Scientific Article

Toothbrush Bristle Wear and Adherence of Streptococcus mutans

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Abstract: ***Purpose:*** *The purpose of this study was conducted to determine if bristle wear impacts the adherence of Streptococcus mutans on toothbrushes and to evaluate whether it affects the extent of adherence at 0, 8, and 24 hours after air-drying.* ***Methods:*** *Sixty toothbrushes—composed of 20 each from 3 different groups and defined by brand, brush trim, and head shape—were used in this study. Bristle wear on half of the toothbrushes was achieved using an orthodontic typodont with metal bands and brackets and evaluated by 4 independent observers. New and worn toothbrushes were inoculated with S mutans, rinsed in tap water, and air-dried for 0, 8, and 24 hours. Four tufts were removed from the brush heads at each time point, placed in saline and vortexed to remove bacteria. Bacteria were aerobically grown on Mitis Salivarius Agar plates until colony-forming units could be counted.* ***Results:*** *The toothbrush group impacts adherence of S mutans on both new and worn toothbrushes at 0, 8, and 24 hours after air-drying, with new toothbrushes harboring significantly more S mutans than worn toothbrushes at 0 hours.* ***Conclusions:*** *The results have implications for the design of toothbrush tufts as well as storage of toothbrushes in the home. (Pediatr Dent 2007;29:243-7)*

KEYWORDS: TOOTHBRUSH BRISTLE WEAR, BACTERIAL ADHERENCE, STREPTOCOCCUS MUTANS

A toothbrush is a mechanical device used to remove dental plaque bacteria and oral debris from teeth. The American Dental Association (ADA) recommends that toothbrushes be replaced after 3 to 4 months of daily use because "worn brushes are not effective at removing plaque bacteria and broken bristles may injure gums."[1] Many people, however, don't follow this advice. They replace their toothbrushes on average between 2½ and 6 months based on the degree of bristle splaying.[2,3]

Toothbrush replacement time is a critical factor in dental hygiene. As toothbrush bristles become worn, the surface area to which microorganisms adhere increases.[4] The most common microorganisms found on toothbrushes are streptococci, lactobacilli, and *Candida albicans*, with the most common caries-causing microorganism being *Streptococcus mutans*.[5-7] While these microorganisms are usually nonthreatening to the body, they do have the ability to cause repeated infections of the mouth and to transmit local and systemic diseases which can pose a significant risk for immunocompromised children and adults.[8-10]

No studies to date have investigated the extent of toothbrush bristle wear over time, nor is there a definitive description of what constitutes a "worn" toothbrush—despite the ADA's toothbrush replacement recommendation.

This study's purpose was to determine if toothbrush bristle wear impacts the adherence of *S mutans* and whether it affects the extent of adherence at 0, 8, and 24 hours after air-drying as measured by the number of recoverable microorganisms.

Methods

Toothbrush bristle wear. Sixty adult toothbrushes were used in this study, comprising 20 each of the following:

1. group A—Crest Triple Effect (Procter and Gamble Company, Cincinnati, Ohio), multilevel bristle trim and oval-shaped head;
2. group, B—Colgate Plus (Colgate-Palmolive Company, Attleboro, Massachusetts), flat bristle trim, and diamond-shaped head; and
3. group C—Oral-B Advantage (Oral-B Laboratories, Iowa City, Iowa), multilevel bristle trim and oval-shaped head (Figure1).

All toothbrush bristles were tufted, soft, and made of nylon. An orthodontic typodont (Rocky Mountain Morita Corporation, Tokyo, Japan) was fitted with metal bands and

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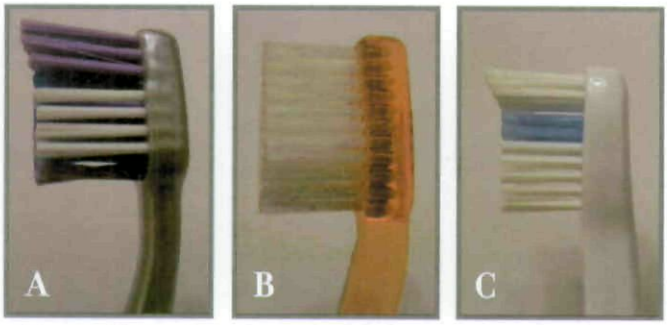

Figure 1. Shows a lateral view of the different toothbrush groups before bristle splaying. Toothbrushes A and C have a multilevel bristle trim, whereas toothbrush B has a flat bristle trim.

brackets, and 4 rubber bands were placed around it to assure constant pressure of the toothbrushes when brushed against the bracketed teeth. To create bristle wear, toothbrushes were placed between the rubber bands and bracketed teeth on the left side of the typodont and 1,500 horizontal forward-and-backward scrubbing motions were made from premolar to molar region. To categorize new and worn toothbrushes, 4 observers were given a written description of a category "3" toothbrush,[4] which required that most tufts overlap and be matted together or that many bristles were bent and curled. Observers also received a photograph of a new and worn toothbrush from each group (Figure 2) and asked to discard any brush with didn't meet the written and verbal criteria.

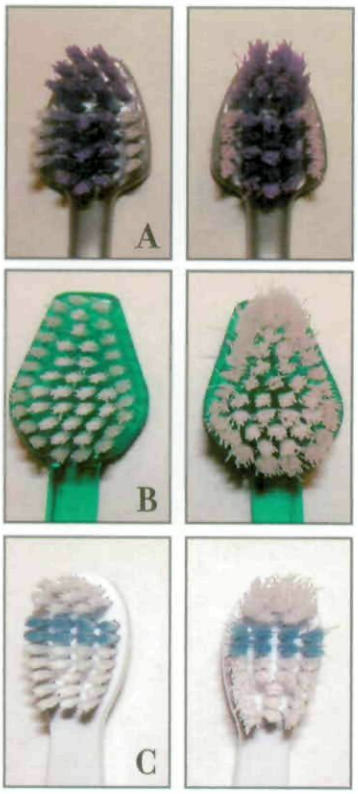

Figure 2. Shows an example of the 3 different toothbrush groups used in this study, with the new toothbrush on the left and a worn toothbrush on the right.

Microbial evaluation. A stock of *S mutans* (ATCC 25175) was used for this study. To ensure its purity and freedom from contamination, 20 mm of the stock solution were pipetted and streaked for singles on Mitis Salivarius Agar plates (Becton, Dickinson and Company, Le Pont de Claix, France). The agar had been prepared as recommended by the manufacturer. The resulting colonies were examined visually to verify that they had the phenotype of *S mutans* and that the culture was free from contamination. Once a pure stock of S mutans was obtained, portion were frozen for future use in the study.

A fresh solution was prepared from frozen stock for each trial. To facilitate the counting of *S mutans* on the agar plates, 1.5 ml of *S mutans* was diluted in 13.5 ml of Phosphate Buffered Saline (PBS), then 100 mm of the diluted sample and 100 mm of Brain Heart Infusion (BHI) media were pipetted into 2 separate wells of a 96-well microplate. An optical reading at 620 nm was used to measure the amount of *S mutans* in the sample compared to the BHI control. To confirm the optical count, 100 mm of *S mutans* was serially diluted from 10^{-1} to 10^{-5}, plated on Mitis Salivarius Agar and grown aerobically in a humidity chamber at 37°C for 2 days until colony-forming units (CFUs) were large enough to be counted.

Ten toothbrushes from each group, 5 new and 5 worn, were inoculated with bacteria by dipping them into a test tube containing *S mutans*, then rinsed for 5 seconds by dipping them into a beaker of nonsterile tap water. Four tufts were plucked at random from each brush head using a sterile hemostat. The hemostat was placed at the interface between the end of the tuft and its insertion point into the brush head, and a twisting hand motion was used to remove the bristles. The tufts were placed in 3 ml of PBS, vortexed for 30 seconds to agitate the bacteria plated on 10^{-1} to 10^{-3} agar plates, and grown aerobically in a humidity chamber at 37°C for 2 days—at which time the CFUs of *S mutans* were counted.

The first group of toothbrushes was then placed vertically in a test tube rack and allowed to air-dry at room temperature for 8 and 24 hours to simulate the normal storage of toothbrushes in the home after brushing. At 8 and 24 hours, 4 more random tufts were removed from the same toothbrushes and the experiment was repeated as above. The same procedure was followed for each of the other 2 toothbrush groups. When all toothbrush groups were tested, the experiment was replicated with an additional 30 toothbrushes—10 from each group. Therefore, statistical analysis was based on 60 toothbrushes.

Statistical analysis.

The mean and standard deviation were calculated for toothbrush bristle splaying on new and worn toothbrushes for each group. The results were displayed on bar graphs. Independent t tests were used to compare bristle splaying between new and worn toothbrushes for the 3 toothbrush groups. Significance for the statistical test was predetermined at $P<.05$.

Means and standard deviations were also calculated for adherence of *S mutans* to new and worn toothbrushes for each group at 3 different time points. The dependent variable was bacterial adherence to toothbrush bristles, and the independent variables were: (1) time; (2) toothbrush group; and (3) toothbrush splaying. The results were displayed on bar graphs. Bacterial data were transformed to log10 to in-

sure a normal distribution. The Stat View program version 1.0 (Abascus Concepts, Berkeley, Calif.) was used to input data. The super analysis of variance (1-factor ANOVA) program was used to test if worn toothbrush bristles harbor more *S mutans* than new toothbrush bristles immediately after contamination with bacteria, at 8 hours and 24 hours after air-drying, as measured by the number of recoverable microorganisms. When significant differences were present, Scheffe's post hoc test was used to determine which means were significantly different from each other.

The sample size of 30 toothbrushes, 5 new and 5 worn per group, was required for at least 80% power to detect a difference of a half log between adherence of *S mutans* on new and worn toothbrushes. A 2-sided α level of .05 was chosen as well as a standard deviation of 0.5.

Results

Toothbrush bristle splaying. A dissecting microscope (Olympus, Center Valley, Penn) was used to look at the tips of the new and worn toothbrush bristles. Resulting photographs (Figure 3) showed that the new bristles (a, b) within a tuft were tightly packed together, while the worn bristles (c, d) were splayed.

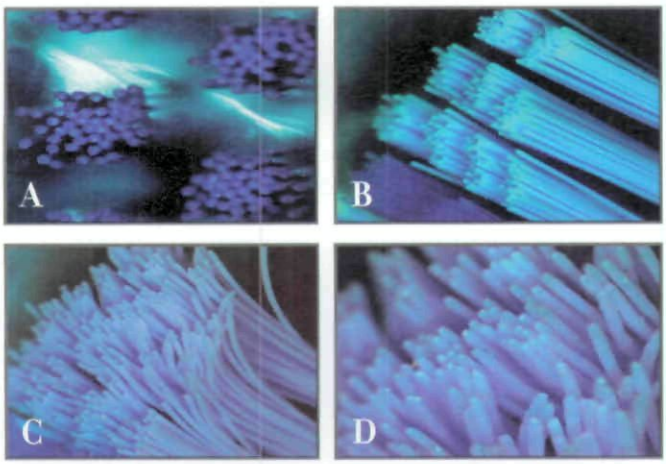

Figure 3. Shows a comparison of new and worn toothbrush bristles using a dissecting microscope at X20 (a, b, c) and X30 (d) magnifications. New toothbrush bristles (a, b) are tightly packed, whereas the worn toothbrush bristles (c, d) are splayed.

Adherence of *S mutans* at different time points by toothbrush group. At 0 hours, the results of the ANOVA (F=8.2; DF=2) comparing the 3 toothbrush groups indicated that there were significant differences ($P<.001$). The results of the Scheffe's post hoc test indicated that the bacterial adherence for group C toothbrushes (mean=4.884±0.293 log CFU/ml) were significantly less than those for groups A and B (5.474±0.338 log CFU/ml and 5.334±0.680 log CFU/ml, respectively). At 8 hours, the results of the ANOVA (F=28.2; DF=2) also indicated that there were significant differences ($P<.001$). The results of the Scheffe's post hoc test indicated that the mean bacterial adherence for groups A, B, and C (4.277±0.658 log CFU/ml, 3.171±1.344 Dg CFU/ml, and 2.055±0.622 log CFU/ml, respectively) were significantly different from each other. At 24 hours, the results of the analysis of variance (F=15.46; DF=2) indicated that there were significant differences at this time interval ($P<.001$). The results of the Scheffe's post hoc test indicated that the mean bacterial adherence for group A toothbrushes (0.156±0.510 log CFU/ml) were significantly less than the adherence found for groups B and C (2.269±1.874 log CFU/ml and 1.543±0.839 log CFU/ml, respectively; Figure 4).

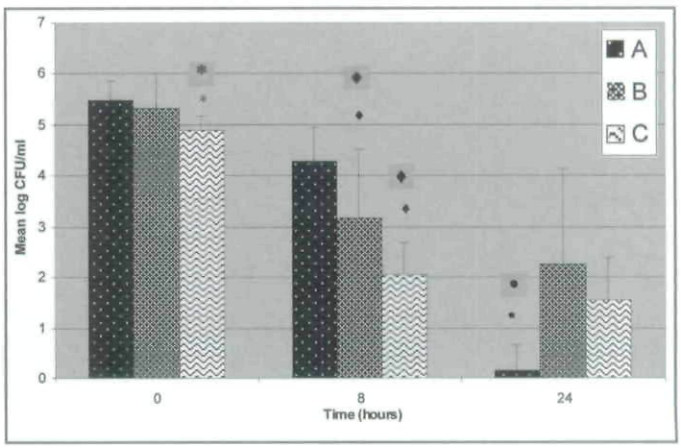

Figure 4. Adherence of *Streptococcus mutans* at different time points by toothbrush group. *$P<.001$ (ANOVA F=8.2; DF=2); ♦$P<.001$ (ANOVA F=28.2; DF=2); •$P<.001$ (ANOVA F=15.46; DF=2). Bacterial adherence was calculated using a visual count of colonies on the agar plates.

Adherence of *S mutans* at different time points by toothbrush splaying. At 0 hours, the results of the t test comparison (t=4.21; DF=1) indicated that *S mutans* adherence to new toothbrushes (mean log CFU/ml=5.370±0.452) was significantly (P=.04) more than *S mutans* adherence to worn toothbrushes (mean log CFU/ml=5.092±0.607; Figure 5). At 8 hours, the results of the t test comparison (t=1.44; DF=1) indicated that *S mutans* adherence to new toothbrushes (mean log CFU/ml=3.368±1.074) was not significantly (P=.24) different than S mutans adherence to worn toothbrushes (mean log CFU/ml=2.968±1.478). Similarly, at 24 hours, the results of the t test comparison (t=2.13; DF=1) indicated that *S mutans* adherence to new toothbrushes (mean log CFU/ml=1.601±1.462) was not significantly (P=.15) different than *S mutans* adherence to worn toothbrushes (mean log CFU/ml=1.044±1.491).

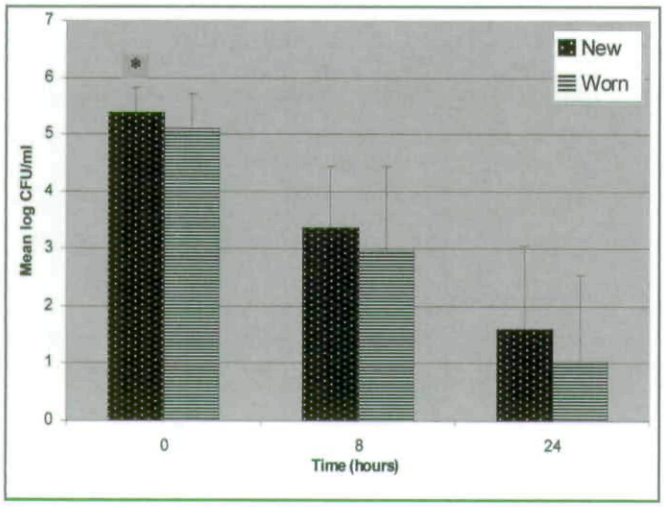

Figure 5. Adherence of *Streptococcus mutans* at different time points by toothbrush splaying. *0 hours–P=.04 (t=4.21; DF=1); 8 hours–t=.24 (t=1.44; DF=1); 24 hours–P=.15 (t=2.13; DF=1). Bacterial adherence was calculated using a visual count of colonies on the agar plates.

Discussion

It is generally believed that daily tooth-brushing causes toothbrush bristles to wear out over time and lose their effectiveness at removing oral microorganisms.[1] Based on a review of the literature, this study was the first to examine adherence of the most common caries-causing microorganism, *S mutans*, to new and worn toothbrushes at 0, 8, and 24 hours of air-drying.

It was hypothesized that toothbrush group, as defined by company brand, brush head trim, and shape, would affect adherence of *S mutans* to new and worn toothbrushes—as measured by the number of recoverable microorganisms. It was found that all 3 toothbrush groups harbored approximately 10^5 CFU at 0 hours, with group C toothbrushes having significantly less adherence than those in groups A and B. At 8 hours, however, adherence of *S mutans* ranged from 10^2 to 10^4 CFU, with group C toothbrushes again having significantly less adherence of *S mutans*. In contrast, at 24 hours, adherence of *S mutans* ranged from almost 0 for group A to 10^2 CFU for groups B and C. Possible explanations for these varying amounts of bacterial adherence may be attributable to differences in brush head shape, brush head surface area, bristle tufting, different bristle lengths, and differing numbers of bristles per tuft.

It was also hypothesized that worn toothbrush bristles would harbor more *S mutans* than new toothbrush bristles after 0, 8, and 24 hours of air-drying—as measured by the number of recoverable microorganisms. These time points were chosen to facilitate the conduct of the study when the laboratory was available. It would have simulated life more closely if 0, 12, and 24 hours were chosen because most people tend to brush their teeth in the morning and then 12 hours later when they go to bed. Contrary to what was expected, new toothbrushes tended to harbor more *S mutans* than worn toothbrushes at 0, 8, and 24 hours after air-drying, with a significant difference seen only at 0 hours. One explanation may be that new bristles tend to be more closely packed than worn bristles, causing bacteria brought into contact with them to stick more easily than on bristles which have splayed and are further apart. Toothbrush manufacturers may want to consider placing bristles within a tuft further apart so that bacteria like *S mutans* do not get trapped, and the ADA might consider guidelines that instruct the general public about toothbrush storage to reduce the transmissibility of microorganisms from toothbrush to toothbrush.

The oral cavity is a complex structure containing teeth constantly bathed in a fluid composed of saliva, bacteria, and other oral debris. The use of a one-time application of a liquid bacterial culture of *S mutans* to measure adherence does not replicate the oral environment in the mouth. In performing this in vitro study, toothbrushes were only inoculated once and then measured over time. In real life, however, people brush their teeth in the morning, at night before bed, and again when they awaken the next day. By doing so, each time they brush their teeth they reinoculate their toothbrushes with their own oral bacteria. Further study in this area should address repeated inoculations of toothbrushes with *S mutans* over time. An in vitro animal study using antibiotically labeled *S mutans* could also be conducted. Specific sites on the bristles where bacteria adhere and colonize over time could be examined. To more closely resemble the oral environment, researchers could investigate adherence of microorganisms in the presence of saliva and toothpaste to see if the antimicrobial properties alter microbial adherence.

This study's results suggest that the toothbrush group, as determined by brand, brush head trim, and shape, impacts adherence of *S mutans* to both new and worn toothbrushes. Furthermore, new toothbrushes harbor more *S mutans* than worn toothbrushes at 0, 8, and 24 hours, with significance seen only at 0 hours. These results are limited, however, because they do not take into account many other factors that go into brushing teeth and the conditions of the oral environment. Further studies need to be done with saliva, toothpaste, and other microorganisms to provide the American Dental Association with better guidelines for toothbrush replacement time.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Further research is required to understand the reasons behind the differences seen in adherence of *Streptococcus mutans* at different time points, with saliva and toothpaste possibly playing a critical role.

2. The results have implications for the design of tooth brush tufts by the manufacturer as well as the storage of toothbrushes in the home.

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Abstract of Science of Literature

Juvenile recurrent parotitis (JRP)

JRP is characterized by recurrent uni- or bilateral swelling of the parotid gland accompanied with pain, fever, and redness of the overlying skin. The etiology and pathogenesis of JRP is unknown. It has been associated with congenital malformation of the ductal system, hereditary and genetic factors, bacterial infection, autoimmune disease and a number of immunodeficiency conditions. This paper retrospectively examined the records of all patients (n=26) under 16 years of age who were diagnosed with JRP over a 3-year period. The authors found that there were slightly more males affected than females, the mean number of episodes per year per patient was 7.2, and the most commonly reported ages of onset were 6 and 10 years. It was rare for both the left and right gland to be affected simultaneously, although 31% of children reported that both glands had been affected at different times. Although the paper did not find a definitive causal diagnosis for all the patients with JRP, sialography and ultrasound commonly showed abnormalities with sialectasis and multiple heterogeneous echos, respectively. A number of patients were found to be anemic or leucocytotic.

Comments: *This study reinforces the idea that JRP is relatively common in our child patients and indicates that no single causal agent has been identified for all presenting cases. This paper also provides an interesting review of the literature and supports the use of conservative treatment.* ***EKM***

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