

Antimicrobial action of sodium hypochlorite and castor oil solutions for denture cleaning – *in vitro* evaluation

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Abstract: The objective of this *in vitro* study was to evaluate the antimicrobial action of sodium hypochlorite (0.25% and 0.50%) and 10% castor oil solutions against specific microorganisms, by counting Colony Forming Units (CFU) of clinically important bacteria and *Candida* species. Acrylic resin specimens (n = 320; Lucitone 550) were obtained from square metal matrices (10 x 10 x 2 mm), sterilized by microwave (650W, for 6 minutes) and contaminated by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida glabrata*. The specimens were immersed for 20 minutes in one of the following hygiene solutions (n = 10/each): A - 0.25% Sodium hypochlorite; B - 0.5% Sodium hypochlorite; C - 10% Castor oil solution; and D (Control) - saline. Adhered cells were suspended and inoculated into a selective solid medium (37°C for 24 h). The Student's t-test ($\alpha = 0.05$) was performed to compare $\log_{10}(\text{CFU}+1)/\text{mL}$ between Groups C and D. The results showed that sodium hypochlorite (0.25% and 0.5%) completely eliminated all detectable microorganisms. The castor oil solution eliminated *B. subtilis* and reduced counts for other strains. Differences between C and D were significant ($p < 0.05$) for all species except for *E. faecalis*. Both sodium hypochlorite solutions (0.25% and 0.5%) were effective in eliminating all microorganisms evaluated, and may be useful as cleaning solutions for complete dentures. The castor oil solution provided moderate efficacy and performed differently on the tested species, with the strongest effect on *B. subtilis* and with non-significant action on *E. faecalis*.

Keywords: Biofilms; Denture Cleansers; Sodium Hypochlorite; *Ricinus communis*; Denture Bases.

Introduction

Despite the widely recommended act of brushing as an efficacious method to remove denture biofilm, this method depends mostly on manual ability, and may have limited efficacy in some cases.¹ A way to compensate this is to use a method associating brushing and immersion in solutions; this has been recommended as an effective hygiene procedure.^{2,3} Thus, immersion in chemical solutions has been considered a feasible alternative for denture wearers who need auxiliary cleaning materials.

Alkaline hypochlorite solutions have demonstrated favorable results for denture hygiene. They act in the organic matrix of the biofilm, present fungicidal and bactericidal properties and can remove stains.^{3,4} The recommendation to use diluted sodium hypochlorite (NaOCl), such as household bleaching solutions, is common.^{5,6} However, these solutions have not only bad taste,⁶ but, most importantly, may damage denture materials, depending on the immersion time and concentration. For instance, they may whiten acrylic resins⁷ and cause corrosion to metal components.^{8,9} Studies have reported the antimicrobial action of NaOCl in concentrations of 1% to 5.25%;^{10,11,12} however, there is no consensus for the most efficacious concentrations and the duration of immersion. Hence, the antimicrobial action of the solution must be evaluated when recommending this product for daily denture hygiene.

It is important to continuously improve existing hygiene methods as well as analyze new formulations to ensure no damage to the denture materials, effectiveness and low cost. In dentistry, compounds made from castor beans (*Ricinus communis*) have been used, because of their biocompatibility and anti-inflammatory activity, as well as their bactericide and fungicide action. A study evaluated a 3.3% castor oil detergent and showed antimicrobial activity against anaerobes and streptococci, both present in pulp necrosis.¹³ Another study reported the effectiveness of a 10% castor oil detergent on the irrigation of root canals,¹⁴ showing the action of the product on microorganisms present in endodontic infections.

A castor oil-based solution has features favoring its use for denture hygiene, based on its potent detergent and antimicrobial action; in addition, it is colorless and has no unpleasant odor. *R. communis* is cultivated in several countries, thus making it available to complete dentures users in the future. However, few studies have been conducted regarding the efficacy of castor oil products as complete denture cleansers. In relation to adverse effects, previous studies evaluated a 2% solution and showed non-significant changes in surface roughness, hardness and color of artificial teeth and acrylic resin.^{15,16} Clinical trials showed that a 2% castor oil immersion solution was effective in denture biofilm removal, compared with an alkaline peroxide solution, but its antimicrobial action was not

evaluated,³ and a 3.3% castor oil mouthwash reduced the clinical signs of denture stomatitis; however, this treatment did not significantly reduce the CFU of *Candida* spp.¹⁷

For this reason, a solution with a 10% castor oil concentration was selected because of its effectiveness against microorganisms present in endodontic infections.¹⁴ Leite *et al.*¹⁸ showed that experimental dentifrice containing 10% *R. communis* oil was effective against *S. mutans*, *S. aureus* and *E. faecalis*. Moreover, the literature has not shown adverse effects in a 10% concentration. Therefore, it is crucial to evaluate the antimicrobial action of these solutions, when applied in immersions of short duration and low concentrations.

The aim of this study was to evaluate the *in vitro* antimicrobial action of NaOCl (0.25 and 0.50%) and castor oil (10%) solutions against bacterial species and *Candida* spp. The null hypothesis of the study was that the tested immersion solutions would have the same effect against the tested microorganisms.

Methodology

Square metallic matrices (10 mm × 2 mm) were invested in type III dental stone (Gesso Rio, Rio Claro, Brazil) and putty condensation silicone (Zetalabor; Zermack, Badia Polesine, Italy), in metallic flasks (Jon, São Paulo, Brazil). After the removal of the matrices, heat-polymerized acrylic resin (Lucitone 550; Dentsply Ind. Com. Ltda., Petrópolis, Brazil) was manipulated, packed and pressed into the dental cast at 1200 kgf for 30 min (Protecni Hydraulic Press, Araraquara, Brazil). The specimens were polymerized by a conventional heating method, according to the manufacturer's instructions (water immersion at 73°C for 90 min and boiling for 30 min), in an electric thermopolymerizing device (Thermocycler T100, Ribeirão Preto, Brazil). The specimens were then deflasked⁸ and immersed in distilled water at 50°C for 24 h to eliminate the residual monomer. The excess of polymerized resin was trimmed with a bur (Maxi-Cut, Maillefer SA, Ballaigues, Switzerland), using a low speed micromotor (Dabi Atlante, Ribeirão Preto, Brazil), and the surfaces were polished using 400- and 600-grit wet/dry sandpapers (Norton, Guarulhos, Brazil), in a horizontal lathe spindle (Arotec, Cotia, Brazil). Three hundred and twenty specimens were

obtained. The specimens were immersed in distilled water and sterilized with microwave irradiation (Panasonic, Kadoma, Japan), Model Perfect, 127V; 800W; 2450MHz, at 650W, for 6 min.¹⁹

An evaluation was made of the efficacy of the hygiene methods against 8 strains from the American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 25175), *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 2001), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas aeruginosa* (ATCC 27853). These microorganisms have been used for controlling and monitoring the antimicrobial activity of various compounds; moreover, these species have been isolated from the oral cavity and dental prosthesis surfaces.^{2,12,20}

Microorganisms were added to saline solution to standardize the selected inoculum. The turbidity of the microbial suspension was verified by spectrophotometer, according to the McFarland scale, and had an absorbance reading from 0.08 to 0.1, at a wavelength of 625 nm. According to our calibration curves, this absorbance corresponds to 10⁶ CFU/mL for yeasts and 10⁸ CFU/mL for bacteria. The culture medium was then inoculated with 1% microbial inoculum.

In the laminar flow cabinet (Pachane, Pa 400-ECO, Piracicaba, Brazil), the acrylic resin specimens were distributed in 24-well tissue culture plates (TPP; Trasadingen, Switzerland), and 1 mL of medium broth containing standardized cell suspension was added to each well. The plates were incubated for 90 min at 37°C at 75 rpm (adhesion period). Afterwards, the surfaces were washed with saline to remove non-adherent microorganisms. Biofilm growth was promoted by adding 1 mL of appropriate sterile medium broth to each well, after which the plates were incubated at 37°C, at 75 rpm for 48 h, under aerobic or anaerobic conditions.

The specimens (n = 10) were randomly assigned to one of the cleansing solutions being evaluated: (A) 0.25% NaOCl (Inject Center, Ribeirão Preto, Brazil); (B) 0.5% NaOCl (Inject Center); (C) 10% castor oil solution (Institute of Chemistry, University of São Paulo, São Carlos, Brazil); (D) control group: 0.85%

Saline (Sodium chloride P.A., Labsynth - Laboratory Products Ltda., Diadema, Brazil).

After incubation, the specimens were removed from the plate and transferred to a tube containing 5 mL of one of the cleansing solutions or saline. The tubes were immersed in the incubator under orbital agitation at 75 rpm, for 20 min, to assure contact of the solutions with all the surfaces. After this immersion period, each specimen was removed aseptically and washed 3 times with saline to remove residual disinfectants and loosely adherent cells. The specimens were then transferred to a tube containing a liquid culture medium (Letheen Broth; Difco Laboratories Inc., Detroit, USA), and the remaining adherent microorganisms were removed from the treated specimens by sonication (Altsonic, Ribeirão Preto, Brazil), for 20 min.

The resultant suspension was vortexed, the initial suspension (10⁰) was diluted from 10⁻¹ to 10⁻³ in a sterile saline solution, and aliquots were plated onto sterile Petri dishes containing specific medium. The solid culture media used were: Mueller Hinton Agar (HiMedia Laboratories Pvt. Ltda., Mumbai, India) for *P. aeruginosa*, *S. aureus*, *E. coli* and *B. subtilis*; Sabouraud Dextrose Agar (HiMedia Laboratories Pvt. Ltda., Mumbai, India) for *C. albicans* and *C. glabrata*; Mitis Salivarius Agar Base (HiMedia Laboratories Pvt. Ltda., Mumbai, India; added to Bacitracin and 20% sucrose) for *S. mutans*; and Tryptone Soya Agar (HiMedia Laboratories Pvt. Ltda., Mumbai, India) for *E. faecalis*. The dishes were then incubated at 37°C for 24 h under aerobic or anaerobic conditions (*S. mutans* and *E. faecalis*).

After the incubation period, the number of colonies in each dilution was counted, and the value of CFUs was obtained, based on a dilution providing 1-300 colonies: CFU/mL = number of colonies × 10ⁿ/q, where: n = absolute value of the dilution (0, 1, 2 or 3), and q = quantity of plated suspension (0.05 mL).

Statistical tests were performed using the SPSS 17.0 program (SPSS Inc., Chicago, USA). CFU values were converted to log₁₀. There were several readings that resulted in zero CFU, thus the microbial count data obtained were expressed as log (CFU+1). The groups were compared by parametric test for independent samples. Since only 2 groups (C and D) resulted in

detectable CFUs, we used Student's t-test. All analyses were performed at a 0.05 significance level.

Results

The results of the CFU/mL for groups A and B (NaOCl) were zero. Table shows the results for Groups C (10% castor oil solution) and D (control group).

Average counts for Group C (10% castor oil solution) were significantly lower than those for Group D (control), regardless of the species ($p < 0.05$), except for *E. faecalis*, which was not reduced by castor oil. *B. subtilis* was undetectable in Group C (10% castor oil solution), similar to the result found for Groups A and B.

Discussion

The results showed that the null hypothesis was rejected, because both NaOCl solutions (0.25% and 0.5%) were effective in eliminating all the microorganisms evaluated, and the castor oil solution provided moderate efficacy and performed differently for the species tested.

In this study, the immersions were performed as an isolated method, to obtain an objective evaluation of the antimicrobial action of each solution, since the association of a chemical method with a mechanical method promotes synergistic activity.^{2,5} The study evaluated the effectiveness of the tested solutions against important microorganisms recommended to assess the antimicrobial action of several disinfectant agents.²⁰

The following important gram positive bacteria were selected: *S. mutans* was chosen for its role as a

primary oral biofilm colonizer and its involvement in the development of caries and gingivitis; *S. aureus* has been associated with infections, such as angular cheilitis, endodontic infections, parotiditis and oral mucositis;²¹ *E. faecalis* has been associated with oral cavity diseases, such as apical periodontitis and endodontic infections;²² *B. subtilis*, a non-pathogenic gram positive bacterium, contributes to biofilm growth and, consequently, to pathogen microorganism adherence.²³ The following gram negative bacteria were selected: *E. coli* is considered transitory in the oral cavity, and responsible for initial yeast adherence to several surfaces;²⁴ *P. aeruginosa* is an opportunistic pathogen that hardly ever causes disease in a healthy immune system; however, it exploits weaknesses of the body in order to establish an infection condition.²⁵ The yeasts selected were *C. albicans*, the fungi species most frequently found in denture biofilm,²⁶ and *C. glabrata*, the second most prevalent Candida species in human beings, and frequently found in biofilm associated with denture stomatitis.²⁷

The results showed that both concentrations of NaOCl applied in immersions of short duration (20 min) were effective, eliminating all microorganisms evaluated. Laboratory studies have reported on the antimicrobial action of NaOCl in higher concentrations and different immersion periods. Rudd *et al.*¹⁰ demonstrated that a 5.25% concentration and a 5 min immersion was an efficient disinfecting agent for complete dentures. A 1% concentration has been effective in disinfecting acrylic resin specimens contaminated with strains of *S. aureus*, *C. albicans*,

Table. Mean values and standard deviation for species, in $\log_{10}(\text{CFU} + 1)/\text{mL}$, average differences among the groups and Student's t-test values for independent samples.

Species	Group D (Control)	Group C (Castor oil 10%)	Difference C-D (95%CI)	p-value
<i>B. subtilis</i>	1.45 (0.85)	0.00 (0.00)	-1.45 (n/a [†])	n/a [†]
<i>C. albicans</i>	2.68 (0.39)	1.66 (0.68)	-1.02 (-1.55 to -0.50)	0.001*
<i>C. glabrata</i>	5.11 (0.22)	1.13 (1.16)	-3.98 (-4.81 to -3.14)	<0.001*
<i>E. coli</i>	3.62 (0.70)	2.09 (1.13)	-1.54 (-2.42 to -0.65)	0.002*
<i>E. faecalis</i>	3.64 (1.02)	3.54 (0.73)	-0.10 (-0.93 to 0.73)	0.805 ^{ns}
<i>P. aeruginosa</i>	6.24 (0.36)	4.44 (0.78)	-1.80 (-2.37 to -1.23)	<0.001*
<i>S. aureus</i>	3.88 (0.69)	2.71 (1.26)	-1.17 (-2.12 to -0.21)	0.019*
<i>S. mutans</i>	5.97 (0.82)	3.70 (0.81)	-2.27 (-3.04 to -1.51)	<0.001*

[†] Test not performed, because all specimens in Group C recorded 0 CFU.

^{ns} no significant difference ($p > 0.05$).

* significant difference ($p < 0.05$).

S. mutans, *P. aeruginosa*, *E. coli* and *B. subtilis*, after 10-15 min of immersion.^{11,12,28} Other studies found that 1.5% concentrations for 20 min and 2% for 5 min have been effective against strains of *C. albicans*.^{28,29} However, it is important to consider the concentration used, because NaOCl may cause alterations in acrylic resin properties.^{7,8}

A 0.5% concentration has proven effective against *C. albicans*, after 10 min of immersion.³⁰ Our results showed the effectiveness of a 0.5% concentration against strains of *C. glabrata* and bacterial species, such as *E. faecalis*, *S. mutans*, *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis*.

The antimicrobial activity results for a 0.25% concentration are relevant, suggesting that NaOCl can be used in lower concentrations. Regarding the adverse effects, Paranhos *et al.*⁷ simulated overnight cleansing for 1½ yr and found alterations in color properties and increased surface roughness of an acrylic resin, using a 0.5% concentration. However, the changes are unlikely to have any clinical significance. Thus, the results showed that both concentrations applied in short-term immersions (20 min) may be useful as auxiliary hygiene agents in controlling complete denture biofilms. Nevertheless, studies on possible adverse effects are needed, since no results for a 0.25% concentration have been reported in the literature.

Just like the NaOCl solution, the castor oil solution showed complete *B. subtilis* elimination, whereas different disinfectants (glutaraldehyde and chlorhexidine) have not proven effective.¹¹ *B. subtilis* is an opportunistic microorganism, not showing sufficient adherent capacity when isolated, but promoting biofilm growth and adherence of other microorganisms.²³ Therefore, its elimination is crucial to denture maintenance and oral health.

The castor oil solution was not effective against *E. faecalis*. Paranhos *et al.*² revealed that the combined

method (brushing followed by immersion) was similar to brushing alone, indicating only a minor effect of the chemical method on this microorganism. Orsi *et al.*¹² noted that *E. faecalis* was the most resistant strain, promoting microbial growth on the internal surfaces of acrylic resin after immersion in chemical solutions. Castor oil solution has been found to provide antimicrobial action against other microorganisms (*C. albicans*, *C. glabrata*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. mutans*), suggesting the solution can be used against complete denture biofilm.

Evaluating the microorganisms individually was a limitation of this study, since antimicrobial activity was evaluated in relation to a simple biofilm. Therefore, further studies involving mixed biofilms should be performed to provide data for microbial interaction. Another aspect to be investigated concerns the possible adverse effects caused to denture materials; thus, the analysis of both products, i.e. hypochlorite and castor oil, must be conducted in the concentrations tested in the study, in order to establish the immersion protocol.

Conclusion

Immersion in sodium hypochlorite solutions (0.25% and 0.5%) is an efficacious method of eliminating pathogens present in denture biofilm. These solutions may prove useful for cleaning complete dentures. Castor oil solution provided moderate efficacy and had a varied effect on different species.

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