Antimicrobial spray for toothbrush disinfection: An in vivo evaluation

Sandra Sato, DDS, MS[1]/Vinícius Pedrazzi, DDS, MS, PhD[2]/
Elza Helena Guimarães Lara, MS, PhD[3]/Heitor Panzeri, DDS, MS, PhD[2]/
Rubens Ferreira de Albuquerque, Jr, DDS, MS, PhD[2]/
Izabel Yoko Ito, MS PhD[4]

Objective: The aim of this study was to evaluate the efficacy of a spray containing an antimicrobial solution for toothbrush disinfection. **Methods and materials:** Three different solutions were sprayed on toothbrush bristles among 30 adults after they had brushed: *(1)* basic formulation (base) plus chlorhexidine; *(2)* base only, and *(3)* sterile tap water (control). Each solution was tested for 1 week. After that, the toothbrushes were collected and sonicated in Letheen Broth, diluted in 10-fold series, and plated on selective and nonselective media for detection of anaerobes, aerobes, streptococci, and gram-negative bacilli. After incubation, the colonies of those microorganisms were counted. Presence of mutans streptococci on the bristles was also confirmed. **Results:** Spray 1 produced a significant reduction in the microbial contamination of toothbrushes for all the microorganisms, spray 2 provided some reduction of contaminants, and spray 3 demonstrated the least antimicrobial effect. **Conclusion:** The antimicrobial spray with chlorhexidine proved to be an effective and practical means for toothbrush disinfection. *(Quintessence Int 2005;36:812–816)*

Key words: antimicrobial agent, bacterial contamination, biofilm, chlorhexidine, disinfection, oral hygiene aids, spray, toothbrush

Concerns regarding instruments for oral cleaning such as toothbrushes and dental floss have always existed. Although methods for toothbrushing are described in the literature, procedures for maintaining the cleanliness of toothbrushes are rarely discussed.

Toothbrushes may be contaminated by some sort of microorganisms found in the oral cavity, (Figs 1a and 1b) such as *Streptococcus mutans*,[1,2] *Actinobacillus actinomycetemcomitans*,[3] and *Candida*.[4,5] Bacteria from the environment, including enterobacteria from the bathroom,[6,7] or other skin commensals,[8,9] can also harbor in these instruments for oral care.

The importance of toothbrush microbial contamination is due to the possibility of transmitting microorganisms to other people, particularly when more than one person uses the same toothbrush. Goh et al[10] observed that the habit of sharing personal articles within the home, such as the toothbrush, was an important mechanism for the transfer of hepatitis B virus.

Besides the cross-infection risk, there is a possibility of patient reinfection when one

[1]Postgraduate Student, Department of Dental Materials and Prosthesis, Faculty of Dentistry, University of São Paulo–Ribeirão Preto, Ribeirão Preto, Brazil.

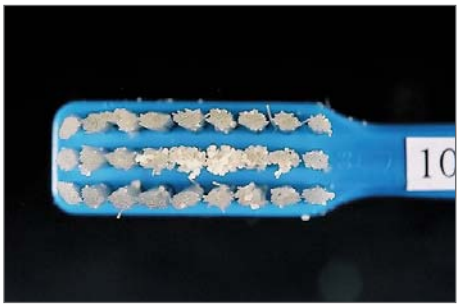
[2]Professor, Department of Dental Materials and Prosthesis, Faculty of Dentistry, University of São Paulo–Ribeirão Preto, Ribeirão Preto, Brazil.

[3]Professor, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, University of São Paulo–Ribeirão Preto, Ribeirão Preto, Brazil.

[4]Professor, Department of Clinical, Toxicological, and Bromatological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo–Ribeirão Preto, Ribeirão Preto, Brazil.

Reprint requests: Dr Vinícius Pedrazzi, Faculdade de Odontologia, Ribeirão Preto–Universidade de São Paulo, Departamento de Materiais Dentários e Prótese, Av. do Café s/n., Bairro Monte Alegre, Ribeirão Preto–SP 14040-904, Brazil. Fax: + 55-16-633-0999. E-mail: pedrazzi@forp.usp.br

This paper is part of a study presented at the 80th General Session of the International Association for Dental Research March 6–9, 2002, in San Diego, CA, USA.



Figs 1a and 1b Demonstration of S mutans biofilm on toothbrush bristles.

uses a toothbrush that harbors pathogenic microorganisms. In an animal model, it was observed that brushing with a self-contaminated toothbrush produced more gingival and other oral lesions than brushing with a sterile toothbrush, and the healing of the ulcerations caused by daily brushing was delayed by the reintroduction of microorganisms.[11] Glass and Shapiro observed that changing the toothbrush at short intervals helped patients achieve elimination of the inflammatory disease symptoms, suggesting that the toothbrush acted as a reservoir for microorganisms capable of producing those diseases.[12] Carvajal et al[13] suggested that toothbrushes contaminated with microorganisms could constitute a bacterial transmission vector. A procedure to reduce the contamination of toothbrushes might be a helpful means to avoid reinfection and cross-infection risks.

Methods for toothbrush decontamination had already been suggested, including ultraviolet radiation,[14,15] immersion in antimicrobial solutions,[16–18] and incorporation of substances in toothbrush bristles for disinfection purposes.[19,20]

The aim of this study was to evaluate the efficacy of a spray containing an antimicrobial solution for toothbrush disinfection.

METHODS AND MATERIALS

Study design

To conduct this study, an approval was obtained from the Ethics Committee at the University of São Paulo at Ribeirão Preto, Faculty of Dentistry (Process #2000.1. 206.58.1). The people who agreed to participate in the study signed the appropriate consent forms.

This study had a single-blind design, in which 30 adults (of both genders) were selected according to the following criteria: must be between 23 and 56 years of age; must have at least 20 teeth; and must not be using any medications, such as antibiotics or antimicrobial substances.

Three solutions were tested: spray 1 was a basic formulation (base) plus chlorhexidine; spray 2 was base only; and spray 3 was sterile tap water (control). Each person tested all 3 solutions.

Each spray was used for 1 week, within the 3 weeks of the study. At the beginning of the week, each volunteer received a new toothbrush (Kolynos Standard, Kolynos), a tube of toothpaste (Sorriso, Kolynos), and one of the 3 sprays to be tested. After cleaning their teeth, the patients were to rinse their toothbrushes under a stream of tap water and then spray the solution 6 times (to standardize the method) on the bristles. Excess solution was removed by shaking the toothbrush. The patient was instructed to rinse the toothbrush with water before each use to eliminate residual solution. After 1 week, the toothbrushes were collected, kept on a rack to avoid contact among the toothbrushes' bristles, and put into a box to be transported to the laboratory. On this occasion, new toothbrushes were supplied with 1 of the remaining sprays, until the 3 sprays were used by all people.

Table 1 Composition of the tested solutions

Components	Spray 1	Spray 2	Spray 3
Propyleneglycol[1]	30.00	30.00	—
Methylparaben (Nipagin)[1]	0.16	0.16	—
Propylparaben (Nipazol)[1]	0.02	0.02	—
Polyvinylpyrrolidone K30[2]	0.02	0.02	—
Ethyl alcohol[1]	10.00	10.00	—
Chlorhexidine digluconate[3]	0.12	—	—
Distilled water (qs) (mL)	100.00	100.00	—
Sterile tap water (mL)	—	—	100.00

[1]Ely Martins, Ribeirão Preto–SP, Brazil.
[2]Goldlab, Ribeirão Preto–SP, Brazil.
[3]Sigma Chemical, St Louis, MO, USA.

Spray composition and culture media

The sprays to be tested were labeled with numbers 1, 2, or 3. Spray 1 contained propylene glycol and distilled water, both vehicles in the formulation for other substances; methylparaben and propylparaben, both preservatives of the solution, to prevent the deterioration of the sprays; polyvinylpyrrolidone K30, a substance to act as a dispersing agent; ethyl alcohol, a solvent; and chlorhexidine digluconate, the active antimicrobial component of the spray. In spray 2, the same components were used, with the exception of chlorhexidine. Spray 3 was the control, containing only tap water subjected to a sterilization process in a steam autoclave. The percentage of these components is presented in Table 1.

The following culture media were used: *(1) K*: blood agar supplemented with 3% to 5% defibrinated rabbit blood, 5 μg/mL hemin, and 1 μg/mL menadione, for anaerobes[21]; *(2) As*: blood agar, with 3% to 5% defibrinated rabbit blood, for total aerobic count; *(3) Ms*: mitis salivarius agar (Difco Laboratories), for total streptococci; *(4) EAM*: eosin–methylene blue agar (Oxoid) for gram-negative bacilli; *(5) CaSaB*: bacitracin sucrose broth, for mutans streptococci biofilm formation; and *(6) SB20*: bacitracin sucrose agar, for mutans streptococci.

The *K* plates were incubated at 37°C in anaerobiosis with Anaerobac (Probac) anaerobic system envelope for 5 days; *As* and *EAM* were incubated aerobically at 37°C for 24 to 48 hours; and *Ms*, *SB20*, and *CaSaB* were incubated at 37°C in microaerophilic conditions under the candle-jar system (*Ms* and *SB20* for 2 to 3 days and *CaSaB* for 3 to 4 days).

Microbial analysis

Each toothbrush was introduced aseptically in a 25 (150-mm test tube, with its bristles completely immersed in 10.0 mL of Letheen Broth (*Calet*) and subjected to sonication for 5 seconds (cell rupture sonicator, Thornton, Inpec Eletrônica). The toothbrushes were then removed from *Calet* and each one was introduced in a test tube containing 10.0 mL of *CaSaB*. The suspensions in *Calet* were 10-fold diluted in phosphate-buffered saline (PBS), and aliquots of 10.0 μL of the dilutions were plated using the drop-plate method[22] in the following media: *K*, *As*, *Ms*, and *EAM*.

After the adequate incubation period, the toothbrushes removed from *CaSaB* had the bristles assessed for mutans streptococci biofilm formation under aseptic conditions using a stereoscopic microscope under reflected light. The colony-forming units (CFU) count was carried out for the other culture media. Confirmation of the presence of mutans streptococci on the bristles was done by transferring some colonies extracted from the toothbrushes to a test tube with 2.0 mL of PBS and some glass beads. The tube was vigorously shaken for 2 minutes in a mixer for colony disintegration, and the suspensions were plated on *SB20*.

Statistical analysis

The original CFU/mL data of the media *K*, *As*, *Ms*, and *EAM* were submitted to the nonparametric Friedman test (n = 30), with a *P* value of .05 chosen as the established significance level. When the Friedman test showed differences among the sprays, the Dunn's multiple comparison procedure was used to compare which pairs of sprays were different at the 5% significance level.

The mutans streptococci biofilm formation data were submitted to the Cochran test, with a chosen *P* value of .05.

RESULTS

Table 2 summarizes the results obtained from the 30 people in the study.

A significant difference was found between the tested sprays for anaerobes (χ^2 = 34.188, P < .0001), aerobes (χ^2 = 28.615, P < .0001), streptococci (χ^2 = 31.022, P < .0001), and gram-negative bacilli (χ^2 = 23.455, P < .0001).

The colonies plated on *SB20* were identified as *Streptococcus mutans*. This procedure confirmed that the biofilm on the bristles was really formed by mutans streptococci. A significant difference in *S mutans* biofilm formation was confirmed for the tested sprays (χ^2 = 44.24, P < .0001).

Table 2 Frequency of positive samples, means and range of microorganisms isolated from the toothbrushes

Microorganisms/ spray type	No. of positive samples (%)	Mean (CFU/mL)	Range (CFU/mL)
Anaerobes			
1[a]	0 (0.0)	0	0–0
2[b]	15 (50.0)	58,862	0–1,585,500
3	25 (83.3)	1,018,356	0–17,700,000
Aerobes			
1[a]	0 (0.0)	0	0–0
2[a]	8 (26.7)	11,180	0–183,000
3[b]	22 (73.3)	2,682,409	0–66,000,000
Streptococci			
1[a]	0 (0.0)	0	0–0
2[a]	11 (36.7)	14,246	0–341,334
3[b]	24 (80.0)	1,601,919	0–27,500,000
Gram-negative bacilli			
1[a]	0 (0.0)	0	0–0
2[ab]	2 (6.7)	704	0–20,600
3[b]	14 (46.7)	1,076,904	0–16,800,000
Biofilm of *S mutans**			
1	0 (0.0)	ND	ND
2	2 (6.7)	ND	ND
3	17 (56.7)	ND	ND

Spray numbers followed by the same superscript letter are not significantly different as determined by Dunn's procedure (P > .05).
*Toothbrush bristles assessed using a stereoscopic microscope under reflected light.
ND = not done.

DISCUSSION

There are many reports of the presence of microorganisms in toothbrushes.[2,6,8,9,23] The risk of cross-infection or reinfection of people because of the presence of microorganisms on toothbrushes makes it relevant to investigate methods of toothbrush care.

This study was performed to assess toothbrush disinfection obtained with an antimicrobial solution sprayed on toothbrush bristles.

The CFU/mL data showed that only spray 1 was able to reduce the contamination to zero (Table 2). Comparing it with spray 2 (base), an equal efficacy of both sprays was observed for the tested microorganisms, except for anaerobes.

Spray 2 proved to be capable of reducing microbial contamination of toothbrushes. This antimicrobial activity can be explained by the presence of substances in the formulation with that property, such as propylene glycol, an alcohol (vehicle); methyl and propylparaben (preservatives); and ethyl alcohol (solvent). It had already been demonstrated that parabens could help in toothbrush decontamination.[24] These components had some other functions in this formulation, but their slight antimicrobial activity was enhanced by the addition of polyvinylpyrrolidone, a film-forming substance. This last component extended the contact time of the spray by the formation of a coating of antimicrobial solution over the toothbrush bristles.

The results of this study showed that the highest degree of microbial contamination was observed with spray 3 (sterile tap water). Tap water was sterilized in this study to guarantee that no microorganism would be present in the spray that could influence the results, and thus mask the real contamination level. In our work, rinsing only with water was not an effective form of toothbrush care; similar results were obtained by Kozai et al.[2]

Other authors tested in vitro preparations for toothbrush decontamination in a spray form. One study demonstrated that activated ethanol and parabens in a spray for toothbrush disinfection were the responsible components for the antimicrobial action.[24] A spray containing cetylpyridinium chloride for toothbrush disinfection was evaluated in vitro, indicating efficacy in toothbrush decontamination, but the total elimination of microorganisms was not achieved.[25]

The results of biofilm formation showed that when using only tap water to clean the toothbrushes, there is still high contamination of the bristles by mutans streptococci. The same finding was observed in a previous study.[18]

The reduction in contamination was highly significant under the conditions of this study, and it seems to justify the use of the spray presented in this paper, with the addition of chlorhexidine, for toothbrush disinfection.

Further studies should be done to assess the efficacy of this spray in eliminating other microorganisms, such as viruses or fungi. Other formulations with different antimicrobials must be studied.

CONCLUSION

The antimicrobial spray with chlorhexidine in its composition might represent an effective and practical means of toothbrush disinfection, and prevent the occurrence of cross-infection or reinfection of patients using a contaminated toothbrush.

REFERENCES

1. Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. Scand J Dent Res 1978;86:412–414.
2. Kozai K, Iwai T, Miura K. Residual contamination of toothbrushes by microorganisms. ASDC J Dent Child 1989;56:201–204.
3. Müller H-P, Lange DE, Müller RF. *Actinobacillus actinomycetemcomitans* contamination of toothbrushes from patients harbouring the organism. J Clin Periodontol 1989;16:388–390.
4. Marcano C. El cepillo de dientes en la ecologia de *Candida albicans*. Mycopathologia 1981;74:135–141.
5. Motzfeld R, Huerta J, Apip A, Araya E, Meller C. Tipo y grado de contaminación por bacterias bucales y leveduras, de cepillos dentales con uso habitual. Rev Fac Odontol Univ Chile 1999;17:9–14.
6. Malmberg E, Birkhed D, Norvenius G, Norén JG, Dahlén G. Microorganisms on toothbrushes at day-care centers. Acta Odontol Scand 1994;52:93–98.
7. Long SR, Santos AS, Nascimento CMO. Avaliação da contaminação de escovas dentais por enterobactérias. Rev Odontol Univ Santo Amaro 2000;5:21–25.
8. Verran J, Leahy-Gilmartin AA. Investigations into the microbial contamination of toothbrushes. Microbios 1996;85:231–238.
9. Taji SS, Rogers AH. The microbial contamination of toothbrushes. A pilot study. Aust Dent J 1998;43:128–130.
10. Goh KT, Ding JL, Monteiro EH, Oon CJ. Hepatitis B infection in households of acute cases. J Epidemiol Community Health 1985;39:123–128.
11. Glass RT, Martin ME, Peters LJ. Transmission of disease in dogs by toothbrushing. Quintessence Int 1989;20:819–824.
12. Glass RT, Shapiro S. Oral inflammatory diseases and the toothbrush. J Okla Dent Assoc 1992;82:28–32.
13. Carvajal E, Gálvez P, Majlis G, Oyarzún A. Presencia de microorganismos en cepillos dentales utilizados en higiene oral habitual. Rev Dent Chile 1995;86: 25–28.
14. Fratto G, Nazzicone M, Ortolani E. Disinfezione degli spazzolini dentali. Ricerca sperimentale. Prev Assist Dent 1990;16:7–10.
15. Glass RT, Jensen HG. The effectiveness of a u-v toothbrush sanitizing device in reducing the number of bacteria, yeasts and viruses on toothbrushes. J Okla Dent Assoc 1994;84:24–28.
16. Feo M. Supervivencia y desinfeccion de *Candida albicans* en el cepillo de dientes. Mycopathologia 1981;74:129–134.
17. Caudry SD, Klitorinos A, Chan ECS. Contaminated toothbrushes and their disinfection. J Can Dent Assoc 1995;61:511–516.
18. Nelson Filho P, Macari S, Faria G, Assed S, Ito IY. Microbial contamination of toothbrushes and their decontamination. Pediatr Dent 2000;22:381–384.
19. Simonetti D'arca A, Capozzi L. Sul potere di autodepurazione dello spazzolino da denti con particolare riguardo al tipo a testina argentata. Riv Ital Stomatol 1983;52:285–292.
20. Suido H, Offenbacher S, Arnold RR. A clinical study of bacterial contamination of chlorhexidine-coated filaments of an interdental brush. J Clin Dent 1998;9:105–109.
21. Tchaou WS, Turng BF, Minah GE, Coll JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials. Pediatr Dent 1995;17:351–355.
22. Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 2001;44:121–129.
23. Glass RT, Lare MM. Toothbrush contamination: A potential health risk? Quintessence Int 1986;17: 39–42.
24. Neal PR, Rippin JW. The efficacy of a toothbrush disinfectant spray-An in vitro study. J Dent 2003;31: 153–157.
25. Meier S, Collier C, Scaletta MG, Stephens J, Kimbrough R, Kettering JD. An in vitro investigation of the efficacy of CPC for use in toothbrush decontamination. J Dent Hyg 1996;70:161–165.