
Survival Rate of Oral Bacteria on Toothbrush and Miswak Stick

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Abstract: Introduction: Oral hygiene aids including toothbrushes if not rinsed in a proper disinfectant can affect oral bacterial translocation and re-infection of the oral cavity due contamination. Aim of the study: The aim of this study was to investigate the survival rate of total oral bacteria on toothbrush and miswak. Material and methods: Totally, 12 young individuals with age range 22-28 years and with 20 more remaining teeth in the oral cavity participated in this study. These individuals were asked to brush one side of their mouth with miswak stick and the other side with a nylon tooth brush (Orange toothbrush No: 106A China). 6 bristles from a tuft of each toothbrush and equivalent amount of fibers from each miswak stick were cut immediately after brushing for 2 min and serially diluted in a nutrient broth. The bacterial suspension was inoculated in agar plates and incubated for overnight. The used toothbrushes and miswak sticks were stored in sterile containers at room temperature and the experiment was then repeated after 24 hours of storage. The survival rates of oral bacteria were then calculated by comparing the total bacterial counts at day one and 24 hours after storage. Results: Miswak sticks harbored an average of 845.6 total oral bacterial counts and 523.7 the toothbrush respectively at a day one. After 24 hours of storage, toothbrush harbors statistically significant $p < 0.05$ more total bacterial counts as compared with miswak stick. Conclusion: The total oral bacterial survival rate on miswak was significantly reduced as compared with a toothbrush, thus the use of miswak after 24 hours can limit the risk for oral bacterial contamination and translocation. This is the first *in vivo* study which shows bacterial survival rate on miswak.

Keywords: Bacterial Carriage, Miswak, Toothbrush, Survival Rate of Bacteria

1. Introduction

The 1999 Consensus Statement on Oral Hygiene has concluded that dental plaque biofilm a primary etiological agent for dental caries, gingivitis and periodontitis; that its regular cleaning and removal on daily basis is recommended for prevention or reduction of these diseases; that miswak chewing sticks may have a role in the promotion of oral hygiene; and that assessment of the effectiveness of miswak chewing sticks requires further research [1]. Thus, the mechanical oral hygiene methods are reliable means of controlling plaque biofilm, provided cleaning and brushing are sufficiently thorough and performed at regular intervals.

Toothbrush after brushing has shown to be heavily contaminated with plaque biofilm. However, depending upon

where we keep toothbrushes after use, the toothbrush can serve as a source for reintroduction of oral bacteria into the mouth [2]. Different microorganisms from storage environments can also be involved, which include enteric bacteria via aerosol near toilet flushing or from contaminated containers or fingers.

It has been recommended that toothbrushes after use should be rinsed in disinfectants like Chlorhexidine gluconate 0.2% before storage [3]. Later, it has been shown that drying of toothbrush in sunlight, table salt to absorb their moisture and/or keeping the brush in a closed container with preparation containing formaldehyde for its disinfection is important procedures [4, 5].

Miswak chewing sticks are used for tooth cleaning by many communities around the world. Reasons for continuing the use of miswak include low cost, availability and or tradition [6]. Moreover, it has been indicated that miswak released pleasant chemicals during chewing and cleaning. A large body of miswak research has not highlighted the mechanism of action of miswak. However, it has mentioned that the chemical components released during cleaning caused oral health promotion and disease prevention. Potential of miswak chemical components releasing during chewing may reduce potential of contamination of miswak [7]. It has been postulated that thiocyanate leaching during cleaning can activate salivary peroxidase thiocyanate hydrogen peroxide antimicrobial system [7].

The cleaning efficacy of miswak is shown to be comparable with that of manual toothbrush or indicated plaque scores to be significantly lower following the use of miswak as compared with the manual toothbrush used without toothpaste. In a controlled study, Kenyan school children brushed their teeth for 5 min at a time using chewing sticks with or without toothpaste [8]. Compared with the baseline data, the brushing under supervision resulted in reduction of plaque deposit proration [9]. Therefore, toothpaste used along with toothbrush did not seem to enhance plaque removal efficiency of the toothbrush.

Toothbrushing generally has only one mode of action, the mechanical removal of dental plaque. However, toothbrushes are also used as a vehicle for supragingival delivery of anti-plaque agents and fluoride incorporated into different toothpastes. The use of toothpaste is such a complementary part of toothbrushing behavior that it cannot be ignored [10]. Thus, toothbrushing with toothpaste was arguably the most common form of tooth cleaning practiced by individuals in developed countries. Chewing sticks are often used as the sole cleansing agent in the developing countries. The Consensus Statement on Oral Hygiene states that tooth brushing and other mechanical methods, including miswak chewing sticks are the most reliable means of controlling plaque, provided that the cleaning is sufficiently thorough and performed in regular base. However, since most studies on miswak lack specific details concerning the oral bacterial contamination it has been difficult to assess the effect of miswak chewing sticks carriage of oral bacteria during storage. The aim of this study was to investigate the survival rate of oral bacteria on toothbrush and miswak sticks using bacterial culture procedures.

2. Materials and Methods

At the dental clinic of the college of dentistry, University of Jazan, 12 young individuals with age range 22-28 years and with 20 more remaining teeth in the oral cavity were asked to brush one side of their mouth with miswak sticks and the other side with a nylon toothbrushes (Orange toothbrush No:106A China). Both oral hygiene devices were then not rinsed with water, and carried in sterilized containers to the laboratory of oral microbiology at the college of

dentistry. 6 bristles of a tuft from each toothbrush and equivalent amount of fibers from each miswak stick were cut immediately after brushing for 2 min and placed in sterile nutrient broth (Oxoid U.K) which composed of 1gram lab-lemco, 2gram yeast extract, 5gram peptone and 5 gram sodium chloride. The medium was prepared by dissolving thirteen grams of dehydrated nutrient broth in one liter of distilled water, mixed up well, the pH was adjusted to 7.4 and the medium was distributed in 5ml amount into test tubes and sterilized by autoclaving at 121°C for 15 minutes. Following serial dilution, the bacterial suspension was used to inoculate nutrient agar (Oxoid U.K) plates for counting of the total bacteria colony forming units. The medium is composed of 1gram lab-lemco, 2gram yeast extract, 5gram sodium chloride, 5gram peptone and 15 gram agar. It was prepared by adding 28gram of dehydrated nutrient agar to one liter of distilled water, and steamed to dissolve completely. The pH was adjusted to 7.54 and the medium was sterilized at 121°C for 15 minute, then aseptically poured into sterile plates (in 20ml amounts) or distilled in 5ml amount into sterile screw-capped bottles and allowed to set in slop position. The used brushes and miswak sticks were stored in sterile containers at room temperature for 24 hours and the same experiment was then repeated. Survival rates were calculated by comparing counts of colony forming units at day one and 24 hours storage. Boiled miswak stick for 2 hours was used as a control.

3. Results

At a day one, miswak harbored more total oral bacteria counts as compared with toothbrush with average total oral bacteria counts, colony forming units (CFU) 845.6 and 523.7 for toothbrush, respectively at 1/10 concentrations. Table 1 shows average numbers of total oral bacterial counts on toothbrush and miswak according to serial dilutions at day one after brushing, this table shows the 3 experiments of both samples. After 24 hours storage toothbrush harbors significantly more total bacterial counts 195.3 CFU and 1.5 CFU for miswak respectively at 1/10 concentrations. Table 2 shows average numbers of total oral bacterial counts on toothbrush and miswak according to serial dilutions after 24 hours of storage. When we used boiled miswak as a control and toothbrush the results at a day one show that toothbrush harbored less total oral bacteria counts as compared with boiled miswak with average total bacteria counts 883.4 and 559.1 for the toothbrush, respectively at 1/10 concentrations. Table 3 shows average numbers of total oral bacterial counts on toothbrush and boiled miswak according to serial dilutions at day one after brushing, this table shows the 3 experiments of both samples. When we used a control, boiled miswak and toothbrush the results after 24 hours storage show that toothbrush harbored average 648.5 CFU less than boiled miswak sticks 1061.4 respectively at 1/10 concentrations. Table 4 Average numbers of total oral bacterial counts on toothbrush and boiled miswak stick according to serial dilutions after 24 hours storage (control).

Table 1. Average numbers of total oral bacterial counts on toothbrush and miswak according to serial dilutions at day one after brushing.

Toothbrush					MiswaK				
Dilutions	1/10	1/100	1/1000	1/10000	Dilutions	1/10	1/100	1/1000	1/10000
Expriment1	343.6	20.1	1.7	0.3	Expriment1	730.6	26.7	3	0.0
Expriment2	805.6	61.3	26.6	3.3	Expriment2	1021	711	153.3	34.3
Expriment3	422	54.5	3.6	1	Expriment3	785.3	253	27	3.6

The p-value is .064144. The result is not significant at $p < .05$.

Table 2. Average numbers of total oral bacterial counts on toothbrush and miswak according to serial dilutions after 24 hours of storage.

Toothbrush					MiswaK				
Dilutions	1/10	1/100	1/1000	1/10000	Dilutions	1/10	1/100	1/1000	1/10000
Expriment1	124.6	6	0.7	0.0	Expriment1	1.7	0.0	0.0	0.0
Expriment2	282.6	42	4	0.0	Expriment2	1.3	0.0	0.0	0.0
Expriment3	178.6	8	0.0	0.0	Expriment3	1.6	0.0	0.0	0.0

The p-value is .006968. The result is significant at $p < .05$.

Table 3. Average numbers of total oral bacterial counts on toothbrush and boiled miswak stick according to serial dilutions at day one after brushing (control).

Toothbrush					MiswaK				
Dilutions	1/10	1/100	1/1000	1/10000	Dilutions	1/10	1/100	1/1000	1/10000
Expriment1	543.6	18.1	1.6	0.3	Expriment1	863.3	434.6	82.6	18.6
Expriment2	705.6	41.3	20.6	3.3	Expriment2	965.3	508.3	92.5	20.7
Expriment3	428	50.5	2.6	1	Expriment3	821.6	435.3	77.3	16.3

The p-value is .011801. The result is significant at $p < .05$.

Table 4. Average numbers of total oral bacterial counts on toothbrush and boiled miswak stick according to serial dilutions after 24 hours storage (control).

Toothbrush					MiswaK				
Dilutions	1/10	1/100	1/1000	1/10000	Dilutions	1/10	1/100	1/1000	1/10000
Expriment1	696.6	14.3	0.0	0.0	Expriment1	1075	475.3	40.1	21.3
Expriment2	718.3	6.6	0.0	0.0	Expriment2	1120	325.6	44.5	28.1
Expriment3	530.5	4.1	0.0	0.0	Expriment3	989	299.3	56.3	24.6

The p-value is .002141. The result is significant at $p < .05$.

4. Discussion

Dental caries and periodontal disease are contagious diseases that can be transmitted through contaminated instruments [11]. The reuse of toothbrush during sometime of a day for tooth cleaning and/or tongue cleansing can be a source of contamination and translocation of plaque bacteria. Careless storage of this device can increase its bacterial carriage during storage because of bacterial growth which can increase the chance of infection and intraoral location after use [12, 13, 14].

The results obtained in this study showed that there were many types of microorganism’s colony forming units that were grown on the toothbrush and miswak. The results show that at day one toothbrush harbored less colony forming units as compared with miswak, this can be explained by that the bristles of toothbrush are smoother than the fibers of miswak which harbored more oral bacteria on the rough surfaces. However, this result has been changed after storage of booth devices for 24 hours. Thus, statistically significant high numbers of colony forming were grown on toothbrush as compared with miswak after storage.

The growth of total bacteria on toothbrush is in agreement with other studies [12, 13]. However, the total counts found

on toothbrush bristles and miswak fibers were dislocated from plaque biofilm during brushing; moreover, other types of microorganisms might also grown as total bacterial colony forming units. Previously, it has been demonstrated that streptococci are the largest numbers of microorganisms found on the toothbrushes that were kept in air for 24 hours, this group represents the most common hygienic measure that is undertaken by the majority of individuals with their toothbrushes without immersing in any antimicrobial solution, and the same results were seen in previous studies [11]. As shown in our results, there was a significant reduction in the mean number of total colony forming units in miswak as compared with toothbrush. The finding of this study is with agreement of our previous observation (Darout *et al.* 2014 unpublished work).

Some *in vitro* studies have shown that miswak extracts inhibited growth of various oral aerobic and anaerobic bacteria as well as *Candida albicans* [15]. Inhibition of *in vitro* plaque formation, growth and acid production of various cariogenic bacteria by such extracts has also been demonstrated. In addition, aqueous extracts of miswak bark, pulp as well as whole miswak were effective against various bacteria including *Streptococcus mutans* [16]. Miswak antimicrobial components may be released during chewing

which has growth inhibitory effect of many species from dental plaque [17, 18]. Our study shows that miswak chewing stick reduces the mean number of colony forming units during storage as compared with toothbrush. It has been shown that there was no noticeable difference in antimicrobial effect between fresh and one-month-old miswak [16]. Miswak contains many chemical components which can be activated while in the mouth by saliva, for instance, Cl⁻ leaching into saliva from miswak may mediate the innate host defense systems in human saliva. Cl⁻, I⁻ and SCN⁻ (pseudohalides) are substrates for salivary peroxidase and/or the myeloperoxidase hydrogen peroxide antimicrobial system. The peroxidase-hydrogen-peroxide-chloride system is a part of the innate host defense that is mediated by polymorphonuclear leukocytes in humans [18]. It has been shown that the latter system was more bactericidal against *Aggregatibacter actinomycetemcomitans* than with the myeloperoxidase-thiocyanate and hydrogen peroxide system. Recently, it has been indicated that the oxidation product of lactoperoxidase and myeloperoxidase with I⁻ and/or Cl⁻ was bactericidal against *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Streptococcus mutans* [19]. The lower levels of *Prevotella intermedia* and *Fusobacterium nucleatum* in the miswak users may be attributed to its Cl⁻ and SCN⁻ content. Oral hygiene aids including toothbrush if not stored in a proper disinfectant can affect oral bacterial translocation and reinfection of the oral, thus the bacterial survival rate on miswak was significantly reduced as compared with a toothbrush, and therefore the use of miswak after 24 hours can limit the risk for oral bacterial contamination and translocation. The use of more advanced bacterial detection technology as DNA-DNA hybridization methods will be more suitable to study the oral bacterial counts on toothbrush and miswak sticks especially after storage for 24 hours.

5. Conclusion

Oral hygiene aids including toothbrushes if not rinsed in a proper disinfectant can affect oral bacterial translocation and re-infection of the oral cavity due contamination. Oral microorganisms on toothbrushes can grow and increase in number after 24 hours. Our study results indicated that, the total oral bacterial survival rate on miswak was significantly reduced as compared with a toothbrush, thus the use of miswak after 24 hours can limit the risk for oral bacterial contamination and translocation. This is the first *in vivo* study which shows bacterial survival rate on miswak.

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