American Journal of Infectious Diseases and Microbiology, 2013, Vol. 1, No. 3, 38-45
Available online at http://pubs.sciepub.com/ajidm/1/3/1

DOI:10.12691/ajidm-1-3-1

Cell Membrane Integrity of Candida Albicans after Different Protocols of Microwave Irradiation

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Received March 27, 2013; Revised May 29, 2013; Accepted May 30, 2013

Abstract **Purpose:** To evaluate the ability of low time microwaveexposureto inactivate and damage cell membrane integrity of *C. albicans*. **Materials and Methods:** Two 200ml *C. albicans* suspensions were obtained. Sterile dentures were placed in a beaker containing Experimental (ES) or Control suspensions (CS). ES was microwaved at 650 W for 1, 2, 3, 4 or 5 min. Suspensions were optically counted using Methylene blue dye as indicative of membrane-damaged cells; spread on Agar Sabouraud dextrose (ASD) for viability assay; or spectrophotometrically measured at 550nm. Cell-free solutions were submitted to content analyses of protein (Bradford and Pyrogallol red methods); Ca^{++} (Cresolphthalein Complexone method); DNA (spectrophotometer measurements at 260nm) and K^{+} (selective electrode technique). Data were analyzed by Student-*t* test and linear regression (α=0.05). In addition, flowcytometry analysis of *Candida* cells in suspensionwas performed using propidium iodide**. Results:** All ES cells demonstrated cell membrane damage at 3, 4 and 5 min,viable cells were nonexistent at 3, 4 and 5 min ES ASD plates and optical density of ES and CS was not significantly differentfor all exposition times. ES cells released highcontents of protein, K^{+}, Ca^{++} and DNA after 2 min exposition when compared to that of the CSs. Similar results were observed with flow cytometry analysiswith regard to the periodsof microwave exposure. **Conclusions:** Microwave irradiation inactivated *C. albicans*after 3min and damaged cell membrane integrity after 2 min exposition.

Keywords: *microwaves, candida albicans, denture stomatitis, disinfection, denture*

1. Introduction

Dental prostheses can be source of infection because pathogenic microorganisms are capable to adhere and survive on acrylic resin surfaces, promoting biofilm formation. For this reason, dentures act as a potential cause of cross-infection transmission between patients and dental personnel. Likewise, as a reservoir of pathogens, intaglio surfaces of acrylic resin dentures enhance the infection potential of microorganisms and can favor the riseof oral infections[1,2].

Oral candidiasis, represented by denture stomatitis in denture wearers, is one of the most common manifestations associated with the use of removable dentures [3]. This condition is mainly caused by microorganisms of the *Candida* genus and affects the palate of approximately 65% of denture wearers. *Candida albicans* represents the predominant species, but the so-called non-*albicans Candida* species are also recognized as important agents of the infection [2]. Management of denture stomatitis is typically drug based but relapse and recurrence frequently require repeated courses of antifungal therapy [4]. Considering that microbial colonization on dental prostheses favor the appearance of denture stomatitis, cleansing and disinfection of dentures is fundamental to prevent and treat this disease [5].

Decontamination of dentures after exposure to microwave irradiation has shown important results for treatment of denture stomatitis [5] and to prevent cross-contamination. While the inhibitory effect of microwaves on microorganisms is under research, how microwave brings about this effect is still a matter of debate among many researchers [6-12]. It is generally thought that the destruction of microorganisms is mainly due to a thermal effect [13,14,15,16]. However, investigations have attempted to ascertain if such irradiation has a non-thermal effect on microorganisms [17,18]. Rosaspina et al. [19] observed changes in microwaved microorganisms, which were not identified after thermal heat of the cells in water bath. Similarly, injury of cells of *Staphylococcus aureus* exposed to microwave irradiation at sublethal temperature has shown to be greater than after conventional heating [18]. Exposition of *Bacillus subtilis* and *Escherichia coli* suspensions to microwave irradiation caused reduction on viable cell counts and increased leaching of DNA and protein, suggesting cell membrane cleavage [12]. It has

been observed that microwave irradiation promoted structural alterations in the cell wall of *C. albicans* adhered to surgical scalpel.

In terms of treating denture stomatitis, 6 min of microwave irradiation regimen(650W, three times a week for 30 days) was originally recommended to disinfect dental prostheses [20]. Using this protocol, Campanha et al. [21] demonstrated that *C. albicans* was inactivated after microwave irradiation and changes in cell membrane permeability of *C. albicans* may be involved in cell inactivation. The authors also found that microwave irradiation promoted release of some electrolytes and nucleic acids from *Candida* cells, and yeasts lost their ellipsoidal morphology [21].

On the other hand, the disinfection protocol of disinfecting dentures with 6 min of microwave irradiation at 650 W may promote adverse effects on physical and mechanical properties of denture materials [22,23]. Consequently, investigations are seeking alternative protocols, such as short exposure times, which could minimize the occurrence of undesirable effects on the material of dental prostheses. Shorter microwave exposure times, e.g. 1 to 5 min of irradiation, have been found to be effective for denture disinfection [24] and microorganisms inactivation including *Candida* species [1,5]. Recent clinical trials showed that microwaving for 3 min(650 W) was an effective method to sterilize complete dentures and also to treat denture stomatitis [25]. Since cell injury caused by the microwave irradiation seems to be proportional to increased temperatures and irradiation times [8], the aim of this study was to evaluate the efficacy of microwave protocols regarding the cell membrane integrity of *C. albicans* after short exposure times.

2. Material and Methods

2.1. Sample Fabrication

Twenty maxillary dentures were fabricated, according to a standardized procedure described in full detail elsewhere [5]. Briefly, a high-viscosity silicone material was used to obtainanimpression of a standard denture. Waxed denture replicas were then obtainedafter initial positioning new sets of artificial teeth inside the silicone impression, pouring molten wax and fully seating a duplicate stone cast onthe mold. After bench cooling, wax-simulated dentures were removed from the silicone and invested in metal dental flasks as usual (Jon 5.5; Jon Produtos Odontológicos, São Paulo, SP, Brazil) with dental stone. Wax was removed and two coats of sodium alginate (Isolak; Classico Dental Products, São Paulo, SP, Brazil) were used as a mould separator. A denture poly(methylmethacrylate) basedresin (Lucitone 550; Dentsply International Inc., York, PA, USA) was prepared according to the manufacturer's instructions by mixing 21 g polymer powder to 10ml monomer liquid. The denture base resin was placed into the moulds and flasks, closed under pressure, and polymerized (Termotron, P100; Termotron Equipamentos, Piracicaba, SP, Brazil) at 73ºC for 90 min followed by immersion in boiling waterfor 30 min at 100ºC. After polymerization, flasks were bench cooled for 30 min and placed in tap water for 15 min. The dentures were carefully removed from the flasks, trimmed using metal burs (Maxi-Cut; Dentsply-Maillefer, Ballaigues, Switzerland) and finished using 360, 400, 600 and 1200-grit abrasive papers (Norton; Saint Gobain Abrasivos Ltda, Guarulhos, SP, Brazil). After finishing, the dentures were polished on a wet rag wheel with pumice slurry followed by tin oxide and individually stored in distilled water for the period needed to stabilize water uptake of denture materials. This pre-saturation was performed in order to minimize the effects of water absorption during irradiation on the infiltration of denture base materials by *C. albicans*. Before using in the experiments, the dentures were steam sterilized.

2.2. Yeast Cell Suspensions and Microwave Irradiation

The microorganism selected was an American Type Culture Collection (ATCC, Rockville, MD)strain of *C. albicans* (10231). The microorganism was cultured in 600 ml of Tryptic Soy Broth (TSB) at 37ºC on a rotating shaker. Yeast cell culture was centrifuged, rinsed in water and resuspended to 10^8 cells ml^{-1} in cold distilled water [26].

The sterile dentures were individually placed in sterile beakers containing 200ml of the yeast suspension, in order to completely cover the denture [27]. Each beaker was separately placed on a rotational plate in a conventional home microwave oven (Sensor Crisp 38, Double Emission System; Brastemp SA, Manaus, Amazonas, Brazil) and irradiated at 650 W for 1, 2, 3, 4 or 5 min (experimental suspensions - ESs) [28,29]. This procedure simulated the disinfection of a contaminated prosthesis so that the acrylic resin denture absorbed part of the microwave energy during irradiation [16]. After cooling, sterile distilled water was added to replace the amount of liquid lost by evaporation during irradiation. Control suspensions (CSs) were prepared identically to the ESs and placed in sterile beakers containing the sterile acrylic resin denture. These beakers were individually kept in the microwave oven for the same period of ESs, without irradiation.

Two ESs and two CSs from each microwave protocol were prepared and treated as described, before determination of yeast cell counts and leakage of proteins, electrolytes and DNA. All experiments were made in duplicates. Sample size was determined by power analysis.

2.3. Yeast Cell Counts

Yeast cell counts were made as previously described by Campanha et al. [21] Samples of suspensions were counted following uptake of methylene blue dye as indicative of yeast cell membrane damage [30]. In this assay, damaged and non-damaged yeast cell membranes were considered as indicative of live and dead yeast cells, respectively. For this purpose, samples of CSs and ESs were both serially diluted in 0.9% NaCl solution from 10^{-1} to 10^{-6} cells m^{-1}. Replicate samples of 0.5ml of each dilution were added to 55µl of 0.05% methylene blue(w/v, prepared in sterile distilled water), mixed carefullyandleft for 5 min at room temperature [30]. Yeast cell concentrations (cells ml^{-1}) were determined by opticalcount in samples of 10µl in a Neubauer chamber. Log_{10}-transformed numbers of damaged and non-damaged yeast cells of each sample were recorded.

Replicate samples (25µl) of ESs and CSs were placed on Agar Sabouraud dextrose (ASD) plates at dilutions of 10^{-1} to 10^{-6}, to determine total viable yeast cells. All plates were incubated at 37ºC for 48h. After incubation, yeast colony counts of each plate were quantified using a digital colony counter (CP 600 Plus, Phoenix Ind Com Equipamentos Científicos Ltda, Araraquara, SP, Brazil). Colony-forming unit per milliliter (CFU ml^{-1}) was determined and $\log_{10}$-transformed [31].

2.4. Leakage of Proteins, Electrolytes and DNA

The suspensions were submitted to analyses for determination of protein, electrolytes (Ca^{++} and K^{+}) and DNA releasedfrom the yeast cells, as previously described by Campanha et al. [21]. ESs or CSs were centrifuged at 1,500g for 10 min and filtered with a 0.45µm acetate cellulose filter. Filtrates were freezedried (LyofilizerFreeZone 6 Liter Benchtop, Labconco Corporation, Kansas City, MU, USA), resuspended in 5ml of sterilized deionized water, frozen and stored until use.

Released protein content from ESs and CSs was quantified by two colorimetric methods: Bradford [12,32] and Pyrogallol red [33]. Microprote system (Doles Reagentes, Goiânia, GO, Brazil), based on the Bradford method, involves binding of Coomassie Brilliant Blue to the protein. This method measures the shift in the absorption spectrum of the dye that occurs when it binds to protein molecules: the absorbance peak shifts from 465 nm to 610nm. The system consisted of a stabilizer solution (Percloric Acid Solution 6.80 mol l^{-1}), Coomassie (Coomassie Brilliant Blue solution 1.2 mmol) and a standard solution (aqueous solution of human albumin 10mg dl^{-1}). Absorbance was read at 610nm (Spectrophotometer 482, Femto Ind. Com. de InstrumentosLtda) and the results were expressed by the following equation, where A is the absorbance at 610nm.

$$\left(A_{test} - A_{\text{stan}\,dant}\right) \times \frac{10}{A_{\text{stan}\,dant} \times A_{blank}} = mg\ dl^{-1}\ protein$$

Sensiprote systems (LabtestDiagnóstica SA, Lagoa Santa, MG, Brazil) uses the Pyrogallol red–molybdate complex method. Binding of the dye (Pyrogallol red–molybdate) to protein causes a shift in the absorption maximum of the dye from 460nm to 600 nm. The system consists of a colour reagent (containing ≥60 µmol l^{-1} of Pyrogallol red; ≥40 µmol l^{-1} of sodium molybdate; 1 mmoll^{-1} of sodium oxalate; 50 mmoll^{-1} of buffer solution, pH 2.5; octylphenol poly-oxyethanol 0.1% and preservatives) and a standard reagent based on 0.05% of sodium azide. Absorbance was read at 600 nm (Spectrophotometer 482, Femto Ind. Com. de InstrumentosLtda) and the results were expressed by the following equation, where *A* is the absorbance at 600nm.

$$\frac{A_{test}}{A_{\text{stan}\,dant}} \times 50 = mg\ dl^{-1}\ protein$$

Amount of Ca^{++} released from ESs and CSs was determined by a colorimetric system(Calcium Liquiform, Labtest Diagnóstica SA), based onthe Cresolftaleine Complex one method [34,35]. The reaction involves binding of Ca^{++} to purple-coloured phthalein in alkaline medium. The system consisted of Reagent 1, containing buffer 920 mmol l^{-1}, pH 12, sodium azide 19 mmol l^{-1}; Reagent 2, containing o-cresolphthaleincomplexone 320 µmol l^{-1}, hydroxyquinoleine 13 mmol l^{-1} and hydrochloric acid 130 mmoll^{-1}; and a standard solution, containing calcium 10 mg/dl and formol 0.1%. Absorbance was read at 570 nm (Technicon Ra-XT; Bayer Diagnostics Manufacturing Ltd, Swords Co, Dublin, Ireland) and the results were expressed by the following equation, where *A* is the absorbance at 570nm.

$$\frac{A_{test}}{A_{\text{stan}\,dant}} \times 10 = mg\ dl^{-1} Ca^{++}$$

For all colorimetric systems described above, deionized water and specific reagents were used for the calibration of the spectrophotometer (blank). Standard curves of linearity supplied by manufacturers were used in order to calculate the concentrations of protein or calcium in the solutions. In order to compensate for increased concentrations in substances due to volume reduction (from 200ml to 5ml) generated by lyophilization process, the obtained results were divided by 40.

The determination of K^{+} contents was carried out using a potassium ion selective electrode (Electrocyte Analyser AVL 9180, AVL Scientific Corporation, Roswell, GA, USA). In order to compensate for increased concentrations due to volume reduction (from 200 to 5 ml) generated by lyophilization process, the results obtained (mmoll^{-1}) were divided by 40.

DNA contents of the samples were determined by reading the absorbance of the filtrates (in replicates) against the appropriated blank (saline solution) at 260nm (Spectrophotometer 482, Femto Ind. Com. de Instrumentos Ltda) [8,12,30]. The spectrophotometer was calibrated with deionized water. The results of two OD readings were transformed in DNA concentration, according to the following formula, where *A* is the absorbance of the filtrates at 260nm and d.f.was the dilution factor (1/40) [8].

$$DNA\ concentration\left(\mu g\ ml^{-1}\right) = A_{260nm} \times 50 \times d.f$$

2.5. Flow Cytometry Analysis

Flow cytometry analysis of *Candida* cells was performed using propidium iodide. Yeast cell culture was centrifuged, rinsed in water and resuspended to 10^{6} cells ml^{-1}in sterile beakers containing 200 ml in cold distilled water [26]. Each beaker was separately placed on a rotational plate in a conventional home microwave oven (Sensor Crisp 38, Double Emission System; Brastemp SA, Manaus, Amazonas, Brazil) and irradiated at 650 W for 1, 2, 3, 4 or 5 min (experimental suspensions - ESs) [28,29]. Control suspensions (CSs) were prepared identically to the ESs. These beakers were individually kept in the microwave oven for the same period of ESs, without irradiation. Propidium iodide solution in PBS (5µl),a marker that only penetrates cells with severe lesions of the membrane, was added to the cell suspensions in order to obtain a final concentration of 1 µg/ml. The samples were then incubated at 35°C for 30 min in the dark. Unstained cells were always included as auto-fluorescence controls.

Cell-associated fluorescence was measured using aFACS-Calibur flow cytometer (Becton-Dickinson Biosciences).

2.6. Statistical Analysis

Original values obtained after cell counting (Neubauer chamber) and after colony countings (ufc/mL) were log transformed before group comparisons. It was calculated the linear regression coefficient between two variables. The linear equation was y= **a** + **b*Time**, where **a** and **b** were the intercept and regression coefficient, respectively, of the prediction equations of released substances or cell counts (y). Average values of the duplicates were submitted to statistical analysis. Pairwise Student's *t*-test was used to evaluate if **a** or **b** were different from zero (5% of significance).In theflow cytometry analysis, expression of mean fluorescence intensity of propidium iodide staining was determined.

3. Results

Results from optical counts using methylene blue dye uptakeas indicative of membrane damaged or non-damaged cells, are illustrated in Figure 1 and 2, respectively. For ESs, damaged cell counts remained similar for all irradiation times tested, exceptfor 1 minperiod (Figure 1). When non-damagecells were considered (Figure 2) all record counts from CS were significant different from zero ($P<0.001$). Non-damaged cell counts also showed similar behavior between ES and CS at 1 min of irradiation, but after 3 min cell counts they were decreased to zero (Figure 2).

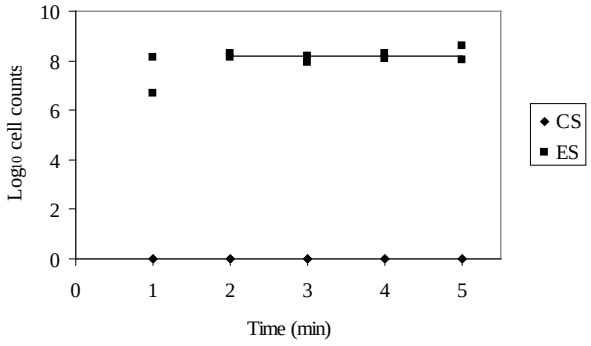

Figure 1. Log_{10}-transformed values of damaged yeast cells of CSs and ESs (Mean of ESs group is represented by the horizontal bar)

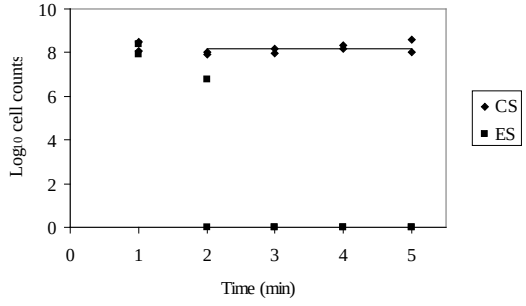

Figure 2. Log_{10}-transformed values (ufc ml^{-1}) of non-damaged yeast cells of CSs and ESs (mean values of CSs group is represented by the horizontal bar)

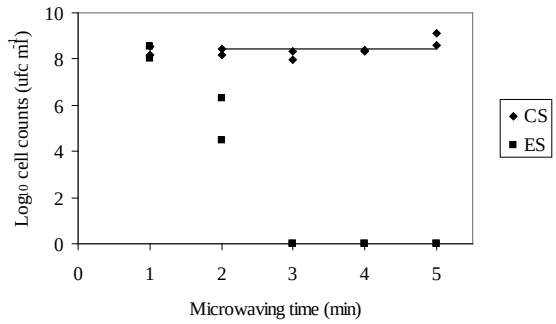

Figure 3. Log_{10}-transformed values of ufc ml^{-1} yeast cells from CSs and ESs (mean values of CSs group is represented by the horizontal bar)

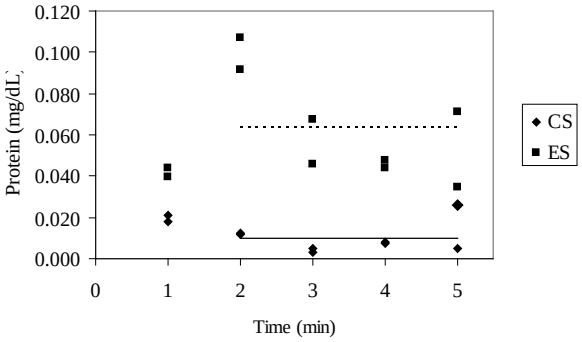

Figure 4. Mean values of protein concentration, as determined by Microprote system (CSs group is represented by the horizontal bar and dashed line represents ESs group)

Figure 3 shows log (ufc ml^{-1}) values obtained from CSs and ESs. Decrease of *C. albicans*viability was dependent on the irradiation time tested. A significant reduction was observed after 2 min of irradiation, and the absence of colony growth (complete killing of the microorganism) was achieved with irradiation during 3 min or more. Substantial microbial growth was observed in all plates of CS groups.

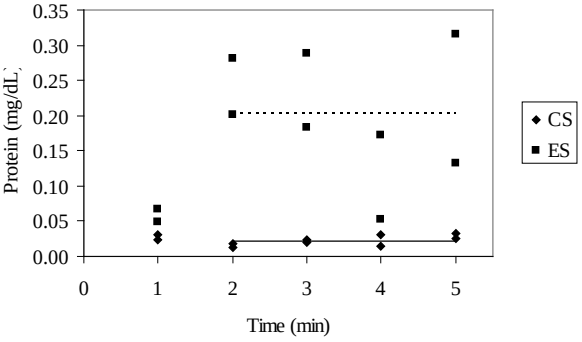

Figure 5. Mean values of protein concentration, as determined by Sensiprote system (CSs group is represented by the horizontal bar and dashed line represents ESs group)

Figure 4 and Figure 5 show mean values of protein concentration obtained from CS and ES groups, as determined by Microprote and Sensiprote systems, respectively. The values of control suspension for protein release were very close to the mean, especially for 2 min to 5 min. For both systems, protein contents released from

ESs were higher than the control when irradiation times of 2 min or higher were considered. There was no evidence of correlation between protein and time using Microprote system (P>0.05) but there was evidence with Sensiproteat some extent(P=0.041).

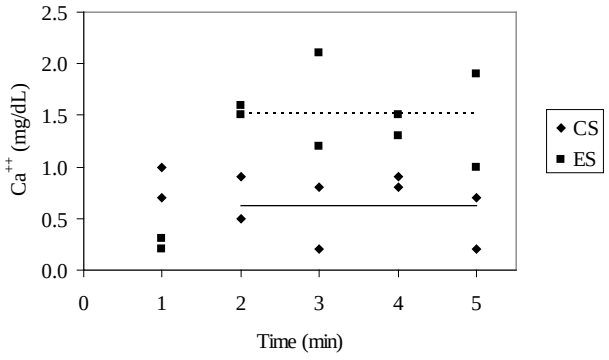

Figure 6. Mean values of Ca^{++} concentration (mean of CSs group is represented by the horizontal bar and dashed line represents ESs group)

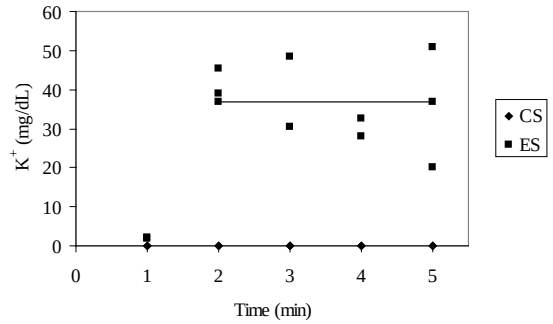

Figure 7. Mean values of K^+ concentration (mean of CSs group is represented by the horizontal bar and dashed line represents ESs group)

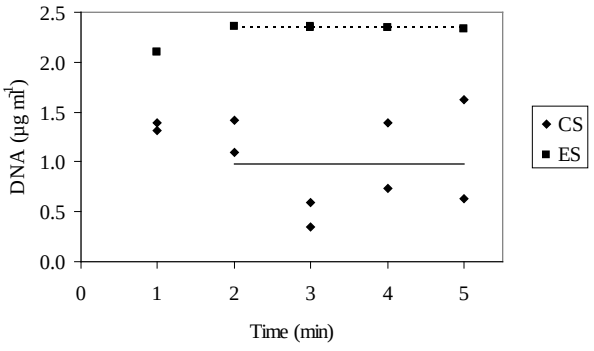

Figure 8. Mean values of DNA concentration (mean of CSs group is represented by the horizontal bar and dashed line represents ESs group)

Figure 6, Figure 7 and Figure 8 show mean values of Ca^{++}, K^+ and DNA concentration obtained from CS and ES groups, respectively. The results suggested that, independent of the time, ESs released more substances than CSs. The results of Ca^{++} release showed similar values of CS and ES only when 1 min time was evaluated. In general, the mean values of ES were 2.5 times higher than the mean values of CS. No signal of K^+ release was found in CS samples, while ES showed high levels of this electrolyte after irradiation times starting at 2 min. Higher levels of DNA were detected in the ES, as compared to CS, regardless of the irradiation time evaluated. After 2 min of irradiation, there was no further increase in the leakage of DNA from ES groups, suggesting that the release of this nucleic acid was not time-dependent.

The Figure 9 shows that similar results to the described above were observed in the flow cytometry analysis. Similar values of CS and ES were found only when 1 min time was evaluated.

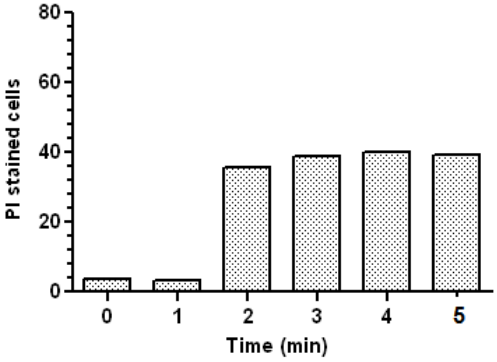

Figure 9. Kinetic study showing propidium iodide stained cells microwaved during 0, 1, 2, 3, 4 and 5 minutes

4. Discussion

When selecting a disinfection procedure, its effect on the physical and mechanical properties of the denture materials shouldbe carefully considered [36,37]. Previous clinical studies showed that microwave disinfection at 650 W for 3 min did not cause detrimental effects on the linear dimensional stability of complete dentures [38]. Thus, the present study was conducted to gain additional understanding on the cellular effects of different exposure times of microwave irradiation on *Candida albicans* cells.

Growth potential is one of the most used parameters for evaluation of destruction or inactivation of microorganisms. In addition, liberation of molecules and intracelular ions such as electrolytes, DNA, RNA, and proteins can help to evaluate cell lysis [39]. Hence, the mechanisms of inactivation of microoganisms by microwavescan be studied at structural and microbial molecular levels by counting the viable cells and release of intracellular substances, as parameters of cell lysis.

Whole cells were not found in the experimental suspension for times greater than 3 min, aftercell count in aNeubauer chamber(Figure 1 and Figure 2). Cells in this suspension lost their refringence characteristic, and became bluish, while still preserved their elipsoidal morphology. According to this methodology, the distinction between intact and non-intactcells is made on the basis of intake of blue methylene dye which is an indirect manner to evaluate the permeability of the membrane and cell walls. Therefore, the obtained results indicate that there was a change of the integrity or permeability of the membrane of cells irradiated for more than 3 min. The results of this study agreed with Mimaet al. [36], who observed changes in cell morphology after microwave irradiation for 3 min under scanning electron microscopy(SEM) analysis.

Regarding the values of CFU ml^{-1}, it was observed that the number was reduced to zero after 3 min (Figure 3). The results of this study are in accordance with other studies. Sanitá et al. [5] proved the hypothesis that simulated complete dentures inoculated with five species of *Candida*, isolated from HIV-infected patients and ATCC strains, could be sterilized by microwave irradiation. The results demonstrated that all dentures contaminated with *Candida* species, regardless of its source (clinical or ATCC), showed uniform sterilization after 3 min of microwave irradiation at 650 W.In the study of Mima et al. [36], SEM analysis indicated that microwave irradiation for 3 min produced changes in cell morphology for sterilized specimens. However, 1 min and 2 min exposition resulted in disinfection of the specimens. In addition, Dovigo et al. [1] found that microwave irradiation for 3 min at 650 W produced sterilization of complete dentures contaminated with *S. aureus* and *Pseudomonas aeruginosa*.

The release of proteins and ions, suggestive of cell membrane damage occurred after 2 to 5 min of irradiation at 650 W (Figure 4, Figure 5, Figure 6 and Figure 7). It would be assumed that the antimicrobial mechanism action of microwaves changed the permeability of cell membrane, and subsequent permeabilization of the membrane caused leakage of intracellular constituents, leading to cell death. Protein molecules are generally very large to pass through undamaged cell membranes [21]. So, loss of protein to the external medium means that the cell was not able to undertake most, if not all, of its essential functions. Ca^{++}, $Na^{++}$and K^{+}ions play specific cell roles and their concentrations in the extracellular environment are maintained through in tramembranous active transport mechanisms. Therefore, ions contents obtained from irradiated cells suggest that the ions were released by vacuole to the cytoplasm, and then to extracellular regions after irradiation.

There was a higher concentration of released nucleic acids (Figure 8)for the same experimental groups (2, 3, 4 and 5 min of irradiation at 650 W), suggesting changes in the cell nuclei. Because nucleic acids(DNA and RNA) are found inside organelles involved by phospholipidic membranes, these molecules can only be released by the microorganism to the extracellular after changing of these membranes [40]. Since DNA is responsible for genetic propagation, the loss of this molecule may be accounted for the lack of growth of the cells from the ESs [40]. The probable explanation is that microwave irradiation accelerates chemical reactions, causing numerous intermolecular collisions making the rotational energy to be converted in thermal energy [9,41].The resulting increase of temperature could cause denaturation of proteins and DNA. These results are in accordance with Lore et al. [42] who observed that, although the microorganism (influenza virus)had been completely inactivated by microwave irradiation, the PCR method was able to detect viral genomic material in the samples. Further investigation is required to determine whether the increase of leakage of electrolytes, proteins and DNA was the cause or the result of microwave-induced cell death. In addition, microwave irradiation has been demonstrated not only to dramatically accelerate a number of organic reactions, but also the activity of various enzymes can be significantly increased when exposed to microwaves [41,43]. However, after 3 or 4 min of irradiation, the temperature rose to above 50ºC, imply that cell inactivation could be a consequence of overheating [41].

Data obtained under the conditions of this study also showed that, microwave irradiation for 1 min at 650 W resulted in survival of the *C. albicans* cells. There were no statistical differences for all parameters used for identification and observation of microorganisms after irradiation for 1 min at 650 W. From this period of irradiation, it was possible to suppose that microwaves did not cause thermal or nonthermal effects on the evaluated microorganisms.

It is known that microwave irradiation is akind of radio frequency energy that excites water molecules, generating heat [42], and it has been applied to inactivateor reduce several species of microorganisms [1,12,20,25]. The explanation about microbial killing with microwave irradiation, however, is not yet completely understood. Two potential explanations have been considered: the thermal effect and non-thermal effect [44]. Regarding the thermal effect, it is believed that microorganisms absorb microwave energy, resulting in thermal death [45]. In addition, Parker [46] found that the effect of microwave irradiation is strongly correlated with water activity. Destruction of microorganisms by the non-thermal effect could be explained by absorption of microwave irradiation by certain biochemical molecules, such as proteins and nucleic acids. So, the interaction between electromagnetic field produced by the microwaves and the cell molecules would lead to cell destruction [47]. It is also suggested that one of the antifungal effect with microwaves is due to the fact that the temperature rise in the microorganism is greater than that of the surrounding medium. In addition, the microorganisms themselves have thermal properties, which are directly involved with microbial destruction [16]. The microorganisms can absorb microwave thermal heat at a much greater rate than the surrounding liquid medium [16]. In addition, it has been suggested that microwaves are not sufficiently strongto directly change chemical bonds and their effects have been attributed to other mechanisms such as dielectric relaxation, ionic conductivity and biopolymer alteration [7]. However, microwave heating is energy conversion and not heating as in a conventional oven, sothe microwave absorbent material exposed to a microwave field converts this energy into heat within itself [47]. In this context, Senna et al. [48] reported that microwave irradiation reaches the microorganism killing temperature more effectively than conventional heating process, which needs to heat the entire environment.

In this study, propidium iodide, a nucleic acid-binding fluorescent probe, was used to evaluate the effect of microwave on cell membranes. Cells with membrane lesions leading to inherent loss of viability internalize propidium iodide, resulting in an increase in red fluorescence [49,50]. The Figure 9 shows the propidium iodide penetration in cells treated with several times in microwave. The results showed a change in the integrity or permeability of the membrane of cells irradiated after 2 min. After the short period of 1 minute in microwave, few cells were propidium iodide positive, i.e. almost 100% cells were viable, with intact membranes.

The methodology of this study did not testthe adhesion of *C. albicans* to the dentures surfaces. Biofilms formed

by *C. albicans*, associated or not with other species of microorganisms, are known to be more resistant to the action of antifungal treatments [51]. A methodology including the development of biofilms should be considered for future investigations. The efficacy of a lower power setting (450 W) was confirmed earlier [48], and has shown to be effective for disinfecting mature *C.albicans* biofilms, at a supposedly safer temperature for denture base acrylic resins. Therefore, the effect of other power densities of microwave irradiation on the inactivation and cell membrane integrity damage of *C. albicans* should be evaluated.

5. Conclusions

The findings of this study demonstrated that microwave irradiation at 650 W for 2 min promoted cell death of *C. albicans* through leakage of proteins, electrolytes and DNA, detectable by different assays. In addition, yeast cell suspensions were 100% inactivated after irradiation for 3 min, suggesting sterilization of the samples. The results provided important insight towards a better understanding of the mechanisms involved in cell death of *C. albicans* after microwave irradiation.

Statement of Competing Interests

The authors have no competing interests'.

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