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An in-vitro evaluation of microwave irradiation and denture cleansers on disinfection and destaining of denture base resin

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ABSTRACT

Purpose: Denture cleansing tablets have been used effectively against stains and *Candida* when soaked for longer time yet complete sterilization against broad range of bacteria is questionable. Microwave sterilization has been shown effective against bacteria and fungus both but not against stains. Our study aims to comparatively evaluate the efficacy of microwave irradiation and denture cleanser on the disinfection and destaining of stained denture.

Materials and methods: Ninety-nine acrylic sheets were stained with *Acacia catechu* and lime and sterilized in UV chamber. Three samples served as negative control and remaining 96 samples were divided in groups A, B, C and D according to contaminating microorganism; three bacteria and one fungus. According to time of irradiation, groups were subdivided into subgroups-1 (2 min), 2(4 min), 3(6 min) and 4 (denture cleanser for 8 h). Microwave irradiation was done either in presence of water or denture cleansing solution. Following procedure samples were scored for staining and then inoculated for 24 h. Then after broth were checked for OD. One sample from each subgroup was inoculated for cfu count.

Results: Effective disinfection for all stained samples achieved with microwave for 6 min in presence of water or denture cleansing solution. Denture cleanser for 8 h was more effective for *Candida albicans*. 100% stain removal was found with irradiation for 6 min in presence of denture cleanser.

Conclusion: 6 min microwave irradiation in presence of denture cleansing solution could achieve complete sterilization even for spore and membrane forming bacteria. Simultaneously it completely destained all the samples.

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1. Introduction

The presence of microbial film on the surface of dentures is an important etiologic factor in denture stomatitis. Despite extensive polishing methods employed at the time of insertion of removable acrylic prosthesis, there remains many surface irregularities. These irregularities are the niche for harbouring microorganisms including viruses, bacteria and fungi[1] in patient's mouth. In absence of proper hygiene the biofilm formed on this surface starts taking up stains together with growth of microorganisms. If not sterilized properly, the appliance becomes main source of infection to patient himself

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and to the operator. Blood and saliva may carry potentially virulent viruses or bacteria that can produce the common cold, herpes, hepatitis B, pneumonia, tuberculosis and AIDS.[2] Denture stomatitis because of *Candida albicans* is common in unhygienic removable prosthesis wearers.[3–5] Various methods had been tried to disinfect infected dentures and toothbrushes which include mechanical, chemical and more recently microwave methods. Brushing acrylic appliances with water and either with soap or toothpaste though has been shown reasonably effective but may cause wearing of dentures, which increases with the increasing diameter of bristles and abrasive toothpastes. Chloroform containing toothpastes causes severe acrylic wear due to chemical dissolution of acrylic resin.[6] Ultrasonic agitation, another means of mechanical cleansing may be effective in presence of disinfectants, but is not a common household appliance.[7] Chemical denture cleansing with oxidants (Fittydent tablets) till now seems to be most effective on new plaque. A study had compared the efficacy of different commercially available denture cleansing tablets to remove stains and *C. albicans* and found that fittydent tablets (group pharmaceuticals, India) not only had greatest stain removal potential but also was significantly effective in removing *C. albicans*.[8] But separate samples of acrylic sheets were taken to test the effectiveness of denture cleansers against stains and *C. albicans*. The acrylic sheets too were of different size for two purposes. While in reality, stained and infected denture is the one object of same thickness in mouth. No study was found to test denture cleansers against whole range of microorganisms and, thereby effectiveness of these to sterilize the denture surface is questionable.

Recently wet microwave irradiation for 6 min has been shown effective against various forms of bacteria.[9] On the other hand dry or wet microwave irradiation alone is not effective against stains. Microwave, now is becoming common household electrical appliance. Patients might be advised to use microwaves to effectively disinfect the denture and thus to reduce denture stomatitis. Stains and *Candida* can be removed effectively by soaking the dentures overnight in denture cleansing solution.[8] But whether better sterilization can be achieved by combining the microwave irradiation with denture cleansing solution is a question to be searched?. Thus this study was undertaken to test the null hypothesis that simulated polished and rough surfaces of denture acrylic, contaminated with three bacteria (*Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Bacillus subtilis*) and one fungus (*C. albicans*) and stained with mixture of *Acacia catechu* (kattha) and calcium carbonate (lime) cannot be destained and sterilized by microwave irradiation in presence of denture cleansing solution.

2. Materials and methods

99 acrylic sheets of dimension 10 × 10 × 3 mm. were fabricated. Wax patterns were invested in denture flasks, dewaxed, packed with the denture base resin (Trevelyan, Dentsply India Pvt. Ltd.), and heat polymerized according to manufacturer instructions. Acrylic sheets were removed from flask after bench cooling. One surface of sheets was polished on buff wheel with pumice slurry (Fig. 1). Other surface was left untouched to simulate intaglio denture surface. All sheets were stored in a sterilized polythene bags containing sterile water at 37 °C.

2.1. Staining of samples

All 99 acrylic samples were dipped in aqueous staining agent prepared with mixture of kattha (water soaked powdered *A. catechu*) and lime in the ratio of 4:1 for two days. These were then dried for 2 h (Fig. 2A,B) and stored in sterilized polythene bags till next step.

2.2. Culturing microorganisms and contaminating the specimens

Three bacteria i.e. *S. aureus*, *B. subtilis*, *P. aerugenosa* and one fungus i.e. *C. albicans* were obtained from microbiology department, Modern Dental College & Research Centre, Indore. Three bacteria were cultured in nutrient broth and one fungus was cultured in sabouraud (SD) broth. Beakers containing broths were incubated for 24 h for bacteria and for 72 h for *Candida* respectively, at 37 °C to a turbidity of 0.5 of the McFarland standard, corresponding to10^7 organism/ml. Now all the stained samples were sterilized under an ultraviolet light for 8 h (Fig. 3) each side and then stored in sterile bags until needed. Out of 99 stained samples, 3 specimens were kept aside to serve as negative control, while remaining 96 specimens were distributed equally into 4 groups for inoculation according to research plan (Fig. 4). 24 stained and sterilized specimens were inoculated in suitable 100 ml broth containing clinical isolates of respective microorganism. Beakers were sealed with foil and incubated for 24 h at 37 °C (Fig. 5). After 24 h incubation three specimens from each group were kept separately in sterilized polythene bags to serve as positive control.

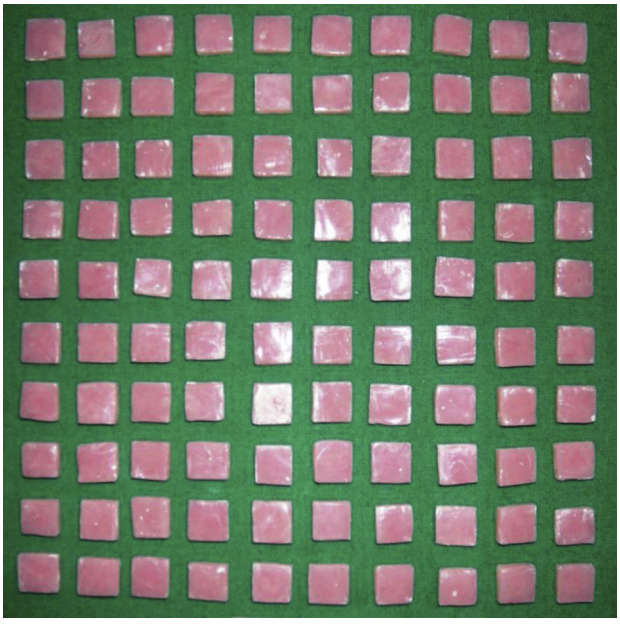


Fig. 1 – Prepared acrylic samples.

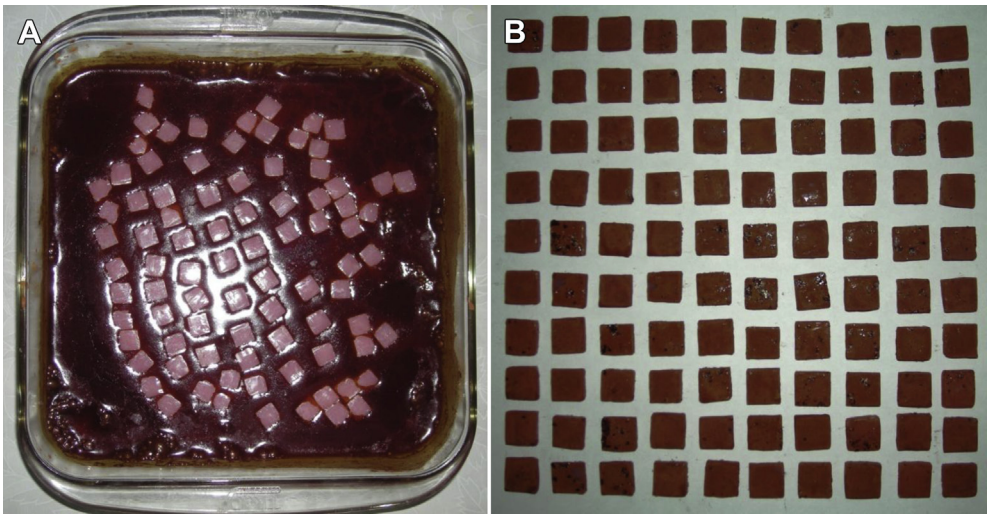


Fig. 2 – A, B Staining and drying of acrylic samples.

2.3. *Sterilization of specimens*

An unmodified domestic microwave (Fedders Lloyd, India, model – 17 S WMN, 700 watts, 50 Hz) was used. For microwave group petri dishes and test tubes were prelabelled according to the time of sterilization (2,4 or 6 min), microorganisms tested (P, B, S OR C), presence of water (MW) or denture cleanser solution (MW + DC). For denture cleanser 8 h subgroup four petri dishes and test tubes were prelabelled as DC P, DC B, DC S or DC C. After contamination procedure is over, the individual beakers were taken in the UV chamber one by one. Three specimens from each of the four beakers representing four microorganisms, were taken in the prelabelled petri dishes for 6 min, 4 min and 2 min sterilization. For denture cleanser subgroup 3 specimens from each of the beaker were immersed in the denture cleansing solution in separate petri dishes. Solution was prepared by dissolving one tablet (Fittydent, Group pharmaceuticals, India) in 200 ml of distilled water (Fig. 6). These petri dishes were kept for 8 h to simulate overnight immersion of denture.

Each time, after microwave irradiation for one particular subgroup, petri dishes were taken into the UV chamber. Irradiated and destained specimens were transferred into the prelabelled test tubes filled with 5 ml of suitable broth. For bacteria nutrient broth and for *Candida* SD broth was used. These test tubes were secured into the prelabelled test tube stand in order to avoid cross mixing and confusion. The all four test tube stands were kept in the incubator at 37 °C for

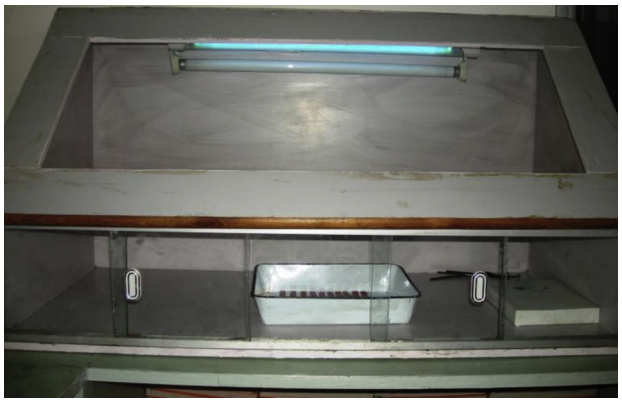


Fig. 3 – Sterilization of samples in UV light.

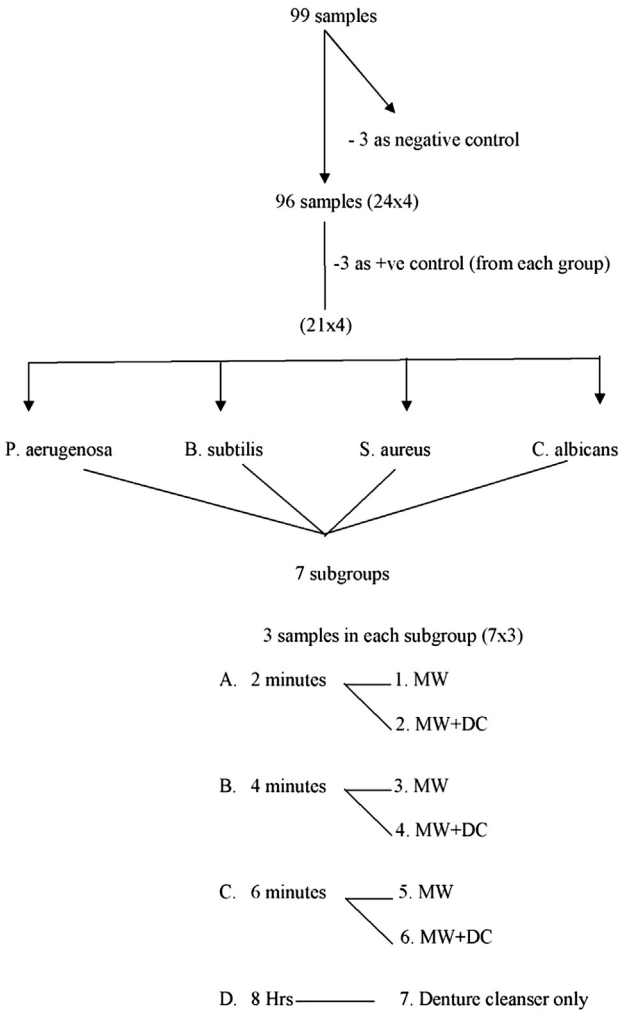


Fig. 4 – Research plan.



Fig. 5 – Contaminating the samples in suitable broth for individual microorganism.

24 h (Fig. 7). After 8 h for denture cleanser group specimens were transferred and incubated in the test tubes in similar way.

After completion of incubation period of 24 h for all the subgroups test tubes were taken out. One by one the 1 ml of broth medium of each test tube was transferred in optical density test tube to check the optical density in spectrophotometer (Electronics, India, model no. 313) (Fig. 8). Now one sample from each subgroup which showed mode OD were selected for cfu count. 50 μL of sample broth was diluted in 5 ml of distilled water to achieve 1/100 dilution were incubated by using pour plate technique in their respective media (i.e. SD media for *Candida* and nutrient media for bacteria). The petri dishes were incubated at 37 °C for 24 h for bacteria and 72 h for *C. albicans* (Fig. 9). For denture cleanser subgroup after 8 h,

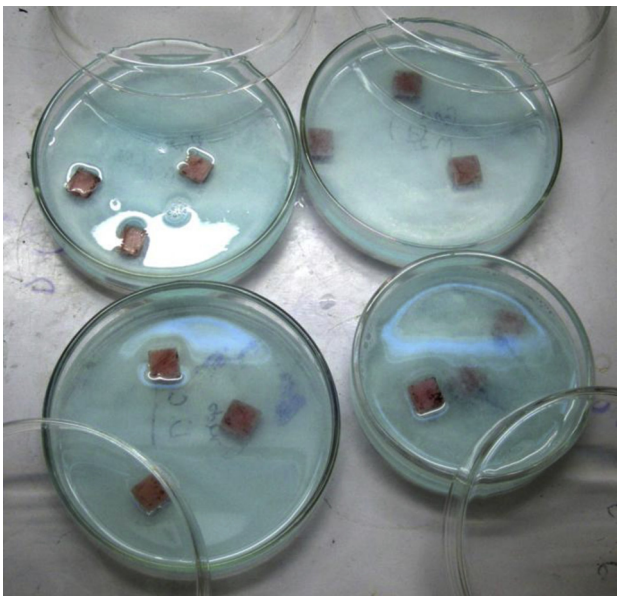


Fig. 6 – Samples immersed in denture cleanser solution for 8 h.

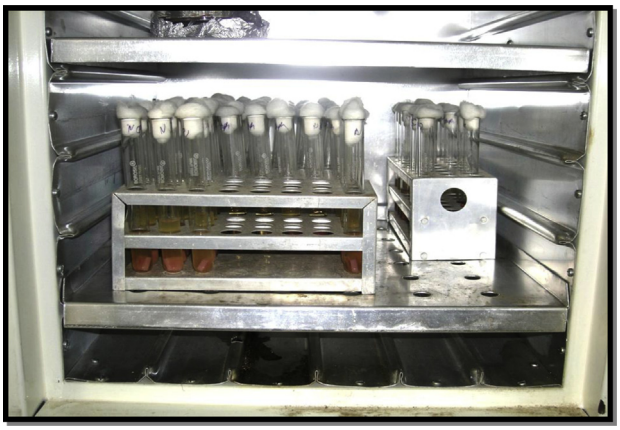


Fig. 7 – Incubation of samples in broth individually.

scoring for stains was done and same procedure was followed to transfer the individual sample in sterilized broth, incubation, recording of OD and cfu count.

2.4. *Scoring for stains*

Each time after irradiation process scoring for staining was done according to the criteria suggested by Thakral et al (Table 1).[10]

3. Results

Scoring for stain removal, OD count and cfu count after sterilization process are shown in Tables 2–4. Simulated specimens of dimensions 10 × 10 × 3 mm contaminated with clinical isolates of *S. aureus*, *B. subtilis* and *P. aerugenosa* and stained with kattha and lime showed complete sterilization after 6 min microwave irradiation in presence of denture cleanser. Complete removal of stains was found in microwave with denture cleanser (MW + DC) subgroup at 4 and 6 min irradiation as compared to denture cleanser (DC) subgroup (Table 2). There was little increase in OD in 2 min in MW + DC subgroup as compared to MW group. OD was found 0 in 6 min in MW +DC subgroup (Table 3).



Fig. 8 – Checking O.D. of broth in spectrophotometer.

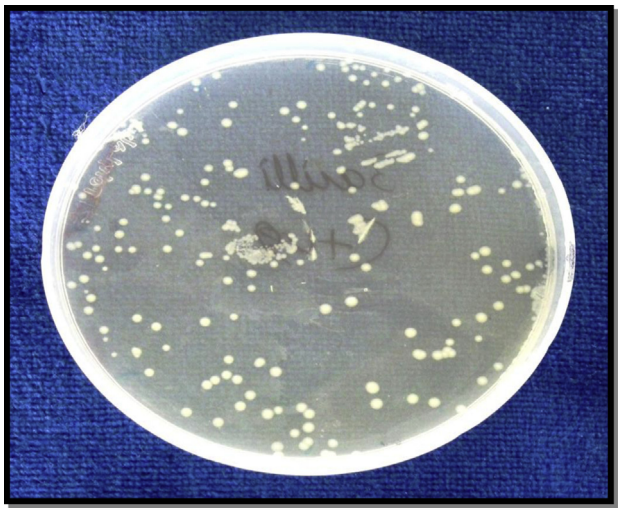


Fig. 9 – Culturing microorganisms in media.

No growth for any of the tested bacteria was observed on plates in MW + DC subgroup irradiated for 6 min. Substantial reduction in *Candida* growth was found as compared to control group (Table 4). Effective disinfection was achieved in denture cleanser subgroup after 8 h immersion. It was more effective for *C. albicans* as compared to bacteria tested (Table 4).

Comparison between subgroups for stain removing efficacy and steriliazation efficacy at different time period is represented in bar diagrams (Figs. 10–12). Bar diagram shows complete sterilization of bacteria but not *Candida* at 6 min. irradiation in MW + DC subgroup.

4. Discussion

The present study showed that simulated stained acrylic specimens contaminated with *S. aureus*, *B. subtilis*, *P. aeruginosa* were effectively sterilized together with complete destaining at 6 min of microwave irradiation (700 watt) in presence of denture cleanser. Specimens contaminated with *C. albicans* also showed consistent sterilization in the same conditions. These findings were not consistent with a previous study by Silva et al.[9] They found mean numbers of cfu/ml for *P. aerugenosa*, *B. subtilis* and *S. aureus* were significantly higher than for *C. albicans*. Reverse was observed in this study. It may be because simulated stained samples as in vivo conditions were taken in this study while Silva et al had used unstained samples.

Kattha with lime was used for staining because it is most widely used in India and consumed with pan and pan masala. This causes heavy and multilayer staining on denture surface which is difficult to brush out. Microorganisms get entrapped between layers which are difficult to sterilize unless layers of stains are removed.

Table 1 – Criteria for scoring of stains on acrylic samples.

No removal of stains	0
Partial removal of stains	1
Almost complete removal of stains	2
Complete removal of stains	3

Rohrer and Bulard[7] observed that acrylic resin dentures contaminated with individual suspensions of four aerobic bacteria and one fungus showed sterilization of all microorganisms after 10 min microwave irradiation at 720 watt. Webb et al[11] soaked denture in sodium hypochlorite before irradiation at 350 watt for 6 min and found it more effective method for denture inoculated with *C. albicans* and *S. gordonii*. But it is important to note that repeated use sodium hypochlorite causes rapid bleaching of denture material.[6,12] Dry microwaving also has been tried which was effective in disinfecting dentures contaminated with *C. albicans*.[7,11,13] On the other hand one other study proved that 5 min irradiation in wet condition resulted in effective sterilization.[14] Perhaps presence of water could kill microorganism even in the micro pores of acrylic resin. Some other studies have also demonstrated that wetting of contaminated sponges and underwears before microwaving was necessary to obtain subsequent disinfection.[15,16]

In this study we have tested the efficacy of irradiation in presence of denture cleansing solution. Denture cleanser has been shown effective in disinfecting the acrylic dentures contaminated with *C. albicans*.[8] At the same time loosening, bleaching and removal of stains occurs from the surface of acrylic resin.[6,8] Denture cleansing tablet (Fittydent) contains alkaline sodium perborate and sodium bi carbonate, when dissolved in water sodium perborate liberates hydrogen peroxide slowly at constant rate.[6] This hydrogen peroxide in basic solution disproportionate into water and oxygen molecule.[17,18] Simultaneously other hydrogen peroxide molecule gives off hydroxyl ions and radicals. Gaseous oxygen molecule is supposed to be responsible for penetrating the subsurface of stains and causing mechanical loosening of it.[6] Application of external heat not only increases the penetration of radicals and ions but also accelerates the production of oxygen.[19] Thus irradiation in presence of denture cleansing solution is supposed to cause destaining as well as effective sterilization. Presence of oxygen itself is lethal for anaerobic bacteria. Radicals produced from hydrogen peroxide causes direct damage to cell wall and DNA.[20] In addition to the presence of activated H_2O_2, irradiation with microwave results in destruction of microorganisms probably from the interaction of the electro-magnetic field with the molecules in the cells and surrounding liquid medium.[21] As microwave has affinity towards water molecule, the H_2O molecule in bacterial cell wall starts oscillating. If these oscillations exceed the elasticity of cell wall, the cell ruptures.[7,9] In our study hydroxyl radical and ions disrupts the cell wall which lowers down the elasticity of it, making it readily vulnerable for destruction with hydroxyl or perhydroxyl radicals. Thus hydroxyl radicals and ions simultaneously could kill live microorganism and bleach the stains chemically together with the rapidly liberated oxygen which could remove the stains mechanically.[6]

The selection of the microorganisms in this study was based on peer-reviewed scientific data regarding concepts of indicator and surrogate pathogen organisms as well their intrinsic microbial resistance. Gram positive *S. aureus*, gram negative *P. aerugenosa*, spore forming *B. subtilis* and fungus *C.*

Table 2 – Scores for stain removal efficacy among different groups.

Bacteria/groups	+ve control	2 min		4 min		6 min		8 h	−ve control
		MW	MW + DC	MW	MW + DC	MW	MW + DC	DC	
P. aerugenosa	0	0	3	1	3	0	3	1	0
	0	1	3	1	3	0	3	2	1
	0	0	2	1	3	1	3	2	1
B. subtilis	1	0	2	2	3	1	3	2	
	1	1	2	2	3	1	3	2	
	0	0	3	1	3	0	3	3	
S. aureus	1	1	3	1	3	1	3	3	
	1	1	3	1	3	1	3	3	
	1	1	3	1	3	1	3	3	
C. albicans	0	1	2	1	3	2	3	2	
	1	1	3	1	3	1	3	2	
	1	1	2	0	3	1	3	1	
Total score	7	8	31	13	36	10	36	26	2

albicans have been recommended as indicator and surrogate pathogens to validate the effectiveness of disinfection procedures.[20]

When optical density was checked at 2 min irradiation there is little increase in OD in MW + DC subgroup (Table 2). It may be because in presence of denture cleanser, stains on the surface of specimen started loosening and got suspended in the broth medium. OD at 4 min irradiation in MW + DC group is less than MW group. It is because sufficient stains were detached from the surface of specimen which started settling down in the medium. OD at 6 min irradiation in MW + DC group is almost zero as compared to MW group perhaps because almost all stains were removed from the surface of specimen and got settled at the bottom of test tube. Supernatant medium thus showed zero readings.

The cfu count at 10^{-2} dilution showing complete sterilization for all the bacteria tested while consistent sterilization for *C. albicans*. In our study growth of fungus was quite high. Perhaps several layers of stains not only entrapped microorganisms but also provided some nutrient to them. The hyphae of fungus thus penetrated deeper into the micropores of acrylic specimens. Nonetheless effect of irradiation in presence of denture cleanser is significant. Overnight soaking in denture cleanser was more effective as compared to the 6 min irradiation for *C. albicans*. Perhaps little longer irradiation in presence of denture cleanser would have killed the *Candida* too. Uludamar et al[22] also found fittydent tablets very effective against *Candida* species.

The present study clearly demonstrates the effect of presence of stains on sterilization procedure. Complete sterilization of stained specimens was not achieved in MW group (6 min) though there was consistent disinfection. While presence of denture cleansing solution not only improved the efficacy of irradiation but also could destain the specimens rapidly without affecting the strength of acrylic. Silva[9] did not find sterilization of unstained dentures contaminated with *P. aerugenosa* and *B. subtilis* when samples were irradiated in presence of water. On the contrary we could find complete sterilization for these bacteria too in MW + DC group. Presence of denture cleansing solution might reduce the time of irradiation for unstained dentures. Silva[9] tested the sterilization on dentures. While in this study we kept 16 specimens in four separate petri dishes at one time so as to approximately simulate the denture surface area. Even then we could achieve the sterilization. Our results support the study by Silva.

There are many studies stating that there were no effect of two cycles of microwave irradiation on flexural strength of four hard chairside reliner resins and one denture base

Table 3 – OD of broth of samples after sterilization.

Bacteria or groups	+ve control	2 min		4 min		6 min		8 h
		MW	MW + DC	MW	MW + DC	MW	MW + DC	DC
P. aerugenosa	0.09	0.01	0.09	0.05	0.01	0.05	0.00	0.15
	0.17	0.04	0.04	0.04	0.01	0.17	0.00	0.11
	0.24	0.03	0.03	0.06	0.01	0.07	0.01	0.13
B. subtilis	0.04	0.01	0.04	0.01	0.04	0.05	0.00	0.05
	0.04	0.05	0.00	0.02	0.01	0.06	0.00	0.09
	0.10	0.04	0.00	0.02	0.01	0.05	0.00	0.07
S. aureus	0.07	0.06	0.06	0.02	0.02	0.03	0.00	0.07
	0.05	0.04	0.08	0.02	0.01	0.04	0.00	0.06
	0.06	0.06	0.08	0.03	0.02	0.05	0.00	0.06
C. albicans	0.06	0.04	0.02	0.02	0.06	0.02	0.01	0.06
	0.06	0.02	0.02	0.02	0.03	0.02	0.04	0.04
	0.04	0.02	0.02	0.02	0.01	0.03	0.02	0.03

Table 4 – Cfu (/2 × 10^3) at 1/100 dilution after sterilization.

Bacteria/groups	+ve control	2 min		4 min		6 min		8 h
		MW	MW + DC	MW	MW + DC	MW	MW + DC	DC
P. aerugenosa	104	11	19	9	12	6	0	16
B. subtilis	184	5	12	4	8	2	0	19
S. aureus	14	7	5	5	2	1	0	12
C. albicans	400	168	80	120	88	112	72	06

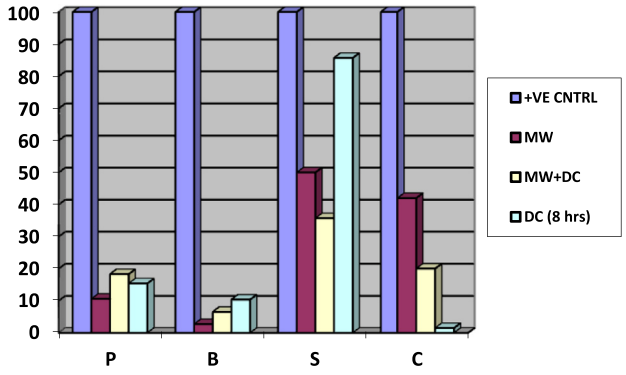


Fig. 10 – Bar diagram representing % cfu in groups after 2 min irradiation.

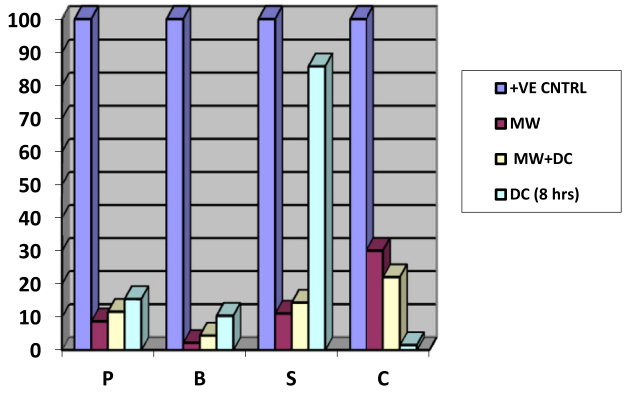


Fig. 11 – Bar diagram representing % cfu in groups after 4 min irradiation.

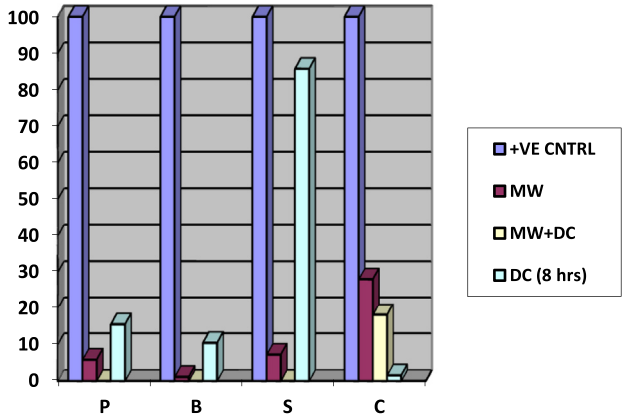


Fig. 12 – Bar diagram representing % cfu after 6 min irradiation.

resin.[22] Also there was no effect of two cycles of microwave disinfection on Vickers hardness number of five brands of acrylic resin denture teeth.[23] Machado et al[24] found that the surface roughness of denture base resin remained unaffected after sodium perborate immersion and microwave irradiation for 6 min at 650 watts. Though, studies had demonstrated that there was no effect in strength of acrylic dentures when immersed in denture cleansing solution overnight, flexural strength and hardness of acrylic specimens after irradiation for different time in presence of denture cleanser is yet to be tested. Future studies are required to test this aspect.

5. Conclusion

Within the limitations of the study, following conclusions could be deduced

- Microwave irradiation for 6 min in presence of denture cleansing solution might be an effective means of sterilization even for membrane forming and spore forming bacteria.
- Stained dentures might effectively be destained rapidly when irradiated for 4 or 6 min in presence of denture cleansing solution.
- Higher mean cfu/ml of the *C. albicans* were observed for positive control stained specimens as compared to other microorganisms.

Conflicts of interest

All authors have none to declare.

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