Comparative evaluation of ultraviolet and microwave sanitization techniques for toothbrush decontamination

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Abstract

Background: Toothbrushes are rapidly contaminated with different microorganisms representing a possible cause of infection or reinfection especially in the periodontal patients under therapy. The purpose of this study was to evaluate the sanitization of toothbrushes previously contaminated by various oral microorganisms using a domestic microwave oven and commercial ultraviolet (UV) light toothbrush sanitizer. Materials and Methods: Thirty male dental graduates were randomly assigned to control or experimental groups and received standardized toothbrushes for home use. Each subject was instructed to use it with the standardized modified Bass technique for 1 week and submit it to the investigator after use. Collected toothbrushes were cultured and analyzed for the number of colony-forming units (CFUs). In the next phase, once again a new set of toothbrush was given to each subject and instructed to use it for one more week and follow the same instructions as given earlier. Subsequently, the used toothbrushes were again collected and were sanitized by microwave irradiation, UV radiation, or were not sanitized (control group). After the sanitization procedure, toothbrushes were again cultured for the number of CFUs. The collected data of the presanitized and postsanitized CFU count were log transformed to normalize their distributions prior to analysis. Furthermore, log CFU data were compared and analyzed by one-way ANOVA, Tukey's post hoc procedure, and paired t-test for the difference in the mean at P<0.05. **Results:** Result showed that after the sanitization procedure, there was a significant (P<0.001) reduction in microbial contamination in both microwave and UV group toothbrushes compared to control group toothbrushes whereas the microbial count in the microwave group was significantly less (P<0.001) compared to the UV group. **Conclusions:** The evidence presented in this study suggests that microwave irradiation is an effective disinfectant agent for bacteria and fungi on toothbrushes.

Key words: Sanitization techniques; toothbrush decontamination; colony forming units

INTRODUCTION

Tooth brushing plays an important everyday role in personal oral hygiene and effective plaque removal. Toothbrushes may become heavily contaminated with microorganisms^[1] and these microorganisms may originate not only from the oral cavity but also

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from the environment where the toothbrushes are stored.^[2] This contamination implicates in the possibility of reinfection of a patient by toothbrushes harboring pathogenic microorganisms. As early as 1920, Cobb *et al.*^[3] reported the toothbrush to be a cause of repeated infections of the mouth. Svanberg *et al.*^[4] found that toothbrushes can be heavily infected by mutans streptococci after 24 h. According to Glass *et al.*^[5] microorganisms not only adhere to and reproduce on used toothbrushes but also have the ability to transmit organisms responsible for both local and systemic diseases.

Procedures for the decontamination of toothbrushes would prevent the risks of reinfection or infection by other pathogenic microorganisms from the environment. Several bactericidal agents have been promoted to reduce the possibility of toothbrush bacterial contamination between uses. These include the use of chlorhexidine,^[6] Brushtox,^[7] and several dentifrices.^[8-10] While all these have shown varying degrees of efficacy, none are widely used as a home-based application. A possible reason for the noncompliance with these methods is that they are time consuming and may result in unwanted product residues.

Recently, few studies indicated that the use of microwave ^[11] and ultraviolet (UV) light^[12,13] is the most effective household method to sanitize the toothbrushes after contamination. Furthermore, due to the ease of use, these techniques may increase compliance in toothbrush bacterial decontamination. However, the extent of bacterial decontamination using the microwave and UV light has not been determined in a clinical setting. Therefore, the present study was designed as an investigator-blinded, controlled, microbiological study to compare the efficacy of microwave and UV light in decreasing toothbrush bacterial contamination.

MATERIALS AND METHODS

Thirty male dental graduates residing in a common hostel (with a common source of water for daily use), with at least 28 teeth and age ranging from 22 to 28 years (mean age 25 \pm 2.6 years) were enrolled into this study. A prior written informed consent was taken from all included subjects. The study was approved by the ethical committee at JSS University, Mysore.

Inclusion criteria included subjects in good general health, who were able to give informed consent and comply with the study protocol, having at least 14 natural teeth per arch, and brushing their teeth twice daily. Exclusion criteria included the clinical evidence of gross caries or periodontal disease, the presence of systemic diseases or conditions that would affect the oral cavity such as uncontrolled diabetes mellitus, use of any medications associated with xerostomia or any antibiotic therapy within 90 days prior to the start of the study protocol.

Subjects were randomly assigned to either control (n = 10; group 3) or experimental (n = 20) groups. Experimental group comprised the microwave group (n = 10; group 1) and the UV group (n = 10; group 2). Before the start of the study, each subject was

given a new, identical multitufted toothbrush with soft nylon tufts (Ajay Quest[®] Toothbrush, Raghav Lifestyle Products, New Delhi, India) with a tube of toothpaste (Colgate TOTAL[™] Toothpaste, Colgate-Palmolive India Pvt. Ltd., Mumbai, India). Two unused toothbrushes (control) were cultured to check for any microbial growth in packed toothbrushes before starting the study. Furthermore, the toothbrush rinsing water from the common tank intended to be used was also subjected to a microbial check before the start of the study. The study was conducted in three phases that included contamination procedures including bacterial culture, sanitization procedures, and postsanitization evaluation.

Contamination procedure

Subjects were trained and/or instructed to use the standardized "modified Bass technique"^[14] for brushing their teeth for 3 min, twice daily for 1 week. Each subject was instructed to rinse the used toothbrush under running tap water without mechanical manipulation for at least 30 s. Subjects were also instructed to keep their toothbrushes in their living room within the provided aerated box at room temperature. After 1 week of use, each toothbrush was collected in a sterile paper bag and sent to the Central Food Technological Research Institute (CFTRI) laboratory, Mysore, within 4 h after collection in the morning. The toothbrushes were promptly delivered to the laboratory for bacterial extraction and cultivation.

Bacterial culture

A standard bacterial culture method was followed in the study. Various selective and nonselective media used in the study included trypticase soy agar for total counts, Mitis Salivarius agar for total streptococci, Mitis Salivarius agar with 2 IU/ml of bacitracin for mutans streptococci, MacConkey agar with 1% lactosec for Escherichia coli and other coliforms, and Rogosa SL agar for lactobacilli. For bacterial extraction, the toothbrushes were individually placed in prelabeled, sterile, 50-ml centrifuge tubes containing 10 ml of the trypticase soy broth (TSB) to immerse the bristles, then vortexed vigorously for 1 min, squeezed against the side of the tube to drain, rinsed with 5 ml TSB, and drained again. A series of undiluted and 10-fold dilutions of each sample were prepared and plated onto the surface of selective and nonselective media. A duplicate series of plates was then incubated aerobically or anaerobically at 37°C for 2-4 days, until colony formation was visible. The number of colonies, measured as colony-forming units (CFUs), was counted using a colony counter.

Sanitization procedure

A new set of standardized toothbrush was once again given to each subject and subjects were instructed to use it for one more week following the same instructions as given earlier. After 1-week usage, the toothbrushes were once again collected and subjected to sanitization procedures. The experimental group toothbrushes were randomly assigned to either the UV light or microwave group. Toothbrushes from the UV light group were sanitized by placing the brush in the UV light toothbrush sanitizer (Violight® tooth brush sanitizer, Violight Inc., India). Sanitization was carried out by placing the brush in the receptacle and exposing the head for 12 min to UV radiation (manufacturer's recommendation, 6 min). Toothbrushes from the microwave group were sanitized by placing the brush in a microwave oven (2450 MHz; Kenstar® microwave oven, Kenstar Kitchen Appliances India Limited, Mumbai). The wet brush was placed on the revolving table along with a glass of distilled water to protect the magnetron. The brush was subjected to microwave radiation at the maximum setting for 5 min. The toothbrushes from the control group were not sanitized.

Postsanitization evaluation

Once again each toothbrush belonging to different groups was collected in a sterile paper bag and sent to the laboratory for further microbial culture and colony count procedure.

Statistical analysis

Data obtained for all the microbial counts were log transformed to normalize their distributions prior to analysis. Logs of the total bacterial count (log CFU) after toothbrush contamination and decontamination were compared and analyzed using one-way ANOVA, Tukey's post hoc procedure for multiple comparisons, and paired *t*-test for the effect of the pre and postsanitization procedure on the microbial count in specific groups. All values were expressed as means and standard deviations. Differences were considered significant at P < 0.05.

RESULTS

Thirty subjects with an age ranging from 22 to 28 years (mean age 25 ± 2.6 years) were enrolled in this study. All the subjects were male and shared the common living environment (common hostel) for more than 4 years. No ethnic/race discrepancy was present between all the included subjects. All the subjects were able to return their toothbrushes on days 7 and 14 in sealed labeled bags as instructed. Bacteria were extracted from the toothbrushes and used to determine CFUs. Unused toothbrushes cultured before the start of the study resulted in negative culture. Moreover, no bacterial growth was observed on culturing the rinsing water obtained from the common tank. The mean microbial growth on toothbrushes in terms of log CFU is shown in Table 1.

One-way ANOVA was used to test for differences in mean microbial CFU counts among three groups of toothbrushes sanitized by their respective methods. The mean microbial CFU count done before the sanitization procedure demonstrated no significant difference, F(2, 27) = 0.344, P = 0.712, among the groups whereas the mean CFU count done after sanitization procedures differed significantly across the all the three groups, F(2, 27) = 267.219, P < 0.001.

Tukey's post hoc comparisons of the three groups after the sanitization procedure indicated that the microwave group (M = 1.49, 95% CI [1.2729, 1.7071]) gave a significantly lower CFU count than the UV radiation group (M = 3.22, 95% CI [2.93, 3.50], P < 0.001) and the control group (M = 5.88, 95% CI [5.49, 6.26], P < 0.001). Comparisons between the UV radiation group and the control groups also showed statistically significant reduction in the microbial CFU count at P< 0.001 [Table 2, Figure 1].

The paired *t*-test was also conducted to analyze the

Table 1: Descriptive statistics of the measured variables of the various groups								
Variables	Groups	Ν	Mean (log CFU)	Std. deviation	Std. error	Minimum	Maximum	
Presanitized	Microwave	10	5.82	0.89	0.28	4.60	7.30	
	UV	10	5.53	0.97	0.30	4.30	6.80	
	Control	10	5.79	0.68	0.21	4.60	6.80	
Postsanitized	Microwave	10	1.49	0.30	0.09	1.20	2.10	
	UV	10	3.22	0.40	0.12	2.60	3.70	
	Control	10	5.88	0.54	0.17	5.40	6.90	

Table 2: Tukey's post HOC comparisons of various groups							
Dependent variables	Groups (I)	Groups (J)	Mean difference (I – J)	Р			
Presanitized	Microwave	UV	0.290	0.734			
		Control	0.030	0.997			
	UV	Microwave	-0.290	0.734			
		Control	-0.260	0.779			
	Control	Microwave	-0.030	0.997			
		UV	0.260	0.779			
Postsanitized	Microwave	UV	-1.730^{*}	0.000			
		Control	-4.390^{*}	0.000			
	UV	Microwave	1.730^{*}	0.000			
		Control	-2.660^{*}	0.000			
	Control	Microwave	4.390^{*}	0.000			
		UV	2.660^{*}	0.000			

*The mean difference is significant at the 0.05 level

microbial CFU count difference between pre- and postsanitization procedures. The results of the paired *t*-test of the microwave group revealed that there were significant differences in log CFU counts, between the pre- ($M = 5.82 \pm 0.89$) and postsanitization procedure ($M = 1.49 \pm 0.303$), with t (9) = 16.18 and P < 0.001. Similarly, the UV radiation group also showed significant differences with t (9) = 6.77 and P < 0.001, between the pre- ($M = 5.53 \pm 0.974$) and postsanitization procedures ($M = 3.22 \pm 0.40$) whereas for the control group, the difference between pre- ($M = 5.79 \pm 0.68$) and postsanitization procedures ($M = -5.8 \pm 0.54$) was not statistically significant, with t (9) = -0.599, P = 0.564.

DISCUSSION

The present study was undertaken to quantitatively microbial growth analvze the after toothbrush contamination and to compare the efficacy of two different sanitization techniques (UV light and microwave irradiation) after the contamination procedure. The result outcome revealed that the contaminated toothbrushes harbored an increased number of aerobic and facultative anaerobic bacteria species. This finding is in accordance with the results of previous studies [15-18] that indicated that an actual risk of recolonization exists after each brushing. In the recent years, there has been an increasing interest in the interrelationship between contaminated toothbrushes and systemic reinfections. Several studies have also stressed [15-17] on the role of contaminated toothbrushes its causation in systemic infections. In this and regard, Brook and Gober suggested that contaminated toothbrushes contributed to the persistence of group A beta-hemolytic streptococci in the oropharynx and to the failure of penicillin therapy in some cases of pharyngotonsillitis.^[15] In another study, Fischer pointed to a relationship between contaminated toothbrushes



Figure 1: Comparison of postsanitized toothbrush groups. *The mean difference is significant at the 0.05 level

and pharyngitis.^[16] Significant bacteremia has also been reported after tooth brushing, especially in patients with severe periodontitis.^[17,18] Therefore, a concern has been raised that the microbial load on toothbrushes might have a significant impact in periodontal patients under therapy.^[17]

Discussions on the modern toothbrushes have suggested the problem of toothbrush construction as a factor of toothbrush contamination. The nylon, multitufted toothbrush has been cited for its design of tufts set too closely to accommodate easy cleaning. The filaments are collected into bundles, bent in half with a metal anchor in the center, and driven into premolded holes in the toothbrush head at a high speed.^[19] In toothbrushes, the bristles can harbor inherent microorganisms, further increasing the bacterial contamination. Several studies have suggested the need for toothbrush disinfection to reduce the number of microorganisms on the bristles using different methods, including UV radiation,^[12,13] microwave irradiation,^[11] boiling water, and chemical agents,^[20] such as Listerine, Plax, Cepacol, and chlorhexidine. In addition, some authors have also attempted to incorporate antimicrobial agents, such as silver,^[21] chlorhexidine,^[22] and others to the toothbrush bristles as a coating layer during the manufacturing process. Despite evidence demonstrating that chemical rinses and dentifrices can reduce the total bacterial load on a toothbrush, these methods are not widely used. Therefore, recently, Devine et al.^[23] raised a need of disinfection methods that are rapidly effective, cost-effective, nontoxic, and can be easily implemented.

The present study was mainly sought to compare two sanitization techniques used for the decontamination procedure. Our result showed that there was a significant reduction in microbial contamination in both microwave and UV groups compared to the control group. Furthermore, our results also showed a significant reduction in the microbial count in the microwave group when compared with the UV group. Our results confirm the findings of Chibebe et al.[11] where previously contaminated toothbrushes when exposed to microwave irradiation at 2450 MHz were reported to get inactivated by the action of microwave. A possible explanation for the effect of microwave irradiation upon formed microbial assemblage on brushes can be validated by the fact that many bacterial species are responsible for biofilm formation on different surfaces like toothbrushes, and microwave irradiation can disrupt the biofilm structure. Data suggest that the destruction of microorganisms by microwave is mainly due to a thermal effect of microwave exposure on the microorganism cellular content resulting in cell lysis.^[24] Another general indication for heat damage is the cell membrane rupture resulting in a leakage of nucleic acid and protein from cells.^[25] In this context, some studies^[24-26] have reported that microwave-injured cells often release ninhydrin-positive material, purines, and pyrimidines into a suspension. The presence of these materials in a suspension, in previous studies, has demonstrated the possibility of damage to cells by microwave at the membrane level.^[24-28]

In the present study, the UV radiation group showed a significant differential reduction in the microbial count compared to the control group. However, the microbial count did not significantly reduce as compared to the microwave irradiation group. Although we exposed toothbrushes for 12 min to UV radiation (6 min, manufacturer's specification), the result was not significant as compared to microwave irradiation. Previous studies^[29,30] have revealed that the longer exposure to UV light is necessary to ensure a complete inactivation of all microorganisms. The long exposure of UV light inactivates microorganisms by damaging the DNA and disrupting the chemical bonds that hold the atoms of DNA together in the microorganism. If the damage is severe enough, the bacteria cannot repair the damage and are inactivated. However, despite long exposure, a previous literature review^[31] has questioned the potential of lowintensity UV radiation in microbial deactivation, and the authors concluded that low-intensity UV rays are not effective against certain microbes and molds. Furthermore, tightly packed bristles and other areas are not in direct exposure and have no chance of disinfection. In the present study, these factors might be the reason for the UV radiation to be less efficient in toothbrush sanitization compared to microwave irradiation.

In contrast to our results, Boylan *et al.*^[13] have reported that a UV light toothbrush holder can effectively reduce an average of 86% total cultivatable bacteria on a toothbrush compared to controls. Our result is not in agreement with this result as our result showed only 42% reduction in the microbial count after the UV sanitization procedure. Therefore, further microbial studies are required to verify the efficacy of the UV light toothbrush holder in the reduction of the microbial content from contaminated toothbrushes.

In the present study, we instructed all the subjects to store their toothbrushes at room temperature in the provided aerated box when not in use. Data suggest that storage conditions of toothbrushes are an important factor for bacterial survival. Dayoub et al.[32] and Meier et al.[33] reported that the number of microorganisms in the toothbrushes kept under aerated conditions was lower than that in toothbrushes stored in plastic bags. Several authors [34-36] have reported that bacterial contamination can be reduced by washing toothbrushes after use, and drying under aerated conditions. Likewise Caudry et al.^[20] and Mehta et al.^[37] have also reported that a wet environment increases bacterial growth and cross-contamination. Therefore, as time increases between one tooth brushing and another, more microorganism development can occur in the toothbrushes stored in a wet/moisture environment.

Our study demonstrated that significant microbial

colonization was present after 1 week of repeated use of toothbrushes. However, data suggest that the time necessary for colonization is contradictory varying from 1 to 30 days.^[19,38] According to Cesco *et al.*,^[39] the colonization on toothbrushes by mutans streptococci occurs in a short time period, since after a single tooth brushing, they found the development of the microorganism in 24% of the cases. Svanberg^[4] reported the presence of mutans streptococci on toothbrushes after 3 days and colonization by mutans streptococci was observed on bristles after five consecutive days of toothbrush use. Our study also showed similar findings where cultivatable microorganisms were present on the bristles after a short-term (1 week) usage with aerated storage conditions.

Our results suggested that microwave irradiation is the better option for the sanitization of tooth brushes as compared to UV radiation. However, further studies are required for determining the optimum temperature and duration for the complete eradication of the organisms and spores, thereby achieving sterilization instead of sanitization. Moreover, the duration of UV sanitization also needs to be reassessed to achieve optimum results. Our results clearly suggest that there is a definite contamination of the toothbrushes after use; hence need arises for either improving sanitization measures or frequent changes of toothbrushes especially after any infections.

CONCLUSIONS

The evidence presented in this study suggests that microwave irradiation is an effective disinfectant agent for the microbiota present on the toothbrushes. It may be an important and efficacious oral health measure not only for the debilitated but also for healthy individuals. Further *in vivo* trials are anticipated on more specific bacteria and biofilm of the oral cavity. However, there seem to be good reasons for the daily use of a toothbrush sanitization even before the results of these further trials are available.

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