Kıl demeti dizaynı ve diş macununun diş fırçalarındaki mikrobiyal kontaminasyona etkisi

The effects of the tuft design and toothpaste on the residual microbial contamination of toothbrushes

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ÖZET

Amaç: Diş fırçaları ilk kullanımlarından itibaren ağız ve çevre yüzeylerde bulunan mikroorganizmalarca kontamine olurlar. Fırça kıllarının dizaynı ve diş macunu kullanımı bakterilerin kıllara tutunmasını etkileyebilir. Çalışmamızda; kıl demeti dizaynının ve diş macununun diş fırçalarındaki çürük yapıcı mikroorganizma kontaminasyonuna etkisinin belirlenmesi amaçlanmıştır.

Yöntem: Bu çalışmada; tükürük *mutans streptokok* düzeyi >10^6 cfu/mL olan toplam 11 diş hekimliği öğrencisi dişlerini bir hafta aralıkla ve birer kez kılları farklı tasarlanmış iki ayrı fırça ile fırçalamışlardır. Deney triklosanlı bir diş macunu kullanılarak tekrar edilmiştir. Fırçalar kullanıldıktan sonra 10 saniye musluk suyu ile yıkanmış ve her fırçadan dörder kıl demeti kopartılarak, kantitatif kültür teknikleri ile total bakteri, *mutans* streptokokları, laktobasiller ve maya yönünden incelenmiştir. İstatistiksel değerlendirme Wilcoxon testi ile yapılmıştır.

Bulgular: Her iki fırça tipinde de streptokoklar; *mutans*, *laktobasil*, *maya* ve toplam bakteri tutuculuğu açısından istatistiksel olarak anlamlı bir fark bulunmamıştır. Macun kullanımı ile fırça

ABSTRACT

Objective: Toothbrushes may become contaminated after a single use by a wide array of microorganisms which are present both in the oral cavity and in the external environment. The retention and survival of microorganisms may depend on brush design and toothpaste usage. We aimed to investigate the effect of the brush tuft design and the use of toothpaste on the cariogenic microbial contamination of toothbrushes.

Method: Totally 11 dental students whose salivary *mutans streptococci* levels were >10^6 cfu/mL, brushed their teeth using toothbrushes with two different tuft design using once at one-week intervals. The experiment was also repeated using a triclosan-containing toothpaste. After use, each toothbrush was rinsed under running tap water for 10 s and four tufts were processed for total bacteria, *mutans streptococci*, *lactobacilli* and yeast counts using culturing techniques. Microbial colony-forming units for a given brush type for the same volunteers were compared using Wilcoxon test.

Results: No statistically significance was found in terms of *mutans streptococci*, *lactobacilli*, yeast and total bacteria adhesion between the two types of toothbrushes (p>0.05). Brushing with toothpaste,

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üzerindeki bakteri sayıları belirgin olarak azalmış (p<0,05), maya sayısında belirgin fark bulunmamıştır (p>0,05).

Sonuç: Ağız mikroorganizmaları diş fırçalarına kolaylıkla kolonize olabilmektedir. Diş macunu diş fırçası kontaminasyonunu belirgin olarak azaltırken, bu çalışmada kullanılan kıl demeti dizaynlarının kontaminasyonda etkisi bulunamamıştır. Diş fırçalarındaki mikroorganizma yükünü azaltmak için geliştirilmeye çalışılan yeni teknolojilere rağmen, triklosanlı diş macunu kullanımının bu konuda etkili ve kolay bir yöntem olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Çürük yapıcı mikroorganizmalar, diş fırçası, diş macunu, kıl demeti dizaynı

significantly decreased the bacteria on toothbrush bristles (p<0.05); while it did not effect the yeast counts (p>0.05).

Conclusion: The oral microorganisms can quickly colonize on toothbrushes. Toothpastes can significantly reduce contamination of toothbrushes but the tuft design of the toothbrushes used in this study does not affect their amount. Despite trying to develop new technologies, the use of triclosan-containing toothpaste seems to be an effective and easy way of reducing microbial load on the toothbrushes.

Key Words: Cariogenic microorganisms, toothbrush, toothpaste, tuft design

INTRODUCTION

Toothbrushes may become contaminated after a single use by a wide array of microorganisms which are present both in the oral cavity and in the external environment (1-3). Microorganisms can remain viable on toothbrush bristles for periods ranging from 4 hours to 7 days (4-6). Therefore, the routine use of contaminated toothbrushes might contribute to disseminate microorganisms within the oral cavity (7).

Caries risk is high at the individuals with high salivary *mutans streptococci* (*MS*), *lactobacilli (LB)* and *yeast* levels. The survival of the cariogenic microorganisms on the brushes of these persons can cause the persistence of caries risk by reinoculation. The retention and survival of microorganisms may depend on brush design and the quality of the plastic and bristles. The influence of brush design and quality of the bristles on the microbial retention was investigated in several studies (7, 8). But there is no study which shows the effect of the tuft design on microbial contamination of toothbrushes.

In response to these reports, several studies have focused on methods of toothbrush disinfection. A simpler way of reducing the microbial load on a toothbrush is by using toothpaste. Recently, antimicrobial toothpastes that contain triclosan have been introduced; triclosan is a compound commonly used for disinfection. To date, there are only few studies on these toothpastes which show their effect on the residual microbial contamination of toothbrushes (4-6).

We aimed to investigate and compare the retention of cariogenic species on two types of toothbrushes manufactured by the same company with different tuft design and the impact of triclosan-containing toothpaste on the microbial contamination.

MATERIAL AND METHODS

Study population

The study was performed at Istanbul University Faculty of Dentistry, in 2008. Totally 11 dental students (five girls, six boys) with a mean age 19.5 years (range 19-21) whose salivary *mutans streptococci* levels were >10^6 cfu/ml were included in this study. Written informed consent was obtained from all volunteers which was approved by the Local

Ethics Committee of Istanbul University Faculty of Medicine No: 2008/1210.

Experimental design

At first, paraffin stimulated whole saliva was collected for five minutes in order to determine the salivary *mutans streptococci* (*MS*), *lactobacilli* (*LB*) and yeast levels of the volunteers.

The study protocol was carried out as a four-stage changeover system with a one-week interval between each stage. A new (unused) toothbrush manufactured by the same company (Colgate-Palmolive Company) was used once. A straight (Colgate Twister) and circular (Colgate 360°sensitive) tuft designed toothbrushes (referred to hereafter as brushes A and B, respectively), and a NaF (0.14w/v) toothpaste containing sodium laurel sulfate, PVM/MA copolymer and triclosan (Colgate Total) were used for the study. The toothbrushes without and with toothpaste were used, respectively. In each stage, the volunteers brushed their teeth for two minutes using Bass' method. After brushing, the bristles were rinsed under running tap water for 10s and transported to the laboratory at room temperature in a dry environment.

Laboratory procedures

Four tufts of each toothbrush were cut and suspended in 1 ml sterile saline for microbial analysis. After homogenized by vortexing for 30s, serial 10-fold dilutions of all samples were prepared. Aliquots of 0.1 ml of appropriate dilutions were then plated on blood agar (trypticase-soy-agar, Merck KgaE, Damstadt, Germany) supplemented with %5 defibrinated sheep for total bacteria; on Mitis Salivarius Bacitracin agar (MSB) (Acumedia Man Inc., Baltimore, Maryland) for *mutans streptococci*; on Rogosa Agar (Merck) for *lactobacilli* and on Sabouraud Dextrose Agar (Merck) for yeast counts (9, 10). MSB Agar and Rogosa Agar plates were incubated in air supplemented with 10% CO_2, while the blood agar plates were incubated anaerobically in GasPak jars (Gas Generation kit, Oxoid Ltd, Basingstone, Hampshire, UK) and Sabouroud Dextrose Agar plates were incubated aerobically. After 48 h incubation at 37 °C, the colonies were counted and results were expressed as the total number of colony-forming units (cfu/ml).

Statistical analysis

Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Microbial colony-forming units for a given brush type for the same volunteers were compared using Mann-Whitney test. The level of significance was defined as $p<0.05$.

RESULTS

All the volunteers harbored high salivary *MS* (>10^6 cfu/ml), *LB* (>10^5 cfu/ml) and *yeast* (>10^3 cfu/ml) levels.

Isolation frequencies for the microorganisms observed in each groups are shown in Table 1. Average mean counts expressed as cfu/ml for the microorganisms for positive samples after the brushing without toothpaste are shown in Table 2. As the measurements for the same volunteers were correlated, no statistical significance was found in terms of *mutans streptococci* (*MS*), *lactobacilli* (*LB*), yeast and total bacteria adhesion between the two types of toothbrushes ($p>0.05$).

Table 1. Isolation frequencies of toothbrush contamination by *mutans streptococci (MS), lactobacilli (LB)* and yeasts after brushing with/without toothpaste (n=11).

	Brush A		Brush B	
	Without paste	With paste	Without paste	With paste
MS	11 (100%)	5 (45.5%)	11 (100%)	1 (9.1%)
LB	11 (100%)	2 (18.2%)	9 (81.8%)	3 (27.3%)
Yeast	4 (36.4%)	1 (9.1%)	6 (54.5%)	0 (0.0%)

MS= Mutans streptococci, LB= Lactobacilli
Detection limit= 10 cfu/mL

The detection frequencies of *Mutans streptococci* (6/22 versus 22/22), *lactobacilli* (5/22 versus 18/22) and yeasts (1/22 versus 10/22) were lower in all types of toothbrushes after brushing with toothpaste (Table 1). The toothbrushes, without distinction, harbored significantly lower numbers of bacteria ($p<0.001$) and yeasts ($p<0.05$) after brushing with toothpaste than brushing without toothpaste using the same brush type (Table 2).

Table 2. Median values for the microorganisms on the brush tufts according to the brush type after brushing with/without using toothpaste (n=11).

	Brush A		Brush B	
	Without paste	With paste	Without paste	With paste
MS	9.000	0 *	0	29.1+69.3 *
LB	1.000	0 *	10	$1.0+3.0x10^{2}$**
Yeast	0	0	20	0
TVC	2.400.000	4.000 *	13.000.000	2.000 *

MS= Mutans streptococci, LB= Lactobacilli,
TVC= total viable count for the bacteria.
*, ** Significantly lower numbers according to the same brush ($p<0.001$, $p<0.05$)

DISCUSSION

According to the results of this study, new toothbrushes harbor a high number of bacteria, including cariogenic bacteria and yeasts, immediately after first brushing. The immediate contamination of toothbrushes has also been shown in previous studies (4-6,11). Bacterial counts tend to increase with repeated use. Used toothbrushes have been shown to reveal bacterial contamination with various microorganisms which colonize in the oral cavity, related to dental status of the patients (2, 12, 13). Müller et al., examined toothbrushes from juvenile periodontitis patients infected by *Aggregatibacter actinomycetemcomitans* and found 69% of the brushes harbored this species (14). Svanberg investigated toothbrushes used by people who were severely infected with *Mutans streptococcus* (15). Toothbrushes were still contaminated with *M. streptococcus* even after being stored in dry air for 24 hours. In our study, we selected the patients with high salivary *MS* level which has an important role in the development and progression of dental caries (16). In this study, all the toothbrushes, without toothpaste, contaminated with *MS* after only one use. Besides two, all the toothbrushes also harbored *LB* (91%), while ten (45%) harbored yeasts. The total cultivable bacteria counts were $>10^{5}$ cfu/ml. These findings were nearly similar for each of the toothbrushes examined in our study.

Manual toothbrushes can be classified according to a number of features including the hardness, shape and type of bristles, as well as handle design. Consumers may have difficulty in selecting a proper toothbrush, owing to a plethora of products that are being marketed with the claims of superiority in size, design, hardness and plaque removal efficacy (17).

The influence of brush design and quality of the bristles on the microbial retention was investigated in several studies. *Porphyromonas gingivalis* and *M. streptococcus* retention were conversely better in two different toothbrushes in an in-vitro study (7). Wetzel et al. showed that the toothbrushes made with the technique of individual in-mold placement of the filaments retained the least amount of microorganism compared to other toothbrushes made with different anchoring techniques (staple-set and in-mold tufting) (8). It has been shown that the anchoring part of the tufts was not the primary niche for microorganisms on the toothbrush (5). In our study, we investigated the toothbrushes with "anchorless" manufacturing technologies. There were no significant differences between the mean numbers of microorganisms attached to and surviving on the two types of tested toothbrushes with different tuft design.

There have been several studies that have examined different ways of reducing contamination of toothbrushes. Some reported decontamination

via antiseptics, others by coating toothbrush filaments with antiseptic substances (3, 4, 6, 18, 19). While coated tufts failed to limit the bacterial contamination, decontamination via antiseptics was reported to be successful in these studies.

A simpler way of reducing the microbial load on a toothbrush is by using toothpaste. There are studies that have shown positive results after use of toothpaste during brushing. Quirynen et al. reported that the use of detergent containing toothpaste had reduced the bacterial survival rate by 2 logs (5). This result was significant, where toothpaste without detergent only had an insignificant bactericidal effect. Toothpastes containing amine fluoride, stannous fluoride and triclosan were also shown to have strong impacts on reducing microbial load of the toothbrushes (4, 6). The results of our study agree with those of the above studies. The mean number of the bacteria attached to the toothbrushes, was significantly reduced after the use of toothpaste with triclosan, which have well known antimicrobial effects. Only six (27%), five (23%) and one (5%) of the 22 toothbrushes revealed *MS*, *LB* and *yeasts* after using toothpaste, respectively. Distinct from those above studies, Warren et al. reported that the isolation frequencies of toothbrush contamination by *A. actinomycetemcomitan*s and *P. gingivalis* were not significantly different between brushing without and with triclosan-containing toothpastes (20).

Toothpaste, in general, has an impact on the bacterial contamination of toothbrushes. The cariogenic microorganisms on toothbrushes are inhibited by triclosan-containing toothpaste.

CONCLUSIONS

We isolated cariogenic microorganisms from toothbrushes after one-time brushing. The brush tuft design of the toothbrushes used in the study did not affect their amount while triclosan-containing toothpaste significantly inhibited the cariogenic microorganisms. Long-term use of a toothbrush with toothpaste may result in findings different from those of our study, which involved one-time brushing with a new toothbrush.

Despite trying to develop new technologies, the use of triclosan-containing toothpaste seems to be an effective and easy way of reducing microbial load on the toothbrushes.

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