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Effectiveness of Six Different Disinfectants on Removing Five Microbial Species and Effects on the Topographic Characteristics of Acrylic Resin

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Keywords
Acrylic resins; disinfection; dental prothesis; disinfectants; infection control; surface properties; surface roughness.

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This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo—Process number 2005/57029-4).

Accepted September 12, 2007

doi: 10.1111/j.1532-849X.2008.00358.x

Abstract

Purpose: The aim of this study was to evaluate the effectiveness of disinfectant solutions (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, tabs of sodium perborate-based denture cleanser, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by *Candida albicans, Streptococcus mutans*, *S. aureus*, *Escherichia coli,* or *Bacillus subtilis* as measured by residual colony-forming unit (CFU). In a separate experiment, acrylic resin was treated with disinfectants to monitor potential effects on surface roughness, Ra (μm), which might facilitate microbial adherence.
Materials and Methods: Three hundred fifty acrylic resin specimens contaminated in vitro with 1×10^6 cells/ml suspensions of standard strains of the cited microorganisms were immersed in the disinfectants for 10 minutes; the control group was not submitted to any disinfection process. Final counts of microorganisms per ml were performed by plating method for the evaluation of microbial level reduction. Results were compared statistically by ANOVA and Tukey's test ($p \leq 0.05$). In a parallel study aiming to evaluate the effect of the tested disinfectant on resin surface, 60 specimens were analyzed in a digital rugosimeter before and after ten cycles of 10-minute immersion in the disinfectants. Measurements of superficial roughness, Ra (μm), were compared statistically by paired *t*-test ($p \leq 0.05$).
Results: The results showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms, followed by 100% vinegar, 3.8% sodium perborate, and tabs of sodium perborate-based denture cleanser. Superficial roughness of the specimens was higher after disinfection cycles with 3.8% sodium perborate ($p = 0.03$) and lower after the cycles with 2% chlorhexidine digluconate ($p = 0.04$).
Conclusion: Within the limits of this experiment, it could be concluded that 1% sodium hypochlorite, 2% glutaraldehyde, 2% chlorexidine, 100% vinegar, and 3.8% sodium perborate are valid alternatives for the disinfection of acrylic resin.

Prosthodontics has been cited as one of the dental specialties that most neglect cross-infection control measures during clinical and laboratory procedures. Cotrim et al[1] related that 52% of dentists interviewed did not believe in the possibility of cross-infection between the dental office and laboratory.

There are several routes of microbial contamination in dental laboratories, including the felt disks and pumice used in the polishing process and contact with contaminated hands. Other forms of contamination occur when prostheses are sent to dental offices for adjustments or repairs, because in certain steps of treatment, these materials may be contaminated by microorganisms from the patient's oral cavity.[1,2]

Microbial adherence capacity is influenced by differences in the surfaces of prostheses.[3,4] Davenport[5] suggested that the roughness in prostheses' surfaces may cause micro traumas in oral tissues, and Williams and Lewis[6] concluded that surface

roughness favors colonization by microorganisms, contributing indirectly to tissue injury.

Several disinfectants have been suggested for the disinfection of prostheses. The best disinfectant should fulfill most of the requirements of the ideal agent while not causing any kind of alteration in the structure of the prosthesis.[7]

Sodium hypochlorite is inexpensive, presents a broad spectrum of activity, and requires a short period of disinfection.[1,2] Rodrigues et al[8] suggested immersion in sodium hypochlorite with 2% active chloride for 30 minutes as the most effective method for the disinfection of acrylic resin prostheses. Additionally, Chau et al[9] observed that besides superficial disinfection of acrylic resin, 1% sodium hypochlorite was also effective in the elimination of microorganisms from the inner surface of the material after 10 minutes. Despite its efficiency as a disinfectant, sodium hypochlorite has some disadvantages, including its corrosive activity on metal surfaces, irritant effect on the skin and other cells, and destruction of cloth, including cotton.[10]

Glutaraldehyde-based disinfectants are often used in dentistry,[11] and their use was first suggested in 1962, after Pepper and Lieberman's studies.[7] The main advantage of these products is that they are not inactivated when in contact with organic materials, are not corrosive, and do not degrade plastics or rubber materials;[12] however, due to their toxicicity, they must be manipulated with care. High antimicrobial activity of glutaraldehyde has been described in the literature, and glutaraldehyde's effectiveness is related to the period of exposure. Angelillo et al[13] demonstrated higher effectiveness of this solution against *Staphylococcus aureus* and *Candida albicans,* and more time needed for *Bacillus subtilis* spore elimination. Silva et al[12] observed that 2% glutaraldehyde was effective against *Streptococcus mutans*, *Escherichia coli*, and *C. albicans* after 10 minutes of immersion and after 20 minutes for *B. subtilis* spores.

In the last few years, chlorexidine has been one of the most studied antimicrobial substances. It is considered the best choice among antiseptics for dental biofilm control, effective for the prevention of dental caries, gingivitis, and stomatitis. Moreover, it is also recommended for hand antisepsis.[14] Antimicrobial activity has been described mainly for Gram-positive bacteria.[7]

Acetic acid is one component of vinegar. This solution has been cited in the medical and food-engineering literature as a promising disinfectant.[15] The use of vinegar as a disinfecting agent of semi-critical articles, control of oral and throat inflammatory processes, and antisepsis of sores is cited.[16] Acetic acid has been used in diluted form as an antifungal and antiprotozoal solution.[12] Nascimento et al[15] cited the effectiveness of white vinegar on *E. coli* and *S. aureus.* Lately, interest in vinegar and other solutions of acetic acid as an antimicrobial solution has increased due to discussions of the toxicity of chlorine and other disinfectants.[15,17]

Tabs of sodium perborate-based denture cleanser are commonly used for prosthesis cleaning and for helping mechanical hygiene. Gornitsky et al[18] verified the existence of antimicrobial activity of these solutions on microorganisms adhered to prostheses,[18] but suggested that the use of prosthesis cleaning agents might be controlled. McCabe et al[19] concluded that these products are complementary to prosthesis hygiene and must be employed in association with mechanical cleaning for more effective biofilm elimination.

Surface roughness is determined by the presence of porosity and other irregularities. In dentistry, the presence of roughness on the surface of restorative and prosthetic materials significantly interferes with the properties of the material and may reduce its durability.[20] Acrylic resin is often employed in dentistry and its material roughness is frequently discussed.[3,4] Pavarina et al[21] observed alteration in acrylic resin superficial roughness after immersion in chlorexidine digluconate and sodium hypochlorite; however, they did not observe alterations after immersion in sodium perborate.

The dental office-prosthesis laboratory connection may represent a potential cross-infection pathway if no effective disinfection procedures are taken.[2] Glutaraldehyde-based solutions are commonly indicated for this purpose, but their use is frequently curtailed due to toxicity.[12] Therefore, other effective alternatives are desirable.

Our group previously studied the disinfection of heat-cured acrylic resin.[22] The aim of the current study was to evaluate the disinfection of cold-cured acrylic resin. The effectiveness of different solutions (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, tabs of sodium perborate-based denture cleanser, and 3.8% sodium perborate) on the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by *C. albicans*, *S. mutans*, *S. aureus*, *E. coli*, or *B. subtilis* was measured by residual CFU. In a separate experiment, acrylic resin was treated with disinfectants to monitor potential effects on surface roughness, Ra (μm), which might facilitate microbial adherence.

Materials and methods

This study was divided into two parts: antimicrobial activity of the disinfectants and evaluation of their effects on superficial roughness.

For the evaluation of antimicrobial activity, 350 standardized acrylic resin specimens were obtained. They were prepared with the aid of an aluminum matrix ($3 \times 0.7 \times 0.2$ cm^3), using colorless, chemically activated acrylic resin (Jet, Artigos Odontológicos Clássico, São Paulo, Brazil, batch/lot 207040). The resin was prepared according to the manufacturer's instructions, by mixing 2.5 parts of the polymer and 1.0 part of the liquid. The components were put into a receiving cover glass (first the liquid and then the polymer) and mixed for a short time with the aid of a stainless steel instrument. The resin remained covered until the dough-like phase. Then the resin was distributed into the aluminum matrix. After curing, the specimens were polished with felt disks (30 seconds each) and pumice and kept in water until use. They were submitted to a standard polishing process and submitted to sterilization by gamma radiation with cobalt 60 (25 KGy/6 hours).

C. albicans (ATCC 18804), *S. mutans* (ATCC 35688), *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), and *B. subtilis* (ATCC 19659) were included for the antimicrobial activity tests.

The following disinfectant solutions were included in the study: 1% sodium hypochlorite (LM Farma, São José dos Campos, Brazil, Lot: 040/107), 2% chlorhexidine digluconate (Manipulário, Taubaté, Brazil, Lot: 0510136), 2% glutaraldehyde (LM Farma, Lot: 140/100), 100% vinegar (Castelo, Jundiaí, Brazil, Lot: 247), tabs of sodium perborate-based denture cleanser (Stafford-Miller, Rio de Janeiro, Brazil, Lot: CTO 261/04), and 3.8% sodium perborate (Manipulário, Lot: 20817).

The specimens were distributed into ten groups for the assays between one microorganism and one disinfectant. The control group (n = 10) was not submitted to a disinfection process.

First, each strain was plated in a specific culture medium: Tryptic Soy Agar (Acumedia, Baltimore, MD, Lot: 0408-144) for *S. mutans*, *E. coli*, and *S. aureus*, and Sabouraud dextrose agar (Becton Dickson, Franklin Lakes, NJ, Lot: 5116433) for *C. albicans*. Cultures were incubated for 24 hours at 37°C (and 5% CO_2 for *S. mutans*) (CO_2 Water Jacketed Incubator, Nuaire, Plymouth, MN). *B. subtilis* spore suspension was obtained according to the methodology proposed by Kuroiwa et al.[23]

The parameters of optical density corresponding to suspensions of 1×10^6 cells/ml of each microorganism were spectrophotometrically (Shinadzu model UV-1203, Kyoto, Japan) observed in sterile saline solution (NaCl 0.85%, Casa Americana, São Paulo, Brazil, Lot: 1784) (*C. albicans* OD = 0.284; *S. aureus* OD = 0.374; *S. mutans* OD = 0.620; *E. coli* OD = 0.324; *B. subtilis* spores OD = 0.178) by the linear regression analysis of the standard curves that correlated the optical density of each microorganism with the value of CFUs per ml in the interval of 10^1 and 10^8 cells/ml. The wavelengths of maximum absorbance used for the spectrophotometric readings of *C. albicans* (530 nm), *S. aureus* (490 nm), *S. mutans* (398 nm), *E. coli* (590 nm), and *B. subtilis* spores (307 nm) were previously established by scanning spectrum in the interval of 100 to 1100 nm.

Then, each sterile acrylic resin specimen was transferred to a tube containing Triptic soy or Sabouraud broth and inoculated with 0.1 ml of the standardized suspension of each microorganism. Tubes were incubated for 24 hours at 37°C (and 5% CO_2 for *S. mutans*). After incubation, each tube was immersed in tubes containing 10 ml of the disinfectant to be tested. After 10 minutes, the specimens were immersed for 2 seconds in sterile distilled water to eliminate any excess of the disinfectant solution. Then, they were transferred to tubes containing 10 ml of sterile saline solution (NaCl 0.85%) and the adhered cells were dispersed.

From this initial suspension, dilutions of 10^{-1}, 10^{-2}, and 10^{-3} in sterile saline solution (NaCl 0.85%) were obtained, and aliquots of 0.1 ml were plated in duplicate on Sabouraud or Tryptic Soy agar. Plates were incubated for 48 hours at 37°C (and 5% for CO_2 for *S. mutans*). After incubation, the numbers of colony-forming units (CFUs) were counted.

In a separate experiment, the effect of the disinfectants on the superficial roughness of acrylic resin was tested. For this purpose, 70 standardized acrylic resin specimens were obtained as described previously and maintained in sterile distilled water until the experiment. Just before the experiment, the specimen was dried with filter paper. Readings of initial superficial roughness, Ra (μm), were performed by digital rugosimeter (mechanical profilometer, precision of the unit = ±0.01 μm; diamond scanning tip, radius 5 μm as per Deutsches Institut für Norming—DIN/Germany) (Hommel Tester T500, Hommelwerke, Villingen-Schwenningen, Germany). Readings were performed at three points (A, superior; B, center; C, inferior) on each specimen (n = 10) with an evaluation distance of 4 mm.

After the initial analysis of superficial roughness, groups of ten specimens were immersed in the disinfectants (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, sodium perborate-based tabs, and 3.8% sodium perborate) for 10 minutes and then stored at room temperature. This disinfection procedure was performed once a day for 10 sequential days. After the disinfection cycles, new readings at the same points cited (A, B, and C) were performed. The aim of performing the disinfection procedure for ten cycles was to simulate the dental clinic routine. This disinfection procedure is repeated several times during treatment (repeated before sending to and after receiving from the dental laboratory). A single cycle would not give a true picture of the disinfection procedure.

Data of microorganism counts were transformed into logarithm values and analyzed by means of one-way ANOVA (parametric approach), $\alpha = 5\%$. Post hoc multiple comparisons were performed according to Tukey test (5%). Differences between the superficial roughness mean values before (x) and mean values after (y) the cycles of disinfection were compared statistically by paired *t*-test ($\alpha = 5\%$).

Results

The results obtained for the antimicrobial effectiveness are shown in Table 1. Statistically significant differences were observed among the final counts of *C. albicans* after the disinfection ($p = 0.0001$). The *p*-values obtained for post hoc Tukey's test are shown in Table 2. No statistically significant difference between the final counts of this microorganism after disinfection with tabs of sodium perborate-based denture cleanser and the control group was observed. Sodium perborate showed higher effectiveness than tabs of sodium perborate-based denture cleanser; however, it was less effective than the other tested disinfectants. Sodium hypochlorite, glutaraldehyde, and chlorexidine showed similar effectiveness, considering that no significant differences among the final *C. albicans* counts were observed after the disinfection with these solutions. Vinegar also showed satisfactory effectiveness against this microorganism.

Regarding *S. mutans*, no statistically significant differences were observed among the final counts after the disinfection with sodium hypochlorite, glutaraldehyde, and chlorexidine. These disinfectants were the most effective against this microorganism, followed by 100% vinegar. Sodium perborate was more effective than tabs of sodium perborate-based denture cleanser against *S. mutans*, but both reduced significantly the counts of this microorganism in relation to the control (Table 3).

Similar results were observed for the tests performed with *S. aureus*. Sodium hypochlorite, glutaraldehyde, and

Table 1 Tukey (5%) HSD multiple comparisons test

	Microorganisms				
Disinfectants	*C. albicans* (Fdf $_{(6;63)}$ = 41.53, $p = 0.0001$) Mean (log CFU/ml)	*S. mutans* (Fdf $_{(6;63)}$ = 132.5, $p = 0.0001$) Mean (log CFU/ml)	*S. aureus* (Fdf $_{(6;63)}$ = 178.9, $p = 0.0001$) Mean (log CFU/ml)	*E. coli* (Fdf $_{(6;63)}$ = 216.8, $p = 0.0001$) Mean (log CFU/ml)	*B. subtilis* (Fdf $_{(6;63)}$ = 57.52, $p = 0.0001$) Mean (log CFU/ml)
Control	4.04 ± 0.38A	4.95 ± 0.07A	6.88 ± 0.14A	4.95 ± 0.07A	3.41± 0.39A
Sodium hypochlorite	0.61 ± 0.81BC	0.00 ± 0.00B	0.71 ± 0.95B	0.00 ± 0.00B	0.47 ± 0.68B
Glutaraldehyde	0.36 ± 0.78B	0.21 ± 0.46BC	0.00 ± 0.00B	0.21 ± 0.46B	2.18 ± 0.47C
Chlorexidine	1.00 ± 1.31BC	0.68 ± 0.94BC	0.77 ± 0.85B	4.25 ± 0.13C	2.33 ± 0.47CD
Vinegar	1.51 ± 0.89CD	0.73 ± 0.70D	3.53 ± 0.83C	1.28 ± 1.12D	2.85 ± 0.33DE
Denture cleanser (perborate-based)	4.17 ± 0.44A	3.60 ± 0.52E	5.30 ± 0.70D	5.85 ± 0.44A	3.48 ± 0.21F
Sodium perborate	2.38 ± 0.29D	2.65 ± 0.29F	5.64 ± 0.45D	2.65 ± 0.29E	3.05 ± 0.30EF

Mean values (log scale) in homogeneous subsets (mean values followed by the same letter in a column are not significantly different; statistical analysis compared the different disinfectants tested) of colony-forming units (CFUs) per ml, obtained after disinfection processes and for control group.

chlorexidine showed high effectiveness followed by vinegar. Sodium perborate-based denture cleanser and 3.8% sodium perborate showed similar activity, and all the groups were statistically different from the control (Table 4).

For *E. coli*, glutaraldehyde and sodium hypochlorite were highly effective, followed by vinegar, 3.8% sodium perborate, and 2% chlorexidine digluconate. All tested disinfectants were more effective than sodium perborate-based denture cleanser, whose results were similar to the control (Table 5).

Sodium hypochlorite was the most effective disinfectant against *B. subtilis*, followed by glutaraldehyde and chlorexidine digluconate (Table 6).

In general, sodium hypochlorite showed the highest antimicrobial activity. Glutaraldehyde and chlorhexidine gluconate showed similar activity and were effective against the tested microorganisms, except *B. subtilis.* Vinegar, sodium perborate, and sodium perborate-based tabs showed no sporicide activity.

Data obtained for the superficial roughness analyses are shown in Table 7. Differences of superficial roughness values (y − x) in chlorexidine digluconate ($p = 0.045$) and sodium perborate ($p = 0.032$) groups were statistically significant. Reduction in the superficial roughness was observed after the disinfection cycles with chlorexidine digluconate. On the other hand, after the cycles in 3.8% sodium perborate, superficial roughness was increased.

Discussion

The possibility of cross-infection between the dental office and laboratory is high. Therefore, the disinfection of prostheses before sending them to and after receiving them from the laboratory is an important step for cross-infection control. Heat-sterilization of acrylic materials is not viable, mainly due to the low ebullition temperature of the monomer that composes the resin.[8] Therefore, disinfection based on chemical substances is essential.

Glutaraldehyde-based solutions are commonly indicated for the disinfection of prostheses.[2] Previous studies and the results of the present study prove its antimicrobial effectiveness[20] and sporicide activity;[10] however, there is concern about its toxicity,[12] and this characteristic is considered a limitation for its use. Based on the clinical need for alternative disinfectants that might be used for this purpose, this study was designed to evaluate the antimicrobial effect of other substances and also to monitor potential effects on surface roughness.

Table 2 Tukey (5%) HSD multiple comparisons test

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Vinegar (1)	–	0.141	0.000	0.773	0.023	0.170	0.000
Hypochlorite (2)	–	–	0.000	0.906	0.990	0.000	0.000
Denture cleanser (3)	–	–	–	0.000	0.000	0.000	0.999
Chlorexidine (4)	–	–	–	–	0.497	0.003	0.000
Glutaraldehyde (5)	–	–	–	–	–	0.000	0.000
Sodium perborate (6)	–	–	–	–	–	–	0.000
Control (7)	–	–	–	–	–	–	–

Results obtained for comparison among *C. albicans* counts after the disinfection process with different substances.

Table 3 Tukey (5%) HSD multiple comparisons test

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Vinegar (1)	–	0.040	0.000	0.999	0.294	0.000	0.000
Hypochlorite (2)	–	–	0.000	0.074	0.972	0.000	0.000
Denture cleanser (3)	–	–	–	0.000	0.000	0.002	0.000
Chlorexidine (4)	–	–	–	–	0.428	0.000	0.000
Glutaraldehyde (5)	–	–	–	–	–	0.000	0.000
Sodium perborate (6)	–	–	–	–	–	–	0.000
Control (7)	–	–	–	–	–	–	–

Results obtained for comparison among *S. mutans* counts after the disinfection process with different substances.

Table 4 Tukey (5%) HSD multiple comparisons test

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Vinegar (1)	–	0.000	0.000	0.000	0.000	0.000	0.000
Hypochlorite (2)	–	–	0.000	0.999	0.209	0.000	0.000
Denture cleanser (3)	–	–	–	0.000	0.000	0.905	0.000
Chlorexidine (4)	–	–	–	–	0.130	0.000	0.000
Glutaraldehyde (5)	–	–	–	–	–	0.000	0.000
Sodium perborate (6)	–	–	–	–	–	–	0.001
Control (7)	–	–	–	–	–	–	–

Results obtained for comparison among *S. aureus* counts after the disinfection process with different substances.

Table 6 Tukey (5%) HSD multiple comparisons test

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Vinegar (1)	–	0.000	0.030	0.117	0.016	0.932	0.069
Hypochlorite (2)	–	–	0.000	0.000	0.000	0.000	0.000
Denture cleanser (3)	–	–	–	0.000	0.000	0.336	0.999
Chlorexidine (4)	–	–	–	–	0.987	0.006	0.000
Glutaraldehyde (5)	–	–	–	–	–	0.000	0.000
Sodium perborate (6)	–	–	–	–	–	–	0.526
Control (7)	–	–	–	–	–	–	–

Results obtained for comparison among *B. subtilis* counts after the disinfection process with different substances.

The selection of microorganisms was based on the pathogenic potential or representative importance for antimicrobial effectiveness evaluation studies. *C. albicans*, in association with other factors (i.e., traumatizing prosthesis, unsatisfactory hygiene conditions, systemic factors), is related to the occurrence of prosthesis stomatitis.[3,4] Therefore, studies in dentistry focus on this microorganism not only as a cross-infection problem, but also as a stomatitis-related factor.[24] *S. mutans* is part of the normal oral microflora, and its presence out of this site may be used as a contamination indicator by oral microorganisms;[25] in the same way *E. coli* is correlated to fecal contamination.[2] *S. aureus* is frequently included in infection control studies because of its important pathogenicity and its resistance to drying, heat, and some groups of disinfectants.[9,10,14] Due to the high resistance to heat and disinfectant agents, *B. subtilis* spores are commonly used in studies that evaluate the effectiveness of disinfection and sterilization processes.[11,13,23] In this study, the microbial species were tested individually to better control the initial and residual counts of microorganisms. Further studies testing several microorganisms simultaneously would generate important data.

The present study focused on chemically activated acrylic resin. The results of a previous study developed by our group[22] aiming to test the same variables on heat-cured acrylic denture base materials were similar to those obtained in this study, suggesting similar performances of these materials even though chemically cured materials contain more porosity. More studies including other types of materials would be of interest.

The results of the present study demonstrated that 1% sodium hypochlorite showed the best antimicrobial effectiveness against the tested microorganisms. These data are in accordance with previous studies analyzing disinfection with this solution.[2,10,18] Despite its high antimicrobial activity, this disinfectant presents serious limitations, such as corrosive activity on metal surfaces.

Chlorexidine digluconate showed high effectiveness against *C. albicans*, *S. mutans*, and *S. aureus* and intermediary activity on *B. subtilis*; however, low performance on *E. coli* was observed. These results are in accordance with those of Guimarães Junior,[7] who cited higher activity of this disinfectant against Gram-positive bacteria.

The use of vinegar as a disinfectant is not frequently discussed in dentistry. In the literature of other areas, it is cited as a promising alternative disinfectant, particularly due to its low toxicity.[15,17] Moreover, the inclusion of this substance in our study was also based on its low cost and easy availability. Interestingly, vinegar was as effective as the most-often employed disinfectants (1% hypochlorite and 2% glutaraldehyde) against *C. albicans*. The antimicrobial effectiveness on *E. coli* and *S. aureus* was previously cited.[15-17] Considering that *C. albicans* is the main etiologic agent of oral candidosis, the possible use of this solution in the disinfection of prostheses might be very promising as a therapeutic and preventive approach, especially considering its low toxicity. Studies on the possible effects on other properties of resin seem to be necessary.

This study showed that although suggested as prostheses cleansers, the commercial tabs tested did not present satisfactory antimicrobial activity. The tested product showed antimicrobial activity only against *S. mutans* and *S. aureus*. These

Table 5 Tukey (5%) HSD multiple comparisons test

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Vinegar (1)	–	0.000	0.000	0.000	0.000	0.0001	0.0001
Hypochlorite (2)	–	–	0.000	0.000	0.965	0.0001	0.0001
Denture cleanser (3)	–	–	–	0.000	0.000	0.0001	0.0030
Chlorexidine (4)	–	–	–	–	0.000	0.0001	0.0459
Glutaraldehyde (5)	–	–	–	–	–	0.0001	0.0001
Sodium perborate (6)	–	–	–	–	–	–	0.0001
Control (7)	–	–	–	–	–	–	–

Results obtained for comparison among *E. coli* counts after the disinfection process with different substances.

Table 7 Results of superficial roughness analyses, Ra (μm), before (initial analysis, x) and after (final analysis, y) the immersion cycles in the disinfectants

Disinfectants	n	Initial (x)	Final (y)	Difference (y – x)	*p*
Hypochlorite	10	0.89	1.02	0.12	0.40
Glutaraldehyde	10	0.67	0.70	0.03	0.78
Chlorexidine	10	0.99	0.74	−0.25	0.04*
Vinegar	10	0.40	0.53	0.12	0.18
Denture cleanser	10	1.06	1.01	−0.04	0.75
Sodium perborate	10	0.56	0.87	0.31	0.03*

Statistical analysis applied to the difference values (y – x); *$p < 0.05$.

results are in accordance with previous studies.[15,23] These results corroborate the conclusion of a previous study[23] that these products are complementary to prosthesis hygiene and must be employed in association with mechanical cleaning for more effective biofilm elimination.

Studies on antimicrobial effectiveness of disinfectants evaluate the reduction of microbial counts;[2,7,10,18,20] however, no cut-off values for interpreting this activity are available. This information would be important for comparing different studies. On the other hand, the establishment of these values requires standardization of methodologies that are different among studies. Moreover, clinically, the cut-off value of acceptable microbial adherence also depends on the varied immunologic conditions of patients, which will generate different responses against the same microbial challenge, not always leading to infectious disease. Therefore, we believe that more discussion on this subject is still necessary.

Results obtained for superficial roughness are in accordance with Pavarina et al,[21] who observed alteration in the acrylic resin superficial roughness after immersion in chlorexidine digluconate and sodium hypochlorite; however, they did not observe alterations after immersion in sodium perborate, observations that were different from those of this study. More studies to explain the mechanism of these agents on resin roughness are needed. Based on the evidence of this study, there did not seem to be strong trends of increasing in microbial counts with increasing surface roughness.

Conclusions

Within the limits of the experiment, it could be concluded that:

1. The most effective disinfectants were 1% sodium hypochlorite, 2% chlorhexidine digluconate, and 2% glutaraldehyde, reducing significantly the counts of the tested microorganisms.
2. Compared to the other tested disinfectants, 100% vinegar and 3.8% sodium perborate showed intermediary effectiveness.
3. A statistically significant ($p = 0.045$) reduction in superficial roughness was observed after the disinfection cycles with chlorexidine digluconate.
4. A statistically significant increase ($p = 0.032$) in the superficial roughness was observed after disinfection cycles with 3.8% sodium perborate.
5. Within the limits of this experiment, it could be concluded that 1% sodium hypochlorite, 2% glutaraldehyde, 2% chlorexidine, 100% vinegar, and 3.8% sodium perborate are valid alternatives for the disinfection of acrylic resin.

Acknowledgments

The authors thank LM-Farma for the donation of sodium hypochlorite and glutaraldehyde solutions.

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