



Evaluation and Comparison of the Effectiveness of Physical Methods of Disinfection on Heat-Polymerized Polymethyl Methacrylate – An *In-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author AUR performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i48B33286

Editor(s):

(1) Syed A. A. Rizvi, Nova Southeastern University, USA.

Reviewers:

(1) Zbigniew Raszewski, Poland.

(2) Alper Ozdogan, Ataturk University, Turkey.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/76374>

Original Research Article

Received 22 August 2021
Accepted 27 October 2021
Published 10 November 2021

ABSTRACT

Introduction: The common relationship of Candida and the ample proof that acrylic resin dentures are related to denture stomatitis in the oral cavity, which can range from simple mucosal lesions to a fatal systemic form. The current study aims to assess and compare the efficacy of microwave irradiation and UV radiation in the disinfection of Candida albicans infection in heat-polymerized polymethyl methacrylate.

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Materials and Methods: A total of 90 specimens were prepared with heat-polymerized acrylic resin, out of which 30 were used for Microwave irradiation, and 30 were used for UV irradiation, and 30 were used for positive control. There were three groups: Group A (Positive Control group); Group B (Microwave irradiation); Group C (UV radiation). After preparing the samples they were chemically sterilized, then infected with *C. Albicans*, and again Group B is disinfected with Microwave irradiation, Group C is disinfected by UV light and Group A is kept as Positive control without disinfection. Turbidity was measured for each group after re-infection of every specimen in CFU/ml.

Results: In the present study, the CFU/ml of *Candida albicans* in the control group was $2.97 \times 10^7 \pm 0.6 \times 10^7$ ($P = .000$), for microwave irradiation, the mean value was 5.3 ± 11.1 ($P = .000$), and for UV radiation, the mean value was 19.3 ± 22.9 ($P = .003$).

Conclusion: According to the results of the current study, microwave irradiation (6 minutes at 650W) is more effective than UV radiation (10 minutes each side at 254nm wavelength) of *Candida albicans* infection on the heat-polymerized acrylic resin in laboratory conditions.

Keywords: Microwave disinfection; UV radiation; polymer; *Candida albicans*; CFU; electromagnetic irradiation.

1. INTRODUCTION

The important goal of dentistry is to provide healing and recover the patients from dental problems. In the event of dental procedures, the dentist has a cardinal role to prevent infection among the patient as well as in dental personnel and technician as a result of cross-contamination. Prevention of cross-contamination is a vital area in the dentistry field over a few decades. Thus there is a huge burden for dental surgeons to prevent infectious diseases such as tuberculosis, viral pneumonia, and herpes among the patients seeking the treatment [1].

The common relationship of *Candida* and the ample proof that acrylic resin dentures are related to denture stomatitis in the oral cavity has been reported several times in the literature [2]. This has resulted in a huge amount of research data pointing to *Candida albicans* as the principal etiologic agent of a disease that can range from simple mucosal lesions to a fatal systemic form. [2-3].

Disinfection or cleaning of dentures encompasses various methods such as mechanical, chemical, and/or a combination of both. The main demerit of the mechanical method, it lacks the capacity to remove a large volume of adherent microorganisms and also elicits scratches on the dental surface [4]. Disinfection using a wide range of chemicals such as vinegar [5], alcohol [6], sodium hypochlorite [7], Glutaraldehyde [8], and chlorine dioxide [9] have been employed in the dental practice. However, the important demerits of these techniques include corrosion, Microwave

energy has been proposed in dentistry as a simple alternative to prosthetic disinfection to avoid the problems of chemical disinfection [10].

Microwave irradiation was initially used to polymerize microwave-activated acrylic resins, but it has since been employed for post-polymerization treatment and has been found to efficiently disinfect [11-13]. In the literature, different microwave irradiation times (3, 5, and 6 minutes) and power (650 and 720 W) settings for denture disinfection have been recommended [14]. Despite its disinfectant efficacy, these techniques have yielded conflicting results, including negative impacts on various physical and mechanical properties as a result of material heating during irradiation, which could impair the polymer structure [15]. Previous research has revealed that the amount of time acrylic resins are exposed to heat during disinfection could be lowered without causing any negative consequences [16]. *Candida albicans* can be disinfected effectively with a one-minute microwave exposure [13]. Only the effects on hardness and flexural strength were validated by a one-minute high-power microwave irradiation exposure [15].

UV radiation has recently become an excellent method of inactivating microorganisms since it is efficient at killing microorganisms and bacterial spores while keeping the material's quality [17]. Against this backdrop, the experimental study was carried out to evaluate and compare the effectiveness of microwave irradiation and UV radiation on the disinfection of heat-polymerized polymethyl methacrylate (PMMA). The null hypothesis is that there was no difference between the efficacy of both physical methods of

disinfection on heat-polymerized polymethyl methacrylate of *Candida albicans* infection.

2. MATERIALS AND METHODS

The present experimental study was conducted at Department of Prosthodontics, Crown & Bridge and Implantology, Modern Dental College & Research Centre, Indore (M.P.) from September 2014 to October 2015. According to Emami E et al. [18] sample size of 30 in each group was determined with 99% confidence interval, 90% power of test, with absolute precision of 0.27.

2.1 Fabrication of Acrylic Specimens

Ninety Specimens of polymethyl methacrylate heat-polymerized denture base resin (Trevalon, Dentsply India Pvt. Ltd., Gurgram, India; Pink colour) were used in this study. To fabricate square acrylic resin specimens, stainless steel die was prepared to simulate the specimen of 10x10x3mm diameter as used by previous study [19]. These metal dies were invested in a conventional brass flask (Varsity flask; Jabbar and company, India) by using dental stone (Dentstone, Neelkanth minichem Pvt. Ltd, Jodhpur, India). After the stone was set, the dies were removed, and separating media (Acralyn-H, Asian Acrylates, Mumbai, India) was applied to the stone mould surface. This was allowed to dry at room temperature. Polymethyl-methacrylate heat cure denture base resin was mixed according to manufacturer's instructions (3:1 polymer monomer ratio by volume). It was packed in the respective mould in the dough stage and then processed using the short curing cycle (74°C for 2 hours and increasing temperature up to 100°C for 1 hour). After curing the flasks were bench cooled overnight before they were deflasked.

The polymerized resin specimens were extracted, and excess resin was removed using a bur from all of the specimens. A cotton wheel and pumice slurry (Local dental market) was used to polish one surface of the resin specimens at slow speed. Finally, all specimens were submerged in distilled water for 48 hours at room temperature to remove any remaining monomers.

2.2 Disinfection of Specimens

All acrylic specimens were sterilized with ethylene oxide gas after being stored in water

(Ambica boiler & Fabricator, Gujrat, India). Five acrylic specimens were evaluated as negative controls to confirm the procedure's efficiency. Acrylic specimens were added individually to 5 mL of sabour dextrose broth (SDB) (Himedia laboratory Pvt Ltd, Mumbai, India) in a 10 mL sterile test tube, which was sealed with foil, fifteen days following disinfection. The test tubes were then incubated for 7 days at 37°C. The broths were tested for microbiological growth after 48 hours and 7 days (turbidity). After 2 and 7 days, there was no turbidity in the broth test tube.

2.3 Inoculants Preparation of *Candida albicans*

The Department of Microbiology in Mumbai provided the *Candida albicans* (ATCC 60193) strain. *C. Albicans* isolates were inoculated in sabour dextrose broth (SDB) to a turbidity of 0.5 McFarland scale = 10^7 CFU/ml for 24 hours at 37°C.

2.4 Infecting Specimens

Each sterile test tube containing the 5ml of sterile SDB received 50 µL of inoculated SDB. For contamination, each sterile acrylic specimen was aseptically placed in one inoculation tube, sealed with foil, and incubated for 24 hours at 37 °C. Following incubation, 30 acrylic specimens were chosen for microwave irradiation, 30 acrylic specimens for ultraviolet radiation, and 30 acrylic specimens that had not been treated were utilized as a positive control.

2.5 Disinfection of Contaminated Specimens

2.5.1 Microwave irradiation

All microwave-irradiated acrylic specimens were transferred to a 600 mL beaker containing 150 mL sterile distilled water in an aseptic manner. each beaker was irradiated at 650 W for 6 minutes on the rotatable plate of an unmodified domestic microwave oven (Havells India Ltd, New Delhi, India, Model e 17 S WMN, 700 watts, 50 Hz). After that, each acrylic specimen was placed in a sterile test tube containing 5ml of SDB and handled in the same way as the positive controls.

2.5.2 Ultraviolet radiation

Each UV-radiated acrylic specimen was transferred aseptically to a sterilized stainless

steel tray and placed in the UV chamber (Sankyo Denki, Japan, G18T8, Japan). All of the infected acrylic specimens were now exposed to UV radiation at a wavelength of 254nm for 10 minutes on each side. Acrylic specimens were individually inserted in a sterile test tube containing 5ml of SDB after 10 minutes of exposure and handled in the same way as positive controls.

2.6 Re-inoculation of Specimen and Colony Counting

The test tube containing positive control, microwave, and UV treated acrylic specimens were vortexed vigorously in a shaker incubator for 1 minute and allowed to stand for 9 minutes, followed by a short vortex to re-suspend any organisms present. To determine the number of micro-organisms in the 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} dilutions, replicate specimens 50 μ L of the suspension were transferred to plates of selective media (Himedia laboratory Pvt Ltd, Mumbai, India) sabourad agar containing 5 μ g/ml Gentamicin. The plates were incubated at 37^o C for 48 hours. After incubation, for 48 hours Candida colony count of each plated acrylic specimen was counted and the number of the colonies according to the dilution ratio was calculated in CFU/ml as used in previous studies [20-22].

2.7 Statistical Analysis

The data were represented as mean and Standard Deviation. The data obtained from the current study were subjected to an independent student t-test for comparison between the groups with statistical software (IBM SPSS Statistics, v20; IBM Corp., Armonk, N.Y., USA)

3. RESULTS

In the present study, the mean value of CFU/ml of *Candida albicans* for the control group was 2.97×10^7 , for microwave irradiation was 5.3, and for UV radiation was 19.3 (Table 1). There was a significant reduction of CFU/ml of *Candida albicans* in microwave irradiation when compared to control ($P = .000$), UV radiation compared to control ($P = .000$), and Microwave radiation compared to UV radiation ($P = .003$) (Table 2).

4. DISCUSSION

The oral cavity is habitat for many different microorganisms, the composition and quantity of

which may change with age of human, health condition. The use of a dental prosthesis is indispensable for the function and esthetic rehabilitation of edentulous patients. Synthetic acrylic resins have a long clinically proven history of use for dentures since they exhibit adequate physical, mechanical, and esthetic properties [23]. However, they are susceptible to microbial adhesion and biofilm forms on the tissue-fitting surface and the outer surface of denture, thereby changing the oral ecosystem which is different from that of natural dentition [24]. Several studies [25-27] were carried out to understand & know the mechanism of adhesion of microorganisms to the prosthesis. The adhesion of microorganisms was found to be dependent on several factors like surface roughness, the free surface energy of acrylic resin base, the activity of microorganisms, saliva, and trauma.

Yeast and bacteria generally colonize dental materials in saprophytic form without producing diseases and candida species have a major role in the development of denture stomatitis [28]. However, some previous study [29] support a different theory that bacteria such as Streptococci, Staphylococci, Neisseria, Actinomyces, Salivarius, and other fungi play a significant role. They can act as a co- adjuvant factor, triggering or perpetuating a disease as in the situation of denture stomatitis, unless there are predisposing host factors such as other diseases or medications, etc. Analysis of the microorganism present in this diseased plaque showed a greater presence of *Candida albicans*.

In removal prosthesis, many disinfectants have been used such as Sodium hypochlorite [7], Glutaraldehyde [8], Chlorine Dioxide [9], and Sodium Perborate Monohydrate [30], etc. The present study was undertaken to quantitatively analyze the candidal growth after acrylic resin specimens contamination and to compare the efficacy of two different commonly used, rapidly effective, cost-effective, non-toxic, easily implemented household disinfection techniques (microwave irradiation and UV radiation) after the contamination procedure.

After the fabrication of acrylic specimens, all specimens were disinfected with ethylene oxide gas. The use of ethylene oxide gas has been advocated by previous studies [20-22,31].

Five additional specimens were examined as negative controls to confirm the procedure's success. Following disinfection, specimens were placed in sterile test tubes with 5 mL of SDB and

Table 1. Mean and SD of colony-forming units of *Candida albicans* in different groups (CFU/ml)

Groups	CFU/ml Mean	Range value		±SD
		Minimum	Maximum	
Group A	2.97 x 10 ⁷	1.96 x 10 ⁷	4.18 x 10 ⁷	0.6 x 10 ⁷
Group B	5.3	0.0	40.0	11.1
Group C	19.3	0.0	80.0	22.9

Table 2. Comparison of *Candida* colony count in the acrylic resin specimens between Untreated, Microwave irradiated and UV radiated group by using t-test

Parameter	Mean	Mean	t-test value	p-value
Distance	CFU/ml			
Control Vs Microwave	2.97 x 10 ⁷	5.3	26.86	0.000***
Control Vs Ultra violet	2.97 x 10 ⁷	19.3	26.16	0.000***
Microwave Vs Ultraviolet	5.3	19.3	3.010	0.003***

*** = Highly-significant

incubated at 37°C for 7 days. The broths were tested for microbiological growth after 48 hours and 7 days (turbidity). At 48 hours and 7 days, there was no turbidity in the broth tubes. It can be correlated with previous studies [20-21]. Organism of current study was strain of *Candida albicans* (ATCC 60193) used, advocated by previous studies [20-21,32]. This fungal agent was adjusted corresponding to 10⁷ organisms/ml in SDB according to the 0.5 McFarland scale, advocated by previous studies [19-21].

In the Group B, acrylic resin specimens were disinfected with the Microwave Irradiation method at 650 W for 6 minutes under moist heat conditions. PMMA acrylic specimens were then kept in a sterile beaker containing 150 ml of distilled water and the water prevents overheating of the acrylic material, the glass transition temperature (T_g) of PMMA ranges from 100-120 °C. After this temperature, the material begins to change its properties and dimensions. The molecules of water play a major role in absorbing the energy from microwave irradiation [33]. Heat is produced by the friction of water molecules, and a rise in temperature can inactivate bacteria [34]. As a result, the outcomes of this investigation might be linked to the presence of water during microwave irradiation. In the post-disinfection culture, all of the specimens in the microwave group showed no or very little growth. The average CFU/ml number is 5.3. Previous research on microwave disinfection of complete dentures [20,35-36], hard [21,37], and soft [38] chair side relines resin, regardless of exposure time and power, have found similar results.

Ultraviolet rays have long been recognized as an effective method for killing microbes [39]. When microorganisms are exposed to UV rays at a particular wavelength (200-280 nm), microorganisms' reproduction capability is destroyed and inactivation occurs at a faster rate [40]. The use of UV rays can be a good choice for disinfection because UV Chambers are also available in most dental clinics. In the current study, UV radiated group, were disinfected by placing the acrylic resin specimens in the UV chamber for 10 minutes on each side with 254 nm wavelengths [40], and the results are according to the previous study [41].

The present results showed that there was a significant reduction in the microbial turbidity in both microwave and ultraviolet groups compared to the control group. Furthermore, our results also showed a significant reduction in the candidal colony count in the Group B when compared with Group C. In fact, statistical analysis revealed significant differences between the Group B & C. This result is supported by previous studies [14,19,41].

Denture disinfection is very essential for the prevention of denture stomatitis or other infectious diseases in the denture wearer patient [42]. Data shows that microwave and UV radiation both can be used for the disinfection of polymethyl methacrylate acrylic resin. Disinfection using microwave irradiation proved to have a more superior quantitative influence on candidal growth as compared to ultra-violet radiation. Microwave irradiation is simpler, less sophisticated, cheaper, more applicable, and easy to use home measure and dental clinic that

still is efficient enough to disinfect acrylic resin materials [43].

Denture bases were shown to be better disinfected by microwave irradiation than UV light, according to present invitro study results. Although further research is needed to determine the optimal temperature and duration for eradicating all organisms and spores, sterilization instead of disinfection will be evaluated of microwave irradiation and UV radiation in the future studies in clinical conditions. Our findings clearly show that a denture base is contaminated after usage, and thus necessitates better disinfection procedures especially after any infections.

5. CONCLUSION

According to current study results, microwave irradiation (6 minutes at 650W) is more effective than Ultraviolet radiation (10 minutes each side at 254nm wavelength) of *Candida albicans* infection on heat-polymerized acrylic resin in laboratory condition.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We conducted our research after taking ethical clearance (S.No. /MPMSU/IEC/2014/1139).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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