







Randomized clinical trial of complete denture cleaning methods in residents of care institutions

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Aim: To evaluate the efficacy of ultrasonic bath and traditional brushing in the hygiene of complete dentures of dependent residents in long-term care institutions (LTCIs). **Methods:** A randomized cross-over clinical study was conducted in 17 maxillary complete denture wearers living in LTCIs. Cleaning protocols were brushing or ultrasonic bath, both applied with neutral liquid soap. Biofilm biomass were estimated by MTT reduction assay and specific microbial load (CFU) of *Streptococcus* spp., *Staphylococcus* spp. and *Candida* spp. were quantified by selective and differential culture media. **Results:** Ultrasound method showed higher percentage reduction of biofilm biomass and specific microbial loads of *Streptococcus* spp. compared to brushing ($p < 0.01$). Reduction of microbial loads of the other microorganisms were not different between cleaning methods ($p > 0.05$). **Conclusions:** The ultrasonic bath proved to be a feasible alternative method for the mechanical cleaning of complete dentures in LTCIs.

Keywords: Dentures. Hygiene. Biofilms. Health services for the aged. Elderly.



Introduction

Denture stomatitis affects approximately 65% of patients with maxillary complete dentures and it is commonly associated to poor oral hygiene¹. The condition may be aggravated by trauma, continuous use of the dentures, allergic reaction to the denture base and cleaning products, hyposalivation, inadequate diet and use of antibiotics^{2,3}. *Candida albicans* is the main etiological agent of this condition or associated to other pathogenic and opportunistic microorganisms, such as *Streptococcus* spp., *Staphylococcus* spp. and *Lactobacillus*⁴⁻⁶.

Systemic dissemination of microorganisms from oral infections associated with biofilm accumulation in complete dentures may cause bacterial endocarditis, aspiration pneumonia and chronic obstructive pulmonary disease in dependent elderly patients⁷⁻⁹. Denture cleaning for biofilm control is essential to reduce the risk of these diseases⁸. Complete dentures can be cleaned using chemical and/or mechanical methods such as hypochlorites, peroxides, enzymes, mouthwashes, brushing, sonic and ultrasonic vibration^{10,11}.

Complete dentures wearers in long-term care institutions (LTCIs) may be unable to maintain hygiene of their dentures due to illness, dementia or low motor coordination, which makes them dependent on caregivers' support¹²⁻¹⁵. Although these professionals consider oral hygiene important, lack of time, work overload and refusal behavior by patients may difficult its adequate performance¹⁶.

Brushing alone or associated with soap, toothpaste or abrasives has been the most commonly reported method for cleaning complete dentures^{8,11,17}. Ultrasonic bath is an accessible and low-cost cleaning method, especially for old and physically compromised patients¹⁸⁻²⁰.

The aim of this study was to compare the efficacy of ultrasonic bath and brushing as cleaning methods of complete dentures in LTCI dependent residents. The outcomes evaluated were the percentage changes of biofilm biomass and specific microbial loads after application of the cleaning methods. The work hypotheses were:

1. There would be no difference between cleaning methods for biofilm biomass;
2. There would be no difference between cleaning methods for specific microbial load;

Materials and methods

The present study was registered in REBEC (RBR-3jj84f) and approved by the Institutional Research Ethics Committee (Protocol Number 2,225,015).

Sample selection

The sample was composed of individuals selected among residents of two LTCIs institutions located in the metropolitan area of the city of Curitiba (Parana, Brazil). All residents were dependent elderly individuals. Inclusion criteria were 60 years of age or older, complete maxillary edentulism and wear of conventional maxillary complete denture for at least one year.

Individuals were excluded if: they wore conventional maxillary dentures that were fractured, repaired or relined; were using or had used antibiotics, antifungal agents or corticosteroids for a period of three months prior to the beginning of the research; were using denture adhesives or dentures with excessive calculus; were smokers.

A sample size calculation for a non-inferiority trial of no differences between one treatment (ultrasound) to another (brushing) indicated a sample size of 13 patients for a power of 80%²¹. The CONSORT 2010 flowchart²² (Figure 1) illustrates the selection of study participants, allocation, monitoring and analysis. The institutions and their residents were invited to participate in the research after presentation of the risks, benefits and objectives of the study. The final sample consisted of 17 patients (4 men and 13 women) with a mean age of 72.5 years, who provided written informed consent to participate in the study. For the incapable patients, the legally designated representative was contacted, given explanations about the research and asked to provide written informed consent.

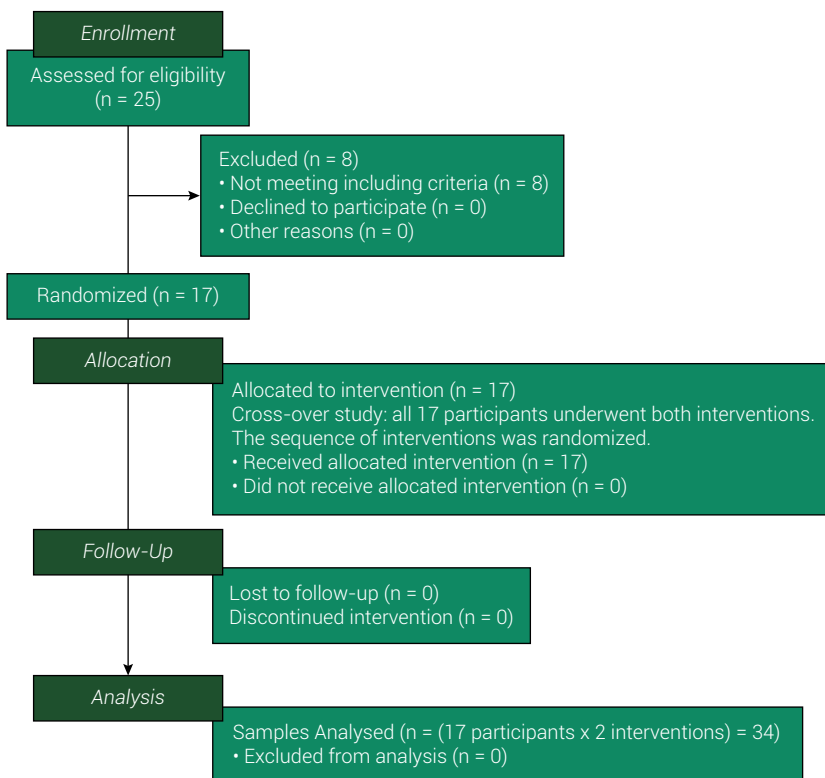


Figure 1. CONSORT 2010 Flow Diagram.

Study design and cleaning methods

This clinical study was randomized, double-blind, controlled, crossover, clinical study. All dentures were exposed to the two interventions, for five days each step.

Intervention 1) Manual brushing with neutral dishwashing soap: brushing once a day, at a fixed time, for two minutes²⁰ using a soft brush specifically for denture cleaning (Twister, Colgate, São Bernardo do Campo, SP, Brazil) associated with neutral dishwashing soap without glycerin (Ype, Goiania, GO, Brazil).

Intervention 2) Ultrasonic bath with neutral liquid soap solution: The dentures were individually placed into identified and closed glass jars containing 100 mL of tap water and one drop of neutral dishwashing soap (Ype), which were subjected to ultrasonic vibration (Cristofoli, Campo Mourao, PR, Brazil) at 160 watts once daily, at a fixed time, for 15 minutes^{19,23}. The dentures were rinsed in running water and returned to the patient.

One of the researchers (RNR) through a lottery system using wrapped cards placed in a plastic jar containing one of two possible sequences of interventions to be tested performed randomization. During the experimental stage, a reminder poster display was fixed on the bedroom wall of each patient with a daily checklist, according to the randomized sequence of cleaning methods. On the bathroom walls, posters were also fixed to emphasize that denture cleaning was not to be performed by anyone other than the previously calibrated caregiver.

Before the research began, alginate impressions of each maxillary complete denture were taken (Jeltrate, Dentsply, Petropolis, RJ, Brazil) and dentures replicated using acrylic resin (Jet Classic, Sao Paulo, SP, Brazil). This procedure was performed so that the replicates could be digitized with a dental scanner (Ceramill map400, Amann Girrbach, Koblach, Austria) and the images were then analyzed using the Solid Edge v20 software (Siemens PLM, Plano, USA) to calculate the total area of the dentures (cm²), which comprised both the external surface of the prosthesis (palate and dental arch) and the internal and retention area.

With the purpose of starting the research with all dentures at the same level of hygiene, they were immersed in 4.2% acetic acid solution for 10 min and then brushed with 2% chlorhexidine digluconate (FGM, Joinville, SC, Brazil) for two min²⁴. The dentures were washed with running water and returned to the patients. They wore the dentures normally for two days, and these were cleaned by brushing once a day. On the third day of the research, possible dietary debris were eliminated by using an indirect vortex of tap water for 30 s, with each denture being placed in a plastic receptacle with a volume of 500 mL. Subsequently, the dentures were immersed in 100 mL of sterile saline solution. The sets were taken to the ultrasonic vessel for 15 min and the suspensions obtained were considered baseline in the microbiological analyses.

After this collection, the dentures were again disinfected by immersion in 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive, Sao Paulo, SP, Brazil) for 15 min, followed by brushing with 2% chlorhexidine gel (Maquira, Maringa, PR, Brazil) for 2 min and returned to the patients, whose dentures were then exposed to the study procedures.

Each of the cleaning methods was implemented for five consecutive days, and after a washout period of one week between each stage, the steps previously mentioned were repeated for all patients prior to beginning with the second intervention, so that all subjects were submitted to both cleaning methods. After apply-

ing each of the methods, the dentures were immersed in identified sterilized glass jars containing 100 mL of saline solution. The sets were taken to the ultrasonic vessel for 15 min and the suspensions obtained were considered “treatment” in the microbiological analyses.

The “baseline” and “treatment” suspensions were sonicated (2840D, Odontobrás, Ribeirao Preto, SP, Brazil) for 20 min for maximum biofilm disintegration. Aliquots of 500 μL were collected for quantification of the specific microbial load. The remaining suspension was used for estimating biofilm biomass by the reduction test of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich M5655, St Louis, MO, USA)²⁵.

Specific Microbial Load Quantification

The following dilution protocol was determined after a pilot trial with a patient's complete denture.

Five hundred microliters aliquots of the suspensions were combined with 500 μL of distilled water and vortexed (AP56, Phoenix, Araraquara, SP, Brazil) at 1500 rpm for 30 s. Aliquots of 100 μL were taken and combined with 900 μL of distilled water and again vortexed at 1500 rpm for 30 s.

One hundred microliter-aliquots of the above-mentioned diluted suspensions were plated in selective and differential culture media Chromocult (Merck, Germany), Manitol Salt Agar (NewProv, Brazil) and Sabouraud Agar Dextrose (NewProv, Brazil). These media allow the presumptive quantification of *Streptococcus* spp. (after confirmation by Gram staining), *Staphylococcus* spp. and *Candida* spp., respectively. Plates containing media for bacterial quantification were incubated at 37°C and plates with yeast media were incubated at 28°C (Orion-502, Fanem, SP, Brazil). All incubations were conducted for 72 h in a normoxic atmosphere.

After the incubation periods, the specific microbial load of the dentures was determined by counting the number of colonies formed (CFU = colony forming units) that presented phenotypes indicating the studied genera, followed by correction for dilution. The specific microbial density was obtained by dividing the numerical CFU values by the area of the dentures (cm^2).

Estimation of biofilm biomass by MTT reduction assay

The sonicated suspensions were centrifuged at 4500 \times g (BE-4004, Bioeng, Curitiba, PR, Brazil) for 10 min. The supernatants were removed and 1.5 mL of sterile water was added. After resuspension and transfer to microtubes, the suspensions were centrifuged at 10,000 \times g (Eppendorf, Hamburg, Germany) for 1 min. After removal of the supernatant, 500 μL of 1 mg/mL^{-1} MTT was added and the microtubes were incubated at 37°C for 3 h. After every one-hour time interval the microtubes were vortexed at 1500 rpm for 30 s. At the end of the incubation, 500 μL of distilled water was added to the microtubes, which were agitated and again centrifuged for 3 min. The supernatants were removed and 200 μL of isopropyl alcohol was added to the sediments. After agitating for 30 seconds, the contents were separated at 10,000 \times g for 1 min. One hundred microliter-aliquots of the supernatants were collected and transferred

to wells of a flat-bottom microplate reader. The optical densities of the formazan crystals formed were measured in a TP-Reader (Thermoplate, GO, Brazil) at 540 nm. The optical density values were divided by the area of the respective dentures. The final results were expressed as OD_{540}/cm^2 .

Estimation of biofilm biomass by MTT reduction assay and specific microbial load were performed by a blinded investigator (APS).

Statistical analysis

Statistical analyses were made with the Statistical Package for Social Science 24.0 (SPSS Inc, Chicago, IL, USA). Data were tested for normality by the Kolmogorov-Smirnov test with Lilliefors significance correlation, and Shapiro-Wilk test. Comparisons between cleaning methods were made using the Mann-Whitney U-test. Comparisons between the percentage change from baseline to treatment of biofilm biomass and specific microbial load count were done with Wilcoxon signed test. All tests were performed at a significance level of 5%.

Results

The means and standard deviation of percentage change of biofilm biomass for brushing and ultrasound methods are presented in Table 1. Ultrasound method showed a significantly higher percentage change of biofilm biomass compared to brushing ($p < 0.05$) (Table 1). Means and 95% confidence intervals of biofilm biomass are shown in Figure 2.

Table 1. Means and standard deviations of percentage change of biofilm biomass.

	Mean (\pm standard deviation)	
Brushing	-23.62% (\pm 51.12%)	$p=0.0004851$
Ultrasound	-70.77% (\pm 16.95%)	

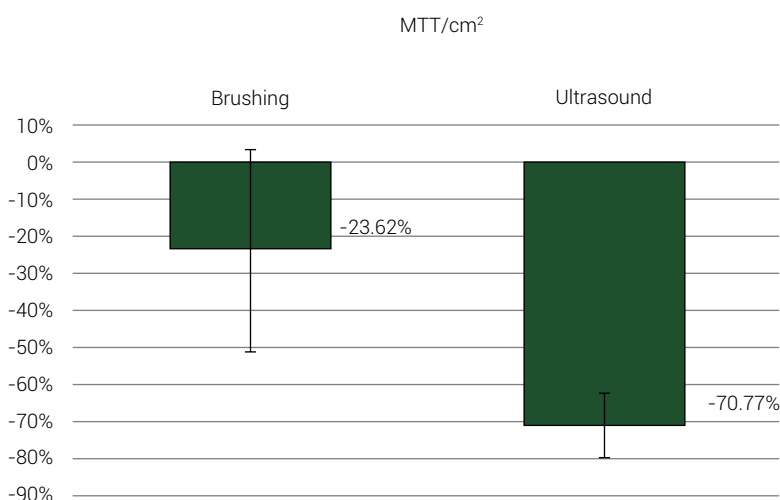


Figure 2. Means and 95% confidence intervals of percentage change of biofilm biomass.

The means and standard deviation of percentage change of specific microbial loads of *Streptococcus* spp., *Staphylococcus* spp. and *Candida* spp. are shown in Table 2. *Streptococcus* spp. was the only group that showed significant reduction in the count of microorganisms in both methods. *Staphylococcus* spp. and *Candida* spp. showed significant reduction in the percentage of microorganisms only in ultrasound method. Means and 95% confidence intervals of specific microbial loads are shown in Figure 3.

Table 2. Means and standard deviations of percentage change of specific microbial load for *Streptococcus* spp., *Staphylococcus* spp. and *Candida* spp. according to cleaning method.

	Cleaning Method	Mean (±standard deviation)	
<i>Streptococcus</i> spp.	Brushing	-23.39% (±45.95%)	$p=0.009$
	Ultrasound	-42.51% (±37.70%)	
<i>Staphylococcus</i> spp.	Brushing	-18.99% (±42.81%)	$p=0.094$
	Ultrasound	-36.54% (±44.07%)	
<i>Candida</i> spp.	Brushing	-16.27% (±35.37%)	$p=0.239$
	Ultrasound	-24.07% (±37.05%)	

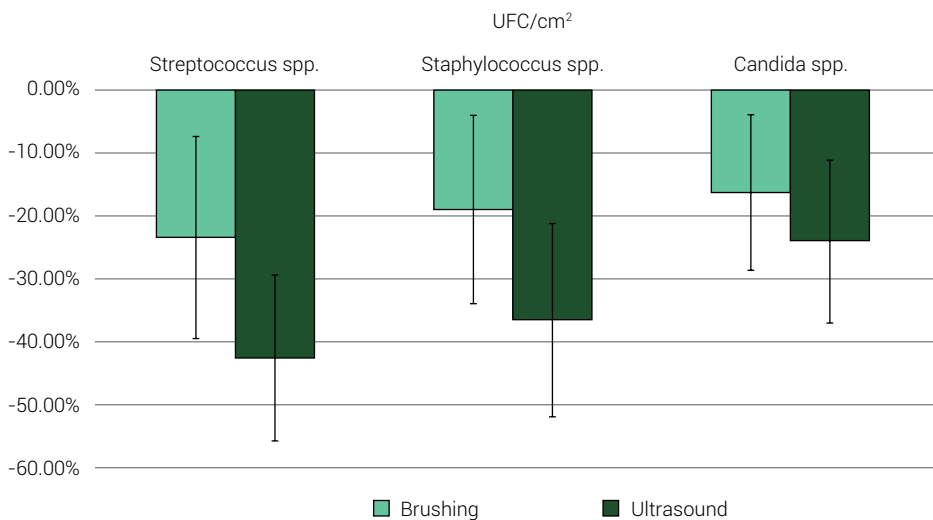


Figure 3. Mean and 95% confidence intervals of percentage change of microbial loads.

Discussion

The current study compared the efficacy of the mechanical cleaning methods brushing and ultrasonic bath, on denture biofilm mass and specific microbial loads. The first null hypothesis was rejected as the positive impact of ultrasonic cleaning on biofilm mass was superior to brushing. The second null hypothesis was confirmed for the *Streptococcus* spp. group, which showed significant changes on the percentage of specific microbial load in both methods, whereas it was rejected for *Staphylococ-*

cus spp. and *Candida* spp. groups, which had significant change on the percentage of specific microbial load only in ultrasound method.

The current study compared the efficacy of the mechanical brushing cleaning and ultrasonic bath methods on denture biofilm mass and specific microbial loads. The first work hypothesis was rejected since the positive effect of ultrasonic cleaning on biofilm mass was superior to brushing. The second work hypothesis was rejected since the *Streptococcus* spp. group showed significant changes on the percentage of specific microbial load in both methods.

The choice of estimating biofilm biomass by the MTT reduction essay was shown to be adequate, since it is a widely used method in different areas of study^{25,26}, and is applicable for quantifying the microbial content covering dental materials²⁷. However, MTT reduction essay in complete denture cleaning methods is innovative. The method of scores based on biofilm staining, although frequently cited in the literature, is limited and not very accurate^{20,24,28–30}. The Polymerase Chain Reaction (PCR), another widely used test for biofilm evaluation in complete dentures^{24,31,32}, does not allow discrimination of living cells from dead cells, since it is based on the indistinct amplification of DNA segments, and may promote erroneous estimation of the microbial load²⁴. On the other hand, the estimation of microbial biomass by the quantification of formazan formed from the MTT reduction is more reliable for estimating the load of living cells²⁶. In the present study, the ultrasound method showed to be more efficient in the reduction of the percentage compared to brushing.

As for the effect of cleaning methods on the percentage of *Streptococcus ssp.* microorganism count, a statistically significant reduction was shown for brushing and ultrasound groups. This reduction has also been previously demonstrated²³, however, the ultrasonic bath was associated with effervescent tablets and applied only once at the end of the research.

In the current study, the counts of *Staphylococcus* spp. and *Candida* spp. were similarly reduced by the two cleaning methods. Another study also reported a decrease in the levels of *Candida* spp. with ultrasound associated to effervescent tablets¹⁹. On the other hand, studies have not observed reduction in *Candida* spp., neither with the method of brushing nor ultrasound, associated or not with effervescent tablets^{23,24}.

A previous study has suggested that *S. mutans* from the biofilm of complete dentures create a glucan barrier that could limit the exposure of other species to chemical cleaning methods, including *Candida* spp³³. Reduced counts of *Candida* spp. were found when bactericidal and/or fungicidal solutions were associated with ultrasonic bath³⁴. Based on these results, it is possible to propose that this association could be effective, since the mechanical cleaning with ultrasound is capable of destabilizing the superficial layers of biofilm on dentures, that contain glucans produced by *Streptococcus spp.* This disruption would expose the *Candida* spp. of deeper layers of the biofilm to the action of chemicals^{33–35}.

The results of the current study comply with these assumptions, since significant reduction in the microbial load was only shown for *Streptococcus* spp., both in the ultrasound and brushing groups. These microorganisms are found more superficially in the biofilm layers and, thus, may be more easily removed. On the other hand, *Can-*

didia spp. and *Staphylococcus* spp., are found in deeper biofilm layers, being positively influenced by the ultrasound method.

Ubiquitous microorganisms, such as *Candida* spp., are present in healthy patients or in those with prosthetic stomatitis. In addition, *Candida albicans* is found in the prostheses of patients with pathological changes^{1,6}. Counts of *Candida* spp. in multi-species biofilm of complete dentures are difficult to reduce even when mechanical cleaning and effervescent tablets are associated^{23,33,36}. In the current study, only the ultrasound was able to reduce the microbial load of *Candida* spp.

Staphylococcus spp. are related to pneumonia that affects many elderly residents in LTCIs⁷. In the current study, they were significantly reduced in concentration when the ultrasound method was applied to the complete dentures. Therefore, it is expected that the method will result in minimizing a possible etiological factor for respiratory diseases related to these microorganisms.

Although brushing with water and toothpaste is the most commonly cleaning method used by complete denture wearers^{8,11,17}, elderly patients may experience a decline in hand dexterity that affects their capacity to brush the dentures effectively¹⁹. Ultrasonic cleaning is not a commonly used technique for denture in nursing homes, probably due to lack of information of caregivers and residents, but could be effective in an institutional home that provides care for the elderly with physical or cognitive deficiencies^{19,37,38}. The effectiveness of ultrasonic cleaning is not dependent on the number of caregivers of the institution, as opposed to the brushing method. The method makes it possible to clean more than one denture at the same time. Other advantages are easy handling of the equipment and low risk of occupational infection to caregivers. In the current study, the ultrasonic bath proved to be efficient in all the analyzed parameters: capability of significantly reducing the biofilm biomass as shown in the MTT assay, as well as of reducing the microbial load for all microorganisms studied groups.

Chemical disinfection is effective when associated with biofilm on denture surfaces^{30,39}. There is a considerable variety of chemicals available for denture cleaning; however, the difficulty in manipulating and the elevated cost of some of these products limits their adoption in LTCIs. As an example, effervescent tablets have demonstrated to be effective for the cleaning of dentures, however, they have an elevated cost, making it difficult to establish their use in underprivileged institutions^{20,40,41}. An ideal cleaning agent for complete dentures should remove organic and inorganic materials, be non-toxic, cost-effective, easy to handle, and reduce biofilm³⁹.

As an example, dishwashing liquid soap, a conventional cleaning product, has proven to present emulsifying action that degrades biofilms. Nevertheless, ultrasonic cleaning has demonstrated to be more effective when associated to chemical agents with these characteristics^{23,35}. The chemical action results from protein solubilization and consequent reduction in microbial adhesion on complete denture surfaces^{40,42}. The advantage over effervescent tablets is centered on non-abrasiveness, low cost and accessibility⁴³.

Finally, it is important to note that, as proposed herein, the prostheses should be somehow stored individually during ultrasonic bath conduction, avoiding possible cross-contamination between residents.

In conclusion, the ultrasound method was more effective than brushing in reducing the biofilm biomass and the count of *Streptococcus spp.* The reduction percentage of the specific count of the microorganisms *Staphylococcus spp.* and *Candida spp* microorganisms did not differ between the two cleaning methods.

The ultrasonic bath proved to be a feasible alternative method for mechanical cleaning of complete dentures in LTCIs.

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