of the potential for transmission of numerous infectious micro organisms during dental procedures have led to an increased concern for, and attention to, infection control in dental practice [1, 2]. ADA councils have stated that materials, impressions, and intra oral prosthesis should be cleaned and disinfected before being handled, adjusted or sent to a dental laboratory [3].

Pilot study was presented at 7th PG Convention of Indian Prosthodontic Society held at Udaipur on February 9th 2008. Completed study was presented at 39th IPS conference held at Dubai on 3rd December 2011 and was awarded best paper presentation.

D. Kalahasti (✉)
Department of Prosthodontics, Vydehi Institute of Dental Sciences & Research Centre, RGUHS, Bangalore, Karnataka, India
e-mail: drdeepthikalahasti@yahoo.co.in

D. Kalahasti
B-101, Sterling Shalom, Kundalahalli colony, ITPL main road, Bangalore 560037, Karnataka, India

V. Hegde
Department of Prosthodontics, Manipal College of Dental Sciences, Manipal University, Manipal, India

K. Kosaraju
Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, India

S. Baliga
Department of Microbiology, Kasturba Medical College, Manipal University, Mangalore, India

N. K. Reddy
Department of Prosthodontics, Sri Venkateswara Institute Of Dental Sciences, Palamoor, Mahaboobnagar, Andhra Pradesh, India

B. Sujatha
Department of Public Health Dentistry, Vydehi Institute of Dental Sciences, Bangalore, Karnataka, India

In Prosthodontics, objects that are potentially contaminated with pathogenic microorganisms are transported between dental laboratory and dental clinic. One common dental procedure that may cause cross-contamination, occurs during impression making and pouring that impression to get positive replica. There is a need to block this potential route for the transmission of infectious disease via the disinfection of dental impressions, gypsum casts delivered to the dental laboratory. The potential for cross-contamination with stone casts is especially prevalent in prosthodontics because of multiple opportunities for the transfer of infectious agents from saliva to casts. Therefore disinfection of these cast should be done after each clinical and laboratory procedures.

Verification of the transfer of micro organisms to dental casts made from artificially contaminated (in vitro) typhodonts and impressions has been documented [4, 5]. Persistent presence of opportunistic pathogens on patient derived impressions and gypsum casts has been documented in a study conducted by Jennings et al. [6, 7]. Certain microbes have been demonstrated to remain viable within gypsum cast materials for >7 days [8]. Powell et al. [9] documented that 67 % of the impressions, dentures, crowns and wax occlusion rims received by four commercial dental laboratories exhibited the presence of potential harmful bacteria. Different methods of disinfection have been tried inorder to reduce or remove the potential for cross contamination by immersion method [10], chemical disinfectant spray technique [11, 12], adding antimicrobial agents to impression material [13–15]. None of these procedures or techniques proved to be efficient in reducing cross contamination.

Microwaves comprise the portion of the electromagnetic spectrum extending from the frequency of 300 to 3,00,000 MHz. Most commercial microwave ovens operate at 2,450 MHz. Microwaves are generated by magnetron, propogated in a strong line along the wave guide and is called as the dominant mode. This radiation when gets absorbed in materials containing water they produce friction of water molecules in an alternating electrical field. The energy thus produced is transformed into heat and it is supposed that microorganisms with high water content can be consequently killed in short time [16]. Microwave fields have different properties when they contain certain types of materials. Metals are microwave reflective and do not heat. Denture base acrylic resins are transparent to microwaves, they neither absorb nor reflect microwave fields and they don't get heated.

Microwave radiation in dentistry has been widely accepted for sterilizing [16, 17] and polymerizing acrylic resins [18] and removing wax from moulds and shortening the dough stage of denture base acrylic resins [19] and for provisional fabrication of facial prostheses [20]. However, the disinfection of gypsum casts through microwave irradiation is seldom reported in literature.

Hence, an attempt has been made in the present study to assess the efficacy of microwave oven radiation in disinfecting dental impressions and gypsum casts.

Objectives of the Study

- To test whether microwave oven irradiation can disinfect gypsum casts effectively.
- To compare the efficacy of microwave oven disinfection with that of chemical disinfection method in disinfecting gypsum casts.

Null Hypothesis

There is no difference in the disinfecting efficacy between microwave disinfectant and chemical disinfectant techniques in disinfecting dental impression materials and gypsum cast.

Research Hypothesis

There is a difference in the disinfecting efficacy between microwave disinfectant and chemical disinfectant techniques in disinfecting dental impression materials and gypsum cast.

Methodology

The present study is an ex vivo, experimental study conducted to check the efficacy of microwave in disinfecting gypsum casts and impressions.

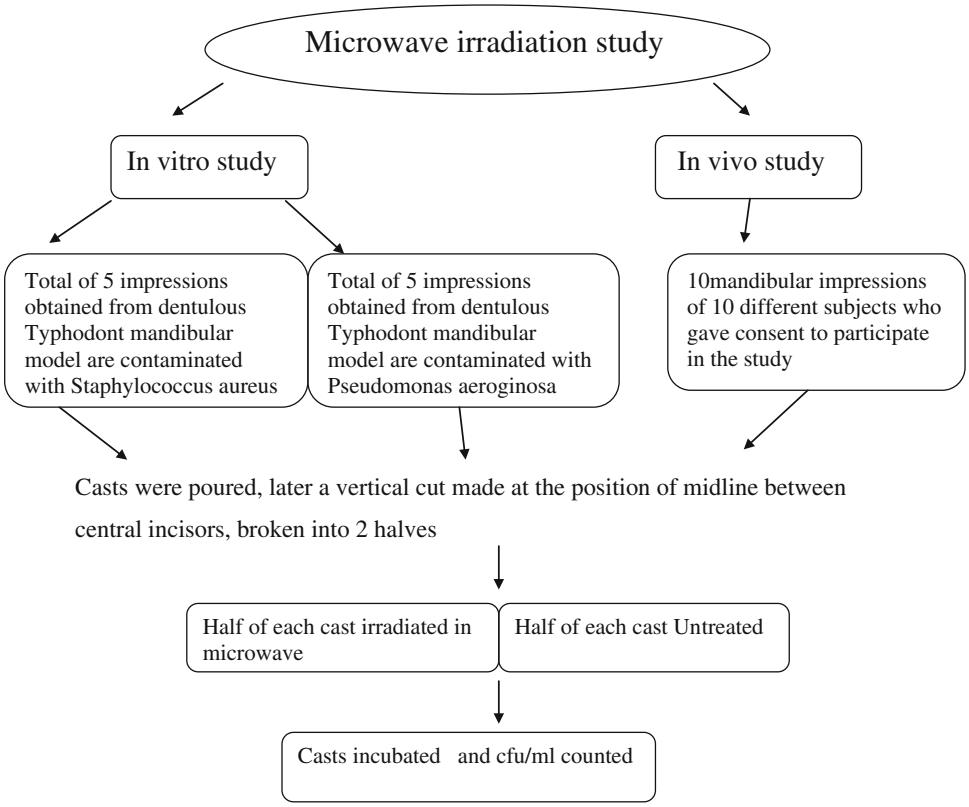
Fig. 1 Materials used for making impression

After obtaining the ethical clearance from Institutional review board and written informed consent from the study participants the present study was carried out. Sample size was determined based on the pilot study conducted on ten study participants. Convenient sampling technique was employed.

Study Design: Schematic Presentation

Study consisted of both microwave irradiation and chemical disinfection (Figs. 1, 2).

Schematic presentation of microwave irradiation study:



Microwave Irradiation Study

Method of In Vitro study

Total of 10 impressions were made with irreversible hydrocolloid (Tropicalgin, Zermack) of a disinfected typhodont model of mandible (Fig. 3). Typhodont model was disinfected by wiping it with 70 % ethanol. Impression material was hand mixed for 30 seconds in a figure of 8 motion (2 scoops of powder mixed with 36 ml of water taken in a cylinder jar provided by manufacturer) and loaded into a flame sterilized stock metal tray (Kalabhai trays). Of these ten impressions that are made from disinfected typhodont model of mandible, five impressions were contaminated by pipetting 1 ml of a suspension of *Staphylococcus aureus* (American type culture collection 29213) and the other five impressions with 1 ml of suspension of *Pseudomonas aeruginosa* (American type culture collection 27853) (Fig. 4). All the suspensions are prepared with turbidity corresponding to Mc farland standard (approximately 3×10^8 colony forming units/ml = 0.5 Mc farland units, represent the number of bacteria) (Fig. 5). After 7 min, bacterial suspension was gently shaken away from the impressions to remove excess liquid, and the casts were poured in type III Gypsum (Kalstone).

Method of In Vivo Study

Total of ten mandibular impressions were made with irreversible hydrocolloid material from ten different consenting dentulous subjects and poured in type III Gypsum without rinsing with in 10 min.

Distilled water was used for both impression material and pouring gypsum casts. The irreversible hydrocolloid material and the gypsum powder were dispensed from commercial packages and were not disinfected before use. The impression and the method of pouring casts were made in accordance with recommendations of the manufacturers.

Fig. 2 Materials used for bacteriological procedures

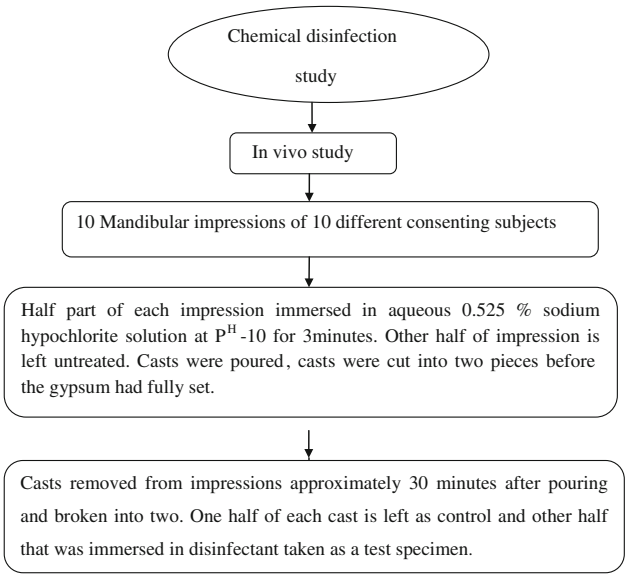
Fig. 3 Making impression of a typhodont dentulous mandibular model

All casts were cut transversely with a sterile surgical blade before gypsum had fully set in order to facilitate subsequent division (Fig. 6). The cut was made at the position of midline between the central incisors. A sterile surgical blade was used to cut each sample. Casts were removed from the impressions approximately 30 min after pouring and broken into two pieces. One half of each cast was irradiated in a microwave and the other unirradiated half was left as a control. The microwave irradiation was performed in a household Electrolux microwave oven type, which was set at 900 W and 2,450 MHz frequency (Figs. 7, 8).

To ensure casts were adequately irradiated on all surfaces, they were first exposed for 1.5 min and subsequently turned upside down and irradiated for the same amount of time. Total time duration that casts were subjected to microwave irradiation was for 3 min. Flame sterilized tweezer was used to turn the casts upside down. Macroscopically, the surfaces of the casts appeared unaffected by the microwave irradiation. No obvious cracks or porosities were observed.

Chemical Disinfection Study

General design of chemical disinfection study is



Ten irreversible hydrocolloid mandibular impressions were obtained from the same ten test subjects who were taken for microwave study. Impression material was hand mixed for 30 seconds using manufacturers recommended water-powder ratio and loaded into autoclaved perforated stock metal tray and impressions are made. Later the impression was removed from patient's mouth and half part of each impression was then immersed in freshly prepared aqueous 0.525 % sodium hypochlorite solution at pH 10 for 3 min. Other half of impression is left untreated to evaluate the amount of micro-organisms carried by the impression. Immediately following disinfection for the prescribed immersion time casts were poured with type III gypsum (Kalstone). Casts were cut transversely at the midline between central incisors before the gypsum had fully set to facilitate subsequent procurement of two halves of the cast. Casts were removed from impressions approximately 30 min after pouring and broken into two exactly at the area where the cut was made. After retrieval from impression, one half of each cast was left as a control and other half of the cast that was obtained from the part of impression which was immersed in sodium hypochlorite was taken as a test specimen. Investigators carried out the study.

Bacteriological Procedures

All casts that were cut into two pieces were placed in wide mouth bottles filled with tryptic soy broth (TSB) (Fig. 9). Tweezers was used to carry casts into bottle (after microwave subjection and chemical disinfectant immersion). All the bottles were incubated aerobically at 37 °C for 6 h

Fig. 4 Infecting the impression with test organisms

Fig. 5 Suspensions of *Staphylococcus* and *Pseudomonas* prepared according to Mc Farland Standard

(Fig. 10). After 6 h of incubation period, TSB aliquots in undiluted, diluted to 1:10, diluted to 1:100 ratio for all the samples were then prepared from the cultures of each of microwave irradiated (test and control), chemical disinfected (test and control) casts (Fig.11).

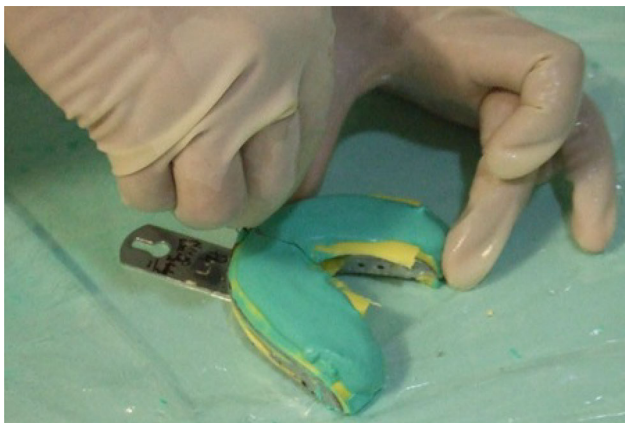


Fig. 6 Partially set gypsum cast divided into two halves by using sharp sterile blade

All dilutions were made with tryptone soy broth. Chocolate agar plates were used for plating the colonies. Each agar plate was divided into four quadrants and are marked for undiluted, diluted 1:10, 1:100 for both test and control samples (Fig. 12). For each quadrant of chocolate agar plate, corresponding undiluted, diluted aliquots was plated by using a loop of diameter 4 mm = 0.01 ml (Fig. 13). The inoculated plates were incubated aerobically at 37 °C for 18 h in an infrared CO_2 incubator (Fig. 14). After incubation of the chocolate agar plates, the cfu/ml for each cast was calculated (Fig. 15).

All the investigations were carried out by two examiners. Investigations related to clinics and laboratory were carried out by one examiner (D.K) and bacteriological investigations by another examiner (K.K).

Statistical Analyses

The data obtained from the study was subjected to statistical analysis using Mann–Whitney U test and Wilcoxon's signed rank test. All computations were conducted with SPSS software (version 11).

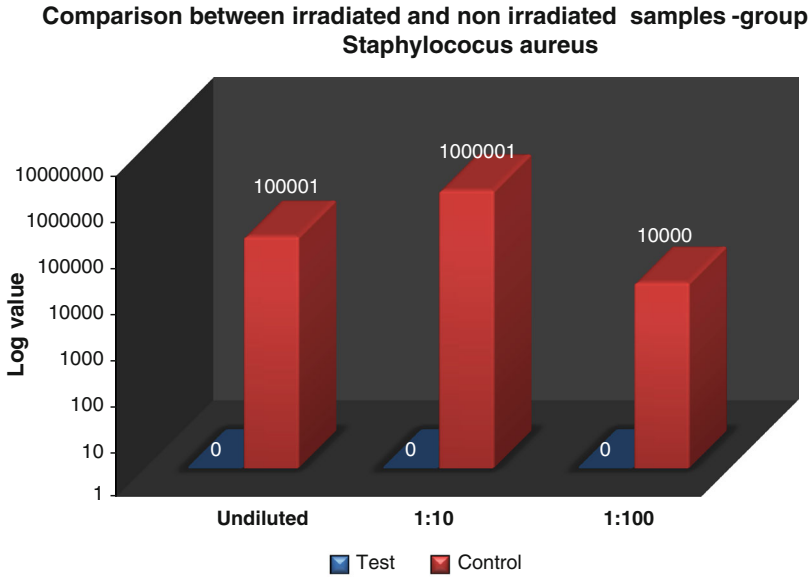
Results

Table 1 represents the *S. aureus* count in cfu/ml using TSB on the irradiated and non irradiated casts obtained from impressions inoculated in different concentrations like undiluted, 1:10, 1:100. Microwave irradiation was found to be highly significant in disinfecting the tested organism in 1:100 concentration at $p < 0.005$, whereas it is significant in both undiluted and 1:10 concentration at $p < 0.05$ with a median value of 0.

Table 2 represents the *Pseudomonas aeroginosa* count in cfu/ml using TSB on the irradiated and non irradiated

Table 1 Representing the *Staphylococcus aureus* count in cfu/ml obtained from TSB in different dilutions (microwave study-in vitro)

Samples		Median	*p* value
Irradiated	Undiluted	0.00	0.007
Non irradiated		1×10^6	
Irradiated	1:10 concentration	0.00	0.017
Non irradiated		1×10^6	
Irradiated	1:100 concentration	0.00	0.005
Non irradiated		1×10^4	



casts obtained from impressions inoculated in undiluted, 1:10, and 1:100 concentrations. Microwave irradiation was found to be highly significant in disinfecting the tested organism in all concentrations at $p < 0.05$ with a median value of 0.

Table 3 represents the efficiency of microwave oven irradiation in disinfecting gypsum casts obtained from ten patients impression when compared to the control group which was not treated by any of the disinfection methods. It was found that microwave oven irradiation was highly effective in disinfecting gypsum casts and the results were highly significant in undiluted, 1:10 and 1:100 concentration at $p < 0.005$ with a median value of 0.

Table 4 represents the efficiency of chemical disinfectant (sodium hypochlorite) in disinfecting gypsum casts obtained from ten patients impression when compared to the control group which was not treated by any of the disinfection methods. It was found that chemical disinfection method was effective in disinfecting casts when compared to control. The results were significant in undiluted and 1:10 concentration at $p < 0.05$ with a median values of 10^6 and 0 respectively.

Discussion

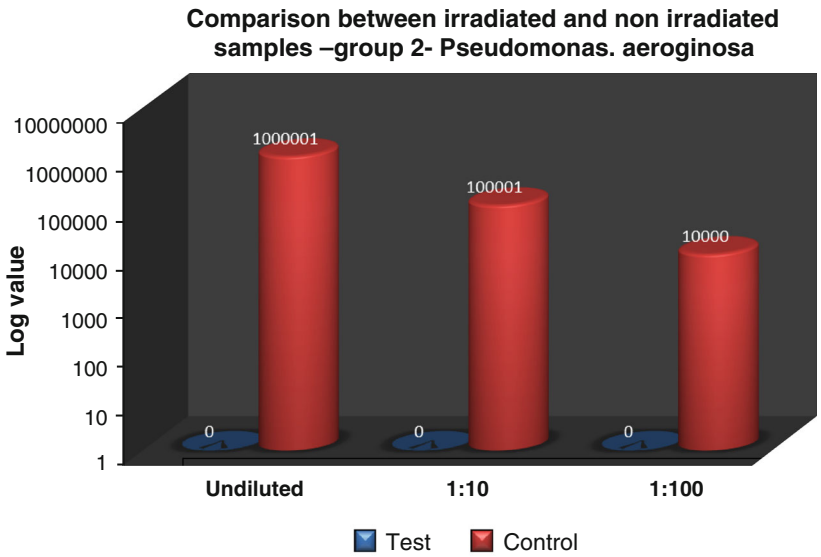
The present ex vivo study was carried out to evaluate the efficacy of microwave oven in disinfecting the dental casts.

Results showed that the microwave oven irradiation is effective in disinfecting gypsum casts and it is more effective as a disinfectant when compared to chemical disinfection.

In the present invitro study the gypsum casts of test group which was disinfected using microwave irradiation,

Table 2 Representing the *Pseudomonas aeruginosa* count in cfu/ml obtained from TSB in different dilutions (microwave study-in vitro)

Samples		Median	*p* value
Irradiated	Undiluted	0.00	0.004
Non irradiated		1×10^6	
Irradiated	1:10 concentration	0.00	0.005
Non irradiated		1×10^6	
Irradiated	1:100 concentration	0.00	0.005
Non irradiated		1×10^4	



the microbial colony count was found to be zero where as it varied between 1.0×10^6 and 1.0×10^4 in the control group.

In the present study the gypsum casts of test group obtained from patients which was disinfected using microwave irradiation, the microbial colony count was found to be zero where as it varied between 1.0×10^6 and 1.0×10^3 in the control group. In chemical disinfection of dental impressions the microbial counts varied between 1.0×10^5 and 5.5×10^3 in the control group, where as it was 1×10^3 to 0.00 in test group.

The present study results are in comparison with the study conducted by Berg et al. [21] in the year 2005, where even the microwave oven irradiation was found to be effective when compared to chemical disinfectant in disinfecting dental gypsum casts.

Chemical disinfectant was considered to be one of the standard effective methods for disinfection, chemical disinfection of impressions have a number of problems associated with their use, as they are time consuming, expensive to perform in dental practice and need to be freshly prepared or have a limited shelf life. Potential bacterial contamination has been detected even after disinfection had been performed. So, in the present study microwave oven irradiation was chosen.

The clinical relevance of microwave oven irradiation in disinfecting gypsum casts is that, this procedure can be performed quickly and repeatedly, without the use of toxic, pungent, or allergenic chemicals. Because casts can become contaminated after the intra-oral adjustments of dental appliances, they must be regarded as the major vehicle for cross-contamination and can be disinfected

Table 3 Representing the efficacy of microwave oven in disinfecting the gypsum casts in comparison to control group in different dilutions

Samples		Median	*p* value
Irradiated	Undiluted	0.00	0.001
Non irradiated		1×10^6	
Irradiated	1:10 concentration	0.00	0.001
Non irradiated		1×10^3	
Irradiated	1:100 concentration	0.00	0.005
Non irradiated		1×10^3	

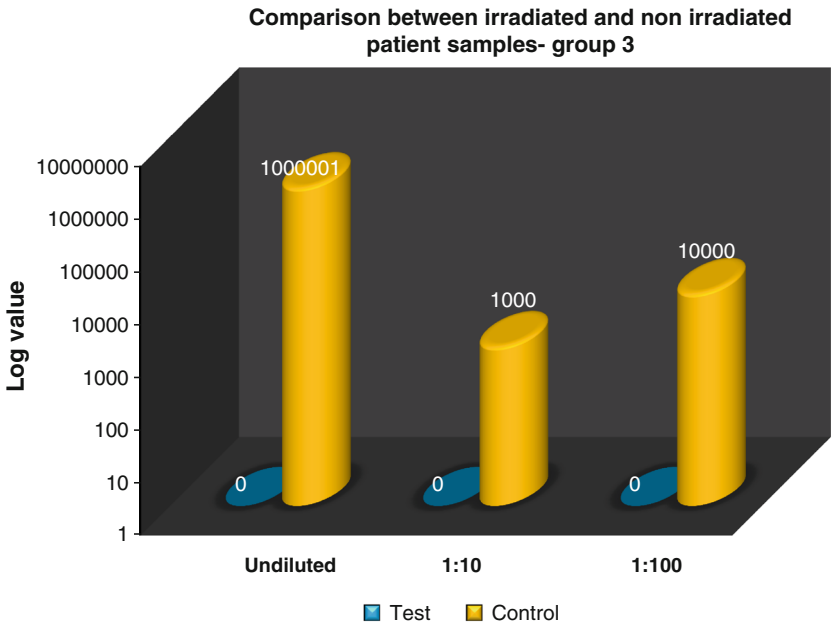


Fig. 7 Commercial household microwave oven used for disinfection procedures

Fig. 8 Gypsum cast subjected to microwave irradiation

Table 4 Representing the efficacy of chemical disinfectant in disinfecting the gypsum casts in comparison to control group in different dilutions

Samples		Median	*p* value
Chemically disinfected	Undiluted	1×10^3	0.041
Non disinfected		1×10^5	
Chemically disinfected	1:10 concentration	0.00	0.024
Non disinfected		5500.00	
Chemically disinfected	1:100 concentration	0.00	0.102
Non disinfected		0.00	

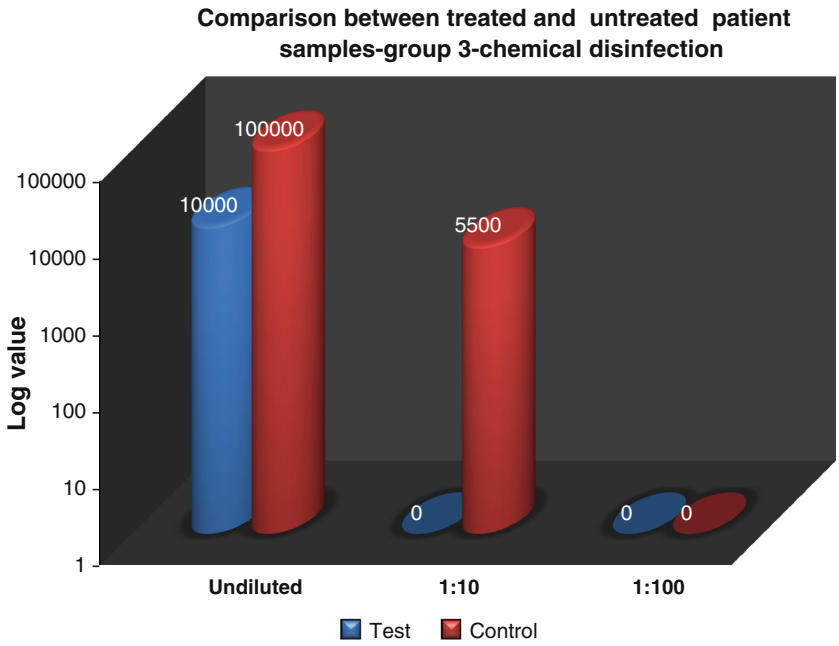


Fig. 9 Wide mouthed bottles with tryptic soy broth contained with samples (*Pseudomonas*, *Staphylococcus*)

using microwave energy throughout all the phases of the dental treatment.

In the present study, *S. aureus*, *P. aeruginosa* were chosen as a test indicators to check the disinfecting efficacy of microwave oven irradiation based on association of analytical chemists guidelines for bactericidal testing of hospital based disinfectant [22] 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. This study was intentionally designed in such a way that maximum amount of bacteria should be transmitted to the casts.

Fig. 10 Bacteriological incubator

The gypsum casts were disinfected using microwave oven irradiation for 3 min as there was chalky appearance of gypsum casts at 5 min and linear cracks, surface defects at 10 and 15 min respectively which was observed during pilot study.

Sodium hypochlorite of concentration 0.525 % and shortest immersion time of 3 min at pH 10 was considered as chemical disinfectant for the present study as it was found to be more effective which was suggested by several authors [23, 24] in their studies. In the current chemical disinfection study, bacterial contamination was harvested from the gypsum casts where as all other previous invivo conventional experimental methods tested impressions by harvesting bacteria from the impression of occlusal surface of molars.

The disinfectant efficacy was evaluated in serial dilutions by macrodilution broth susceptibility to quantitate the lowest concentration of an antimicrobial agent in

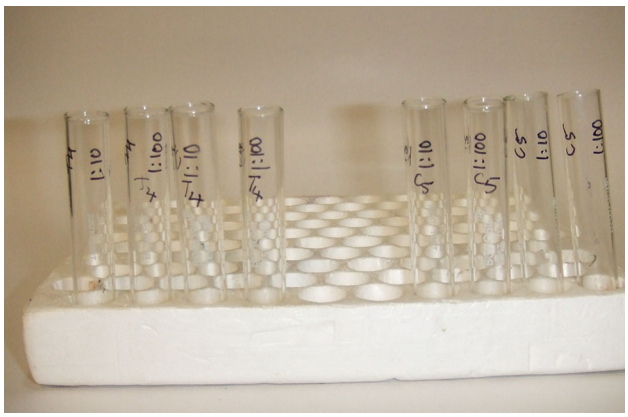


Fig. 11 Suspensions of both control and test in serial dilutions

micrograms per millilitre that inhibits visible invitro growth of microbes-the minimum inhibitory concentration [25]. Macrodilution broth susceptibility serves as a reference method. Procedure involves serial dilutions of anti microbial agent that are made in broth (or in agar) and bacterial suspension is added to it. It is expressed as micrograms of disinfectant per millilitre.

An investigation of bactericidal activity of microwave oven set at 2,450 MHz at 325, 650, 1,400 W on suspensions of various non-sporogenic bacteria; including *S. aureus*, *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 suspensions when a 1,400 W setting was used for 10–20 min [26].

From this study effective reduction of bacteria on the dental cast was found after 3 min of microwave irradiation in a microwave oven set at 900 W. Disinfection here can be performed quickly, repeatedly without the use of chemicals. Significant decrease in total counts of bacteria was observed after microwave irradiation.

Recommendations

1. Efficacy of microwave irradiation on fungi and viruses can be investigated before commenting on the effectiveness of microwave as a disinfectant.

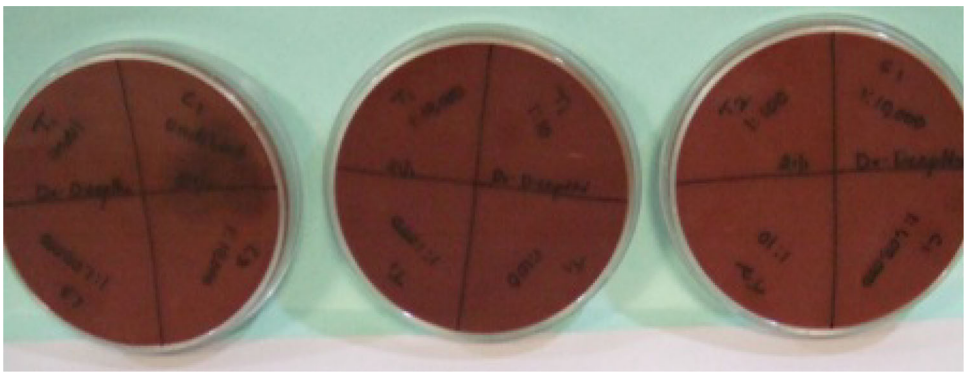


Fig. 12 Chocolate agar plates divided into four quadrants for streaking of samples contained in soy broth

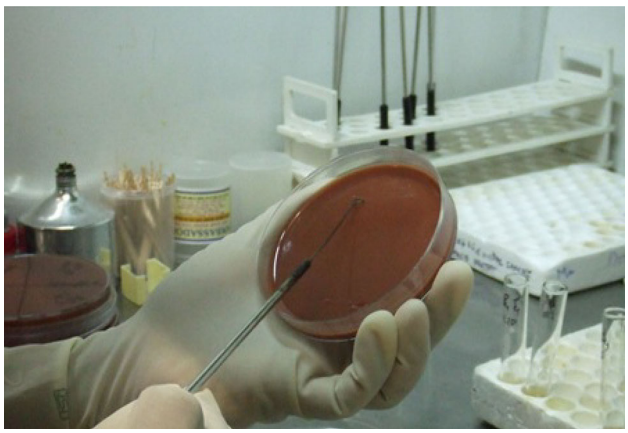


Fig. 13 Streaking of samples contained in tryptic soy broth over divided chocolate agar plates

2. The possible effects of microwave irradiation in surface detail quality, dimensional accuracy, wet compressive strength of dental casts after single and multiple irradiations can be investigated.

Conclusion

- Microwave oven irradiation is effective in disinfecting gypsum casts, it is considered to be more effective in disinfecting dental gypsum casts when compared to chemical disinfection.

Fig. 14 Infra red carbondioxide incubator

Routine use of microwave for disinfection of cast could be recommended and used in between procedures to prevent cross contamination in the dental clinics.

Fig. 15 Chocolate agar plates with colonies in control sample

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Conflict of interest None.

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