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Evaluation of two alternative methods for disinfection of toothbrushes and tongue scrapers

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Abstract: *Objective:* The aim of this study was to investigate the effectiveness of two alternatives methods for the disinfection of oral cleaning devices. *Methods:* One type of toothbrush and two types of tongue scrapers (steel and plastic) were tested in this study. Sixteen specimens of each group were cut with standardized dimensions, contaminated separately with *Candida albicans*, *Streptococcus mutans* and *Staphylococcus aureus* and incubated for 24 h. After this, oral cleaning devices were washed in saline solution to remove non-adhered cells and divided into two groups (n = 8), one irradiated in microwave and other immersed in 3.78% sodium perborate solution, and evaluated for microbial recovery. The values of cfu of each group of microorganism after disinfection were compared by Kruskal–Wallis and Dunn non-parametric test, considering 95% of confidence. *Results:* The toothbrush harboured a significant larger number of viable organisms than the tongue scrapers. The steel tongue scraper was less susceptible to adhesion of the three oral microorganisms. The time required to inactivate all contaminating microorganisms using microwave oven was 1 min and, for the immersion in 3.78% sodium perborate solution, was 2 and 3 h, respectively, for *C. albicans* and *S. mutans/S. aureus*. *Conclusion:* Microwave irradiation proved to be an effective alternative method to the disinfection of tongue cleaners and toothbrushes.

Key words: disinfection; microwaves; sodium perborate; tongue scrapers; toothbrushes

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Introduction

Oral cavity harboured over 700 different bacterial species, besides fungi and transient microorganisms, that may or may not cause infectious diseases (1). The anatomical features of tongue dorsum promote the accumulation of food remnants, exfoliated cells, saliva components that can act as substrates to metabolism and growth of these microorganisms (2, 3). Volatile molecules are end products of bacterial metabolism that contribute to oral malodour, by putrefaction of sulphur-containing proteins, peptides and amino acids (2, 3). Tongue cleaning using a toothbrush or tongue scraper is recommended to remove oral debris and reduce microorganism proliferation (3, 4). After use, cleaning devices gets contaminated and, if are not disinfected, may be a reservoir of microorganisms that maintain their viability for a significant amount of time, ranging 24 h to 7 days (5–9). Microbial survival promotes reintroduction of potential pathogens in the oral cavity or dissemination to other individuals when cleaning devices are stored together or shared (10, 11). Consequently, this contamination may cause septicaemia and induce respiratory, gastrointestinal, cardiovascular and renal problems when carried pathogenic microorganisms (12).

Some studies have suggested the need for toothbrush disinfection to reduce remaining microbiota using different methods, including chemical agents (5, 13–17) and ultraviolet (UV) radiation (18). UV sanitizers eliminate almost 100% of the pathogens in <3 min (10, 18), but they are still very expensive to be indicated for general population. Among the chemical agents, chlorhexidine gluconate solutions (0.12%) have proved efficient toothbrush disinfection eliminating *Streptococcus mutans* (5, 16), *Candida albicans*, *Staphylococcus aureus* and *Streptococcus pyogenes* in 10 min (16). Antiseptic solutions such as sodium perborate, indicated for the cleansing of prostheses and orthodontic appliances, have demonstrated a significant reduction in some pathogenic microorganisms in relation to control without antimicrobials, but much less effective than chlorhexidine (19). Tongue scrapers are widely used in routine oral hygiene practices and present good results in reducing bacterial load and production of volatile sulphur compounds that cause halitosis (3, 17). However, no study has investigated the efficacy of disinfection methods of these devices when colonized by oral pathogens.

Microwave irradiation is extremely effective on microbial elimination and has been used to sterilize removable dentures contaminated with *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida albicans* after irradiation for 6–10 min (20–22). For toothbrushes and tongue scrapers, microwave irradiation could be a practice and low-cost disinfection method. The aim of this study was to investigate the effectiveness of two alternatives methods for disinfection of oral cleaning devices.

Materials and methods

Specimens

Steel (Berinox, São Carlos, SP, Brazil) and plastic (‘‘Odonto-B’’, Odontobrindes, São Paulo, SP, Brazil) tongue scrapers and adult toothbrushes (Colgate-Palmolive Ind. e Com. Ltda., São Bernardo do Campo, SP, Brazil) were tested. Both scrapers and toothbrushes were cut into 1-cm-long specimens and sterilized with ultraviolet light for 15 min. Each group was composed by sixteen specimens.

Microorganisms and growth media

Streptococcus mutans (ATCC 25175) was cultured in brain heart infusion broth (BHI, Acumedia, Michigan-EUA) anaerobically in candle jars at 37°C for 24 h. *Candida albicans* (ATCC 18804) and *Staphylococcus aureus* (ATCC 6538) were cultivated in Mueller–Hinton broth (MH, Acumedia, Michigan-EUA) aerobically at 37°C for 24 h. Microbial cultures were harvested for 10 min at 3500 *g*, and the pellet was washed twice in sterile phosphate-buffered saline. The microorganisms were then suspended in culture media and adjusted to about 10^7 colony-forming units (cfu) per ml, which was estimated using a spectrophotometer (BioPhotometer Eppendorf, AG 22331 Hamburg, Germany) and confirmed by plating in specific culture media for 24 h. Growth curves of each microorganism were obtained to determine the optic density (Abs = 600 nm) that corresponds to 10^7 cfu ml^{-1}.

Biofilm production

After UV exposure, two specimens were inserted in culture medium and maintained for 48 h to verify the efficacy of sterilization. Standard suspensions of 10^7 CFU ml^{-1} of *C. albicans*, *S. mutans* or *S. aureus* in culture medium were added separately in sterile 24-well plates. One specimen of the tongue cleaner fragments was inserted in each well to be contaminated, totalizing 16 specimens per group. The plates were then incubated in candle jars for *S. mutans* and aerobically for *C. albicans* and *S. aureus* at 37°C on an orbital shaker to allow the microorganisms to adhere to the specimens. After 24 h, specimens were transferred to another well and washed twice in sterile PBS to remove unattached microorganisms.

Specimen disinfection

The tongue cleaners were divided in two groups with eight specimens each and submitted to a two methods of disinfection: microwave irradiation and immersion in 3.78% sodium perborate solution. Other eight specimens were used as control and do not submitted to disinfection methods (time 0). For the first technique method, introduced by Neppelenbroek *et al.* (23), contaminated specimens were individually immersed in 200 ml of sterile distilled water and irradiated for 30 s and 1 min at 650 W in a domestic microwave oven (Sensor Crisp 38 DES; Brastemp, Manaus, AM, Brazil) to identify the minimum amount of time needed to disinfect the specimens by microwave irradiation. For the chemical disinfection, developed by Pavarina *et al.* (19), specimens contaminated with each microorganism were immersed in separate aliquots of perborate solution for 1, 2, 3 and 6 h to determine the minimum amount of time needed for disinfection in this solution. After the physical and chemical disinfection trials, specimens were washed three times in sterile phosphate-buffered saline, transferred to tubes containing 4.5 ml of sterile PBS and sonicated for 20 min to release the adhering microorganisms. The resultant suspension was serially diluted and spread on Sabouraud dextrose agar for *C. albicans*, mannitol-salt agar for *S. aureus* and sucrose-bacitracin (SB-20) agar for *S. mutans*. Plates were incubated for 48 h at 37°C in the atmospheric conditions described above. After this period, colonies were counted, and data obtained were converted into UFC ml^{-1}. The results obtained for each tongue cleaner group in different times were submitted to Kruskal–Wallis and Dunn non-parametrical tests at 95% confidence level, using the statistical program SPSS Statistics 17.0 (IBM Inc., Chicago, IL, USA).

Results

Tables 1 and 2 show medians (range) obtained for microbial counting after microwave and chemical method applications, respectively. Time 0 represents data obtained for non-disinfected specimens (control group). The toothbrush carried a larger number of viable microorganisms than either of the tongue scrapers ($P \le 0.05$). Stainless steel scrapers were less colonized by all tested microorganisms when compared to plastic scrapers ($P \le 0.05$). All microorganisms were inactivated after 1-min microwave exposure. *S. mutans* and *S. aureus* were eradicated from the specimens after 3 h of immersion in 3.78% sodium perborate. For *C. albicans*, this disinfectant was effective after 2 h.

Discussion

Tongue harbours a bacterial coating that may be a source for volatile sulphur compounds that are the major components of the oral malodour, mainly associated with gingivitis and periodontitis (24). To cleaning this site, tongue scrapers have been developed with specific forms to adjust to the anatomy of the tongue and gained new importance among the oral hygiene devices. Although most of the patients use toothbrushes to clean the tongue, yet it has been stated that using a regular toothbrush for tongue cleaning is inferior for removing debris and microorganisms from the tongue compared to scraping tools (25, 26). Some investigators showed the efficacy in reducing volatile sulphur compounds of tongue scrapers associated or not with toothbrushes when compared to toothbrush alone (17, 26, 27). Anyway, both tongue scrapers and toothbrushes may carry large quantities of viable pathogenic microorganisms after use. In this present study, the toothbrush carried significantly higher numbers of viable organisms than the tongue scrapers. Variation in microbial adhesion to the specimens may be attributable to morphological differences and the adhesion-specific features of each strain (27). Previous studies have described microbial adhesion to tongue cleaners and toothbrushes (6, 28, 29). Spolidorio *et al.* (6) demonstrated that a toothbrush's surface provides favourable conditions for microbial adhesion, thus actuating as a reservoir for pathogens. Toothbrushes may be contaminated by streptococci for long periods, making them potential vehicles for bacterial transmission (29, 30). Scrapers are made from various materials, including stainless steel- and polystyrene-based injection-moulded plastic. Saliva is capable of contaminating metal or plastic for a considerable length of time (28). This study showed that *C. albicans*, *S. aureus* and *S. mutans* can adhere in both types of tongue scrapers, but the stainless steel was less susceptible to microbial colonization. Strongest microbial adhesion is related to roughness and hydrophobicity of material surface (31). It was suggested that because steel is considered less rough and hydrophobic when compared to plastic material, it causes less bacterial colonization.

Disinfection of plastic and metal tongue cleaners by microwave irradiation for 1 min at 650W was successful for all tested microorganisms, because no microbial growth was seen in any of the experimental groups. Neppelenbroek *et al.* (22, 23) and Silva *et al.* (32) evaluated this method to disinfected complete dentures and observed that appropriate microwave irradiation was effective not only for disinfection but also for sterilization of acrylic resins. Studies evaluating this method for tongue cleaners, including scrapers and toothbrushes, were not found. The mechanism by which this irradiation method eliminates microorganisms is still unclear, but some authors have hypothesized that the heat generated causes sterilization (33–36). However, other studies have suggested that additional factors may also be responsible for disinfection (20, 21, 34, 37). For example, specific microwave frequencies may be absorbed by certain important biological molecules, such as nucleic acids, causing cell death. Other possible mechanisms include changes in the selective permeability and molecular resonance of cell membranes, with the latter resulting in cleavage (20, 37).

Table 1. Median **(range) of microorganisms (UFC $ml^{-1} \times 10^4$) surviving after exposure to microwave radiation**

Time (s)	Plastic scraper			Steel scraper			Toothbrush		
	Ca	*Sm*	*Sa*	*Ca*	*Sm*	*Sa*	*Ca*	*Sm*	*Sa*
0	0.34 (0.3 – 0.4)^a	0.4 (0.3–0.5)^a	12 (1.9–26)^a	0.12 (0.1–0.16)^a	0.1 (0.06–0.2)^a	0.22 (0.18–0.24)^a	1.3 (1.2–6.2)^a	190 (160–210)^a	940 (150–2700)^a
30	0 (0–0.3)^b	0 (0–0.5)^b	1.5 (0.1–2.5)^b	0 (0–0.1)^b	0 (0–0.18)^b	0 (0–0.2)^b	1.55 (0.8–3)^b	1.05 (0.6–1.9)^b	0.08 (0.016–0.27)^b
60	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c

Different superscript letters in the columns denote statistical difference in the frequency of microorganisms among the tested periods (Kruskal–Wallis and Dunn tests, *$P \le 0.05$). Ca, C. albicans; Sm, S. mutans; Sa, S. aureus.*

Table 2. Median **(range) of microorganisms (UFC $ml^{-1} \times 10^4$) surviving after immersion in 3.78% sodium perborate solution**

Time (h)	Plastic scraper			Steel scraper			Toothbrush		
	Ca	*Sm*	*Sa*	*Ca*	*Sm*	*Sa*	*Ca*	*Sm*	*Sa*
0	0.34 (0.3 – 0.4)^a	0.4 (0.3–0.5)^a	12 (1.9–26)^a	0.12 (0.1–0.16)^a	0.1 (0.06–0.2)^a	0.22 (0.18–0.24)^a	1.3 (1.2–6.2)^a	190 (160–210)^a	940 (150–2700)^a
1	0 (0–0.7)^b	0.4 (0.3–0.5)^a	12 (1.9–26)^a	0 (0–0.1)^b	0.1 (0.06–0.2)^a	0.22 (0.18–0.24)^a	2.9 (0.12–5)^b	190 (160–210)^a	940 (150–2700)^a
2	0 (0)^c	1.03 (0.12–3)^b	2 (1.6–2.5)^b	0 (0)^c	0.03 (0–1)^b	0.06 (0–0.8)^b	0 (0)^c	21^b (2.4–160)	1.26(0.18–4.3)^b
3	0 (0)^c	0 (0)^d	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c	0 (0)^c

Different superscript letters in the columns denote statistical difference in the frequency of microorganisms among the tested periods (Kruskal–Wallis and Dunn tests, *$P \le 0.05$). Ca, C. albicans; Sm, S. mutans; Sa, S. aureus.*

Immersion of tongue cleaners in 3.78% sodium perborate solution has been also investigated (19). It has been reported that products based on alkali peroxides, such as sodium perborate, liberate oxygen on contact with water, resulting in effervescence that has a mechanical cleansing effect. Additionally, these substances are powerful oxidizing agents, resulting in potent antimicrobial effects (38). In this study, two tongue scrapers and a toothbrush were successfully disinfected by immersion in sodium perborate for 2 h for *C. albicans* and 3 h for *S. mutans* and *S. aureus*. Results from earlier studies have showed similar chemical products based in alkaline peroxides, effectively disinfecting dentures after more than 30 min of immersion (39, 40). Another study has reported that biofilm formation on the surface of complete dentures was greatly reduced following immersion in 3.78% sodium perborate for 10 min (19). Paranhos *et al.* (41) also evaluated the immersion of acrylic specimens in an alkaline peroxide solution followed by brushing with a dentifrice and observed a reduction in CFU for biofilms of *S. aureus, S. mutans* and *P. aeruginosa*. Recently, Komiyama *et al.*(16) evaluated 0.12% chlorhexidine digluconate, 50% white vinegar, a triclosan-containing dentifrice solution and a perborate-based tablet solution for the disinfection of toothbrushes. These investigators observed that sodium perborate was the less effective against *Streptococcus mutans*, *Streptococcus pyogenes*, *Staphylococcus aureus* or *Candida albicans*. Triclosan and chlorhexidine solutions reduced significantly all tested microorganisms, and vinegar reduced some of them. Other studies proved the high efficacy of chlorhexidine to clean toothbrushes (15, 16), but this substance is considered expensive, thus limiting its widespread use by population (16). For tongue scrapers' disinfection, no chemical solution was tested yet.

It was concluded that the steel tongue scraper is less susceptible to the adhesion of the tested microorganisms (*C. albicans, S. mutans* and *S. aureus*). This study also determined that microwave irradiation proved to be an effective alternative method to the disinfection of tongue cleaners and toothbrushes.

Conflict of interest

The authors declare that they have no conflict of interests.

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