

Reduction in bacterial contamination of toothbrushes using the Violight ultraviolet light activated toothbrush sanitizer

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ABSTRACT: Purpose: This two armed, self-controlled, investigator blinded, clinical study tested the efficacy of an ultraviolet (UV) light toothbrush holder (Violight) to decrease toothbrush bacterial contamination. **Methods:** 25 subjects were randomly assigned to control or experimental groups and received two toothbrushes for home use on either even or odd days. The control group rinsed both toothbrushes after use in cold tap water with no mechanical manipulation. The experimental group rinsed one toothbrush in cold running water while storing the other toothbrush in the Violight toothbrush holder after use. The toothbrushes were returned after 2 weeks use in sealed plastic bags and were analyzed for the number of colony forming units (CFU) of *S. mutans*, *S. salivarius*, lactobacilli, *E. coli*, and other coliforms, and total bacterial counts by culture. An additional analysis of the total bacterial profile was performed using denaturing gradient gel electrophoresis (DGGE). **Results:** The Violight toothbrush holder reduced total CFU by an average of 86% (ANCOVA, $P=0.037$). In addition, a tendency was noted for a reduction in total bacterial population as detected by DGGE. (*Am J Dent* 2008;21:313-317).

CLINICAL SIGNIFICANCE: These results suggest that the Violight toothbrush holder can decrease bacterial contamination of toothbrushes between uses.

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Introduction

The uninterrupted accumulation of oral bacterial plaque is strongly associated with an increased risk for dental caries, the development of gingivitis and the promotion of oral malodor.¹⁻³ Mechanical disruption of bacterial plaque through the use of toothbrushes, or a combination of toothbrushing, interproximal hygiene procedures and use of oral mouth rinses has been regarded as an optimal means of preventing plaque accumulation and the promotion of overall oral health. However, bacteria remain on the toothbrush after use and may provide a potential source for recontamination of the oral cavity on the next use. One study⁴ has reported a bacteremia due to a viridans streptococci (*S. oralis*) strain that was cultured from the patient's oral cavity and from the subject's toothbrush suggesting that colonization of toothbrushes with oral microflora may be associated with bacteremia in some patients. Environmental enteric bacterial contamination of toothbrushes resulting from bathroom aerosols has also been reported^{5,6} which may also contaminate faucets, combs and hairbrushes.⁷

Several bactericidal agents have been promoted to reduce the possibility of toothbrush bacterial contamination between uses. These include the use of chlorhexidine,⁸ Brushtox,⁹ and several dentifrices.¹⁰⁻¹² While all of the above have shown varying degrees of efficacy, none are widely used as a home based application. A possible reason for non-compliance with these methods is they are time consuming and may result in unwanted product residues.

Ultraviolet (UV) light is bactericidal to a wide array of bacterial species including antibiotic resistant species¹³⁻¹⁵ and has been extensively used in water purification systems. Violight^a (Fig. 1) is a toothbrush holder that uses UV light for bacterial decontamination. The toothbrush is inserted into the device and a button is pressed to deliver a 10-minute UV

irradiation dose. The design of the Violight toothbrush holder prevents exposure to UV light when in use. Due to its ease of use, Violight may increase compliance in toothbrush bacterial decontamination. However, the extent of bacterial decontamination using the Violight toothbrush holder has not been determined in a clinical setting. Therefore, the objective of the present investigator-blinded, self-controlled, clinical study was to determine the efficacy of Violight in decreasing toothbrush bacterial contamination.

Materials and Methods

Subject recruitment and study design - The study design was reviewed and approved by the New York University School of Medicine Institutional Board of Research Associates. Subjects were recruited from the patient pool seeking dental care at the New York University College of Dentistry and from advertisements in local media. Inclusion criteria included subjects be ≥ 18 years of age, in good general health, able to give informed consent and comply with the study protocol, have at least 10 natural teeth per arch, and brush 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 7 days prior to the start of the study protocol.

Subjects were randomly assigned to either the control or the experimental groups. Each subject was provided with two toothbrushes of different color (green and silver) for home use on either odd or even days. The control group was instructed to rinse both brushes after use in cold running water without any mechanical manipulation. The experimental group was instructed to rinse one brush (green) in running water without mechanical manipulation and treat the second toothbrush (silver) with



Fig. 1. Violight toothbrush holder storing toothbrushes. The device can be used to store toothbrushes after the treatment cycle is completed.

the Violight toothbrush holder (Fig. 1) after every use. The treated brush was stored in the Violight toothbrush holder between uses in the subject's room at home where they typically brush their teeth (*i.e.* typically their bathroom). Subjects were instructed to start the study the evening after enrollment and to follow their regular oral hygiene regimen. Subjects returned the first toothbrush in a pre-labeled sealed plastic bag on day 13 of the study and the second toothbrush in a pre-labeled sealed plastic bag on day 14 of the study. Toothbrushes were returned to the Bluestone Center for Clinical Research within 1 hour after use in the morning. The toothbrushes were promptly delivered to the laboratory for bacterial extraction and cultivation (see below). After extraction, the toothbrushes were stored at -20°C .

Bacterial culture - Methods used for bacterial culture followed standard techniques.¹⁶ Media used for bacterial culture were purchased from Difco^b unless otherwise noted. Media included Trypticase Soy Agar^c for total counts, *Mitis salivarius* agar^c for total streptococci, *Mitis salivarius* agar with 2 IU/ml of bacitracin^c for mutans streptococci, MacConkey agar with 1% lactose^c for *E. coli* and other coliforms, and Rogosa SL agar^c for lactobacilli.

For bacterial extraction, the toothbrushes were individually placed in pre-labeled, sterile 50 ml centrifuge tubes containing 10 ml of trypticase soy broth^c (TSB) to immerse the bristles, then vortexed vigorously for 1 minute, 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 non-selective 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 (CFU), was counted using a colony counter.

Polymerase chain reaction (PCR) and denaturing gradient gel

electrophoresis (DGGE) assays - PCR and DGGE assays were used to identify both cultivable and non-cultivable bacteria present on four pairs of randomly selected toothbrushes from the 25 pairs of clinical samples. The toothbrushes were washed by agitation in 5.0 mL of phosphate-buffered saline (PBS) and centrifuged at $126,800 \times g$ for 30 minutes. The supernatant was discarded and the cell pellet was re-suspended in 0.5 ml TE buffer (10 mM Tris-Cl, pH 7.5, and 1 mM EDTA). The total bacterial genomic DNA was isolated by means of a DNA purification kit (MasterPure^d) with modifications as previously described.^{17,18}

A nested-PCR approach was used to first amplify the entire 1500-bp 16S-rRNA locus for all extracted bacterial DNA samples with a set of universal 16S rRNA PCR primers¹⁹ followed by a second amplification of a hyper-variable region (~300 bp) of the 16S-rRNA locus.¹⁹ In the first PCR, each 50 μL reaction mixture contained a standardized 100 ng of the total genomic DNA, 200 μM of each dNTP, 50 pmole of universal primers 16S-8f and 16S-1492r (19), 1.5 mM MgCl_2 , 5 μL of 10X PCR buffer II, and 2.5 U of Taq DNA polymerase.^c In the second PCR, a specific set of universal bacterial 16S rRNA primers (prbac1 and prbac2)²⁰ was used with a 40-nucleotide GC-clamp to facilitate the DGGE analysis.²¹⁻²³ All PCR procedures were performed with the GeneAmp PCR System 9700.^e PCR conditions and reagents were as described elsewhere.^{17,18}

A standardized 20 μL of each PCR-amplified product was separated on gradient gels as previously described.^{17,18} A 40% to 60% linear DNA denaturing gradient was formed in an 8% (w/v) polyacrylamide gel. PCR products were directly loaded in each lane and were run along with known species-specific DGGE reference markers.¹⁷ After electrophoresis, the gels were rinsed and stained for 15 minutes in 0.5 $\mu\text{g}/\text{mL}$ ethidium bromide. The DGGE images were digitally captured and recorded.^f The DNA fingerprints of the DGGE were compared and analyzed by means of Fingerprinting II Informatix^g software as previously described.^{17,18}

Statistical analysis - Data were entered and checked into a password-protected data set and analyzed using SPSS for Windows^h (Version 13). All bacterial counts were log transformed to normalize their distributions prior to analysis. An ANCOVA was performed to compare the differences in bacterial levels between the control and treatment groups using as a covariate the log of the total bacterial count on the first (cold water rinsed) toothbrush. The ANCOVA was adjusted for the individual specific CFU counts in the untreated situation and compared the second untreated toothbrush in the control group to the treated (Violight) toothbrush in the treatment group. The differences in total and each specific bacterial counts were also expressed as a percent reduction in CFUs comparing the Violight treated brush to the untreated, cold water rinse brush. Group characteristics were compared by Mann-Whitney-Wilcoxon test for continuous variables and Fisher's exact test for categorical measures. A value of $P < 0.05$ was accepted for statistical significance.

Results

Twenty five subjects (20 females, five males) aged 21-65 years (28 ± 10 ; mean \pm standard deviation of the mean) were enrolled in this study consisting of seven Caucasians, 10 African-

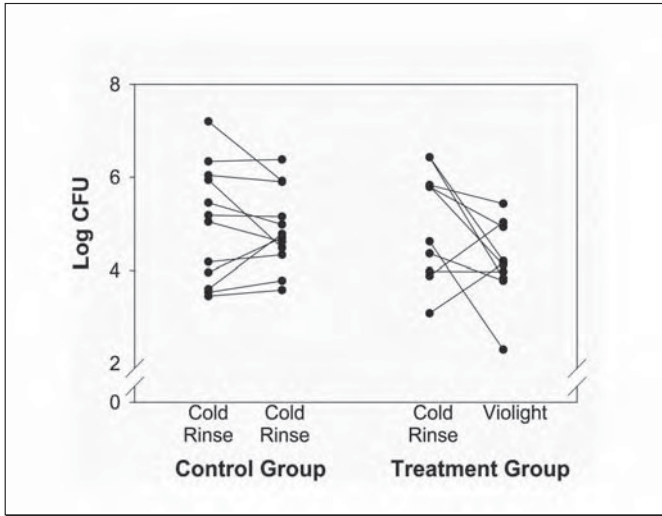


Fig. 2. Changes in CFU of bacterial levels on the paired toothbrushes for each subject. Each pair of circles connected by a line represents one subject. The left hand group represents the control subjects and the right hand group represents the treatment subjects whose second day toothbrush received Violight treatment. Seven of the 10 treatment subjects show reductions in CFU for the toothbrush treated with the Violight as compared to toothbrushes treated with cold water.

Table 1. Descriptive statistics for log CFU.

Group	N	Toothbrush #1		Toothbrush #2		Adjusted value Toothbrush #2	
		Mean	SD	Mean	SD	Mean	SD
Control	12	4.99	1.25	4.88	0.85	4.89	0.75
Treatment	10	5.02	1.18	4.17	0.86	4.17	0.75

Statistics for the two toothbrushes in each treatment group and for toothbrush 2 adjusted for the log CFU values on Toothbrush 1 based on an ANCOVA. Toothbrush 1 was always a cold water rinse brush (regardless of group). Toothbrush 2 was cold water rinsed in the control group and Violight treated in the treatment group. The data are for log transformed CFU's. Visually looking at the difference in mean values, there was little difference in brush 1 and 2 in the control group but a reduction in the treatment group.

Table 2. ANCOVA. Total CFU.

Source	Type III sum of squares	df	Mean square	F	P value
Baseline	4.008	1	4.008	7.155	0.015
Group	2.822	1	2.822	5.038	0.037
Error	10.643	19	0.560		

The dependent variable was the log CFU on Toothbrush 2. The independent factor was group and the covariate was the log CFU on Toothbrush 1 (a baseline value).

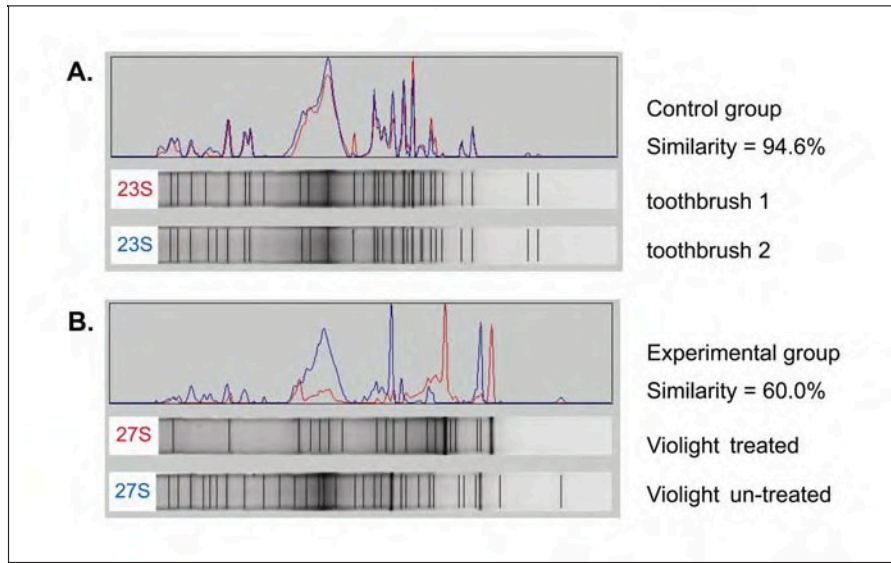


Fig. 3. Comparison of DGGE profiles between the controls (A) and experimental toothbrushes (B). The pairwise similarity was 95% for the controls, suggesting no changes in bacterial population between the two samples. The similarity was 60.0% for the experimental pair, indicating a 40% reduction in the bacterial population after Violight treatment.

Americans, four Hispanics, two Asians and one other ethnic group. One subject failed to return and was excluded from the final data analysis. The control and treatment groups were balanced for age ($P=0.295$), ethnicity ($P=0.805$), and gender ($P=0.332$).

Twenty-four of the 25 subjects were able to return their toothbrushes on day 13 and 14 in sealed labeled bags as instructed. Bacteria were extracted from the toothbrushes and used to determine CFU or for DGGE analysis. *Streptococcus mutans* was cultured from only two subject's toothbrushes and lactobacilli were cultured from only one subject's toothbrush, therefore no further analyses were performed for these two species. Log transformation of *E. coli* and *S. salivarius* and

subsequent ANCOVA analysis revealed no statistically significant differences between the experimental and control groups ($F=1.05$, $df=1$, $P=0.32$). However, an 86% reduction in the CFU of the total bacterial counts was observed between the Violight and control groups. An approximate 10-fold mean reduction from 104,713 to 14,791 CFU was observed in the Violight treated group (Table 1). ANCOVA was used to adjust for the individual specific CFU counts in the untreated situation compared between the second untreated toothbrush in the control group to the treated (Violight) toothbrush in the experimental group. As reported in Table 2, there was a statistically significant difference ($F=5.04$, $df=1$, $P=0.037$) in the log CFU of the total bacterial counts between the control and experi-

mental groups. Finally, a comparison of the difference between the toothbrushes for both the control and experimental groups was determined for each individual. The results in Fig. 2 show that the number of bacterial counts on the treated toothbrushes decreased in the experimental group compared to that in the control group.

To further determine the efficiency of the above bacterial extraction method and the Violight treatment, a pair of toothbrushes were analyzed using DGGE. No bacterial DNA samples were detectable by PCR after extraction for culture, suggesting the extraction procedure was highly efficient. A second two pairs of toothbrushes from two subjects in the control group were directly examined for bacterial population profile using DGGE without cultivation. The comparison was conducted between the toothbrushes before and after regular water rinsing, and the two bacterial profiles obtained were found to be highly similar (94.6% concordance) (Fig. 3). A third pair of toothbrushes was obtained from a subject in the experimental group. The bacterial profile analysis showed that the similarity was only 60% (Fig. 3) suggesting a reduction in bacterial contamination may occur after using the Violight toothbrush holder.

Discussion

The paired toothbrush design used in this study was developed to determine if the Violight toothbrush holder can reduce the bacterial load remaining on toothbrushes after use. This study, using both conventional bacterial culture methods and more sensitive PCR-based non-culture methods, demonstrates that the Violight group had an average reduction of 86% in the bacterial load (Fig. 2, left side, Fig. 3 bottom). This finding was further supported by an approximate 40% decrease in bacterial population by the DGGE analysis.

Decreasing the total bacterial load in the oral cavity, as well as decreasing specific bacterial species, is a fundamental therapeutic approach designed to decrease the incidence and severity of gingival inflammation, caries and oral malodor. Although few studies have examined whether bacteria transferred to a toothbrush can be a source for oral re-colonization, studies have shown that bacteria from the oral cavity and other sources can be found on toothbrushes and use of various chemical products can decrease the bacterial load on the toothbrush.⁹⁻¹² Despite evidence demonstrating that chemical rinses and dentifrices can reduce the total bacteria load on a toothbrush, these methods are not widely in use. Possible reasons for poor compliance include ease of use, the need for additional procedures after brushing or unwanted product residues. The results of this study suggest that a UV light toothbrush holder can effectively reduce by an average of 86% total cultivatable bacteria on a toothbrush. This result was supported by the DNA-based DGGE technique. Since the device requires no additional input from the subject other than pushing a button to activate the device, it is possible that the ease of use of the Violight toothbrush holder will increase patient compliance while reducing between use bacterial load on toothbrushes.

It is important to note the limitations of the present study. First, bacterial culture demonstrated that one subject had an

increase in the total cultivatable bacteria with the Violight treated toothbrush compared to the untreated toothbrush (Fig. 2). One possibility for this anomalous result could be that the subject had poor compliance in treating the toothbrush with Violight toothbrush holder prior to delivering it to the laboratory. Although subjects were asked if there were any problems using the Violight toothbrush holder, the study design did not include a questionnaire or any other means to evaluate that the treated toothbrush was indeed treated before being returned for bacterial analysis. Second, as *S. mutans* and lactobacilli are associated with caries initiation and progression, we were interested to determine the effect of the Violight toothbrush holder on these species. However, this study observed low detection rates for both *S. mutans* and lactobacilli. However, both species are known to have low retention rates on toothbrushes.^{24,25}

E. coli is not generally considered to be a common resident of the oral cavity, yet it and other common enteric bacteria have been found in the oral cavity by other studies.^{26,27} The presence of *E. coli* is more common in patients with periodontal disease, but not exclusively so.²⁶ *E. coli* and other enteric bacteria are found as pathogens in elderly subjects with hospital acquired pneumonia.²⁷ Despite some association with disease, it is important to remember that many strains of *E. coli* are avirulent, so that the mere presence of these bacteria may not necessarily be an indicator of disease.

Finally, reducing the bacterial load and population in the oral cavity is generally thought to be beneficial to oral health by reducing caries, gingivitis and oral malodor, as well as promoting general health. Yet, data in support of this contention are limited.⁴ While this study demonstrates that the Violight toothbrush holder reduces both the bacterial load and overall population on toothbrushes, this result should not be extrapolated to conclusions about the effect of the Violight toothbrush holder on oral or general health. Further clinical studies will be required to determine the effect of reduction of toothbrush bacterial load and diversity on oral and general health.

- a. Violight Inc., Elmsford, NY, USA.
- b. Difco, Detroit, Michigan, USA.
- c. Sigma, St. Louis, MO, USA.
- d. Epicenter, Madison, WI, USA.
- e. PE Applied Biosystems, Foster, CA, USA.
- f. Alpha Innotech Corporation, San Leandro, CA, USA.
- g. Bio-Rad Laboratories Inc., Hercules, CA, USA.
- h. SPSS, Chicago, IL, USA.

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