ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Retention of Microorganisms in Commercially available Toothbrushes with different Tuft Anchoring Technology – A Comparative SEM based study

Dania Ibrahim AlMejmaj[1], Budour Jamal AlShayea[1], Rawan Abdullah AlDaiji[1], ShrutiBasavaraj Nimbeni[2*]
1. Intern College of Dental Sciences, Mustaqbal University, Buraidah Al Qassim, 52547, Saudi Arabia
2. Division of Pediatric Dentistry, Department of Preventive Dentistry, Mustaqbal University, Buraidah Al Qassim, 52547, Saudi Arabia

***CORRESPONDING AUTHOR**
Dr. ShrutiBasavarajNimbeni, Division of Pediatric Dentistry, Department of Preventive Dentistry, Mustaqbal University, Buraidah Al Qassim, 52547, Saudi Arabia
Email: pedoshruti1@gmail.com, Phone +966507434032

ABSTRACT
Purpose: This study aimed to compare the retention of microbes in two different types of commercially available toothbrushes manufactured with different tuft anchoring technology.
Materials and Methods: 60 toothbrushes of which 30 were manufactured by in-mold tufting technology and 30 were manufactured by staple-set tufting technology respectively, were distributed among 30 patients with carious lesions and poor oral hygiene. The patients used these toothbrushes for brushing under unvarying conditions. The toothbrushes were subsequently collected and examined for the presence of streptococci and lactobacilli in the brushes. This examination was done at 3 different time frames. The toothbrushes were also examined under the Scanning Electron Microscope for the presence of spaces that could act as a potential reservoir for microorganisms.
Results: In our study, it was seen that immediately after brushing, more microorganisms were retained in toothbrushes manufactured by in-mold tufting technology compared to toothbrushes manufactured by staple – pin tufting technology as determined by paired t-test. Though microorganisms were retained in both the brushes after 2 hours and 8 hours of brushing, the difference was not statistically significant. More colonies of streptococci were found compared to L. bacilli.
Conclusion: All commercially available toothbrushes retain a substantial amount of microorganisms irrespective of their manufacturing technology. There is a need for the fabrication of toothbrushes with better bristle anchoring techniques to reduce the retained microbial load in the toothbrush.
Keywords-Contamination, In-mold fusion tuft anchoring, Lactobacilli toothbrush, Streptococcus mutans, Staple-pin tuft anchoring

1. INTRODUCTION

Toothbrushes are the most used devices to maintain oral hygiene. They help in the removal of plaque and food debris on the oral cavity.[1]Numerous microorganisms are lodged on the toothbrush after use which can survive for a variable amount of time.[2]Toothbrushes can also get contaminated by the bacteria disseminated by aerosols from lavatory flushing or via soiled fingers and pseudomonads originating from the restroom and moist areas.[3]In a study conducted bySnezanaPesevskaet al, more than 50 different types of microorganisms were detected in the toothbrushes after use of which the more predominant ones were Escherichia coli, streptococci, Klebsiella, Enterobacter cloacae, Serratia and Pseudomonas aeruginosa.[4]It was also learnt that toothbrushes stored in bathrooms with attached toilets carried more microorganisms compared to the bathrooms with no toilets.[5]Toothbrushes laden with pathogenic microbes can cause sepsis, cardiovascular diseases, and damages to vital organs.[6]
Toothbrushes spread microorganisms by retaining them between the bristles and in the spaces available in the toothbrushes.[7]Toothbrushes are manufactured by 3 main technologies. One of them is staple set tufting which is the conventional technique. The bristles are fastened to the toothbrush head with a metal anchor in a predrilled hole.[8]This creates a lot of space where the microbes can enter by capillary action and multiply.[9]
Anothertechnique is in-mold tufting technology wherein the tuft of bristles are heated and pressed into the mold in the toothbrush head.[9]The toothbrushes manufactured by this technology were assumed to retain fewer microorganisms as there was less space in the tufts to hold on to microorganisms.[10]Very few companies are employing this technology in manufacturing toothbrushes.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

The third technology for bristle anchoring is the in-mold placement of individual filaments. Here, individual bristles are placed into the mold in the toothbrush head with a synthetic material. This technique is most successful in retaining the least number of microorganisms as there are no tufts.[7]The toothbrushes manufactured by this technology are not marketed. Only a prototype was tested for retention of microorganisms by Wetzel et al in 2005.[7]Thus, our study aimed to compare the retention of microorganisms in staple set toothbrushes and in-mold tufted toothbrushes at different intervals of time after brushing.

2. METHODS

In the present study, we assessed 2 different types of toothbrushes with different anchoring techniques for retention of caries causing microorganisms such as Streptococcus mutans and Lactobacilli at different intervals of time. Ethical approval was taken from the General Directrateof Health Affairs –Al Qassim. (Application No- 1442-254800) regional prior to beginning the study.

2.1 TOOTHBRUSHES

60 toothbrushes were examined, of which 30 brushes were manufactured by staple- set tufting technology (TOOTHBRUSH A) (Jordan toothbrush classic medium Duplo) and 30 were manufactured by in-mold tufting technology (TOOTHBRUSH B) (Oral-B Pro-Health). Brands of the toothbrushes were of no significance in the study.

Before beginning the study a photographic analysis of the toothbrushes was done under the scanning electron microscope. 5 brushes were selected from each group and sterilized chemically using Leit-C (Sigma- Aldrich, USA). The whole length of the bristles in the toothbrush head were gently cut and the brush heads were sputtered with gold powder using Leica EM ACE200 (Leica EM ACE Coaters, Germany) to form a film of 100 to 150 nanometer thick.

Photographs were taken using thescanning electron microscopy (JEOL-JSM; 6460LV, Tokyo, Japan) at 20KV accelerating voltage according to the method described by Althauset al.[10]

2.2 SUBJECTS

30 healthy individuals in the age of 20 to 45 years with dental caries and chronic gingivitis and periodontitis, who walked into the dental unit of Mustaqbal University College of Dental Sciences Buraidah, were screened according to the DMFT[11] and Periodontal Index[11] for dental caries and periodontal status and based on severity they were selected for the study.

The study participants were tutored to brush their teeth properly using the Bass technique[12] in the demonstration room of the dental clinics in the university. Two toothbrushes were given to each participant to clean the maxillary and mandibular teeth on one side with toothbrush A and on the other side with toothbrush B. This training was done for 3 minutes and each individual used a pea-sized quantity of fluoridated toothpaste. Next, the toothbrushes were washed under 50 ml of tap water by the standardized protocols by the investigator. The toothbrushes were vortexed 10 times in a beaker filled with water. The toothbrushes, after one use, were sent for microbiological examination at 3 different intervals i.e. immediately after use, 2 hours after use, and 8 hours after use. The brushes were dried with air by placing them discretely at 5 cm distance on an absorbent tissue paper in a sterilized boxes at room temperature. The boxes were not ventilated to prevent cross-contamination of microorganisms.

2.3 MICROBIOLOGICAL ANALYSIS:

For isolation of the microorganisms, the heads of the toothbrushes were dipped in 15 ml of Sputasol solution and placed in an ultrasonic device. Sputasol solution consists of 0.02 g potassium chloride, 0.1-gram dithiothreitol, 0.78 g sodium chloride, 0.02 g potassium dihydrogen phosphate, 0.112 g disodium hydrogen phosphate.Centrifugation of 1 ml of the bacterial suspension was done. 800-micron liters of supernatant suspension was discarded. The remaining 200-micron liters were shaken with the pellet. 20-micron liters of the solution was inoculated on different agar Petri dishes i.e. mitis salivarius bacitracin agar, a selective growthmedia for S. mutans, and rogasa agar media, a selective growth media for Lactobacilli. The microbial load for 20 µL of the suspended solution was tallied and later calculated for the extracted volume of 1 mL of suspended solution. The Petri dishes were sent for incubation at 37^0 C for 48 hours.Colony counter apparatus was used for counting the colonies of viable microorganisms.

2.4 STATISTICAL ANALYSIS:

We compared the microbial colonies in toothbrush A and toothbrush B in all groups for significant differences. We also compared the retention of microbes after 0 hours, 2 hours, and 9 hours respectively. Statistical analysis of the collected data was done using SPSS 19 (SPSS, NY).[11]

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

3. RESULTS

3.1 Analysis of the toothbrush

Characteristics of the toothbrush heads: In the present study 2 types of toothbrushes were used to assess the microbial retention after brushing. Figure 1 illustrates the heads of the two toothbrush types. The head of toothbrush A had 37 tufts with each tuft having 72+/- 29 bristles and the bristle diameter being 172+/- 9µ. The head of toothbrush B had 33 tufts with 52+/- 6 bristles in each tuft and the bristle diameter being 193+/- 8µ.

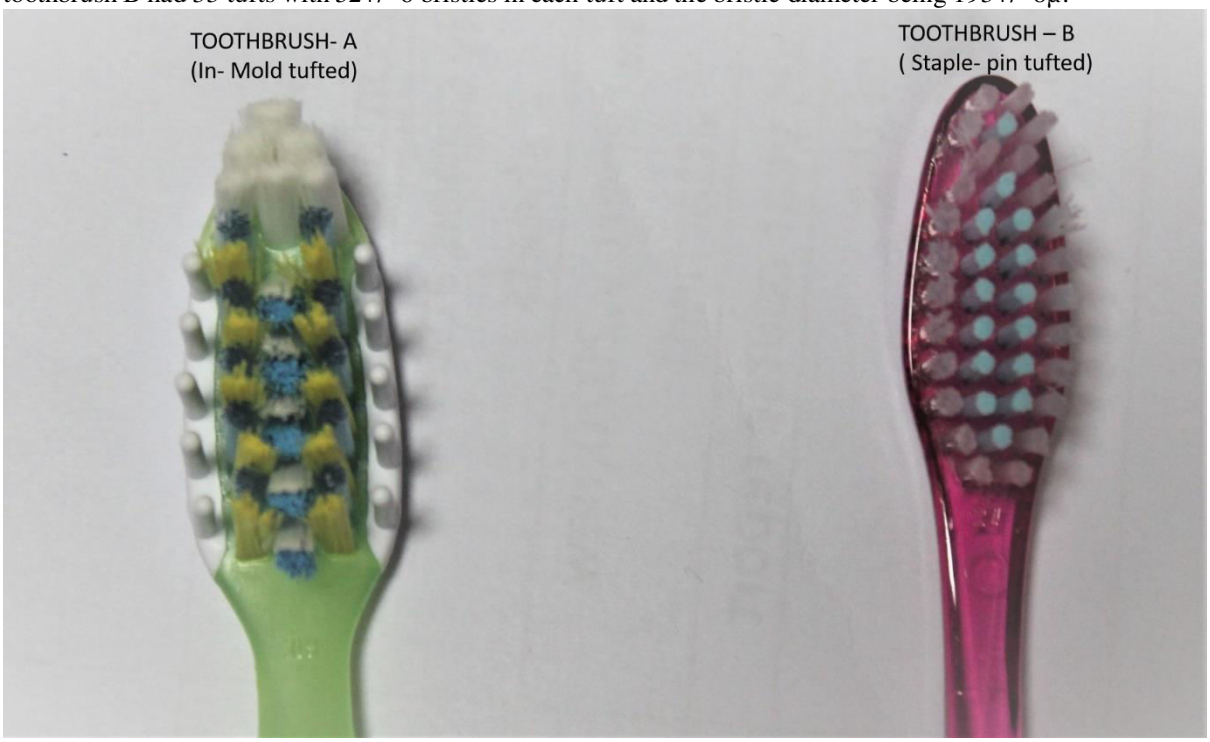

Figure 1: Types of toothbrushes used in the study

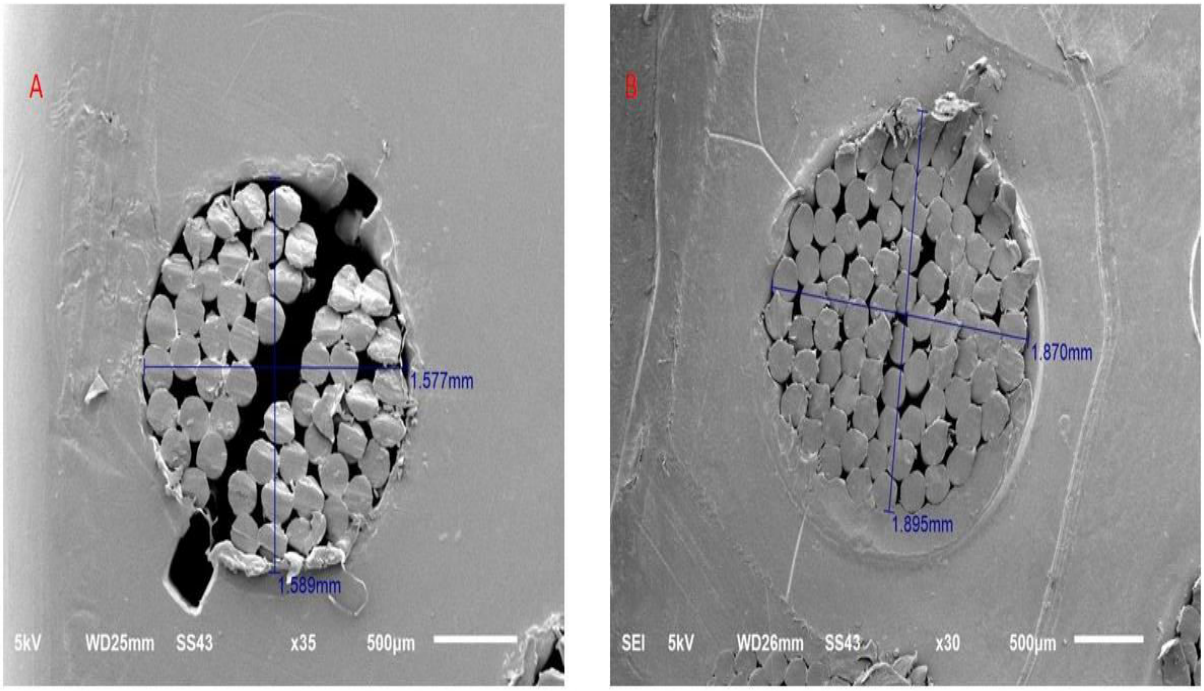

Figure 2: Cross section of tuft under SEM. (A) Staple pinned tuft. (B) In-mould fused tuft

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Tuft anchoring- The bristle tufts were anchored in two ways. In staple-set tufting, as shown in Figure 2B, a cross-section of the tuft was taken and examined under the digital scanning electron microscope. It was observed that the bundles were not secured tightly to the synthetic material. Some gaps and holes formed a part of the outer surface of the brush. A rectangular space was seen where the metal pin was used to staple the pin to the brush head. The gaps were more visible under the scanning electron microscope (Figure 2B).In in-mold tufting, only a few gaps were visible. The scanning electron microscope analysis exposed close-fitting filament anchoring (Figure 2A).

3.2 Microbiological analysis

In our study, it was seen that immediately after brushing, more microorganisms were retained in Toothbrush B compared to Toothbrush A as determined by paired t-test (Table 1). Though microorganisms were retained in both the brushes after 2 hours and 8 hours of brushing, the difference was not statistically significant (Table 1). More colonies of streptococci were found compared to L. bacilli.

Table 1: Mean number of microbial colonies at 0 hours, after 2 hours of brushing, and at 8 hours of brushing.

Zero Hour						
Variables	Mean ± SD	Paired Mean ± SD	Confidence Interval		t-value	p-value
			Lower bound	Upper Bound		
Toothbrush A	108.80 ± 93.93	45.40 ± 42.84	14.74	76.05	3.351	0.009*
Toothbrush B	63.40 ± 59.50					
Two Hours						
Variables	Mean ± SD	Paired Mean ± SD	Confidence Interval		t-value	p-value
			Lower bound	Upper Bound		
Toothbrush A	63.90 ± 59.70	4.53 ± 50.33	-31.70	40.30	0.270	0.793
Toothbrush B	59.6 ± 64.56					
Eight Hours						
Variables	Mean ± SD	Paired Mean ± SD	Confidence Interval		t-value	p-value
			Lower bound	Upper Bound		
Toothbrush A	11.5 ± 30.04	2.90 ± 31.74	-19.81	25.61	0.289	0.779
Toothbrush B	8.6 ± 12.39					

A paired t-test determined that the mean number of microbial colonies in toothbrush A and toothbrush B differed statistically significantly at immediate (zero) ($t = 3.351$, $P = 0.009$). It was also seen that the mean number of microbial colonies in toothbrush 1 and toothbrush 2 is not statistically significant after 2 hours ($t = 0.270$, $P = 0.793$) and 9 hours ($t = 0.289$, $P = 0.779$).

When a GroupWise comparison was done it was seen that the filament anchoring system and the drying time had a significant effect on the microbial contamination of the brushes. The toothbrushes retained a significantly fewer number of microorganisms after 8 hours compared to the microorganisms retained after 2 hours and zero hours (Table 2).

Table 2: GroupWise comparison of microbial retention in the toothbrushes by one – way ANOVA

Toothbrush A	Mean ± SD	F-value	P-value	Tukey's Post-hoc test
Zero	108.80 ± 93.93	5.353	0.011*	9 hrs> Zero >2 hrs
2 Hours	63.90 ± 59.70			
8 hours	11.5 ± 30.04			
Toothbrush B	Mean ± SD	F-value	P-value	Tukey's Post-hoc test
Zero	63.40 ± 59.50	3.573	0.042*	9 hrs> Zero >2 hrs
2 Hours	59.6 ± 64.56			
8 hours	8.6 ± 12.39			

There was a statistically significant difference between groups was determined by one-way ANOVA ($F = 3.573$, $p = .042$). A Tukey post hoc test revealed that at 8 hours least number of microbes were found compared to the same at 0 hours and 2 hours.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

4. DISCUSSION

Toothbrushes have a vitalrole in the maintenance of dental health and hygiene. Toothbrushes can cause transmission of diseases and upsurge the possibility of getting infection as they can act as reservoirs for microbes in healthy andmedically compromised individuals[14]. Contamination is defined as the retention and persistence of infectious organisms on fomites. Generally, contamination of toothbrushes happens after the initial use and intensifies with recurrent use[15]. Brushes acquire microbes from the environment, mouth, hands, aerosols, and containers. Microorganisms get attached to the toothbrush, they multiply, and persist in the toothbrushes from where they can get transmitted to the humans, causing disease and morbidity. [16, 17]

Toothbrushes are manufactured with different designs and materials. The toothbrush bristles with different consistency and diverse tufting pattern can be made. They can be manufactured out of plastic and wood. Many studies have been conducted on the retention of microorganisms based on the toothbrush design, filament type, color of the bristles, and bristle pattern. It was reported by Efstratiou et al that the type of filaments used in the manufacture of toothbrushes affected the retention of bacteria.[2]

Goldschmidt MC reported that toothbrushes with closely arranged bristles retained more bacteria.[18]This finding was true in our study also. In in- mold toothbrushes, the bristles are very closely packed and anchored in the head of the toothbrush, thus more colonies of bacteria were found to be retained in the toothbrush at zero hours (fig -1). Whereas in Staple pin toothbrushes due to the metal pin there is space created between the bristles which allowed air to dry the moisture and fewer microbes were retained at zero hours (fig-2). After drying for 2 hours and 8 hours, though the number of microbial colonies in the brushes was statistically insignificant, the colony count was still higher in in-mold tufted toothbrushes compared to staple- pin tufted toothbrushes. This finding was similar to the report of Glass et al that spacing between the bristles allows the air to circulate and dry the moisture which is the most important medium for the retention of bacteria.[19] Mehta et al reported that moisture and oral debris caused the retention of microorganisms in the toothbrush.[20]

According to a study conducted by Wetzel et al, there was no significant difference in the retention of microbes between staple-pin tufted toothbrushes and in-mold tufted toothbrushes.[5]The findings of our study contradicted the findings of Wetzel et al since there were more bacteria retained in in-mold tufted toothbrushes compared to staple – pin tufted toothbrushes at zero hour. However, the conclusion of the study was similar to our study. There is a need to conduct a similar study on a larger scale to get more significant results.

5. CONCLUSION

In conclusion, it was seen that anchoring of closely packed bristles increased the retention of bacteria in the brush. Though both the types of brushes retained microorganisms, in-mold tufted brush retained more microorganisms than staple- pin brushes. There is a need to invent a new and economic method of anchoring bristles in the toothbrush heads like the filament anchoring technology wherein single filaments are anchored in the toothbrush head which reduced adherence of microorganisms to the bristles**.**

ACKNOWLEDGMENTS

Nil

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

DIA and BJA contributed to design a study. RAA contributed to database searched. SBN contributed to draft manuscript. All authors read and approved final manuscript.

REFERENCES

1. Creeth JE, Gallagher A, Sowinski J, Bowman J, Barrett K, Lowe S, Patel K, Bosma ML. The effect of brushing time and dentifrice on dental plaque removal in vivo. J Dent Hyg 2009;83:111-116.

2. Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos IA, Panis V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. J Dent 2007;35:331-337. doi: 10.1016/j.jdent.2006.10.007.

3. Frazelle MR, Munro CL. Toothbrush contamination: a review of the literature. Nurs Res Pract 2012;2012:420630. doi: 10.1155/2012/420630.

4. Pesevska S, Ivanovski K, Mindova S, Kaftandzieva A, Ristoska S, Stefanovska E, et al. Bacterial contamination of the toothbrushes. J Int Dent Med Res 2016; 9(1):6-12.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

5. Contreras A., Arce R., Botero J.E. , Jaramillo A., Betancourt M. Toothbrush Contamination in Family Members. Rev. Clin. PeriodonciaImplantol. Rehábil. Oral 2010; Vol. 3(1); 24-26.
6. Nascimento AP, Watanabe E, Ito IY. Toothbrush contamination by Candida spp. and efficacy of mouth rinse spray for their disinfection. Mycopathologia 2010; 169:133-138. doi: 10.1007/s11046-009-9239-z.
7. Wetzel WE, Schaumburg C, Ansari F, Kroeger T, Sziegoleit A. Microbial contamination of toothbrushes with different principles of filament anchoring. J Am Dent Assoc. 2005; 136 (6):758-806. doi:10.14219/jada.archive.2005.0259
8. Warren DP, Goldschmidt MC, Thompson MB, Adler-Storthz K, Keene HJ. The effects of toothpastes on the residual microbial contamination of toothbrushes. JADA 2001;132:1241-5.
9. Krause E, Bluecher H, Edlauer L, inventor(s). (In-mold tufting of toothbrushes). German patent DE 355645. Patent issued June 30, 1922.
10. Althaus D, Kockapan C, Wetzel WE. Bristle end rounding and anchoring in childrens' toothbrushes [in German]. Schweiz Monatsschr Zahnmed 1990;100:159-64.
11. World Health Organization. Oral health Surveys – Basic Methods. 3rd ed., Springer: Berlin; 1987.
12. Crews K, O'Hara J, Gordy F, Penton N. The Bass technique: Charles Cassidy Bass' legacy. Miss Dent Assoc J 1995;51:18-20.
13. IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.
14. Glass RT. The infected toothbrush, the infected denture, and transmission of disease: a review. Compendium. Jul 1992;13(7):592, 594, 596-598
15. Bonten M, Hayden, M, Nathan, C., van Voorhis, J., Matushek, M., Slaughter, S. et al. Epidemiology of colonization of patients and environment with vancomycin-resistant enterococci. The Lancet. 1996;348:1615- 1619
16. CDC. In CDC's National Center for Infectious Diseases. Available at: http://www.cdc.gov/ncidod/dhqp/hai.html, 2009
17. ADA. ADA statement on toothbrush care: Cleaning, storage, and replacement. Available at: http://www.ada.org/1887.aspx. Accessed 1/27, 2009.
18. Goldschmidt MC, Warren, D. P., Keene, H. J., Tate, W. H., & Gowda, C. Effects of an antimicrobial additive to toothbrushes on residual periodontal pathogens.The Journal of Clinical Dentistry. 2004;15(3):66-70.
19. Glass RT. Toothbrush types and retention of microorganisms: How to choose a biologically sound toothbrush. . Journal - Oklahoma Dental Association. 1992;82(3):26-28.
20. Mehta A, Sequeira, P. S., & Bhat, G. . Bacterial contamination and decontamination of toothbrushes after use. . The New York State Dental Journal. 2007;73(3):20-22.