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Disinfection protocols to prevent cross-contamination between dental offices and prosthetic laboratories

Ana Carolina Rodrigues Danzi Salvia[a], Fernanda dos Santos Matilde[a], Francine Cristina Silva Rosa[b], Estevão Tomomitsu Kimpara[a], Antonio Olavo Cardoso Jorge[a], Ivan Balducci[a], Cristiane Yumi Koga-Ito[a,*]

[a] *São José dos Campos Dental School, Univ. Estadual Paulista (UNESP), Avenida Engenheiro Francisco José Longo, 777, São José dos Campos, SP 12245-000, Brazil*
[b] *Health Multidisciplinary Institute, Federal University of Bahia (UFBA), Avenida Olívia Flores, 3000, Vitória da Conquista, BA 45055-090, Brazil*

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Summary Control of cross-contamination between dental offices and prosthetic laboratories is of utmost importance to maintain the health of patients and dental office staff. The purpose of this study was to evaluate disinfection protocols, considering antimicrobial effectiveness and damage to the structures of prostheses. Solutions of 1% sodium hypochlorite, 2% chlorhexidine digluconate, 50% vinegar and sodium perborate were evaluated. Specimens were contaminated *in vitro* with standardized suspensions of *Candida albicans*, *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* spores. Disinfection by immersion for 10 min was performed. Final counts of microorganisms were obtained using the plating method. Results were statistically compared by Kruskal—Wallis ANOVA and Dunn's test. The surface roughness of 40 specimens was analyzed before and after 10 disinfection cycles, and results were compared statistically using Student's *t* test. The solution of 50% vinegar was as effective as 1% sodium hypochlorite and 2% chlorhexidine against *C. albicans*, *E. coli* and *S. mutans*. The sodium perborate

* Corresponding author at: São Paulo State University, São José dos Campos Dental School, Microbiology and Immunology, Avenida Engenheiro Francisco José Longo, 777, São José dos Campos, SP 12245-000, Brazil. Tel.: +55 1239479069; fax: +55 1239479010.
E-mail addresses: ana.salvia@fosjc.unesp.br (A.C.R.D. Salvia), fernanda.sma@gmail.com (F.d.S. Matilde), drfransilva@yahoo.com.br (F.C.S. Rosa), estevao@fosjc.unesp.br (E.T. Kimpara), olavojorge@fosjc.unesp.br (A.O.C. Jorge), ivan@fosjc.unesp.br (I. Balducci), cristiane@fosjc.unesp.br, cykogaito@yahoo.com.br (C.Y. Koga-Ito).

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solution showed the lowest antimicrobial effectiveness. Superficial roughness increased after cycles in 1% sodium hypochlorite ($p = 0.02$). Solutions of 1% sodium hypochlorite, 2% chlorhexidine and 50% vinegar were effective for the disinfection of heat-polymerized acrylic specimens. Sodium hypochlorite increased the superficial roughness.

Introduction

There is growing concern about cross-contamination between dental clinics and prosthetic laboratories [1]. During dental treatment, prostheses are transported from one place to another several times. The lack of adequate disinfection is harmful for dental office staff, patients and laboratory staff. Prostheses are considered semi-critical articles and must be subjected to high-level disinfection or sterilization. However, because acrylic resins are thermo-sensitive materials, the use of chemical disinfectants is necessary [2,3]. These substances should be non-toxic, as the aim is patient and operator safety [2].

Several substances have been suggested for the disinfection of prostheses. Sodium hypochlorite is inexpensive and has broad-spectrum antimicrobial activity. However, its corrosive activity on metallic surfaces and irritative effect on the skin might limit its applications [4,5]. The effectiveness of 2% chlorhexidine digluconate for the disinfection of chemically activated acrylic resin specimens has been previously reported [6]. Glutaraldehyde has been cited as highly effective for disinfection; however, its toxicity is considered an important limitation for its clinical use [4].

Acetic acid is one component of vinegar. Acetic acid has been used in diluted form as an antibacterial, antifungal and antiprotozoal agent [7,8]. The interest in acetic acid as an antimicrobial solution has been increasing due to its low toxicity [6]. Sodium perborate-based tabs are commonly recommended for prosthesis cleaning and for improving mechanical hygiene. Previous studies have demonstrated the effectiveness of this product on microorganisms that are adhered to prostheses [4].

Surface roughness is determined by the presence of porosity and other irregularities in the material [9]. The surface roughness of restorative and prosthetic materials interferes significantly with their properties and may reduce their durability [10]. Additionally, increased superficial roughness enhances the adhesion of microorganisms and biofilm formation [6,10,11].

The effectiveness of the disinfection of chemically activated acrylic resin has been previously evaluated [5]. However, little data on heat-cured resin are available in the literature. According to Paranhos et al. [12], 0.5% and 1% sodium hypochlorite did not influence the color stability, surface roughness and flexural strength of this resin.

Although there is a consensus that there is the need for prosthesis disinfection to avoid cross-contamination between dental offices and laboratories, a protocol for such disinfection has not yet been determined. Antimicrobial effectiveness with minimal effect on the prosthesis material is required. The aim of this study was to evaluate the antimicrobial efficacies of 1% sodium hypochlorite, 2% chlorhexidine digluconate, 50% vinegar and sodium perborate-based tabs on heat-polymerized acrylic and to verify their effects on the surface roughness of the resin.

Materials and methods

Specimens

A total of 200 standardized heat-polymerized acrylic resin specimens were generated using gypsum molds (3 cm × 0.7 cm × 0.2 cm) that were filled with acrylic resin (Clássico; Clássico Artigos Odontológicos Ltda, São Paulo, SP, Brazil) following the manufacturer's instructions. After filling the molds, the flasks (Teflon VIPI STG; Dental Vipi Ltda, Indústria e Comércio de Material Odontológico, Pirassununga, SP, Brazil) were submitted to a hydraulic bench press (1.25 ton) for 15 min before polymerization. The microwave oven was scheduled for three cycles, according to the manufacturer's instructions: 3 min/power 40%; 4 min/power 0% and 3 min/power 90%. The specimens were all finished and polished using felts disks for 30 s, abrasive paper and pumice and were kept in water until use. Then, they were sterilized by gamma radiation with cobalt 60 (25 Kgy/6h; Embrarad, Cotia, SP, Brazil).

Antimicrobial activity assessment

Candida albicans was grown in Sabouraud dextrose broth (Difco, Detroit, Michigan, USA), and bacterial species were cultured in Tryptic soy broth (Difco, Detroit, Michigan, USA). Then, they were incubated for 24 h at 37 °C, with 5% CO_2 for *Streptococcus mutans* (CO_2 Water Jacketed Incubator, Nuaire, Minnesota, USA). A *Bacillus subtilis* spore suspension was obtained according to Kuroiwa et al. [13]. The specimens were contaminated *in vitro* by immersion in 10 ml Tryptic soy broth inoculated with 0.1 ml of standardized suspensions containing 1×10^6 cells/ml of *C. albicans* (ATCC 18804), *S. mutans* (ATCC 35688), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538) and *B. subtilis* spores (ATCC 19659) in sterile saline solution (NaCl 0.9%), the concentrations of which were verified spectrophotometrically (Shinadzu model UV-1203, Kyoto, Japan). The parameters of optical density and wavelength adopted for each microorganism were as follows: *C. albicans* (0.284/530 nm); *S. aureus* (0.374/490 nm); *S. mutans* (0.620/398 nm); *E. coli* (0.324/590 nm); and *B. subtilis* spores (0.178/307 nm).

The specimens were randomly distributed into 20 groups ($n = 10$). The disinfectants used were 50% vinegar (Castelo, Jundiaí, SP, Brazil) (in sterilized distilled water), sodium perborate-based denture cleanser (Corega Tabs, Stafford-Miller, Rio de Janeiro, RJ, Brazil), 1% sodium hypochlorite (LM Farma, São José dos Campos, SP, Brazil) and 2% chlorhexidine digluconate (Manipulário, Taubaté, SP, Brazil). After the incubation period of 24 h for *in vitro* contamination, each specimen was immersed in a tube containing 10 ml of the disinfectant to be tested. After 10 min, the specimen was washed for 2 s with sterile distilled water to eliminate the excess disinfectant solution. Then, each specimen was transferred a tube containing 10 ml sterile saline solution (NaCl 0.85%), and the adhered cells were dispersed from this initial suspension. The specimens from the control group were immersed in sterile distilled water for 10 min. The dilutions 10^{-1}, 10^{-2} and 10^{-3} were obtained in 0.85% NaCl. Aliquots of 0.1 ml of these suspensions were plated on Sabouraud dextrose or Tryptic soy agar and incubated for 48 h at 37 °C (and 5% CO_2 for *S. mutans*). The colony forming units (cfu) were counted, and the values of cfu/ml were calculated.

Superficial roughness evaluation

Forty specimens were obtained as described previously and maintained in distilled water until the experiment. The specimens underwent a surface roughness test using a digital rugosimeter (Germany Hommel Tester T500; mechanical profilometer, precision of the unit = ±0.01 μm; diamond scanning tip, radius 5 μm as per Deutsches Institut für Norming — DINI Germany). Readings were performed at three points (A = superior; B = center; C = inferior) on each specimen, with an evaluation distance of 4 mm. After the initial analysis, groups of 10 specimens were immersed in the disinfectant for 10 min and stored at room temperature. This procedure was repeated once a day for 10 sequential days. After the disinfection cycles, a final reading at the same points were performed. Values of roughness were expressed in Ra.μm.

Data on microorganism counts (expressed in values of cfu per specimen) were analyzed using two-way analysis of variance (ANOVA) at the 95% confidence level and the Kruskal—Wallis test ($\alpha = 5\%$). *Post hoc* multiple comparisons were performed according to Dunn's test ($\alpha = 5\%$). Differences between the superficial roughness mean values before and mean values after the disinfection cycles were compared statistically by paired *t* tests ($\alpha = 5\%$). For all tests, p values < 0.05 were considered indicative of statistical significance.

Results

Analyses of the obtained data on the antimicrobial effectiveness of the tested substances are shown in Table 1. The most effective substances for the disinfection of specimens contaminated by *C. albicans* were 50% vinegar and 2% chlorhexidine digluconate, followed by 1% sodium hypochlorite.

Counts of microorganisms obtained after disinfection with sodium perborate-based tabs were similar to the control group.

All of the disinfectants were effective against *S. mutans*; counts of this microorganism reached zero in all conditions. For *S. aureus* and *B. subtilis*, 1% sodium hypochlorite and 2% chlorhexidine digluconate were the most effective disinfectants, and no significant differences were observed between them. A solution of 50% vinegar was not as effective as 1% sodium hypochlorite and 2% chlorhexidine, but the final counts were statistically lower than the control.

Additionally, 1% sodium hypochlorite, 2% chlorhexidine digluconate and 50% vinegar were the most effective disinfectants against *E. coli*. Final counts of *E. coli* in the control group and the group exposed to sodium perborate-based tabs were not significantly different.

Table 1 Median values of colony forming units per specimen (cfu/specimen) of the tested microorganisms and homogeneous groups[a] obtained by Dunn's test (5%) after Kruskal—Wallis test[*].

Groups	Median counts (cfu/specimen)				
	C. albicans	*S. aureus*	*E. coli*	*S. mutans*	*B. subtilis*
Control	843 ^{AB}	249,750 ^{A}	230,000 ^{A}	5800 ^{A}	1450 ^{A}
Sodium perborate	875 ^{*A}	142,400 ^{AB}	55,250 ^{AB}	0 ^{B}	1553 ^{A}
50% vinegar	0 ^{C}	6025 ^{BC}	0 ^{C}	0 ^{B}	880 ^{AB}
2% chlorhexidine	0 ^{C}	307 ^{C}	100 ^{BC}	0 ^{B}	56 ^{BC}
1% sodium hypochlorite	102 ^{BC}	8.25 ^{C}	0 ^{C}	0 ^{B}	0 ^{C}

Df: degree of freedom; kw: Kruskal—Wallis.
$^{A, B, C}$ indicates statistically similar values.
[a] Medians.
[*] *C. albicans*: kw = 36.69; df = 4; $p = 0.0001$; *S. aureus*: kw = 39.05; df = 4; $p = 0.0001$; *B. subtilis*: kw = 36.69; df = 4; $p = 0.0001$; *S. mutans*: kw = 31.24; df = 4; $p = 0.0001$; *E. coli*: kw = 43.82; df = 4; $p = 0.0001$.

The results of superficial roughness obtained before and after the disinfection cycles are shown in Table 2. Superficial roughness (Ra.μm) increased after cycles in sodium hypochlorite ($p = 0.02$). No differences were observed after cycles with 50% vinegar ($p = 0.066$), 2% chlorhexidine digluconate ($p = 0.415$) and the sodium perborate-based denture cleanser ($p = 0.067$).

Discussion

Several substances have been suggested for prosthesis disinfection. The ideal disinfectant should be inexpensive, effective against pathogenic microorganisms, non-toxic and harmless to the structure of the prosthesis [2,3].

The selection of the microorganisms included in this study was based on the pathogenic potential or importance for antimicrobial effectiveness evaluation studies. The inadequate cleaning of dentures may favor the multiplication of *Candida* spp. and bacterial species, which may be related to denture stomatitis [3—5]. These microorganisms may also be reservoirs for disseminated infections [14,15].

C. albicans is the most frequently isolated yeast from the oral cavities of patients with denture stomatitis. This microorganism has capacity to adhere and proliferate upon both soft and hard tissue surfaces [16,17]. *S. mutans* may also be isolated from dentures. The production of acid and extracellular polysaccharides due to hydrolysis of sucrose facilitates their adhesion to dental and prosthesis surfaces [10]. *E. coli* may colonize mucosal surfaces and can also cause disseminated infections [18]. *S. aureus* is related to a number of oral infections, such as osteomyelitis and stomatitis [17]. *B. subtilis* is considered to be non-pathogenic, but it is frequently used as an indicator to test sterilization efficacy due to the resistance of the spore form [18].

In this study, 1% sodium hypochlorite performed well against the tested microorganisms. This finding is in accordance with a previous study [5] on chemically activated resin. Additionally, 2% chlorhexidine digluconate showed very good activity against all of the tested microorganisms except *E. coli*. These results are in accordance with the reports of Silva et al. [5], who found that the activity of this disinfectant is higher against Gram-positive bacteria.

A solution of 50% vinegar exhibited good antimicrobial activity when compared to the other disinfectants tested. Interestingly, this substance was as effective as 1% sodium hypochlorite and 2% chlorhexidine digluconate against *C. albicans*, *E. coli* and *S. mutans*. These results seem to be very promising considering that vinegar is very inexpensive and non-toxic and may be easy to use. However, long-term studies on the effects of vinegar on the properties of acrylic resin are necessary.

Tabs of sodium perborate-based denture cleanser exhibited low antimicrobial activity. However, its effectiveness when associated with mechanical cleaning procedures could still contribute to proper hygiene for prostheses [14].

Regarding the applicability of these findings to the daily dental practice, the effect of these substances on the resin must be considered because previous studies have also reported damage to dentures caused by the disinfection procedure [3,5,8]. The present study showed that despite its good antimicrobial activity, sodium hypochlorite increased the superficial roughness. This observation is not in accord with a previous study that reported no alterations in the surface roughness of heat-cured acrylic resin after cycles of disinfection with 1% sodium hypochlorite [16]. Furthermore, the absence of significant changes in the properties of

Table 2 Comparison of superficial roughness before and after disinfection cycles with the tested disinfectants (values in Ra.μm; confidence interval, CI = 95%, $\alpha = 5\%$).

Comparison of superficial roughness	Disinfectants			
	Sodium hypochlorite	Vinegar	Chlorhexidine digluconate	Sodium perborate
Before	0.252 ± 0.087	0.85 ± 0.038	0.250 ± 0.094	0.278 ± 0.135
After	0.29 ± 0.119	0.218 ± 0.040	0.276 ± 0.079	0.326 ± 0.119
Before × after	CI (95%):	CI (95%):	CI (95%):	CI (95%):
	0.007 to 0.068	−0.069 to 0.002	−0.092 to 0.041	−0.100 to 0.004
	$t = 2.83$	$t = 2.09$	$t = 0.86$	$t = 2.08$
	$p = 0.020$	$p = 0.066$	$p = 0.415$	$p = 0.067$

materials used for lining dentures after disinfection with 2% sodium hypochlorite was previously reported [8].

Comparing the results obtained in this study with those obtained for chemically activated resin [5], differences in the responses of the resins could be observed regarding the effects on superficial roughness. It was observed that heat-polymerized resin superficial roughness increased with cycles in sodium hypochlorite. This result differs from that observed for chemically cured resins, which were affected only by 3.8% sodium perborate [5].

The outcomes of this paper indicate the antimicrobial effectiveness of 1% sodium hypochlorite, 2% chlorhexidine digluconate and 50% vinegar. It is expected that the systematic use of these substances for prosthesis disinfection used in dentistry may contribute to improved infection control and may minimize the risk of cross-contamination. Future studies should evaluate the effects of these solutions on other properties of acrylic resins.

Conclusion

According to the results, 50% vinegar was as effective as 1% sodium hypochlorite and 2% chlorhexidine digluconate against *C. albicans*, *E. coli* and *S. mutans* for the disinfection of heat-polymerized acrylic resin. Sodium perborate-based tabs exhibited antimicrobial activity only against *S. mutans*. The 1% sodium hypochlorite solution increased prosthesis superficial roughness.

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Competing interests

None declared.

Ethical approval

Not required.

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