Viability of Streptococcus Mutans Toothbrush Bristles

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ABSTRACT

Purpose: Employing microbial culture, the purpose of this study was to assess in vitro the viability of *Streptococcus mutans* on toothbrush bristles relative to the drying time.
Methods: Forty-five toothbrushes were soaked in a suspension containing *S mutans* (ATCC 25175) in a 1,720.000 cfu/mL concentration (0.5 McFarland scale) for 4 minutes, rinsed in sterile tap water, and assigned to 9 groups. Group 1 toothbrushes were immediately incubated in CaSaB CaSaB (bacitracin sucrose broth—selective enrichment broth) culture medium for 4 days. Toothbrushes from groups 2 to 9 were kept at room temperature for 4, 8, 12, 24, 36, 48, 60, and 72 hours, respectively, and subsequently incubated in CaSaB culture medium.
Results: It was observed that micro-organisms were present on toothbrushes of groups 1 to 3, ranging from 50 to 100+ cfu. From the 12-hour drying period on, there was no growth of *S mutans*. Regarding the *S mutans* cfu, the results were expressed in scores and submitted to the Kruskal Wallis statistical test. It was observed that groups 1 to 3 were similar to each other ($P>.05$) and differed significantly ($P<.001$) from other groups, which, in turn, behaved similarly ($P>0.05$). From the 12-hour drying period on, there was a statistically significant decrease in the number of *S mutans* cfu ($P<.01$).
Conclusion: It may be concluded that *Streptococcus mutans* remained viable on the toothbrushes' bristles for up to 8 hours. (J Dent Child 2008;75:29-32) Received January 15, 2007 Last Revision March 27, 2007 | Revision Accepted March 28, 2007.

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Transmission of *Streptococcus mutans* occur through direct contact (saliva) or indirect contact,[1] which include fomites such as spoons,[2] cups, toys, or contaminated toothbrushes.[1,3]

Toothbrushes are manufactured free of micro-organisms.[4,5] After a single use, however, toothbrushes may become contaminated by a wide array of bacteria,[4,5,7-13] viruses,[14,15] yeasts, and fungi,[16,17] present both in the oral cavity and in the external environment.[18] Retention and survival of cariogenic micro-organisms on the toothbrushes' bristles represent a possible cause of recontamination of the mouth.[19] Therefore, the routine use of contaminated toothbrushes may disseminate micro-organisms within a person's oral cavity or between different individuals.[3,20]

The purpose of this in vitro study was to investigate, employing microbial culture, the viability of *Streptococcus mutans* on toothbrushes' bristles relative to the drying time.

METHODS

Forty-five toothbrushes (Johnson Jr, Johnson & Johnson, Sao Paulo, Brazil) were taken from their original packages and soaked in a suspension containing *Streptococcus mutans* (strain ATCC 25175) in a 1,720.000 cfu/mL concentration (0.5 McFarland scale) for 4 minutes. Thereafter, the toothbrushes were rinsed in sterile tap water for 5 seconds by the same operator and assigned to 9 groups (N=5). Immediately after rinsing, the group 1 toothbrushes (control) were submitted to microbiological processing. Toothbrushes

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from groups 2 to 9 were kept at room temperature for 4, 8, 12, 24, 36, 48, 60, and 72 hours, respectively, in a closed custom-made container to avoid contact between them, but allowing air circulation for drying, and were subsequently submitted to microbiological processing.

To investigate whether the toothbrushes presented contamination deriving from manufacturing and packaging processes, 5 unused toothbrushes (additional control) were taken from their original packages and submitted to microbiological processing.

Table. Number of Streptococcus Mutans Colonies/Biofilms of the Toothbrush Bristles After Different Drying Periods*

Toothbrush	Group 1 (control)	Group 2 (4 hs)	Group 3 (8 hs)	Group 4 (12 hs)	Group 5 (24 hs)	Group 6 (36 hs)	Group 7 (48 hs)	Group 8 (60 hs)	Group 9 (72 hs)
1	100+	100+	82	0	0	0	0	0	0
2	100+	100+	88	0	0	0	0	0	0
3	100+	100+	50	0	0	0	0	0	0
4	100+	100+	100+	0	0	0	0	0	0
5	100+	85	0	0	0	0	0	0	0

** 100+=intense bacterial growth, with confluent colonies, not allowing for an accurate counting of the number of colonies/biofilms colonies.*

MICROBIOLOGICAL PROCEDURES

After the drying periods, the toothbrushes of each group were individually and vertically placed into 25x150 mm test tubes containing 10.0 mL CaSa B (bacitracin sucrose broth—selective enrichment broth prepared by the modification of Jensen and Bratthall,[21] medium specific for mutans streptococci without trypan blue for 3 to 4 days at 37°C. Care was taken to avoid contact between the bristles and the test tube walls. The toothbrushes were withdrawn and rinsed in the broth with gentle shaking to remove planktonic microbiota, leaving sessile bacteria adhered as "spike" or "mushroom-like" colonies/biofilms. The toothbrush bristles were carefully analyzed by one experienced examiner from all sides and angles. Sessile colonies/biofilms of *S mutans*, based on colony morphology, were counted under aseptic conditions with a stereomicroscope (Nikon, Tokyo, Japan) under reflected light.

The number of *S mutans* colonies/biofilms on the surface of the bristles was expressed according to a ranked scale, as follows:

1. score 0=no colonies/biofilms were detected, indicating absence of micro-organisms;
2. score 1=1 to 50 colonies/biofilms;
3. score 2=51 to 100 colonies/biofilms;
4. score 3=over 100 colonies/biofilms (intense bacterial growth, with confluent colonies, not allowing an accurate counting of the number of colonies/biofilms).

The examiner was blinded to the group being examined.

The results were submitted to statistical analysis via the Kruskal Wallis nonparametric test at a 5% significance level, using 8.1 GMC statistical software package (available at: "www.forp.usp.br/restauradora/gmc/gmc.html").

RESULTS

As the drying time increased, the number of colonies/biofilms decreased. The table shows that micro-organisms were present on all toothbrushes of group 1 (control) and 2 (4-hour drying) and on 4 out of 5 group 3 toothbrushes (8-hour drying) with numbers ranging from 50 to 100+ (Figures 1 and 2). From the 12-hour drying period on, there was no *S mutans* growth. Groups 1 to 3 were statistically similar to each other ($p>0.05$) and differed significantly ($P<.001$) from the other groups, which, in turn, behaved similarly ($P>.05$). From the 12-hour drying period on, there was a statistically significant decrease in the number of *S mutans* cfu ($P<.01$) regarding groups 1 to 3 ($P<.01$).

There was no bacterial contamination on the 5 unused toothbrushes after incubation at 37°C for 20 days.

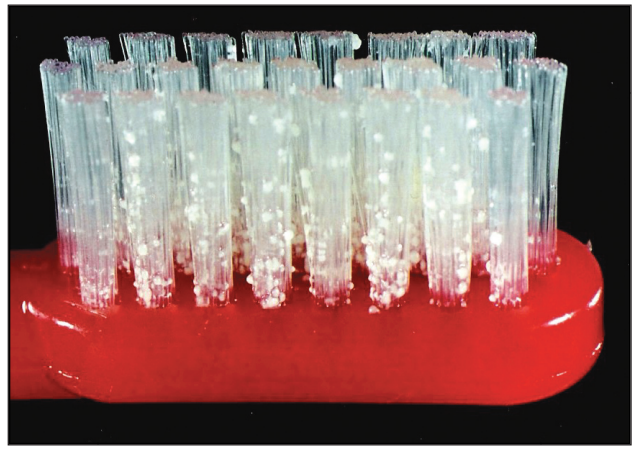

Figure 1. Toothbrush representative of group 1. Presence of a great number of Streptococcus mutans colonies/biofilms on the toothbrush bristles, after microbial culture.

Figure 2. Toothbrush representative of group 3. Presence of a great number of Streptococcus mutans colonies/biofilms on the toothbrush bristles, after microbial culture.

DISCUSSION

Toothbrushes are mainly used to disorganize and remove the bacterial biofilm. When bacteria survive on toothbrush bristles, they may reinoculate the oral cavity of the original host. This can pose a significant risk of dissemination for certain patients, such as immunosuppressed individuals, organ transplant recipients, and patients with cardiopathies in whom transient bacteremia occurred after routine brushing with contaminated toothbrushes which favor the occurrence of bacterial endocarditis.[11,24-26]

Furthermore, toothbrushes belonging to different members of the same family occasionally may be in direct contact when stored in the same toothbrush holder or put together in bathroom drawers or cabinets.[1] In addition, salivary contact is hardly controlled among children staying in daycare centers, kindergartens, and other institutions that shelter children at an early age,[6] where toothbrushes can be inadvertently changed or shared. These facts justify the importance of assessing the viability of micro-organisms in toothbrushes in order to propose methods for their elimination. How long *S mutans* remain viable on toothbrush bristles after use is still a controversial subject.

Kozai et al[4] reported that *S mutans* were present on toothbrushes used by children at high levels (2.55x104 cfus) even 6 hours after use and exposure to the air. Spolidório et al,[22] investigated the viability of *S mutans* on toothbrushes made of opaque and transparent materials by the dilution method and observed that, after 8 hours, the number of micro-organisms decreased to 0. Nelson-Filho et al[13] showed in situ that *S mutans* were present on 93% of toothbrushes used by children 4 hours after exposure to air at room temperature, while Wetzel et al[19] reported contamination of toothbrushes even after an 8-hour drying period. Other studies found that the contamination of toothbrush bristles with *S mutans* decreased with time and that the toothbrushes might be infected by this micro-organism even 24 hours after usage.[3,17]

The present controlled in vitro study indicates that toothbrushes can be heavily infected with *S mutans* for 8 hours. Considering that micro-organisms were detected on toothbrush bristles even after this drying period, it is possible that multiple strains could be replanted in the oral cavity by the wfurther usage of the same toothbrush, which would increase the risk to caries disease, especially in children. This result strongly implies that air-drying of toothbrushes may be an incomplete method for disposing of micro-organisms and highlights the need for toothbrush disinfection after brushing procedures.

Further in vivo studies should be undertaken to determine the viability of different mutans streptococci strains with different levels of pathogenicity on toothbrush bristles, including *Streptococcus sobrinus*, and different types of bacteria, viruses, and fungi under clinical conditions. In conclusion, the results of the present study showed that toothbrushes can retain *S mutans* and ensure their survival for at least 8 hours.

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