International Journal of Dental Hygiene

Official Journal of the International Federation of Dental Hygienists

ORIGINAL ARTICLE

LA Turner
GB McCombs
WL Hynes
SL Tolle

A novel approach to controlling bacterial contamination on toothbrushes: chlorhexidine coating

Authors' affiliations:
Lisa A. Turner, *Gayle B. McCombs*, *S. Lynn Tolle*, School of Dental Hygiene, Old Dominion University, Norfolk, VA, USA
Wayne L. Hynes, Department of Biological Sciences, Old Dominion University, Norfolk, VA, USA

Correspondence to:
Lisa A. Turner
School of Dental Hygiene
Old Dominion University
Norfolk
VA, USA
Tel.: +1 757 735 0159
Fax: +1 757 735 0159
E-mail: 5turners@verizon.net

Dates:
Accepted 11 November 2008

To cite this article:
Int J Dent Hygiene **7**, 2009; 241–245
DOI: 10.1111/j.1601-5037.2008.00352.x
Turner LA, McCombs GB, Hynes WL, Tolle SL. A novel approach to controlling bacterial contamination on toothbrushes: chlorhexidine coating.

Abstract: *Purpose:* This project was conducted to determine the effectiveness of chlorhexidine-coated toothbrush filaments in reducing quantities of bacteria. *Materials and methods:* An Institutional Review Board (IRB)-approved, two-group, double-blind, randomized, post-test only study was conducted. Sixty-four individuals utilized control and experimental toothbrushes, for 30 days. At the end of the study toothbrushes were returned and transported to the laboratory for analysis. Microorganisms were detached from the filaments by sonification and vortexing then plated on Mitis Salivarius (MS) (selective) and trypticase soy agar (TSA) 5% Sheep Blood (non-selective) media. Inoculated plates were incubated aerobically for 24 h at 37°C. After incubation, bacterial colony-forming units (CFU) were determined. Data were analysed using Wilcoxon and Kruskal–Wallis tests. *Results:* Fifty-nine toothbrushes were returned for analysis; experimental (n = 31) and control (n = 28). Data from TSA media revealed a mean CFU for the control group of 5.41×10^5 compared with 6.28×10^5 for the experimental group. Data from MS agar resulted in a mean CFU for the control group of 4.32×10^5 compared with 4.20×10^5 for the experimental group. *Conclusion:* Results revealed no statistically significant difference in the quantity of bacteria surviving on toothbrush filaments between control and experimental groups, on both selective and non-selective media, after 30 days.

Key words: bacteria; chlorhexidine; toothbrush

Introduction

The paradigm shift from restorative-based dentistry to a model of prevention has generated research interests in the treatment

and prevention of oral disease through the use of adjunctive antimicrobial therapeutics. Research has substantiated the effectiveness of chemotherapeutic agents such as chlorhexidine, povidone iodine, stannous fluoride and topical antibiotics on the destruction of pathogenic oral bacteria (1–7). Incorporating antimicrobial agents into oral care products such as rinses and dentifrices may be a means to reduce the risk of infection. The purpose of the present study was to examine the effectiveness of chlorhexidine coated toothbrush filaments in reducing the quantity of bacteria remaining on toothbrush filaments after 30 days of use.

Toothbrushes have the potential to serve as a reservoir for oral microbial flora (8), including pathogenic organisms, which creates a nidus for the spread of disease (1, 7, 9, 10). Variations in toothbrush design, care, and storage, as well as environmental factors such as splashing and aerosol droplets from both toilets and sinks creates a potential for cross-contamination to uncovered toothbrushes (11). On the other hand, covering a toothbrush during storage has been found to prolong drying time thus extending the proliferation of organisms (4, 12).

Researchers have identified specific bacteria found on contaminated toothbrushes, their lifespan, and the ease with which they are spread to other brushes and objects such as the orifice of the toothpaste tube (1, 13, 14). Oral bacteria such as *Streptococcus mutans* and other opportunistic microorganisms, coupled with variation in toothbrush design, can create an environment where bacteria can proliferate, contaminate other objects and spread to humans.

Numerous studies explored the susceptibility of certain oral bacteria such as *S. Mutans* to chlorhexidine (3, 6, 15–17) with varying levels of success. *S. mutans*, a caries-causing organism, is cited frequently as a key pathogen spread by toothbrush contamination (1, 18), and has been recovered from contaminated filaments even after 6 h drying time (19). The spread of *S. mutans*, as well as other pathogenic oral bacteria such as *Lactobacilli*, *Streptococcus sobrinis* and periodontal pathogens creates a potential health risk especially in those individuals whose health is already compromised. After studying toothbrush rinsing habits and residual bacteria on filaments, Kozai *et al.*, concluded that although rinsing and drying times affected bacterial quantity, most individuals did not rinse long enough to sufficiently remove bacteria from toothbrushes. Therefore, results of this study suggested a supplemental method of disinfection, such as immersion in an antimicrobial rinse.

In an effort to control bacterial proliferation on toothbrushes researchers have been challenged to develop a cost-effective, convenient method of reducing this threat. Over the years, numerous methods of toothbrush sanitization have been put forward, such as exposure to ultraviolet light and microwaves, disinfectant tablets, and immersion in solutions such as Clorox and antimicrobial agents, all with varying levels of effectiveness (1, 5). Therefore, the present study tested the effectiveness of chlorhexidine-coated toothbrush filaments in controlling bacterial quantity.

Studies have shown that contaminated toothbrushes not only harbour, but also transmit, both viruses and bacteria that can cause localized, systemic and oral infections (1, 11). The American Dental Association (ADA) position statement on toothbrush care supports the theory that bacteria proliferate on toothbrushes occurs. Furthermore, the ADA recommends that family toothbrushes be kept separately in an effort to prevent cross-contamination, as many oral and environmental microorganisms establish themselves on brushes. Similarly, the Centers for Disease Control (CDC) published a fact sheet on the use and handling of toothbrushes (20). This information suggests that people who are immunosuppressed 'may need to seek alternative means of oral hygiene', because even after rinsing toothbrushes thoroughly with tap water, they 'can remain contaminated with potentially pathogenic organisms.' Consequently, the CDC suggests that the likelihood of toothbrush cross-contamination in group and school settings is particularly high, perhaps because of lack of proper handling and storage.

The bathroom is considered by many to be the most contaminated room in the house. Toothbrushes that are placed in close proximity to the toilet and/or sink, shared or stored in a cluster may become a breeding ground for pathogenic microorganisms. Researchers postulate that contaminated toothbrushes have the potential to compromise the health of immunosuppressed individuals, re-infect people with chronic periodontal disease, and may also re-introduce bacteria into the mouths of healthy individuals. Researchers have proposed links between lingering colds, sore throats and the flu and contaminated toothbrushes. Considering the evidence that suggests oral bacteria may play a role in heart attacks, diabetes and premature births, it is prudent to consider ways to reduce or prevent organisms from establishing and proliferating on toothbrushes.

Study population and methodology

The experimental toothbrush evaluated in this study was designed to reduce the bacterial load on toothbrush filaments, thereby minimizing the opportunity for the spread of pathogenic microorganisms. According to the manufacturer, the amount of chlorhexidine released into the oral cavity while

brushing remains limited, thus providing no systemic effect (21). The total aerobic bacterial count and the number of oral streptococci present were evaluated in order to determine whether the chlorhexidine-coated toothbrush filaments were effective at decreasing oral and other aerobic bacteria.

An IRB-approved, two-group, double-blind, randomized design was utilized. The experimental group used toothbrushes with chlorhexidine-coated nylon filaments, whereas the control group used identical brushes, provided by the manufacturer, without chlorhexidine coated filaments (21).

Toothbrushes with chlorhexidine-coated filaments served as the experimental variable. The nylon filaments on the brushes were coated and dried with a bactericidal coating during manufacturing. The coating consisted of a polymer with cation exchange capacity and a cationic bactericide, one part chlorhexidine to 100 parts polymer by weight. The coating ranged from 0.1 to 10.0 μ thick. The insolubility of this coating in water accounts for the 30-day length of antibacterial ability suggested (21).

Participants were recruited via flyers posted throughout the university and through campus-wide email announcements sent to faculty, students and staff. At screening, an oral examination was conducted to determine eligibility. Individuals were excluded if they: (i) used any antibiotic or antiseptic mouthrinse in the last 30 days, (ii) were pregnant, (iii) presented with severe periodontal disease or caries, (iv) had a systemic disease such as diabetes or (v) were immunocompromised. A convenience sample of 64 adults (48 females and 16 males) were enrolled. Five female participants withdrew from the study for various reasons (four control and one experimental) resulting in a final sample size of 59.

At baseline, participants were randomly assigned to one of two equal groups, the experimental (n = 32) or the control (n = 32). Both groups were provided with a study toothbrush, a ventilated toothbrush cover designed for the study brushes, and Crest Regular Mint dentifrice (Procter & Gamble, Cincinnati, OH, USA). Individuals were supplied with the same dentifrice to control for ingredients that may have affected the bacterial growth on the brushes (22). Each toothbrush was colour-coded for identification by the participants and by researchers. Toothbrushes were not sterilized prior to study initiation; however, brushes remained in the original unopened package until distributed to participants. Individuals were provided with both written and verbal instructions and were advised to follow their normal oral hygiene procedures. Participants were advised to only use the toothbrush and toothpaste provided and to refrain from using other products such as mouthrinses and antigingivitis toothpaste, which could affect the survival rate of bacteria. Individuals were instructed to rinse their toothbrush with tap water for 30 s and to cover the brush with the ventilated cap provided when not in use. Participants conducted their unsupervised portion of the trial at home.

Although research suggests that covering toothbrushes extends drying time and encourages proliferation of bacteria, the covers were used in this study to protect the toothbrushes from environmental bacterial contamination, in addition to control for cross-contamination from other toothbrushes stored in close proximity (4, 9, 12).

Laboratory methods

Prior to the start of the study, a pilot test was conducted to validate laboratory methods and determine appropriate dilutions. Serial dilutions were plated on trypticase soy agar (TSA) and Mitis Salivarius (MS) agar and incubated for 24 h at 37°C. Plates which yielded colonies of approximately 30–300 colony-forming units (CFU) were deemed to be accurately quantifiable.

On the last day of the study, participants used their toothbrush in the morning prior to delivering them to the researchers in sealed packages. Samples were transported to the laboratory for analysis within 4 h. Fifty-nine of the 64 toothbrushes distributed at the inception of the trial were collected and analysed (28 control and 31 experimental).

In the laboratory, each toothbrush was placed in a vial, submerged in sterile saline, capped, vortexed for 30 s, and then sonicated in an ultrasonic bath. Dilutions were plated onto TSA and MS agar. Samples were then incubated in aerobic conditions at 37°C for 24 h.

Mitis Salivarius agar was chosen because this selective medium isolates streptococci, and to prevent these microorganisms from being inhibited by other fastidious organisms (23). TSA 5% sheep blood, which has also been shown to grow streptococci, was selected as the non-selective medium to enumerate total aerobic bacteria. After incubation, CFU were counted by means of visual inspection under magnification by the primary researcher and one research assistant.

Plates which contained between 30 and 300 colonies easily identifiable colonies, were selected for analysis. If plates grew colonies too numerous to count, the result was recorded as such and was included in the analysis in rank order representing the highest ranked value. Results reported reflect the samples analysed from dilution 10^{-3} (n = 78), which represented the majority of the samples quantified (TSA = 42 and MS = 36) (Table 1).

Table 1. **Total samples analysed for TSA (sheep blood) and Mitis Salivarius**

Media	Control	Treatment	Samples analysed
TSA blood agar	19	23	42 plates
MS agar	13	23	36 plates
Total	32	46	78 plates

TSA, trypticase soy agar; MS, Mitis Salivarius.

Results

A total of 59 toothbrushes (28 control and 31 experimental) were returned and analysed. Nineteen toothbrushes were included from the control group and 23 from the experimental group, yielding a total of 42 TSA plates. Results expressed in Fig. 1 indicate a larger number of bacteria found on the experimental toothbrushes. However, data analysis using both Kruskal–Wallis and Wilcoxon tests revealed no statistically significant difference in the total quantity of bacteria present on the experimental toothbrushes when compared with the control brushes after 30 days use.

Thirteen toothbrushes from the control group and 23 from the experimental group yielded a total of 36 MS agar plates. Results revealed in Fig. 1 indicate slightly more bacteria found on the control toothbrushes. Data revealed no statistically significant difference in the quantity of oral streptococci present on the experimental toothbrushes when compared with the control brushes after 30 days use.

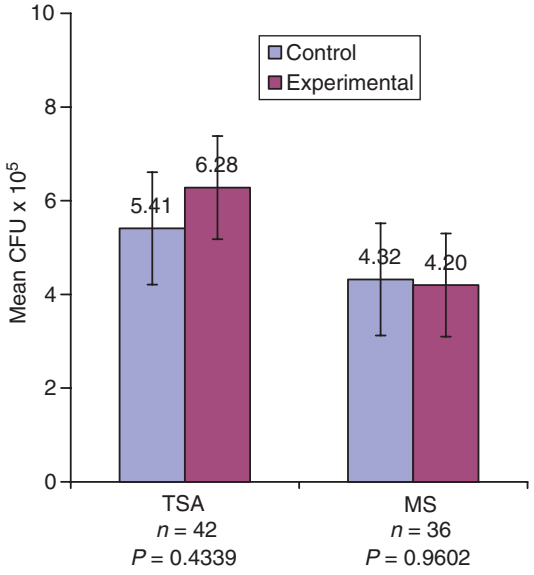

Fig. 1. Comparison of mean CFU values for TSA and MS agar using Kruskal–Wallis test ($P = 0.05$).

Discussion

Toothbrush sanitization is by no means a new idea and the premise has been researched for many years. The debate lingers concerning the necessity and effectiveness of various toothbrush disinfection methods, suggesting that there is more research that needs to be accomplished. Manufacturers continue to develop new products and introduce toothbrush sanitizers and disinfection methods into the marketplace, leaving dental professionals and consumers to ponder their merits. The present study was designed to test the effectiveness of a toothbrush with chlorhexidine-coated filaments, aimed at controlling bacterial growth.

The intent of this novel product design is to reduce the pathogenic bacterial load present on the toothbrush filaments, thereby decreasing the possibility of reinfection and cross-contamination. The manufacturer of the experimental toothbrush suggests that the effectiveness of the chlorhexidine-coated filaments extends over a 30-day period, after which time the toothbrush should be replaced. Researchers postulate that the effects of the antimicrobial filaments occur at higher levels at the inception of its use, and diminish over time. Therefore, the experimental toothbrush may be more effective at preventing bacteria from multiplying on the filaments, at day 7 or 14. Laboratory results from the present study indicate no statistically significant difference between control and experimental brushes after 30 days use. However, additional research is needed to substantiate effectiveness of the chlorhexidine coating at various time points.

Current literature supports the theory that bacterial contamination of toothbrushes is certain (1, 5, 9, 19, 24), and that a means to disinfect brushes is recommended to prevent cross-contamination, as well as growth and translocation of bacteria (5, 19). Likewise, the susceptibility of oral bacteria to chlorhexidine is well documented (3, 6, 15, 16). The amount of chlorhexidine incorporated into the filaments of the experimental toothbrush provides antibacterial activity only on the filaments and offers no systemic effect. The amount of chlorhexidine released into the oral environment, as a result of using the antimicrobial toothbrush, does not impact pathogenic oral microorganisms; therefore, the concept of antimicrobial filaments presents a plausible premise.

In this study, researchers kept the protocol as close to 'real-world' conditions as possible. During previous laboratory trials conducted, the activity of the coating was substantiated during small-scale trials with and without the use of toothpaste (21). During this study, a larger sample was enrolled (64 participants), and participants were provided with a widely used,

over-the-counter, mint flavoured fluoride toothpaste. No added ingredients with antimicrobial activity were present. The toothpaste contained sodium lauryl sulphate (SLS) (approximately 1%), a common inactive ingredient found in toothpaste, which acts to disperse the ingredients in the toothpaste. Previous unrelated studies have demonstrated an interaction between SLS and chlorhexidine. As an anionic compound, SLS has the ability to inactivate the positively charged chlorhexidine for a limited time period. Whether an interaction occurred between SLS and the chlorhexidine-coated toothbrush filaments remains to be tested. Future studies may consider using an SLS-free toothpaste in order to achieve the full activity of the chlorhexidine coating.

Further contemplation should be given to vulnerable people like those who are immunosuppressed or who reside in group environments. A toothbrush harbouring fewer bacteria may prove beneficial to populations at risk for contracting illnesses and could decrease the possibility of bacteremias associated with using a contaminated toothbrush (25–27). In addition to reducing the quantity of *S. mutans* surviving on the toothbrush, the antimicrobial activity of the filaments may also decrease the overall number of other bacteria present.

Acknowledgements

Sunstar Americas Inc., Chicago, Illinois, provided funding and materials to aide in the success of this study. Special acknowledgement goes to research assistant, Tonesia Smith and to Dr Dayanand Naik and Jayesh Srivastava for conducting statistical analysis.

References

1 Caudry SD, Klitornos A, Chan ECS. Contaminated toothbrushes and their disinfection. *J Can Dent Assoc* 1995; **61:** 511–516.
2 DenBesten P, Berkowitz R. Early childhood caries: an overview with reference to our experience in children. *J Calif Dent Assoc* 2003; **31:** 139–143.
3 Emilson CG. Susceptibility of various microorganisms to chlorhexidine. *J Dent Res* 1977; **85:** 255–265.
4 Mehta A, Sequeira PS, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. *N Y State Dent J* 2007; **4:** 20–22.
5 Meier S, Collier C, Scaletta MG, Stephens J, Kimbrrough R, Kettering JD. An *in vitro* investigation of the efficacy of CPC for use in toothbrush decontamination. *J Dent Hyg* 1996; **70:** 161–165.
6 Sekino S, Ramberg P, Uzel NG, Socransky S, Lindhe J. Effect of various chlorhexidine on salivary bacteria and de novo plaque formation. *J Clin Periodontol* 2003; **30:** 919–925.
7 Suido H, Offenbacher S, Arnold R. A clinical study of bacterial contamination of chlorhexidine-coated filaments of an interdental brush. *J Clin Dent* 1998; **9:** 105–109.
8 Taji SS, Rogers AH. The microbial contamination of toothbrushes. A pilot study. *Aust Dent J* 1998; **43:** 128–130.
9 Kettering JD, Garcia M, Tan-Habaradas D, Thomas CJ. Toothbrush storage and potential transfer of opportunistic microorganisms. *J Dent Hyg* 2001; **75:** 336.
10 Quirynen M, DeSoete M, Pauwels M, Gizani S, Van Meerbeek B, Van Steenberghe D. Can toothpaste or a toothbrush with antibacterial tufts prevent toothbrush contamination? *J Periodontol* 2003; **74:** 312–322.
11 Kagan LJ, Aiello AE, Larson E. The role of the home environment in the transmission of infectious diseases. *J Community Health* 2002; **27:** 247–267.
12 Borso H, Crump R, Schelling M. The effect of toothbrush covers on bacterial retention. *J Dent Hyg* 2004; **78:** 4.
13 Abraham N, Cirincione U, Glass R. Dentists' and Dental Hygienists' attitudes toward toothbrush replacement and maintenance. *Clin Prev Dent* 1990; **12:** 28–32.
14 Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. *Scand J Dent Res* 1978; **86:** 412–414.
15 Takeuchi H, Senpuku H, Matin K *et al.* New dental drug delivery system for removing streptococci from the oral cavity: effect on oral microbial flora. *Jpn J Infect Disinfection* 2000; **53:** 211–212.
16 Yang Y, Sreenivasan PK. An ex-vovi multiplexed antibacterial test on oral microflora. *Oral Microbiol Immunol* 2005; **20:** 180–185.
17 Featherstone JDB. The science and practice of caries prevention. *J Am Dent Assoc* 2000; **131:** 887–899.
18 Palomari-Spolidorio DM, Goto E. Viability of *Streptococcus mutans* on transparent and opaque toothbrushes. *J Dent Hyg* 2003; **77:** 114.
19 Kozai K, Iwai T, Miura K. Residual contamination of toothbrushes by microorganisms. *J Dent Child* 1989; **56:** 201–204.
20 *Center for Disease Control, Factsheet*. http://www.cdc.gov/oralhealth/infectioncontrol/factsheets/toothbrushes.htm (accessed 19 August 2008).
21 Iwai T, Ebisudani K, Yokosuka N. *United States Patent Number: 5,633,083*. Assignee: Sunstar Kabushiki, Toray Monofilament Co. Application no. 27668.
22 Warren DP, Goldschmidt MC, Thompson MB, Adler-Storthz K, Keene HJ. The effect of toothpastes on the residual microbial contamination of toothbrushes. *J Am Dent Assoc* 2001; **132:** 1241–1245.
23 Verran J, Leahy-Gilmartin A. Investigations into the microbial contamination of toothbrushes. *Microbios* 1996; **85:** 231–238.
24 Glass RT. Toothbrush types and retention of microorganisms: how to choose a biologically sound toothbrush. *Oral Pathol* 1992; **82:** 26–28.
25 Kennedy HF, Morrison D, Tomlinson D, Gibson BES, Bagg J, Gemmell CG. *Gingivitis and Toothbrushes: Potential Roles in Viridians Streptococcal Bacteremia*. Vol 46, issue 1, pp 67–70. January 2003, The British Infection Society: Elsevier Science Ltd.
26 Martin M. Is there a link between tooth brushing and infective endocarditis? *Int Dent J* 2003; **53:** 187–190.
27 Silver JG, Martin AW, McBride BC. Experimental transient bacteremias in human participants with varying degrees of plaque accumulation and gingival inflammation. *J Clin Periodontol* 1977; **4:** 92–99.