

### MICROWAVE DISINFECTION OF ACRYLIC RESINS FOR LABORATORY EXPERIMENTAL APPLICATIONS

### DESINFECÇÃO POR MICRO-ONDAS DE RESINAS ACRÍLICAS PARA APLICAÇÕES EXPERIMENTAIS EM LABORATÓRIO

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### ABSTRACT

Disinfection of acrylic resin specimens can be achieved through microwave irradiation. However, inappropriate settings could lead to physical distortions or impair the decontamination process. This study aims to propose a rapid and reliable microwave disinfection regimen for acrylic resin specimens, in compliance with certified sterility norms, for laboratory experimental applications. Specimens were submitted to monospecific biofilm formation of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Thioglycolate fluid medium was used for the qualitative disinfection test during 14 days of incubation at 35° C. Surface roughness, Knoop microhardness, flexural strength, and dimensional stability were also evaluated. Irradiation protocols were 3 or 5 min at 450 W or 650 W in 250 mL of distilled water. Statistical analyses were performed considering  $\alpha$ =0.05. All irradiation regimens were able to disinfect the specimens exposed to *C. albicans*. Only the regimen 450 W for 3 min did not achieve disinfection of the specimens exposed to *S. aureus* and *E.coli*. Surface roughness, dimensional stability, and flexural strength were not altered by any regimen (*p*>0.05). Microhardness decreased in all regimens, except 450 W for 3 min. Irradiation with 450 W for 5 min in 250 mL of water

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is an optimal disinfection procedure for acrylic resin specimens as it combines optimal potency and exposure time.

KEYWORDS: Acrylic resins. Microwave. Disinfection. Biofilm.

### RESUMO

A desinfecção de amostras de resina acrílica pode ser alcançada através de irradiação de microondas. No entanto, configurações inadeguadas podem levar a distorções físicas ou prejudicar o processo de descontaminação. Este estudo tem como objetivo propor um regime de desinfecção por micro-ondas rápido e confiável para amostras de resina acrílica, em conformidade com as normas de esterilidade certificadas, para aplicações experimentais em laboratório. Os espécimes foram submetidos à formação de biofilme monoespecífico de Staphylococcus aureus, Escherichia coli e Candida albicans. O meio fluido tioglicolato foi utilizado para o teste qualitativo de desinfecção durante 14 dias de incubação a 35°C. A rugosidade da superfície, microdureza Knoop, resistência à flexão e estabilidade dimensional também foram avaliadas. Os protocolos de irradiação foram de 3 ou 5 min a 450 W ou 650 W em 250 mL de água destilada. As análises estatísticas foram realizadas considerando α=0,05. Todos os regimes de irradiação foram capazes de desinfetar os espécimes expostos a C. albicans. Apenas o regime de 450 W por 3 min não atingiu a desinfecção das amostras expostas a S. aureus e E. coli. A rugosidade da superfície, estabilidade dimensional e resistência à flexão não foram alteradas por nenhum regime (p>0,05). A microdureza diminuiu em todos os regimes, exceto 450 W por 3 min. A irradiação com 450 W por 5 min em 250 mL de água é um procedimento de desinfecção ideal para amostras de resina acrílica, pois combina potência e tempo de exposição ideais.

PALAVRAS-CHAVE: Resinas acrílicas. Micro-ondas. Desinfecção. Biofilme.

### INTRODUCTION

Acrylic resin specimens are vastly used for laboratory experimental studies simulating the conditions found in the oral cavity and, similar to acrylic dentures, these specimens face numerous challenges concerning convenient and affordable sterilization methods. Several techniques are implemented in the disinfection and sterilization and of these materials, especially chemical methods, such as ethylene oxide, chlorhexidine gluconate, sodium hypochlorite, among others. In addition to high operational cost, some of these methods could also alter properties such as stability, roughness, and color (MACHADO et al., 2011; POLYCHRONAKIS et al., 2018; VASCONCELOS et al., 2013). Typical laboratory sterilization methods, such as steam autoclaving, cannot be appointed for acrylic dentures sterilization, as temperatures exceeding 71 °C may cause plastic deformation (POLYCHRONAKIS et al., 2018; WAGNER; PIPKO, 2015).

Microwave irradiation has been considered a more practical and faster approach for the sterilization of acrylic resin specimens. It has been suggested that the elimination of microorganisms is possibly due to alterations caused by radio frequency waves on cytoplasmatic structures rather than by the heat generated during microwaving (ROHRER; BULARD, 1985). However, this method still regarded with caution because, under certain irradiation parameters, it could alter physical and mechanical characteristics (BASSO et al., 2012; HAMOUDA; AHMED, 2010). Three variables appear to play a major role in the outcomes: irradiation time, potency, and amount of water (AL-SAADI, 2014; SEO et al., 2007). In early studies, the irradiation time of 6 min was declared efficient in sterilizing both acrylic specimens and acrylic dentures (NEPPELENBROEK et al., 2003; SILVA et al., 2006).



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Nevertheless, several authors pointed out that this parameter might cause considerable distortions in dimensional stability, flexural strength, surface roughness, microhardness, and shear bond strength (CAMPANHA et al., 2005; MACHADO et al., 2012, 2011; CAMPOS et al., 2009; PAVAN et al., 2005; SEO et al., 2007). In more recent studies, shorter irradiation time (3 – 5 min) with potency varying from 450 to 650 W did not result in mechanical or physical changes (AL-SAADI, 2014; BASSO et al., 2012; KONCHADA et al., 2013; CAMPOS et al., 2009; PAVAN et al., 2005; XEDIEK CONSANI et al., 2014).

Moreover, these irradiation regimens were also reported to be capable of eliminating microorganisms associated with denture contamination, such as *Candida albicans*, *Staphylococcus aureus* and even *Bacillus subtilis* spores (AL-SAADI, 2014; MIMA et al., 2008; RIBEIRO et al., 2009; SENNA et al., 2012; SILVA et al., 2013). Nonetheless, literature still lacks a rapid and reliable disinfection procedure for acrylic resins to be used in laboratory testing applying certified methods, such as provided by the Code of Federal Regulations and the United States Pharmacopoeia. Fluid thioglycollate medium (FTM), one of the cultivation media recommended by the mentioned norms, allows for the assessment of disinfection, in which the clear, translucent medium becomes visibly turbid, indicating viable microorganisms.

Therefore, the aim of the study was to test some of the irradiation regimens suggested by the literature to determine which was the most effective in the disinfection of previously contaminated acrylic specimens when submitted to certified protocols. Furthermore, we investigated whether the dinsinfection of the specimens occured with no physical and/or mechanical alterations as to establish a fast and reliable irradiation protocol for laboratory experimental applications.

### MATERIALS AND METHODS

#### Experimental design

This research is divided in two distinct sections: the first section consists of microbiological experiments testing the effectiveness of microwave energy in the disinfection of acrylic resin specimens against three representative microorganisms: *Staphylococcus aureus* (gram + bacteria), *Escherichia coli* (gram – bacteria) and *Candida albicans* (yeast). In the second section, we evaluated the effect of microwave energy on surface roughness, Knoop microhardness, flexural strength, and dimensional stability. For both stages, the potency/time irradiation regimens were: E1 (450 W for 3 min), E2 (450W for 5 min), E3 (650W for 3 min), E4 (650W for 5 min).

#### Specimen manufacture

Both microbiological and mechanical experiments were performed with thermo-polymerizable acrylic resin specimens (OndaCryl, Artigos Odontológicos-Clássico, São Paulo, Brazil), commonly used for denture bases. Metallic matrices of different shapes and sizes were inserted in stone casts inside a muffle, according to each test employed. After casting, the matrices were removed from the muffle and the molds were filled with acrylic resin manipulated following the manufacturer's recommendations. Finishing and polishing were done with water sandpapers (Microgrit P240, P400, P600, P1200, and P2500) and the specimen dimensions were confirmed using a digital caliper (Model CD-6 " CSX-B, Mitutoyo Sul Americana LTDA, Suzano, Brazil). Specimens were then



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cleaned, immersed in distilled water, and kept in an incubator (TE-393/1, Tecnal, Piracicaba, Brazil) at  $37 \pm 1^{\circ}$ C for 1 week to allow the release of residual monomer, with distilled water being replaced daily. The specimens were then sterilized with ethylene oxide and stored for one week to allow the release of any residual gas.

### Microbiological procedures

Fifty-four acrylic resin disks (10×3 mm; n=3/group) were exposed to Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC<sup>®</sup> 8739), and Candida albicans (ATCC<sup>®</sup> 10231). The same procedures were executed for each microorganism strain individually. Optical density (O.D.) was measured with a spectrophotometer (Ultrospec 1100 pro, Amersham Biosciences GmbH, Freiburg Germany) in Brain Heart Infusion (BHI) broth (Kasvi, São José dos Pinhais, Brazil) for S. aureus and E. coli and with Sabouraud Dextrose broth (Kasvi, São José dos Pinhais, Brazil) for C. albicans after 18h of incubation at 37 °C. For an inoculum dilution corresponding to 1x10<sup>6</sup> cells/mL, the absorbances registered ranged between 0.08 and 0.10 at a wavelength of 652 nm for S. aureus, 1.43 at 600 nm for E.coli, and 0.28 at 530 nm for C. albicans (AL-SAADI, 2014; BASSO et al., 2012; DANTAS et al., 2014; HAMOUDA; AHMED, 2010; NEPPELENBROEK et al., 2003; SILVA et al., 2006). Specimens were immersed in 1.5 mL of inoculum in individual wells of a sterile 24-well tissue plate (K12-024 tissue plate, Kasvi, São José dos Pinhais, Brazil) and incubated at 37 °C for 48 h to promote biofilm growth on the specimen surface (CAMPANHA et al., 2005; CONSANT et al., 2008; GONÇALVES et al., 2006; IZUMIDA et al., 2011; MACHADO et al., 2009; MIMA et al., 2008; SENNA et al., 2012; SILVA et al., 2013). After incubation, each specimen was rinsed inside an individual well of another sterile 24-well plate containing 1.5 mL of autoclaved distilled water and transferred with flambed tweezers into sterile reagent bottles containing 250 mL of distilled water. Each specimen was then randomly assigned to one of the following groups as shown in table 1 (ASLANIMEHR et al., 2018; BASSO et al., 2010; DOVIGO et al., 2009; MOJARAD et al., 2017; SANITA et al., 2009; SENNA et al., 2012; SILVA et al., 2013).

### Disinfection procedure

Specimens were individually immersed in 500 mL reagent bottles containing 250 mL of distilled water and placed, without lid, in a microwave oven (BMY45, Brastemp, São Paulo, Brazil) with the potency and time of the corresponding experimental group, as described in table 1. The amount of water was determined in a pilot study (data not shown) as the least amount which did not boil after 5 min at the highest tested potency (650 W). After irradiation, each specimen was rinsed again and individually placed in transparent glass test tubes containing 5 mL of fluid thioglycolate medium (FTM - K25-1533, Kasvi, São José dos Pinhais, Brazil). The test tubes were vortexed and incubated for 14 days at 35 °C, being monitored daily for turbidity of the culture medium, following the recommendations by the Code of Federal Regulations and the United States Pharmacopoeia (Code of Federal Regulations, Title 21 Food and Drugs, Part 610.12 Sterility testing; The United States Pharmacopeial Convention , USP, Chapter 71; Dixon et al., 1999)



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### Table 1. Microwave irradiation potency and time for each experimental group.

Time / Potency	No irradiation	450 W		650 W		
No irradiation No biofilm	Positive Control Group (ethylene oxide sterilization)					
No irradiation S. aureus biofilm E.coli biofilm C. albicans biofilm	Negative Control Group					
3minuteirradiationS. aureus biofilmE.coli biofilmC. albicans biofilm		Experimental Group (E1)	1	Experimental (E3)	Group	3
5 minute irradiation S. aureus biofilm E.coli biofilm C. albicans biofilm		Experimental Group (E2)	2	Experimental (E4)	Group	4

Surface roughness test

Using a surface roughness reader profilometer (T 1000, Hommel Tester, Hommelwerke GmbH, Villingen-Schwenningen, Germany) five measurements were performed on 40 specimens (12×12×2 mm; n=10/group) to obtain the mean surface roughness (Ra, µm) to observe whether the surfaces of the specimens were standardized before starting the experiment. Roughness values were compared before and after irradiation, comparing values within the same group and among different groups (IZUMIDA et al., 2011; MACHADO et al., 2012, 2011, 2009; CAMPOS et al., 2009; SARTORI et al., 2006; VASCONCELOS et al., 2013).



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### Microhardness test

Knoop Microhardness measurements (kg/mm<sup>2</sup>) were obtained with a microdriometer (FM-ARS 9000, Future-Tech corp., Kawasaki-City, Japan). Forty specimens (14×3 mm; n =10/group) were subjected to a calibrated vertical load of 25 g for 5 seconds. For each measurement, five random indentations were performed, and the mean value of the control group was calculated for later comparison with the means of the experimental groups (CAMPANHA et al., 2005; CONSANT et al., 2008; GOIATO et al., 2013; MACHADO et al., 2009; VASCONCELOS et al., 2013).

#### Flexural strength test

For flexural strength measurements (MPa), a three-point bending test was conducted on 25 rectangular shaped specimens (64×10×3 mm; n=5/group) using a universal testing machine (DL200 EMIC, Instron Ltda., São José dos Pinhais, Brazil). The specimens were mounted in the testing device with 50 mm distance between two vertical supports. A vertical load was applied midway between the supports with 5 mm/minute crosshead speed until the fracture of the specimen. The maximal load at fracture was recorded. The obtained data were calculated according to the following formula:  $s = \frac{aFd}{2wh^2}$ , where F is the applied load (N) at the highest point of the load-

deflection curve, d is the span length (50 mm), w is the measured width of the test specimen, and h is the measured thickness of the test specimen (PAVARINA et al., 2005; SHAFEEQ et al., 2016).

#### Dimensional stability test

Forty specimens (14×3 mm; n=10/group) were identified and marked with diamond-tipped burs in peripheral points forming a triangle of approximate sides. Standardized photos were taken using a light microscope (DM1000 LED, Leica do Brasil Importação e Comércio Ltda, São Paulo, Brazil) and a smartphone (Galaxy S7, f/1.7, 12 MP Samsung Electronics Co. Ltd, Seoul, South Korea). All photographs were taken placed next to a millimeter scale. The segments were measured with ImageJ Launcher software (National Institutes of Health, Bethesda, MD, USA), calculating the length (mm) of each segment, determined in the photos obtained before and after disinfection (CONSANI et al., 2008; POLYCHRONAKIS et al., 2018, 2014).

#### Data analysis

For statistical analysis, the software GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, USA) was used. The data obtained from the mechanical tests were submitted to the D'Agostino & Pearson normality test. Intragroup comparisons, before and after microwave disinfection, were performed with t-paired tests for normal data and Wilcoxon for non-normal data. Intergroup comparisons were performed with one-way ANOVA and Tukey tests for normal data, and Kruskal-Wallis and Dunn for non-normal data. All analyses considered a 95% confidence interval.



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### RESULTS

In the experiments with *S.aureus* and *E.coli*, all the negative controls (contaminated specimens that did not receive microwave irradiation) exhibited visible turbidity after 24 h. Specimens from the E1 group (450 W for 3 min) also demonstrated turbidity. After 48 h, each of the three test tubes from this group became visibly turbid, with a visual aspect similar to the negative control group. After 14 days, no other group exhibited turbidity.

The negative control group with *C. albicans*, which did not undergo microwave irradiation, exhibited turbidity after 24 h of incubation. During the 14-day follow-up, only one test tube of the E1 group (450 Wfor 3 min) showed turbidity. No further test tube exhibited changes in its visual aspect, including the positive control (specimens sterilized with ethylene oxide), assuring that the specimens were sterile before the experiment.

The results for surface roughness (Table 2) showed that specimens were properly standardized and randomly distributed among the experimental groups before the experiment (p=0.4423) and remained similar after microwave irradiation (p=0.1584). The results within each group demonstrated no significant statistical difference within the same group after irradiation (p=0.6183, p=0.3673, p=0.1523, p=0.2930, respectively).

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Regimen	450 W 3 min	450 W 5 min	650 W 3 min	650 W 5 min	p
Initial condition	0.17 (±0.04)	0.18 (±0.04)	0.18 (±0.02)	0.20 (±0.051)	0.442
Treated sample	0.17 (±0.04)	0.19 (±0.04)	0.19 (±0.03)	0.22 (±0.05)	0.158
p	0.618	0.367	0.152	0.293	

Table 2. Surface roughness (Ra,  $\mu$ m) before and after microwave disinfection (mean and  $\pm$ SD).

One-way ANOVA (α=0.05).

The Knoop microhardness values before irradiation showed no statistical difference among the specimens was observed (p=0.3402). Lower and statistically different hardness values within the same groups (E2, E3, and E4) after irradiation were detected (p=0.038, 0.0048, 0.0039, respectively), except for the group with the lowest potency and irradiation time (E1)(Table 3).

Table 3. Knoop microhardness (kg/mm<sup>2</sup>) before and after microwave disinfection (mean and ±SD).

Regimen	450 W 3 min	450 W 5 min	650 W 3 min	650 W 5 min	р
Initial condition	16.91 (±0.62)	17.14 (±0.75)	17.63 (±1.11)	17.31 (±0.57)	0.340
Treated sample	16.85* (±0.71)	15.66 (±0.99)	16.42 (±0.85)	16.25 (±0.75)	0.030
p	0.776	0.004	0.005	0.004	

\*versus 450 W 5 min (one-way ANOVA/ Tukey; α=0.05)

Table 4 shows the results for the flexural strength assessment in mean and standard deviation values, with non-irradiated specimens (control) and irradiated specimens (450 or 650 W for 3 or 5 min) showing no significant statistical difference.



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Table 4. Flexural strength (MPa) of the non-irradiated and irradiated samples (mean and ±SD).

		<u> </u>			
(±7.43)	(±7.37)	(±11.60)	(±7.93)	(±5.576)	0.841
58.23	57.23	56.04	57.86	52.83	
Control	450 W 3 min	450 W 5 min	650 W 3 min	650 W 5 min	p

One-way ANOVA ( $\alpha$ =0.05).

Dimensional stability (table 5) in the intragroup analysis showed no statistically significant difference (p>0.05) before and after irradiation.

Table 5. Linear dimensional change evaluation of the segments (mm) before and after microwave disinfection

Regimen	450 W 3 min	450 W 5 min	650 W 3 min	650 W 5 min	p
Initial condition	3.88*	4.51	4.93	4.90	< 0.001
	(±0.86)	(±0.98)	(±1.14)	(±0.87)	
Treated	3.82**	4.53	4.97	4.89	< 0.001
sample	(±0.87)	(±1.08)	(±1.20)	(±0.97)	
p	0.092	0.847	0.463	0.774	

\*versus 650 W 3 and 5 min (Kruskal-Wallis/ Dunn); \*\*one-way ANOVA/ Tukey ( $\alpha$ =0.05).

### DISCUSSION

Although microwave irradiation achieved disinfection of the acrylic resin specimens, not all evaluated properties remained unchanged. Temperature, potency, resin brand, polymerization cycle, and amount of water demonstrated a major role in the outcomes of experimental studies involving microwave sterilization. Higher temperatures could lead to higher diffusion, loss, and polymerization of residual monomer molecules (BASSO et al., 2012; IZUMIDA et al., 2011). This process could be responsible for increasing internal stress and other changes in mechanical properties (IZUMIDA et al., 2011). Moreover, it is believed that microwave irradiation prompts vibration of water molecules inside microorganisms, disorganizing their biological structures, among other unknown interactions (RIBEIRO et al., 2009; SANITÁ et al., 2009).

Specimens undergoing irradiation cycles without water immersion were more prone to shape distortion and less effective in killing the tested microorganisms, suggesting that the amount of water also influences the outcome (Al-Saadi, 2014; Hamouda and Ahmed, 2010). In most studies, the amount of water is usually found between 150 and 250 mL. In irradiation cycles under 5 min adjusted to 650 W or less some changes in the physical and mechanical properties were observed in reservoirs containing 150 mL of distilled water (CONSANT et al., 2008) whereas by using 200 mL better results were obtained (CAMPANHA et al., 2005; GONÇALVES et al., 2006; MACHADO et al., 2009). During microwave irradiation, water might boil, which has been previously reported (AL-SAADI, 2014; MIMA et al., 2008; NEPPELENBROEK et al., 2003). Boiling temperature is a critical factor as temperatures slightly above 100 °C could reach the glass transition temperature of the resin, leading to dimensional and mechanical distortions. In several studies (BASSO et al., 2010; CAMPANHA et al., 2005; GONÇALVES et al., 2006; MACHADO et al., 2009; MIMA et al., 2008; NEPPELENBROEK et al., 2010; CAMPANHA et al., 2005; GONÇALVES et al., 2006; MACHADO et al., 2009; MIMA et al., 2008; NEPPELENBROEK et al., 2010; CAMPANHA et al., 2005; GONÇALVES et al., 2006; MACHADO et al., 2009; MIMA et al., 2008; NEPPELENBROEK et al., 2010; CAMPANHA et al., 2005; GONÇALVES et al., 2006; MACHADO et al., 2009; MIMA et al., 2008; NEPPELENBROEK et al., 2003; RIBEIRO et al., 2009; SANITÁ



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et al., 2009; WAGNER; PIPKO, 2015), 200 mL of water was considered a safe amount for irradiation. However, especially in groups exposed to higher potencies and longer irradiation times, a previous pilot study (data not shown) indicated that water would eventually reach boiling temperature. Using 250 mL and allowing the microwave oven to cool off between sessions prevented water from boiling.

According to the Code of Federal Regulations and The United States Pharmacopoeia, specimens should remain in incubation for at least 14 days in a liquid culture media to allow slow-growing microorganisms to develop, which could be overlooked in the other methods with shorter incubation time. In accordance with the abovementioned regulations, FTM was selected because of its versatile properties that allow several microorganisms to grow. A similar methodology was previously reported only by Dixon et al. (1999), which could be partially responsible for the outcomes with lack of microorganism growth after irradiation.

The most used potency/time regimen in recent studies was 650 W for 3 min (DANTAS et al., 2014; MIMA et al., 2008; RIBEIRO et al., 2009; SANITÁ et al., 2009; SILVA et al., 2013). A study by Mima et al., (2008) showed that the use of shorter irradiation time such as 2 min at 650 W using 200 mL of sterile distilled water was capable of eliminating *C. albicans* from contaminated acrylic specimen whereas a minimal of 3-minute exposure was necessary to eliminate *S. aureus*, *P. aeruginosa* and *B. subitilis* under the same testing conditions. Ribeiro et al. (2009) reported that 2-minute irradiation at 650 W would be enough only for reducing colony forming units (CFU) counts and 3-minute irradiation using the same parameters was necessary to completely eliminate *Candida* spp., *Staphylococcus* spp. and *mutans streptococci*. The results of this work demonstrated consistent turbidity in the groups tested with *S. aureus* irradiated for 3 min at 450 W, which could be partially explained by the lower potency used. In most studies investigating the efficacy of microwave irradiation against *S. aureus*, 650 W for 3 min or more appeared to be effective (MIMA et al., 2008; NEPPELENBROEK et al., 2003; SILVA et al., 2006). This could also be attributed to the different microbiological assessment and the different amount of water employed in these studies, which was 200 mL.

Despite limited data on the antimicrobial activity against *E. coli*, most studies suggest that the parameter 650 W for 3 min is effective against gram-negative bacteria (DOVIGO et al., 2009; MOJARAD et al., 2017). The work by Senna et al. (2012) concluded that 3-minute irradiation time at an even lower potency (450 W) was enough to sterilize acrylic contaminated specimens with *C. albicans*. However, the study employed an incubation period under 14 days and a lesser amount of water (200 mL), which could partially explain the slightly different results concerning the E1 group (450 W for 3 min), in which one of the test tubes exhibited turbidity during the experiments with *C. albicans*, thus requiring further investigation. The specimens contaminated with *C. albicans* exhibited no turbidity for the other irradiation regimens, which is also in accordance with several other studies (ASLANIMEHR et al., 2018; MIMA et al., 2008; NEPPELENBROEK et al., 2003; RIBEIRO et al., 2009; SANITA et al., 2009; SENNA et al., 2012; SILVA et al., 2013, 2006).

Surface roughness has been described as an overly sensitive property to microwave irradiation (MACHADO et al., 2012, 2011, 2009; CAMPOS et al., 2009; SARTORI et al., 2006). This could be related to the release and dilution of residual monomer, which could eventually cause water sorption, leading to a more irregular surface (IZUMIDA et al., 2011; MACHADO et al., 2012). A surface roughness threshold of 0.2 µm has been



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proposed as ideal for avoiding greater bacterial adhesion (MACHADO et al., 2009; SARTORI et al., 2006). The results of this work remained very close to the value of 0.2 µm for all parameters tested. Machado et al., (2009) reported that even 7 cycles of 6-minute-irradiation at 650 W did not alter the surface roughness to values far above 0.2 µm. Some other studies revealed that 6 min of irradiation at a potency varying from 650 to 690 W would increase roughness either at the first irradiation or at further repetitions (IZUMIDA et al., 2011; MACHADO et al., 2012, 2011; CAMPOS; KOCHENBORGER; SILVA; TEIXEIRA, 2009; SARTORI et al., 2006), which suggests that a lower potency or irradiation time should be more secure. The results of the present study indicated that 3 min of microwave exposure at 650 W or 5 min at 450 W were effective for sterilization without any significant increase in surface roughness. The work by Campos et al., (2009) also revealed that acrylic resins that undergo microwave polymerization (Onda-Cryl) tend to demonstrate higher discrepancy in surface roughness than resins cured in a hot water bath (QC-20) when exposed to microwave disinfection cycles. Nevertheless, despite reports of statistically significant roughness changes, it is still unclear whether these results would have any clinical implications (CAMPOS et al., 2009).

The effects of microwave irradiation on the microhardness of acrylic resin are still very controversial. The results presented in many studies have shown variations in absolute values, often statistically significant (*p*<0.05) (CAMPANHA et al., 2005; CONSANT et al., 2008; MACHADO et al., 2009). However, some authors consider these variations acceptable (CONSANI et al., 2008; IZUMIDA et al., 2011; SEO et al., 2007) and others suggest that these changes in the surface of the material could lead to easier wear over time (CAMPANHA et al., 2005; CONSANT et al., 2008; XEDIEK; CONSANI et al., 2014).The study by Consani et al., (2008) hinted at a higher decrease in hardness values for microwave-polymerized acrylic resins (Onda-Cryl) compared to acrylic resins cured in a hot water bath (QC-20) for specimens subjected to 3-minute irradiation at 650 W, whereas hardness values for QC-20 did not exhibit statistical difference compared to the non-irradiated group. In the present study, only the group with the lowest potency and shortest irradiation time (450 Wfor3 min) did not show a statistically significant differences compared to the specimens before irradiation. All the other groups presented statistically differences compared to baseline; however, it is still not clear whether these changes would be considered relevant considering that all values were above 15 Knoop, which is deemed clinically acceptable (GOIATO et al., 2013).

The effects of microwave energy on the flexural strength of acrylic resin specimens also appear to be related to the irradiation regimen as well as the brand and type of curing of the acrylic resin, with irradiation cycle repetitions and heat-cured resins demonstrating lower fracture values (PAVARINA et al., 2005; SHAFEEQ et al., 2016). In this study, no significant difference was found after the irradiation cycles, which is in accordance with studies using similar parameters (CONSANT et al., 2008; KONCHADA et al., 2013; PAVARINA et al., 2005). Hamouda et al., (2010) stated that microwave oven disinfection caused significant changes in the flexural strength of the acrylic resin specimen when a potency of 720 W was used.

Dimensional stability also follows the pattern observed for the other mechanical properties evaluated in previous studies which reported that brand and polymerization/curing cycle could influence the results (BASSO et al., 2010; GONÇALVES et al., 2006). A study by Polychronakis et al.,



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(2014) showed that microwave disinfection with complete dentures immersed in water exhibited greater dimensional changes when compared to those not immersed, suggesting that disinfection cycles would be more effective if carried out in a dry state. However, this could affect the sterilization of the specimens, which have been reported to be more effective when specimens were immersed in water (MIMA et al., 2008; NEPPELENBROEK et al., 2003; RIBEIRO et al., 2009). All tested groups in this study demonstrated minor variations after microwave irradiation, which were considered statistically irrelevant (p>0.05). When considering the dimensional change in percentage, all groups analyzed in this work remained between 0.02% and 1.54% of distortion in the segment length. Values ranging up to 1% of dimensional change are considered clinically acceptable water (MIMA et al., 2008; NEPPELENBROEK et al., 2003; RIBEIRO et al., 2009). Studies using irradiation time above 5 min or potency values higher than 650 W showed a higher tendency for dimensional distortions (AL-SAADI, 2014; POLYCHRONAKIS et al., 2014; SEO et al., 2007; WAGNER; PIPKO, 2015), whereas studies with shorter irradiation time (under 6 min) and potency values ranging from 420 to 650 W suggested that dimensional changes were not relevant (BASSO et al., 2010; CONSANI et al., 2008; PAVAN et al., 2005; POLYCHRONAKIS et al., 2018). Nevertheless, it is still debatable whether these changes will be negatively perceived when taken into account the variations in size, as even larger samples such as dentures could suffer larger asymmetric distortions (CONSANI et al., 2008; SEO et al., 2007). Repeated microwave cycles could also increase dimensional distortions over time (POLYCHRONAKIS et al., 2018).

Although it has been described as a safe irradiation regimen, the 650 W for 3 min protocol has also shown controversial results. Increasing potency would be impractical as higher potencies could adversely affect acrylic resins (SENNA et al., 2012). In the work by Al-Saadi (2014) it is highlighted that specimens with larger biofilm areas might require even longer irradiation exposure. Irradiation regimens with longer exposure times are more likely to cause physical and mechanical distortions unless a lower potency is applied (CAMPOS et al., 2009). In our investigation the regimen 450 W for 5 min achieved disinfection of the specimens without relevant alterations, thereby fitting the requirements for lower potency and appropriate exposure time.

The present study evaluated the outcomes of microwave irradiation on acrylic resin specimens to validate and establish a disinfection protocol, limiting its reproducibility to laboratory experiments that require sterility of specimens. Standardization for clinical investigations, employing this technique, is particularly challenging because each denture is individually designed for a single patient. Therefore, homogeneous heat dissipation would be difficult to achieve, resulting in different shape and size distortions. The lack of comparison between other thermopolymerizable acrylic resin brands and the evaluation of other physical and mechanical properties such as impact strength were also limiting factors, although we were able to evaluate the main properties. The findings of our study could serve as a guideline for further investigations regarding the disinfection and sterilization of acrylic resin specimens and the future establishment of a clinical protocol.



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### CONCLUSIONS

Within the limitations of this *in vitro* study, it was concluded that a microwave irradiation regimen consisting of 450 W for 5 min immersed in 250 mL of distilled water could be considered reliable and fast approach for disinfecting acrylic resin specimens for laboratory experimental purposes.

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