

Effectiveness of single use over multiple use toothbrushes on negative oral microflora of plaque

Rohan Sachdev¹, Kriti Garg², Garima Singh³, Ankit Mehrotra⁴, Kriti Nigam⁵

¹UWA School of Population and Global Health, University of Western Australia, Perth, Australia, Departments of ²Oral Medicine and Radiology and ³Pedodontics, Rama Dental College, Kanpur, Uttar Pradesh, ⁴Department of Prosthodontics, MPDC INSTITUTE Kanpur, Kanpur, Uttar Pradesh, ⁵Private Dentist, Gurugram, Haryana, India

ABSTRACT

Context: Role of toothbrush in decreasing oral microflora. **Aims:** To evaluate the effectiveness and compare the negative oral microbial flora of dental plaque after the use of a self-contaminated multiple use toothbrush and that of a single-use toothbrush. **Settings and Design:** Sixty healthy individuals without any systemic conditions. **Methods and Materials:** The study conducted with 60 participants who were free from any systemic or oral disease and without any adverse habits. In these participants, plaque samples were collected after 1 month use of a self-contaminated multiple use toothbrush. Each participant was given a set of 30 new toothbrushes and a toothpaste tube and instructed to use one toothbrush everyday and discard it after use. The plaque samples were collected on seven days interval and cultured on Mitis Salivarius agar. The colonies were identified and their count was recorded. **Statistical Analysis Used:** Student *t* test was applied. **Results:** *Streptococcus mutans*, *S. sanguis*, *S. milleri*, and *Candida* were identified from the samples. A highly marked decrease in their numbers was found after the use of a single-use toothbrush over multiple use toothbrushes. **Conclusions:** As a self-contaminated multiple use toothbrushes can aid microorganisms to grow rapidly into the oral cavity, it may be preferable to change the toothbrush as frequently as possible.

Keywords: Microflora, oral cavity, plaque, toothbrush

Introduction

Tooth brushes are the most common gadget used to clean oral cavity and it aids in maintaining proper oral hygiene as well. Toothbrushes help in cleaning teeth mechanically, remove plaques and food debris and prevent dental decay, gingival inflammation as well as various other oral diseases.^[1,2] While cleaning oral cavity with toothbrush, the toothbrush gets infected with various microbes present in the oral cavity.^[3] Oral microflora contamination of the toothbrushes affects not only the oral health but also the overall health of the individual.^[4] After brushing, cleaning with plain water

may not eliminate all the oral microorganisms from toothbrush bristles.^[5] Storage and poor maintenance of tooth brush mainly in the moist conditions may increase the accumulation of residual oral microflora colonies to grow more rapidly and may act as a helping aid to reintroduce microflora into the oral cavity on further use.^[6,7] Thus the possibilities of occurring various oral diseases has been increased in past few years due to multiple use of self-contaminated tooth brushes.^[8] The aim of this study was to evaluate the effectiveness and comparison of the negative oral microbial flora of dental plaque after the use of a self-contaminated multiple use toothbrush and that of a single-use toothbrush.

Subjects and Methods

The study was conducted at a dental college Kanpur, Uttar Pradesh, India in the month of June to August 2019. Total 60

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Address for correspondence: Dr. Kriti Garg,
117/K-68, Sarvoadya Nagar, Kanpur - 208 005,
Uttar Pradesh, India.
E-mail: drkriti_garg@rediffmail.com

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participants were included in the study, with equal number of 30 males and females of age range 18–25 years and who were free from any systemic or oral disease and without any adverse habits of smoking, tobacco chewing and alcohol and were also not given any antibiotics during the study time period.

After obtaining ethical clearance from Institutional Ethics Committee (Ethical approval was given on 25th MAY 2019 by the Commettii.), verbal consent was taken from each participant; a proper oral prophylaxis was performed on every individual. Then, each participant was given a new toothbrush and toothpaste tube and instructed to use the same every day for 4 weeks. All were instructed to keep clean and store the toothbrush like their regular practice. At the end of 1 month, the dental plaque sample was collected, and every participant was asked to gargle his or her mouth with normal saline to remove all food debris. With taking care of all aseptic measures, the plaque was collected from the buccal sulcus of the right side mandibular first molar tooth using a sterile paper point so that the standardized length of the paper point (colored area) touched the tooth for 10 sec. This sample was immersed in 1 ml of phosphate-buffered saline (PBS). These plaque samples were vortexed for 15 sec and immediately subcultures were performed on Mitis Salivarius (MS) agar and Sabouraud's Dextrose agar (SDA) taking 5 ml of plaque in PBS. The inoculated agar plates were incubated at 37°C aerobically for 48 hours. The colonies of oral microflora were identified on the sample based on colony morphology. The colonies of oral microflora with similar shape and size were counted using a colony counter and their numbers were recorded.

During the second month of study, every participant was provided with a set of 30 new toothbrushes and a new toothpaste tube. All participants were instructed to use one toothbrush every day and also to avoid touching the opening tip of the toothpaste tube or the bristles with their fingers and discard the toothbrush it after a single use. All plaque samples were collected on every seventh day for four weeks and sent for culture. Based to the colony morphology identified, the isolates were speciated on MS agar and their numbers were recorded.

The results of the first month end samples and the every week for 4 week samples of the second month study period were analyzed for statistical analysis using student *t* test and *P* value was calculated.

Results

Three common species of Streptococci (*S. mutans*, *S. sanguis* and *S. milleri*) were identified from the oral microbial samples collected in 2 months duration. Candidia species were also included in the study sample as they can be the important oral microflora colony present in the toothbrush, as stated by Taji *et al.*^[6]

Table 1 indicates the comparison of three different species of Streptococci and Candida between the first months of self-contaminated multiple use toothbrushes and that of

single-use toothbrush use for second month. It shows a very highly significant decrease in the number of microorganisms. The analysis showed a *P* value of 0.001 for *S. mutans*, 0.0061 for *S. sanguis*, 0.0057 for *S. milleri* and for *Candida* 0.0052.

Table 2 shows the comparison of the mean numbers of colony levels of Streptococci and *Candida* after use of “single-use” toothbrush and there is a decrease in the mean value of oral microflora with changing the toothbrush every day as compare to multiple use toothbrush.

Discussion

The plaque is the most common etiologic factor in development of various oral diseases and the cleaning of food debris is the most important act to maintain good oral hygienic. Cleaning of oral cavity and removal of plaque can be achieved with various oral hygiene devices, of which toothbrush is the commonly used now a days. Various studies stated that after brushing and also during improper storage, the toothbrush may get contaminated with some microbes.^[5,9]

Nelson *et al.* in 2000 have shown that *Candida*, Staphylococci, Corynebacteria, Pseudomonas, and Coliforms can colonize and Christersson LA *et al.* in 1985 stated that group A beta hemolytic Streptococci can colonize on a toothbrush. Studies also show that viruses and fungi can contaminate used toothbrushes.^[10,11] The daily-use toothbrushes are commonly stored in the bathrooms and in open areas thus may get infected by aerosols from the toilet as well as by contaminated fingers of the individuals.^[6]

Various factors may be the source of contamination of the toothbrush, and this contaminated toothbrush can be the cause of various infections entry in the oral cavity and may alter the

Table 1: Comparison of oral microflora after using a self contaminated multiple use toothbrush with that of single use toothbrush for four weeks duration

Oral microflora in four week duration	Mean deviation	Standard deviation	<i>t</i>	<i>p</i>
<i>S. mutans</i>	2.263	2.7007	4.9979	0.0001
<i>S. sanguis</i>	1.938	2.3456	3.1057	0.0061
<i>S. milleri</i>	1.622	2.0381	2.9374	0.0057
<i>Candida</i>	2.396	2.7836	3.0015	0.0052

Table 2: Comparison of oral microflora colonies of plaque in single use multiple and toothbrush for four weeks durations

Oral microflora in four week duration	Mean number of colonies	
	Single use tooth brush	Multiple use toothbrush
<i>S. mutans</i>	21.24	27.91
<i>S. sanguis</i>	14.95	18.68
<i>S. milleri</i>	14.87	17.68
<i>Candida</i>	15.27	19.71

oral microflora. In the present study, three different species of Streptococci – *S. mutans*, *S. milleri*, *S. sanguis* on MS agar and also *Candida* species on SDA were isolated from dental plaque which was found similar to study done by Pal V in 2009 except the presence of four species of Streptococci.

Various Studies performed and literature available has main focus in evaluating the contamination of toothbrushes, disinfecting them with various solutions and in knowing the effectiveness of the disinfecting solution, but the present study was performed to evaluate the difference in the quantity of oral microorganisms in the plaque after using a self-contaminated multiple use toothbrush and single-use toothbrush.^[12]

Studies done in past show various time periods from 44 hours to 30 days for the growth of oral microflora mainly Streptococci species on the toothbrush.^[4,8] Streptococci organisms mostly found on tooth surfaces, it does not uniformly colonize on all tooth surfaces but, instead, localizes on certain surfaces. Christerson *et al.* stated that *S. mutans* can be transmitted from colonized to noncolonized areas through the toothbrush bristles.^[11] *S. milleri* showed a significant reduction in the mean value when compared with multiple use toothbrush with single use in the present study [Table 1]. The reduction in *S. sanguis* was not as significant as that seen in *S. mutans*, results were found similar to the study done by Pal V except the presence of *S. mitis* in the study with a significant reduction in mean number.^[5]

In 1979, Parker *et al.* reported that *S. mutans* and *S. sanguis* exhibit similar habitat relationship, which lead them to grow in oral cavity in similar numbers.^[13] In present study *S. mutans* was slight raised in number as compare to *S. sanguis* which was not in consistent with the study results of Pal V where there was a decrease in the number of *S. sanguis* in comparison with *S. mutans* because the number of *S. mutans* was decreasing [Table 2].^[5] *S. milleri* is the major organism present in the gingival crevice fluid. The present study showed the presence of this organism in few numbers in the sample after the use of a self-contaminated multiple use toothbrushes and there was a decrease following the use of a “single–use” toothbrush. Results were found similar to study done by Pal V.^[5]

Glass *et al.* and Taji *et al.* has been reported presence of *Candida* colonies on the toothbrush and its bristles.^[6,14] In the present study samples *Candida* colony was found along with Streptococci colonies and the present study shows reduction in *Candida* mean numbers in plaque samples by changing the toothbrush, the *Candida* count in the plaque decreased significantly with single use toothbrush over the multiple use toothbrush, which was similar to study done by Pal V in 2009.^[5] Thus, in the present study, it was noticed that the number of oral microbe colonies present on the plaque decreased significantly by changing the toothbrush after every use as compared to multiple use toothbrush.

Conclusion

Modern lifestyle leading to various changes in food and drinking habits now-a-days, which may cause occurrence of plaque accumulation and initiation of oral cavity diseases. Toothbrushes are the most conveniently used devices that help to clean the oral cavity and thus help in preventing oral diseases as well as on the other hand causing infections. Therefore, it is advisable to change toothbrushes at regular intervals, at least once in a month to decrease the oral microflora growth and also, a cost-effective simple method of disinfecting toothbrushes should be implemented. As a famous saying is there that “Mouth is the mirror of the body”, so it is our basic duty that we should clean our oral cavity as much as possible and store our daily use toothbrushes in hygienic conditions.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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