Microwave radiation is effective at disinfecting dental stone surfaces without changing their physical properties

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The aims of this study were to evaluate the effectiveness of different microwave radiation regimens for disinfection of type IV dental stone surfaces and to assess the influence of these regimens on surface roughness and dimensional change following disinfection. Three hundred cylindrical (20 × 2-mm) test specimens were made in type IV stone and divided into subgroups of 20 according to the microorganisms tested (*Staphylococcus aureus, Escherichia coli,* or *Candida albicans*) and the 900-W microwave radiation protocol (cycles of 3, 5, or 7 minutes; a positive control; or a negative control). To test physical changes, 80 test specimens were made with the same dimensions except that they had 2 parallel and symmetrical indentations measuring 8 × 4 mm. These specimens were divided into 4 subgroups of 20 each (a subgroup for each radiation time and a negative control). The mean dimensional change and roughness data were analyzed by mixed models for repeated measures and Tukey-Kramer tests. Disinfection was analyzed with descriptive statistics. For *E coli* and *C albicans*, all radiation times proved effective at sterilizing the test specimens. For *S aureus*, sterilization was achieved with 5 and 7 minutes of exposure; however, colonies were observed in 10 Petri dishes (50%) exposed to 3 minutes of microwave radiation. No statistically significant difference in dimensional change or surface roughness was observed for any radiation regimen ($P > 0.05$).

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Oral microorganisms can be transmitted via dental impressions and dental casts, which are handled by various dental professionals, such as laboratory technicians, dentists, and dental assistants.[1] Highly pathogenic microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans, Pseudomonas aeruginosa, Staphylococcus* species, and *Streptococcus* species, can be transmitted via dental casts.[2] Although many disinfectant solutions used on dental casts have proved effective, some studies have demonstrated significant deleterious changes in physical properties when such substances are used.[3-7] In this context, microwave radiation for disinfection of dental casts stands out as a simple, cost-effective, and noninvasive approach in comparison to chemical techniques.[8]

Microwaves are electromagnetic waves with frequencies varying between 300 MHz and 300 GHz and wavelengths ranging from 1 mm to 1 m, respectively.[9] The interactions between electromagnetic fields and biological processes are constantly being investigated.[10]

According to Jacob et al, the interaction between microwave radiation and matter is explained by the dielectric polarization caused by the wave's electric field, which occurs when particles are attached to a compound.[11] Polarization occurs in both the atom (atomic nucleus and electron cloud) and the molecule as a whole. Polar molecules (dipoles) undergo rotation within the electric field, producing heat.

The mechanism by which microwave radiation causes the death of microorganisms is still a subject of research and controversy in the literature. Some studies have indicated that the antimicrobial activity of microwave radiation is explained solely by its thermal effects, in particular intracellular protein aggregation caused by heat.[12,13] Other studies, however, have shown that microwaves may have additional, nonthermal effects on matter and biological processes.[11,14]

The effectiveness of microwave disinfection on dental casts has been corroborated by several studies.[8,15-19] This disinfection has been accompanied by gains in physical properties, such as increased tensile and compressive strength, and no change in dimensional stability.[18,20] The number of studies on the subject is, however, limited, and other changes in physical and mechanical properties of dental stone have not been explored. Therefore, the objective of the present study was to evaluate the effectiveness of different microwave radiation regimens for disinfection of type IV dental stone surfaces and the influence of these regimens on the physical properties (surface roughness and dimensional changes) after disinfection. The null

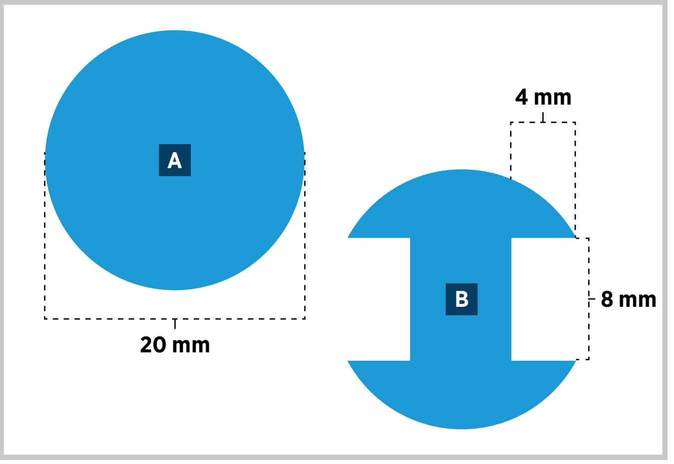

Fig 1. Dental stone specimen dimensions. A. Disinfection group. B. Physical change group.

hypothesis tested was that different microwave radiation times would not achieve disinfection of type IV dental stone casts and would not alter their physical properties.

Materials and methods

Experimental design

The experimental units consisted of 380 type IV stone specimens, divided into a disinfection group (n = 300) and a physical changes group (n = 80). The response variables were bacterial count, surface roughness, and dimensional changes of the stone casts. The factors studied were microwave disinfection protocols on 5 levels: positive contamination; negative contamination; and disinfection for 3 minutes, 5 minutes, or 7 minutes.

The factors studied were assigned to experimental units at random, forming the following subgroups (n = 20): disinfection, positive control; disinfection, negative control; disinfection with 3-minute exposure; disinfection with 5-minute exposure; disinfection with 7-minute exposure; physical changes, control (no exposure); physical changes with 3-minute exposure; physical changes with 5-minute exposure; and physical changes with 7-minute exposure. Each of the 5 disinfection subgroups was repeated separately for 3 organisms: *Escherichia coli, S aureus,* and *C albicans.*

Specimen preparation

Industrial rubber matrices (Redelease)—1 matrix for the disinfection group and 1 matrix for the physical changes group—were used for preparation of the stone specimens, producing many specimens with the same dimensions (Fig 1). Three hundred cylindrical specimens, measuring 20 mm in diameter and 2 mm in thickness, were made for the disinfection group. For the physical change group, 80 specimens were produced with the same outer dimensions as the disinfection group but with 2 parallel and symmetrical indentations of 8 × 4 mm.

All specimens were made with special type IV stone (Durone, Dentsply Brasil). The powder-liquid ratio was 100 g of powder to 19 mL of water, as specified by the manufacturer. The stone was initially mixed in a rubber mortar and then inserted in a vacuum chamber (Polidental) for 25 seconds at –690 mm Hg.[21]

The stone mixture was poured in the aforementioned rubber matrices over a stone vibrator. After crystallization time (1 hour), the specimens were removed from their matrix.[22] The specimens were then divided into the subgroups described previously.

Disinfection testing

For specimen contamination, strains from the ATCC collection were used: a gram-positive microorganism (*Staphylococcus aureus*; ATCC 25923), a gram-negative microorganism (*Escherichia coli*; ATCC 25922), and a fungus (*Candida albicans*; ATCC 10231). The suspensions were adjusted using the McFarland nephelometric method (Probac do Brasil); that is, McFarland standard 1 and McFarland standard 6 were used for bacteria and fungus, respectively, in a suspension of 300 mL. All microorganisms had individual suspensions and contamination protocols; that is, the species used in this study were not mixed.

The specimens in this group were autoclaved (Phoenix Luferco), and manipulation of sterile specimens took place in a laminar flow hood (Filtracom Sistemas e Componentes). The stone specimens were carefully removed from their sterile package using sterile forceps and placed in sterile beakers, where the stone specimens received the contaminated suspension (50 ml for 10 specimens). The immersion time was 15 minutes. The specimens were then removed from the suspension and dried on sterile gauze for 4 minutes (2 minutes per side).

Microwave irradiation was performed in a microwave oven (Brastemp Maxi with 30-L capacity and 30-cm internal plate, Brastemp) at 900 W and 2450 MHz. The specimens were placed in sterile Petri dishes and then placed in the microwave oven. The dishes were arranged so that all specimens received the same amount of radiation.[9]

Exposure time was 3, 5, or 7 minutes for all irradiated groups. Each side of the specimen received half the radiation time, so that the specimen as a whole was uniformly exposed to microwaves.[15] The negative control subgroup was not exposed to the contaminated suspensions or treatments in the microwave oven. The positive control subgroup was exposed to the suspensions but did not undergo the microwave cycles. To prevent possible damage to the magnetron, a beaker containing 300 mL of water was placed in the center of the rotating plate.

The Petri dishes containing the disinfection specimens were returned to the laminar flow hood for sample collection following microwave cycles. Sterile swabs soaked in saline were used. Samples were taken from all surfaces of the specimens and seeded into culture media: brain-heart infusion for bacteria and Sabouraud agar for *C albicans*. All culture media were placed in a microbiological incubator (ACB Labor) for 24 hours at 37°C under aerobic condition.

The Petri dishes were removed and analyzed under a stereomicroscope to observe the presence or absence of colony growth.

Physical changes testing

The specimens from the physical changes group underwent the same process of microwave irradiation described for the disinfection group. The negative control subgroup was not subjected to any microwave irradiation.

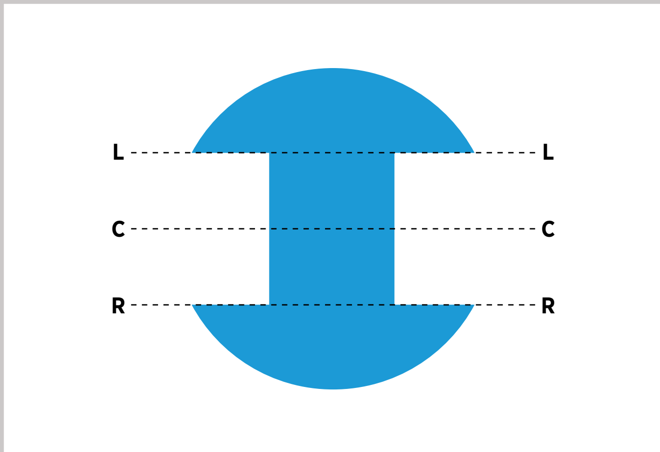

Fig 2. Points measured to examine dimensional change. L, left; C, center; R, right.

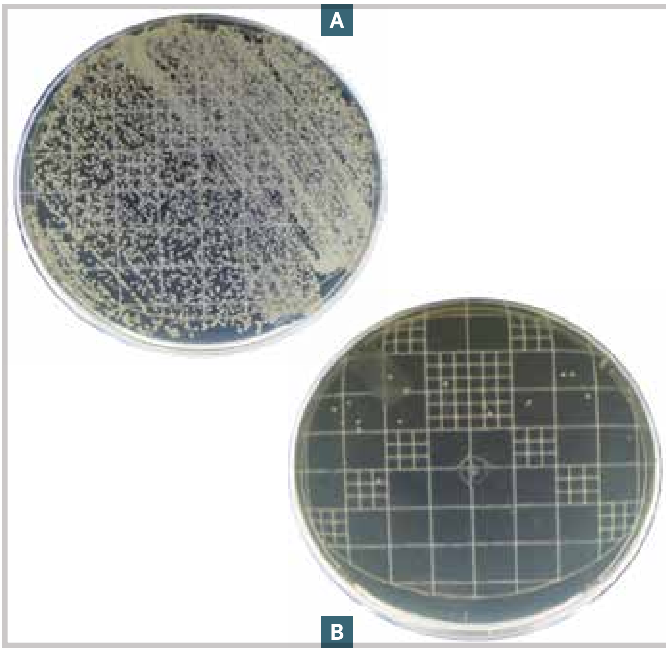

Fig 3. Growth of *Staphylococcus aureus* in Petri dishes. A. Positive control subgroup. B. Subgroup microwaved for 3 minutes.

Table 1. Number of Petri dishes with positive and negative growth according to disinfection method.

		Growth	
Microorganism	**Time (n = 20)**	**Positive**	**Negative**
Staphylococcus aureus	Positive control	20	0
	3-Min disinfection	10	10
	5-Min disinfection	0	20
	7-Min disinfection	0	20
	Negative control	0	20
Escherichia coli	Positive control	20	0
	3-Min disinfection	0	20
	5-Min disinfection	0	20
	7-Min disinfection	0	20
	Negative control	0	20
Candida albicans	Positive control	20	0
	3-Min disinfection	0	20
	5-Min disinfection	0	20
	7-Min disinfection	0	20
	Negative control	0	20

Table 2. Mean (SD) surface roughness (Ra) of type IV dental stone specimens before and after microwave disinfection.

	Ra	
Subgroup (n = 20)	**Baseline**	**Endpoint**
Control	0.68 (0.16)^{Aa}	0.67 (0.15)^{Aa}
3-Min disinfection	0.67 (0.10)^{Aa}	0.68 (0.11)^{Aa}
5-Min disinfection	0.63 (0.08)^{Aa}	0.63 (0.08)^{Aa}
7-Min disinfection	0.65 (0.14)^{Aa}	0.65 (0.13)^{Aa}

Means followed by the same letter are statistically similar. Lowercase letters compare data in rows and uppercase letters compare data in columns.

Surface roughness

Surface roughness tests were performed with a profilometer (TR200, Beijing TIME High Technology) before exposure (baseline) and after exposure (endpoint) to microwave radiation. The mean roughness was calculated from 4 measurements—2 vertical, 1 horizontal, and 1 oblique—with 5.00 × 0.25-mm cutoffs and Gauss filter.

Dimensional change

The specimens were measured with a digital caliper (Mitutoyo America) before exposure (baseline) and after exposure (endpoint) to microwave radiation. A single operator made 3 consecutive measurements: 2 measurements at the ends of the indentations and 1 measurement in the center (Fig 2).

Statistical analysis

The variable disinfection was analyzed with descriptive statistics. After exploratory data analysis regarding the means of dimensional changes and surface roughness, the physical change data were analyzed using mixed models for repeated measures and Tukey-Kramer test for multiple comparisons. All analyses were performed with SAS software (release 9.2, SAS Institute) and a significance level of 5%.

Results

Bacterial count

For *E coli* and *C albicans*, all radiation times (3, 5, and 7 minutes) were sufficient to sterilize the test specimens (Table 1). For the gram-positive bacteria (*S aureus*), sterilization occurred

Table 3. Mean (SD) dimensions (in mm) of type IV dental stone specimens before and after microwave disinfection.

Measurement point*	Subgroup (n = 20)	Dimension	
		Baseline	Endpoint
Left	Control	11.81 (0.06)^{Aa}	11.80 (0.06)^{Ba}
	3-Min disinfection	11.79 (0.08)^{Aa}	11.79 (0.08)^{Ba}
	5-Min disinfection	11.80 (0.08)^{Aa}	11.79 (0.08)^{Ba}
	7-Min disinfection	11.80 (0.06)^{Aa}	11.80 (0.06)^{Ba}
Center	Control	11.76 (0.07)^{Aa}	11.77 (0.10)^{Aa}
	3-Min disinfection	11.77 (0.08)^{Aa}	11.77 (0.08)^{Aa}
	5-Min disinfection	11.75 (0.08)^{Aa}	11.75 (0.08)^{Aa}
	7-Min disinfection	11.78 (0.08)^{Aa}	11.78 (0.08)^{Aa}
Right	Control	11.80 (0.10)^{Aa}	11.80 (0.08)^{Aa}
	3-Min disinfection	11.79 (0.09)^{Aa}	11.79 (0.09)^{Aa}
	5-Min disinfection	11.79 (0.09)^{Aa}	11.78 (0.08)^{Aa}
	7-Min disinfection	11.82 (0.07)^{Aa}	11.82 (0.07)^{Aa}
Mean	Control	11.79 (0.07)^{Aa}	11.78 (0.07)^{Ba}
	3-Min disinfection	11.78 (0.08)^{Aa}	11.78 (0.08)^{Ba}
	5-Min disinfection	11.78 (0.08)^{Aa}	11.78 (0.08)^{Ba}
	7-Min disinfection	11.80 (0.06)^{Aa}	11.80 (0.06)^{Ba}

*See Fig 2 for an illustration of the measurement points.

Means followed by the same letter are statistically similar. Lowercase letters compare data in rows and uppercase letters compare data in columns.

with 5 and 7 minutes of exposure to microwave radiation. However, bacterial growth was detected in 10 Petri dishes (50%) after 3 minutes of exposure. Despite the high number of dishes exhibiting positive growth in this subgroup, the number of colony-forming units was small when visually compared to the number in the positive control subgroup (Fig 3).

Surface roughness

No statistically significant difference was found among treatments between baseline and endpoint values for the variable roughness ($P > 0.05$) (Table 2).

Dimensional change

No statistically significant difference was observed among the groups for any of the treatment regimens investigated ($P > 0.05$) (Table 3). At the endpoint, a lower mean value at the left measurement site was observed when compared to baseline ($P < 0.05$). The mean dimension was also lower at the endpoint ($P < 0.05$). No statistically significant difference between baseline and endpoint values was observed for the other measurements ($P > 0.05$).

Discussion

The radiation exposure times selected for this study were based on previous studies that used a microwave oven with the same power (900 W) and reported high-power disinfection of type IV dental stone with 5 minutes of exposure.[16,17,19] Therefore, in the present study, longer (7 minutes) and shorter (3 minutes) exposure protocols were also tested. Additionally, the greater the power of the microwave oven, the less time needed for the same amount of radiation to be delivered.[12]

Most domestic microwave ovens used for dental cast disinfection have been reported to have a power of 900 W.[16-20] In view of the times and power levels studied, the time of 5 minutes at a power of 900 W was the most effective protocol for disinfecting/sterilizing dental casts. Berg et al, using cycles of 5 minutes at a power of 900 W, were also able to sterilize dental stone casts, despite using different experimental methods from those used in the present study.[16,17] Moreover, the shorter exposure time (3 minutes) in the present study resulted in a high degree of disinfection of the specimens, corroborating the results of another in vitro study.[8] Thus, the null hypothesis for the disinfection group was rejected.

Other chemical disinfection methods for dental stone have previously been studied, including incorporation of chemicals in the stone mixture and application of substances on the outer surface of the set dental stone.[3-7,18,19,23,24] It has been shown, however, that the addition of such agents to dental stone may alter mechanical and surface properties.[4-6] These changes reportedly can be inhibited by incorporating additives such as gum arabic and calcium hydroxide at the time of mixing.[4,5,25] A different study, however, demonstrated that, even with the addition of such substances, incorporation of 2% glutaraldehyde solutions and 0.525% sodium hypochlorite in the mixture led to a decrease in hardness of type III and IV dental stones.[6] The application of disinfectants to the outer surface also decreased hardness values and increased linear dimensions.[7]

It is important to highlight that glutaraldehyde can induce several toxic reactions in the body, such as allergic contact dermatitis, eye irritation, conjunctivitis, irritation of the nasal and pharyngeal tracts, sore throat, and asthma.[26] Due to its low vapor pressure (2.3 kPa at 20°C), glutaraldehyde is extremely volatile and should be handled in a cool environment while the user wears personal protection equipment.[26,27] Despite the antimicrobial efficacy demonstrated by some of these substances, there seems to be no scientific consensus on the use of chemical disinfectants in dental stone as a protocol for cross-contamination control.[3,19,23]

Disinfection of stone casts in a domestic microwave oven has been validated by other studies as more effective than chemical disinfection.[8,16-19] No reports of deleterious changes in physical properties have been identified to date.[18,20,21]

The present study found no dimensional changes in the stone surfaces evaluated, corroborating other studies in the literature.[8,18,20] Although significant drops in the values at the left measurement point and the overall dimension were observed when dimensional change was evaluated after sterilization ($P < 0.05$), the difference was very small in decimal values. The mean value of the left measurement changed from 11.80 mm at baseline to 11.79 mm after microwaving, and the mean value of the overall dimension was reduced from 11.79 mm at baseline to 11.78 mm after disinfection. It is unlikely that such changes would have a clinically relevant effect on cast accuracy, especially because the control cast (without disinfection) demonstrated the same pattern of alteration, so the null hypothesis for the dimensional changes group was accepted.

The microwave oven is a relatively low-cost, safe, durable, and easy-to-operate device that poses no significant risks to the operator and is of great value to cross-infection control in the context of prosthetic laboratories. Time can be optimized by disinfecting several casts in the oven at the same time without compromising the elimination of microorganisms.[17] The total radiation time corresponds to the sum of the times needed to expose each surface. In the present experiment, for example, the recommended exposure of 5 minutes at 900 W corresponded to 2.5 minutes of exposure on each side of the stone cast.

Conclusion

For the microorganisms used in this study, 900-W microwave radiation for 5 and 7 minutes promoted sterilization of specimens; high-level disinfection was achieved in 3 minutes with no change in dimensions or surface roughness. The findings demonstrate that routine disinfection of dental casts in microwave ovens can be safely applied by dental professionals without jeopardizing the accuracy of the prosthetic working cast.

Author information

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References

1. Leung RL, Schonfeld SE. Gypsum casts as a potential source of microbial cross-contamination. *J Prosthet Dent.* 1983;49(2):210-211.
2. Egusa H, Watamoto T, Abe K, et al. An analysis of the persistent presence of opportunistic pathogens on patient-derived dental impressions and gypsum casts. *Int J Prosthodont.* 2008;21(1):62-68.
3. Ivanovski S, Savage NW, Brockhurst PJ, Bird PS. Disinfection of dental stone casts: antimicrobial effects and physical property alterations. *Dent Mater.* 1995;11(1):19-23.
4. Abdelaziz KM, Combe EC, Hodges JS. The effect of disinfectants on the properties of dental gypsum, part 2: surface properties. *J Prosthodont.* 2002;11(4):234-240.
5. Abdelaziz KM, Combe EC, Hodges JS. The effect of disinfectants on the properties of dental gypsum, 1: mechanical properties. *J Prosthodont.* 2002;11(3):161-167.
6. Roy SM, Sridevi J, Kalavathy N. An evaluation of the mechanical properties of Type III and Type IV gypsum mixed with two disinfectant solutions. *Indian J Dent Res.* 2010;21(3):374-379.
7. Kumar RN, Reddy SM, Karthigeyan S, Punithavathy R, Karthik KS, Manikandan R. The effect of repeated immersion of gypsum cast in sodium hypochlorite and glutaraldehyde on its physical properties: an in vitro study. *J Pharm Bioallied Sci.* 2012;4(Suppl 2):S353-S357.
8. Robati Anaraki M, Lotfipour F, Moslehifard E, Momtaheni A, Sigari P. Effect of different energy levels of microwave on disinfection of dental stone casts. *J Dent Res Dent Clin Dent Prospects.* 2013;7(3):140-146.
9. Vollmer M. Physics of the microwave oven. *Phys Educ.* 2004;39(1):74-81.
10. Banik S, Bandyopadhyay S, Ganguly S. Bioeffects of microwave—a brief review. *Bioresour Technol.* 2003;87(2):155-159.
11. Jacob J, Chia LHL, Boey FYC. Thermal and non-thermal interaction of microwave radiation with materials. *J Mater Sci.* 1995;30(21):5321-5327.
12. Fujikawa H, Ushioda H, Kudo Y. Kinetics of *Escherichia coli* destruction by microwave irradiation. *Appl Environ Microbiol.* 1992;58(3):920-924.
13. Woo IS, Rhee IK, Park HD. Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. *Appl Environ Microbiol.* 2000;66(5):2243-2247.
14. Rosaspina S, Salvatorelli G, Anzanel D, Bovolenta R. Effect of microwave radiation on *Candida albicans. Microbios.* 1994;78(314):55-59.
15. Rohrer MD, Bulard RA. Microwave sterilization. *J Am Dent Assoc.* 1985;110(2):194-198.
16. Berg E, Nielsen O, Skaug N. High-level microwave disinfection of dental gypsum casts. *Int J Prosthodont.* 2005;18(6):520-525.
17. Berg E, Nielsen Ø, Skaug N. Efficacy of high-level microwave disinfection of dental gypsum casts: the effects of number and weight of casts. *Int J Prosthodont.* 2007;20(5):463-464 [erratum: 2007;20(6):575].
18. Goel K, Gupta R, Solanki J, Nayak M. A comparative study between microwave irradiation and sodium hypochlorite chemical disinfection: a prosthodontic view. *J Clin Diagn Res.* 2014;8(4):ZC42-ZC46.
19. Meghashri K, Kumar P, Prasad DK, Hegde R. Evaluation and comparison of high-level microwave oven disinfection with chemical disinfection of dental gypsum casts. *J Int Oral Health.* 2014;6(3):56-60.
20. Hasan RH. The effect of microwave disinfection on tensile strength of dental gypsum. *Al–Rafidain Dent J.* 2008;8(2):213-218.
21. Robati Anaraki M, Moslehifard E, Aminifar S, Ghanati H. Effect of microwave disinfection on compressive and tensile strengths of dental stones. *J Dent Res Dent Clin Dent Prospects.* 2013;7(1):42-46.
22. Hersek N, Canay S, Akça K, Ciftçi Y. Tensile strength of type IV dental stones dried in a microwave oven. *J Prosthet Dent.* 2002;87(5):499-502.
23. Mansfield SM, White JM. Antimicrobial effects from incorporation of disinfectants into gypsum casts. *Int J Prosthodont.* 1991;4(2):180-185.
24. Hall BD, Muñoz-Viveros CA, Naylor WP, Sy J. Effects of a chemical disinfectant on the physical properties of dental stones. *Int J Prosthodont.* 2004;17(1):65-71.
25. Abdelaziz KM, Attia A, Combe EC. Evaluation of disinfected casts poured in gypsum with gum arabic and calcium hydroxide additives. *J Prosthet Dent.* 2004;92(1):27-34.
26. Takigawa T, Endo Y. Effects of glutaraldehyde exposure on human health. *J Occup Health.* 2006;48(2):75-87.
27. Ballantyne B, Jordan SL. Toxicological, medical and industrial hygiene aspects of glutaraldehyde with particular reference to its biocidal use in cold sterilization procedures. *J Appl Toxicol.* 2001;21(2):131-151.