Scientific Article

Efficacy of Microwaves and Chlorhexidine on the Disinfection of Pacifiers and Toothbrushes: An In Vitro Study

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Abstract: ***Purpose:*** *The purpose of this study was to evaluate, in vitro, the contamination of toothbrushes and pacifiers by Streptococcus mutans, and the efficacy of microwave and chlorhexidine for their disinfection.* ***Methods:*** *Sixty pacifiers and 60 toothbrushes were contaminated with S mutans and then divided into groups according to the disinfection protocol: Group 1—chlorhexidine solution; Group 2—microwave sterilization; and Group 3—sterile tap water. The devices were evaluated microbiologically as to the formation of S mutans colonies/biofilms and were examined by scanning electron microscopy. The results were submitted for statistical analysis by Friedman's test at a 5% significance level.* ***Results:*** *The results of both types of evaluation showed a large number of S mutans colonies/biofilms after spraying with sterile tap water, and chlorhexidine spraying and microwaving were effective in eliminate colonies/biofilms. Groups 1 and 2 were statistically similar to each other (P>.05) and differed significantly from Group 3 (P<.05).* ***Conclusions:*** *The 0.12% chlorhexidine solution spray and 7 minutes of microwave irradiation were effective for disinfection of pacifiers and toothbrushes. (Pediatr Dent 2011;33:10-3) Received July 29, 2009 / Last Revision January 26, 2010 / Accepted March 10, 2010*

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Toothbrushes are free from micro-organisms after the manufacturing process.[1,2] They can, however, be contaminated after only one tooth-brushing during 30 seconds to 4 minutes,[3,4] by different types of bacteria,[2,5] viruses,[6,7] and fungi[8,9] present in the oral cavity or the environment.[10] As public health policies emphasize the concepts of prevention and biosecurity, it is important to disseminate the idea that the toothbrushes should be stored, disinfected, and replaced properly. There are, however, few studies that have evaluated toothbrush contamination and disinfection methods.[2,9,11-13]

Similarly, some authors[14,15] have demonstrated that because the silicone or latex pacifiers are in permanent contact with saliva and the oral microflora, they constitute a site for the growth of micro-organisms. The literature also emphasizes that the use of pacifiers is associated with the occurrence of otitis media,[16] dental caries,[17] and intestinal parasites as protozoan cysts and helminthes eggs or larvae[15]. Indeed, these pacifiers can be considered a vehicle of contamination and microbial transmission in children and disabled patients.

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While studies related to the contamination of pacifiers can be found in the literature, there are no studies referring to the use of disinfection methods to eliminate contamination by oral micro-organisms. From a social point of view, these methods of disinfection should be effective, simple, and inexpensive.

Some studies have suggested the importance of the disinfection of toothbrushes to reduce the number of micro-organisms present on the bristles using UV radiation,[13] electrolyzed water,[18] and chemical agents such as Listerine, Plax, Cepacol,[10] and chlorhexidine.[12,19] Although there are a few antimicrobial agents that could be used for disinfection, chlorhexidine is still considered the "gold standard."

Recently, several studies have evaluated microwave efficacy for the disinfection of dental devices and materials such as prosthesis,[20] acrylic resins,[21] and composites.[22] Microwave irradiation is claimed to be a simple, effective, and inexpensive disinfection method.

The purpose of the present study was to evaluate, in vitro, the contamination of toothbrushes and pacifiers by *Streptococcus mutans* and the efficacy of microwave and chlorhexidine spray on their disinfection.

Methods

Sixty silicone pacifiers (Kuka Baby, São Paulo, Brazil) and 60 toothbrushes (Johnson Jr, Johnson & Johnson, São Paulo) were taken from their original packages and soaked in a suspension containing *S mutans* (strain ATCC 25175) at 1,720,000 cfu/mL concentration (0.5 McFarland scale) for 5 minutes.

Immediately after the contamination process, the pacifiers and toothbrushes were randomly assigned to 3 groups of 20 specimens each, according to the following protocols described: Group 1- spraying 4 times with 0.12% chlorhexidine solution (Periogard, Colgate/Palmolive, Divisão da Kolynos do Brasil Ltda, São Bernardo do Campo, São Paulo, Brasil); Group 2 - disinfection in a microwave oven (Sharp Carousel, São Paulo, Brasil – 1100 watts) adjusted to potency level 7 (corresponding to 70% of full power) for 7 minutes; and Group 3 - spraying 4 times with sterile tap water.

The microwaving parameters used for disinfection of toothbrushes were established based on the results of a pilot study in which different potencies and periods of exposure to microwaves were evaluated. In the pilot study, 9 pacifiers were contaminated by a known *S mutans* concentration (ATCC 25175) before exposure to microwave cycles of 7, 8, and 9 minutes. Two microwave potencies were compared (7 and 10). The best results for preventing the growth of micro-organisms were obtained with 7 minutes of exposure to microwaves at potency 7.

The toothbrushes and pacifiers were transferred from the contaminated solution to empty open glass containers (10 specimens per container) and taken to the microwave oven for 7 minutes at potency 7. The containers were arranged at a distance to avoid contact among them, and the toothbrush bristles and silicone pacifiers were placed in a vertical position.

After the different disinfection protocols, all toothbrushes were maintained in a closed, custom-made container to avoid contact among them at room temperature for 4 hours. All pacifiers were immediately sent for microbiological evaluation.

Microbiological analysis. After 4 hours, the toothbrushes of each group were individually and vertically placed into 25 x 150 mm test tubes containing 10.0 mL CaSa B (Bacitracin Sucrose Broth—selective enrichment broth specific for *S mutans* without trypan blue)[2,23] for 10 days at 37°C. Each pacifier was individually placed in Borel tubes containing 25 mL CaSa B and incubated in the same way as the toothbrushes. Evaluations of the surfaces for the presence of bacteria were made at different time intervals, 4, 7 and 10 day after incubation. At each time interval, the toothbrushes and pacifiers were withdrawn, rinsed in the broth, and gently shaken to remove planktonic microbiota, leaving sessile bacteria adhered as "spike" or "mushroom-like" colony/biofilms. The toothbrush bristles and silicone pacifier surfaces were carefully analyzed on all sides, and sessile *S mutans* colonies/biofilms, based on colony morphology, were counted under aseptic conditions with a stereo-microscope (Nikon, Tokyo, Japan) with reflected light by a blinded examiner.The microbiological results were analyzed statistically with Friedman's non-parametric test at a 5% significance level using GMC statistical software 8.1 (available at "http://www.forp.usp.br/restauradora/gmc/gmc.html").

Scanning electron microscopy (SEM) analysis. After microbiological processing, 4 representative toothbrushes and 4 representative pacifiers of each group were fixed in 4% glutaraldehyde in cacodylate buffer, pH 7.4, at 37°C. Two bristle tufts from each toothbrush and a portion of the silicone from each pacifier were removed, post-fixed with 1% osmium tetroxide, dehydrated in ascending ethanol grades, and critical-point dried with liquid carbon dioxide. Subsequently, they were mounted on stubs, sputter-coated with gold, and examined with a scanning electron microscope (Zeiss, DSM 940A, Jena, Germany) operated at 15 kV. SEM micrographs were made to evaluate the presence or absence of contamination.

Results

In the first evaluation (after 4 days), the presence of *S mutans* was observed in 4 (20%) of 20 pacifiers (sterile tap water), and the number of colonies/biofilms ranged from 64 to uncountable. In the second evaluation (after 7 days), *S mutans* were present in 5 pacifiers (25%), and the number of colonies/biofilms ranged from 9 to uncountable. In the third evaluation (after 10 days), these micro-organisms were observed in 6 pacifiers (30%), and the number of colonies/biofilms ranged from 4 to uncountable. Considering the 3 evaluations, *S mutans* were present in 15 (75%) of the 20 pacifiers analyzed. When 0.12% chlorhexidine spray and microwave were used (Groups 1 and 2), colonies/biofilms were absent in 100% of the cases, in all 3 evaluation intervals. Figures 1A-B are representative of the presence of *S mutans* on pacifiers from Group 3.

Groups 1 and 2 were statistically similar to each other ($P>.05$) and differed significantly from Group 3 ($P<.05$).

When microbiological culture was positive by detection of colonies/biofilms under stereomicroscopy in Group 3, *S mutans* biofilm was observed adhered to the surface of the silicone pacifiers on SEM analysis. After no detection of colonies/biofilms on silicone surface from Groups 1 and 2 by stereomicroscopy, SEM examination showed no micro-organisms or only sparse micro-organisms.

Figure 1. *Streptococcus mutans* colonies/biofilms on microbial culture (A) and scanning electron microscope (B) on Group 1 pacifiers.

In relation to the toothbrushes, after the first evaluation, *S mutans* colonies/biofilms were observed in all cases (100%) of Group 3 (sterile tap water), with an uncountable number of colonies/biofilms in all toothbrush bristles evaluated (Figure 2A) at all evaluations. On toothbrushes from Group 1 (0.12% chlorhexidine solution) and Group 2 (microwave), colonies/biofilms were absent in 100% of the cases in all 3 evaluations.

According to the statistical analysis, it was observed that Groups 1 and 2 were statistically similar to each other ($P>.05$) and differed significantly from the Group 3 ($P<.05$).

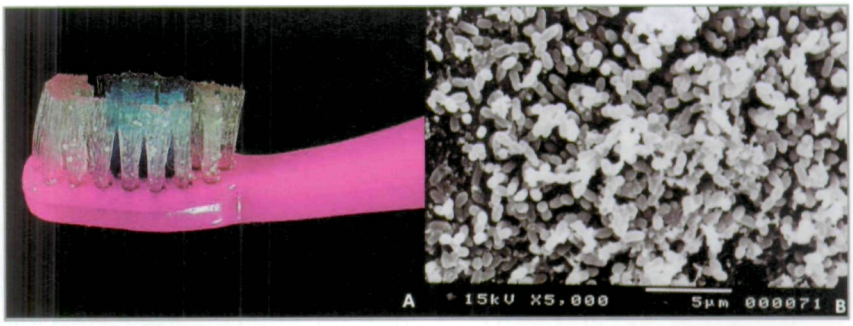

Figure 2. *Streptococcus mutans* colonies/biofilms on microbial culture (A) and SEM (B) on Group 1 toothbrushes.

When microbiological culture was positive by detection of colonies/biofilms under stereomicroscopy (Group 3), *S mutans* biofilm was observed adhered to the toothbrush bristles on the SEM analysis (Figure 2b). When colonies/biofilms were not observed by stereomicroscopy (Group 1 and 2), no micro-organisms or only sparse micro-organisms were observed on SEM examination.

Discussion

In the present in vitro study, there was *S mutans* contamination on 100% of Group 3 toothbrushes, which were sprayed with sterile water. This result shows that the methodology is correct and can be used as a control in *in vitro* tests to evaluate the efficacy of different disinfection protocols (physical and chemical) before the in vivo evaluations. The present study's findings are similar to those of in vivo studies by Motzfeld et al.,[24] Nelson-Filho et al.,[12,25] and Quirynen et al.,[11] which described an intensive contamination by *S mutans* on adults' and children's toothbrushes after use.

Several studies have suggested the importance of disinfection in reducing the number of micro-organisms on toothbrush bristles. In the present study, the 0.12% chlorhexidine spray showed 100% efficacy for toothbrush disinfection, eliminating all colonies/biofilms in this group. These results agree with Nelson-Filho et al.[2,12] and Saravia et al.,[2] who observed absence of *S mutans* growth in toothbrushes after the use of a 0.12% chlorhexidine solution. We agree with Moshrefi[26] in that chlorhexidine is the "gold standard" antimicrobial, compared to other agents used for dental biofilm control.

Although the microwave efficacy has been evaluated only in protheses,[20] acrylic resins,[21] and composites,[22] in the present study this method was also effective in eliminating micro-organisms from toothbrushes. It is important to highlight that in the present study efficacy was evaluated at a single power parameter (potency level 7 - 70% from 1100 watts), and the results could not be generalized to other microwaves with different parameters. Dental practices must take this into account when they will choose power levels and microwave models.

Pacifier nipples, made of silicone or latex, are in permanent contact with saliva and, therefore, oral microflora. For this reason, their surface is a preferential site for the growth of biofilms.[27,28] As biofilm micro-organisms are components of the oral microflora, it may be assumed that they also attach to pacifier nipple material.[15] A few authors have searched for specific microbial species on pacifiers. Pedroso and Siqueira[29] demonstrated that pacifiers were an important source of infection by intestinal parasites. Mattos-Graner et al.[28] found a relationship between the use of pacifier and yeast proliferation in the oral cavity. According to the American Academy of Pediatrics, gastrointestinal infections and oral colonization with Candida albicans are more common among pacifier users. Comina et al.[15] observed 80% of biofilm colonization on pacifier nipples and stated that the pacifiers can be seen as potential reservoirs of pathogens.

In the present study, 75% of Group 3 pacifiers were contaminated with *S mutans*, which is the primary etiologic agent of dental caries. According to Ollila et al.[30], prolonged pacifier-sucking is a possible risk factor for dental caries in children. Moreover, micro-organisms from the environment are able to adhere to pacifiers, as children often drop their pacifiers on the floor and do not take good care of them. This contact with a wide range of microbial species might boost biofilm formation on the surface of pacifier nipples. Therefore, pacifiers can be contaminated by *S mutans* after use and should be disinfected.

We agree with Comina et al.[15] in that a correct disinfection of pacifiers is important to limit contamination. Hospital nurseries give strict instructions for the disinfection and sterilization of feeding bottles, but they do not give similar instructions for pacifiers. Strict rules of hygiene and an efficient antibiofilm cleaning protocol should be established to answer the worries of parents concerning the safety of pacifiers. Regarding this issue, we observed that simply cleaning pacifiers with water (Group 3) was not able to control the microbial contamination.

On the other hand, the use of chlorhexidine solution or microwaves as disinfections methods resulted in 100% elimination of *S mutans*. It was not possible to compare our results to the literature, however, because there are few papers addressing microbial contamination on pacifiers. Similarly, there is no paper related to pacifier disinfection with 0.12% chlorhexidine or microwaving. Further in vitro studies and clinical trials are needed at other research levels to evaluate disinfection methods with different types of micro-organisms.

Conclusions

1. *S mutans* colonies/biofilms were observed in on 100% of pacifiers and toothbrushes (Group 3) that were sprayed with sterile water.

2. When 0.12% chlorhexidine spray and microwave were used colonies/biofilms were absent in 100% of the cases, in all 3 evaluation interval.
3. Chlorhexidine solution spray (0.12%) and 7 minutes of microwave irradiation were effective for disinfection of pacifiers and toothbrushes.

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