Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.38995-39000, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

AN IN –VIVO STUDY ON REDUCTION OF BACTERIAL CHALLENGE AND BRISTLE PROTECTION WITH ANTIBACTERIAL TOOTH BRUSH (MISWAK HERB) IN COMPARISON WITH NANO-SILVER TOOTH BRUSH

***,[1]Mathew Asok, [2]Amira Hafez Azouz, [3]Nader Ahmed, [4]Ahmed Al- Radaideh, [5]Shibu Thomas, [6]Sudhir Varma and [7]Nisha T Varghese**

[1, 5, 6]Faculty, College of Dentistry, Ajman University of Science and Technology Al Fujairah
[2]General Practioner, Ajman University of science and technology Al Fujairah
[3]Faculty, College of pharmacy, Ajman University of science and technology Al Fujairah
[4]Faculty, College of requirements Ajman University of science and technology Al Fujairah
[7]Resident, Hamdan bin Mohammed College of dental medicine Dubai

ARTICLE INFO

Article History:
Received 29th June, 2016
Received in revised form
22nd July, 2016
Accepted 17th August, 2016
Published online 30th September, 2016

Key words:

Miswak,
Silver nanotechnology,
Streptococcus mutans,
Mitis salivarius.

ABSTRACT

Background: Oral health is an integral part of general health. It directly and indirectly reflects the overall well-being of an individual, thus maintaining oral hygiene becomes a crucial factor.Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. Regular toothbrush care and maintenance are also important considerations for sound oral hygiene. This study was designed to evaluate the antibacterial bristles self-protection among the Miswak stick, toothbrush with silver nanotechnology and regular toothbrush (as a control group).

Materials and Methods: Thisstudy was conducted in vivo by randomly selected 30 volunteers without any reported systemic diseases and habits.Theyare divided into 3 group 10 in each group first group was given Miswak stick, the 2nd group was given regular toothbrush, and the last group was given silver care nano-silver coated toothbrush. Each subject was instructed to brush their teeth for 3min after signing a patientconsent. Part of the bristles was collected in different time duration to evaluate the antibacterial bristles protection among different kinds of brushes.

Results: It was shown that the silver care toothbrush has the maximum bacterial reduction in the period of 24hrs followed by Miswak stick and the last was the regular toothbrush which showed minimum bacterial reduction.

Conclusions: The present report indicates that silver tooth brush had the highest antibacterial self-bristles protection and the Miswak had moderate effect and the regular toothbrush had minimum effect.

Citation: Mathew Asok, Amira Hafez Azouz, Nader Ahmed *et al*. 2016. "An in –vivo study on reduction of bacterial challenge and bristle protection with antibacterial tooth brush (Miswak herb) in comparison with nano-silver tooth brush", *International Journal of Current Research*, 8, (09), 38995-39000.

INTRODUCTION

Oral health is an integral part of general health. It directly and indirectly reflects the overall well-being of an individual, thus maintaining oral hygiene becomes a crucial factor (Karibasappa, 2011). Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. Appropriate toothbrush care and maintenance are also important considerations for sound oral hygiene (Svanberg, 2011). Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene. Oral hygiene aids have been in use since Sumerian times.

Corresponding author: Mathew Asok,
Faculty, College of Dentistry, Ajman University of Science and Technology Al Fujairah

Brushing teeth is the primary mode of oral hygiene practice. In earlier days, chewing sticks like Miswak, Neem and Babul were the sole oral hygiene aids used by different populations. In 1844, the first toothbrush was manufactured by hand and patented as a three-row brush of serrated bristles with large tufts by Dr. Meyer. L. Rhein (Karibasappa, 2011). The ADA recommends that consumers replace toothbrushes approximately every 3–4 months or sooner if the bristles become frayed with use (Sun *et al.,* 2005). In the case of the toothbrush; the pH of the toothbrush is usually irrelevant because most of the microbes come from the mouth, and are conditioned to survive a wide range of pH levels (Larsen, 2010). Due to the basic chemical properties in toothpaste, the pH of the toothbrush may become more basic. Thus, if the optimal growth of bacteria is at low pH then the toothpaste will disrupt the community, but at the same time has very minimal

effect on those who favor higher pH. The pH of a toothbrush is strictly determined by the chemical residues left behind after brushing which is the toothpaste itself with other minor factors (Schrack *et al.,* 2013). The pH level then changes again if the toothbrush is thoroughly washed with tap water. The pH will generally fall within 6-8, the pH of tap water. Once the toothbrush dries, the microbes that would normally not survive within pH of 6 to 8 are no longer affected by the basicity of the water. The common understanding that the pH level has a great effect on bacteria is true but also misleading. Biofilms can be defined as communities of microorganisms attached to a surface. It is clear that microorganisms undergo profound changes during their transition from planktonic (free-swimming) organisms to cells that are part of a complex, surface-attached community. During micro-colony formation, cells undergo developmental changes which give rise to the complex architecture of the mature biofilm. Paramount among these changes is the production of the exo-polysaccharide (EPS) matrix, one of the hallmarks of a mature biofilm. Toothpaste resulted in lower tooth brush contamination than without toothpaste use although the use of tooth paste will not appeared to exhibit complete inhibition of pathogenic bacterial contamination of the toothbrush (Schrack *et al.,* 2013).

In addition, a toothbrush's niche can be affected by another toothbrush in close proximity. This is called cross contamination and occurs in a communal environment, since a typical household uses a holder to store multiple toothbrushes. Bacteria living on one toothbrush can thus be transferred to another nearby toothbrush through contact. The toil*et al*so harbors a community of bacteria that can be partially transferred onto the toothbrush. Results show that during the first two hours after flushing, bacteria are mostly found in a limited area near the toilet. Mehta *et al*. found that the use of a cap for toothbrush storage increased bacteria survival (Mehta *et al.,* 2007). Efstratiou *et al*. found that filament type affected bacterial retention Toothbrushes with bristles that are frayed and arranged closely together trapped and retained more bacteria (Efstratiou *et al.,* 2007). Contamination was the lowest in soft and round, clear, two bristle row toothbrushes. Streptococcus mutans is facultative, anaerobic, Gram-positive coccus - shaped bacterium commonly found in the humanoral cavity and is a significant contributor to tooth decay (Wikipedia, 2013). The polysaccharide capsule has been identified as an important virulence factor for the group B streptococci (GBS), which are the leading cause of neonatal sepsis, pneumonia and meningitis in the United States and western Europe (Timothy and Mitchell, 2003). The beneficial effects of Miswak in respect of oral hygiene and dental health are partly due to its mechanical action and partly due to pharmacological actions. (Isolated benzyl-isothiocyanate from Salvadora persica root.The alkaloid present in Salvadora persicais Salvadorine, which yields trimethylamine on hyrolytical cleavage exerts a bactericidal effect and stimulatory action on the gingiva. Studies have indicated that Salvadora persica contain substances that possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria which are frequently found in the oral cavity. It was observed that the chewing sticks they used were harvested one month earlier, and suggested that using more fresh sticks will give better result (Al Bagieh *et al.,* 1997). A comparison of alcohol and aqueous extract of Miswak was also made. It was found that alcoholic extract is more effective than aqueous extract for antibacterial activity (Masoumeh *et al.,* 2012). The formulations of mouth washes available are found to cause a reduction in plaque. It can be used as endodontic irrigation solution. Abo Al Samh, evaluated, *in vitro,* the effect of different concentrations of Miswak extract on L929 cell-line in tissue culture and compared the results with sodium hypochlorite (NaOCI).They found a concentration dependent morphological change of L929 cell-line when exposed to Miswak extract and NaOCI (Abo Al-Samh *et al.,* 1997). Historically, silver metal has been used widely across the civilizations for different purposes. In ancient Indian medical system (Ayurveda) silver has been described as therapeutic agent for many diseases. There is an increasing use of silver as an efficacious antibacterial and antifungal agent in wound care products and medical devices including dental work and catheters (Humberto Lara *et al.,* 2011; Nowack and Harald, 2011). Metallic silver has also been used for surgical prosthesis and splints, fungicides, and coinage. Soluble silver compounds, such as silver salts, have been used for treating mental illness, epilepsy, nicotine addiction, gastroenteritis, stomatitis, and sexually transmitted diseases, including syphilis and gonorrhea. Additionally, $AgNO_3$, as eye drops, have been utilized to prevent gonococcal ophthalmic neonatorum in newborns by paediatricians for centuries (Humberto Lara *et al.,* 2011).

Other agents derived from silver, such as silver sulfadiazine (AgSD) cream, have been used by surgeons, as topical treatments to heal burn wounds, for the past 60 years. Utilizing these topical treatments, applied directly to the burn site, erythema decreased, while the expression of matrix metallo-proteinases (MMPs) increased.Silver nanoparticles (AgNPs), having a long history of general use as an antiseptic and disinfectant, are able to interact with disulfide bonds of the glycoprotein/protein contents of microorganisms such as viruses, bacteria and fungi.Both silver nanoparticles and silver ions can change the three dimensional structure of proteins by interfering with S-S bonds and block the functional operations of the microorganism (Humberto Lara *et al.,* 2011; Lara *et al.,* 2010). Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-100 nm[13] .

Although researchers and scientists are still exploring the specifics behind how the nano-sized silver (AgNP) releases silver ions, the nanoparticles, which are non-ionic, allow for these silver ions to be delivered more effectively and they provide a larger surface area for release. The biocidal potency of a silver additive is therefore directly related to the potential for releasing silver ions. It should be noted, however, that most commercial applications of nano-silver involve embedding the particles within a matrix such as a plastic or a coating (Lara *et al.,* 2010). As the size of silver metal is decreased from bulk through to micrometer-sized particles through to nano sized particles, the potential for releasing silver ions increases because of increasing surface availability per mass of silver and because both the solubility and dissolution kinetics of silver may vary as a function of size as silver metal size decreases. Recently, it has been suggested that nanoparticles bind with a viral envelope glycoprotein and inhibit the virus by

binding to the disulfide bond regions of the CD4 binding domain within the HIV-1 viral envelope glycoprotein gp120. This fusion inhibition was later elegantly demonstrated by Lara and colleagues in their latest report (Lara *et al.,* 2010). It is generally understood that Ag, in various forms, inactivates viruses by denaturing enzymes via reactions with sulfhydryl, amino, carboxyl, phosphate, and imidazole gr oups. It is generally understood that Ag, in various forms, inactivates viruses by denaturing enzymes via reactions with sulfhydra, amino, carboxyl, phosphate, and imidazole groups. However, it is necessary to design studies in vivo to increase therapeutic benefit and minimize adverse effects. Argyria is a condition characterized by a bluish-gray discoloration of the skin. The toxicity of silver is considered to be relatively low and toxic effects on humans other than Argyria are only observed at very high concentrations (Sun *et al.,* 2005).

MATERIALS AND METHODS

This clinical study was conducted in the student clinics of Ajman University of science and technology Al Fujairah. Thirtyvolunteers were selected with the agerange of 20-45 years with the following criteria. The inclusion criteria of the volunteers followed was such that they were nonsmokers without any habits, didn't had any systemic disease, and without any history of drugs. These 30 volunteers were divided into 3 groups, 10 in each group. Group A were given miswak stick, group B were given regular toothbrush and group C were given silver care tooth brush to use. Figure 1-3. Each subject was instructed to brush for 3 min without a tooth paste after signing a consentform. The silver care® tooth brush is manufactured by Spazzolificio Piave, Italy.

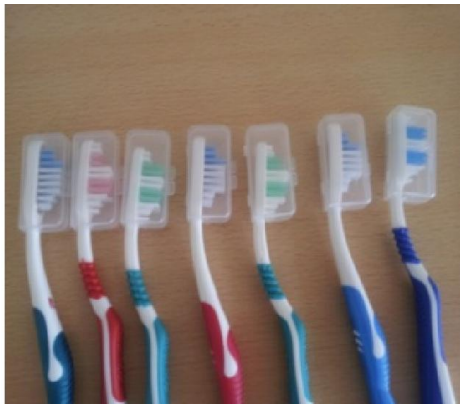


Fig. 1. Silver care tooth brush

Fig. 2. Miswak sticks

Fig. 3. Nylon tooth brushes

Figure 4. Extraction with saline

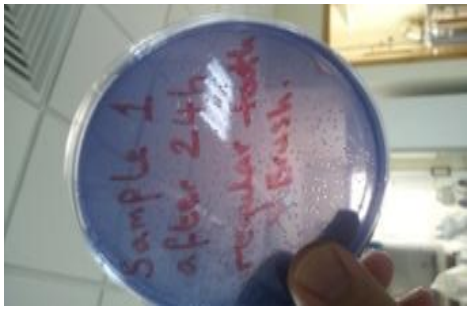


Figure 5. Spreading on the mitis salivarius selective medium

Figure 6. CFU counting with colony counting meter

The bristles are coated with nano silver particles and get activated by the contact with water molecules. This project was approved by the ethical committee of AjmanUniversity of science and technology, UAE. The miswak sticks, silver care toothbrush and regular nylon tooth brush were collected immediatelyand part of the bristles were removed from each of brush using a sterilized scissorThe bristles were soaked in 1mm of sterile distilled water for 15 min. Fig 4 0.3ml of the distilled water was loaded in separate newly prepared mitis salivarius agar by a sterile spreader for equal distribution of the sample on the mitis salivarius agar. Fig 5.

Chart A – Comparison of percentage of bacterial reduction

	Toothbrush	storage	Immediate	After 24h	reduction	percentage
1	Regular b.	Covered	7615	6013	1607	21%
2	Regular b.	covered	3536	3041	495	13.99%
3	Regular b.	covered	4095	4326	-231	- 5.64%
4	Regular b.	covered	2080	1838	242	11.63%
5	Silver care b.	uncovered	3120	1012	2100	67.30 %
6	Silver care b.	covered	4275	1083	3192	74.66%
7	Silver care b.	covered	2860	750	2110	73.77%
8	Silver care b.	uncovered	3120	780	2340	75%
9	Silver care b.	uncovered	7020	2156	4864	69.28%
18	Silver care b.	uncovered	3250	823	2427	74.67%
11	Silver care b.	covered	4276	1141	3135	73.31%
12	Silver care b.	covered	7427	1340	6086	81.94%
13	Silver care b.	uncovered	4160	260	3900	93.75%
14	Silver care b.	covered	2535	610	1925	75.93%
15	Regular b.	covered	4340	4510	-170	-3.91%
16	Regular b.	uncovered	3835	3621	214	5.58%
17	Regular b.	uncovered	2145	1970	175	8.15%
18	Regular b.	uncovered	3055	2880	175	5.72%
19	Regular b.	uncovered	5720	5501	219	3.82%
20	Regular b.	uncovered	3120	2989	131	4.19%
21	Miswak s.	ventilated	2210	1003	1207	54.61%
22	Miswak s.	ventilated	2600	1253	1347	51.80%
23	Miswak s.	ventilated	1365	585	780	57.14%
24	Miswak s.	ventilated	1625	460	1165	71.69%
25	Miswak s.	ventilated	2080	1105	975	46.87%
26	Miswak s.	Non-vent.	2795	1425	1370	49.01%
27	Miswak s.	Non-vent.	1170	260	910	77.77%
28	Miswak s.	Non-vent.	1820	446	1380	75.82%
29	Miswak s.	Non-vent.	2405	1690	715	29.72%
30	Miswak s.	Non-vent.	1885	947	938	49.76%

Table 1. The values obtained from the CFU counting are statistically compared between 3 groups

Descriptive Statistics						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Miswak	10	715	1380	1078.70	78.318	247.665
Regular	10	-231	1607	285.70	160.701	508.180
Silvercare	10	1925	6086	3207.90	434.005	1372.444

The entire 30 immediate samples were loaded in the incubator for 24 hours. The storage of the brushes was done in two ways by segregating them as covered and uncovered. The storage of miswak was divided to ventilated and unventilated container to see the effect of humid environment and drying on the growth of bacteria. It was done to evaluate the relation between the bacterial reduction in the bristles and the different storage condition of the tooth brushes. Same procedure has been repeated on these brushes after 24hours to compare the amount of bacterial reduction among the brushes and miswak in the period of 24h post application. Allthe sampleswere kept in the incubator for 24 hour. Counting of the colonies was performed for each sample twice (immediate load and the other (24 hour sample) by the colony counter device. Figure 6

RESULTS

Chart a shows the comparison of 3 different groups and clearly shows the amount of bacterial reduction in percentage. Table 1 shows the statistical comparison of 3 different groups. Table (2) showing One-way ANOVA: results for the type of brushes.In the ANOVA table, the p-value (0.047) for Type of brushes indicates that there is sufficient evidence that not all the means are equal when alpha is set at 0.05, which means that there is sufficient difference among the mean values of CFU values from different brushes. Moreover; to explore the differences among these means, we examine the multiple comparison results.

Table 2. One-way ANOVA between types of brushes

Source	DF	SS	MS	F	P
Type	2	45720033	22860016	31.17	0.047
Error	27	19799986	733333		
Error	29	65520019			

S = 856.3 R-Sq = 69.78% R-Sq(adj) = 67.54%

Table 3. Hsu's MCB (Multiple Comparisons with the Best)

Level	N	Mean	Standard deviation
Miswak stick	10	1078.1	246.9
Regular brush	10	285.2	506.7
Silver care brush	10	3208.8	1372.0

Table 3. Shows the mean and standard division of the three brushes for the MCB

Table 4. Shows the Intervals of the three brushes. Hsu's MCB (Multiple Comparisons with the Best) compares each mean with the best (largest) of the other means. We compares the

means of Miswak stick and regular brush to the silver care brush mean because it is the largest. The silver care brush may be best because the corresponding confidence interval contain positive values. No evidence exists that Miswak stick and regular brush is the best because the upper interval endpoints are 0, the smallest possible value. Grouping Information Using Tukey Method is attempted below in the tables to follow.

Table 4. Mean and standard deviation of 3 different groups

Level	Lower	Center	Upper
Miswak stick	-2753.1	-2130.7	0.0
Regular brush	-3546.0	-2923.6	0.0
Silver care brush	0.0	2130.7	2753.1

Table 5. Tukey analysis of 3 different brushes

Type	N	Mean	Grouping
Silver care brush	10	3208.8	A
Miswak stick	10	1078.1	B
Regular brush	10	285.2	B

Table (5) shows the mean and grouping information using Tukey Method for the three brushes. Means that do not share a letter are significantly different. Tukey 95% Simultaneous Confidence Intervals are being used. All Pairwise Comparisons among Levels of type

Individual confidence level = 98.04%
Type = miswak stick subtracted fromfrom regular and silver care tooth brush.

Table 6. Tukeyanalysis between regular and silver tooth brush

Type	Lower	Center	Upper
Regular brush	-1743.4	-792.9	157.6
Silver care brush	1180.2	2130.7	3081.2

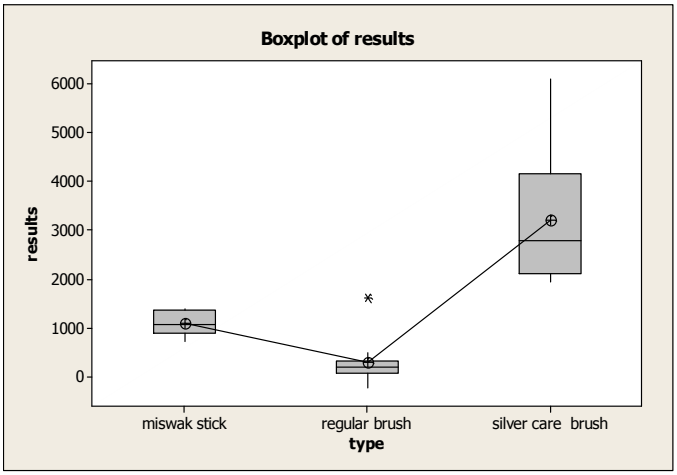
Table (6) shows comparison between the confidence interval of regular and silver toothbrush.

Table 7. Tukey analysis of silver care tooth brush

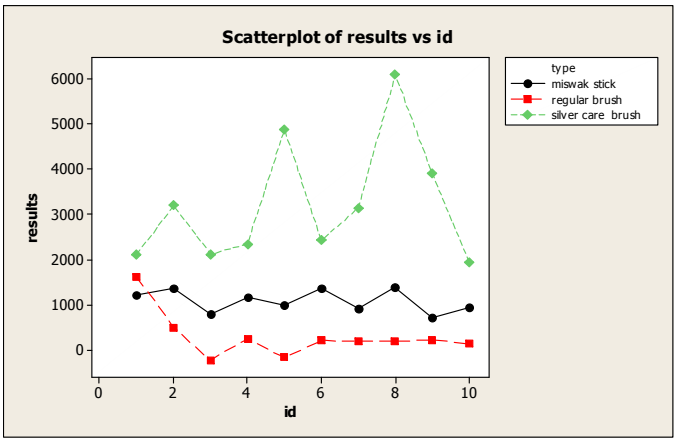
Type	Lower	Center	Upper
Silver care brush	1973.1	2923.6	3874.1

Table 7 shows comparison of tukey analysis of values with regard to silver care tooth brushsustracting from regular tooth brush. Tukey's test provides grouping information and 2 sets of multiple comparisons of confidence intervals. The grouping table shows that group A contains silver care brushwhile group B contains Miswak stick and regular brush which means that Miswak and regular brush have similar mean. The confidence interval for the silver brush mean present in the positive sides which indicate it has the best effect compared with the negative side where we can find in the Miswak and regular brush which are approximately have the same mean.

Graph 1 box plot, shows that the silver care brush had the highest mean, followed by miswak and the lowest mean is the regular toothbrush.



Graph 1. Boxplot of 3 diffrerent groups



Graph 2. Scatter plot of 3 different groups

Graph 2 showing scatter plots of results vs. volunteers in the samples taken which shows that the silver brush is having the highest amount of bacterial reeducation in all of the 10 samples, followed by miswak and then the regular nylon tooth brush.

DISCUSSION

Darmani *et al*. in 2006 examined the effects of aqueous extracts of miswak on the growth of the various cariogenic microorganisms including Streptococcus mutans. The result showed inhibition in growth of Streptococcus mutans which is similar to our result (Darmani *et al.,* 2006). It wasalso found by Padma K Bhatin 2012 that miswak extract had very significant detrimental effect on both the dental caries causing micro-organisms at the tested conditions. It had shown significant reduction of microbial count as compared to toothbrush and saline in the present study. (Padma *et al.,* 2012; Al-Bayaty *et al.,* 2010) had also found the miswak extract as an effective antimicrobial agent which is comparable to our study results. Chewing sticks were effective in reducing plaque and gingival inflammation. Where properly used, miswak had been reported to be as effective as tooth brushing (Edbatwa, 2006). Furthermore, a study by Darout *et al.,* 2000 showed that aqueous miswak extracts contained potential antimicrobial anionic components in addition to chloride and sulphate, which

were thiocyanate and nitrate. They hypothesized that thiocyanate leaching out from miswak, while in the oral cavity, may lead to an elevated level of salivary thiocyanate. This, in turn, may enhance the efficacy of the salivary hydrogen peroxide–peroxidase-thiocyanate system, a known antimicrobial component of human saliva. In the present study, we concluded that silver nano brush had the maximum effect of bacterial reduction, followed by miswak which had moderate bacterial reduction and then the regular tooth brush which had a very minimal bacterial reduction .Regarding the miswak stick doesn't show any differences in bacterial reduction in case of storage in ventilated or non- ventilated plastic container. Silver care toothbrush doesn't show any differences between the covered & uncovered brush in bacterial reduction. Regular toothbrush showed differences between the covered & uncovered brush.The covered brushes showed less amount of bacterial reduction & in two samples it showed bacterial growth not the bacterial reduction. This finding supports other studies stating that brushes which are covered with humid environment will favor bacterial growth. Miswak showed that using the non-ventilated containerwill maintain the miswak fresh up to two and half month, but the miswak stick in the ventilated container becomes dry within two weeks.The study was limited to small sample size and limited to streptococcus mutans in selective mitis salivarius media. The study can be extended to a larger population with more microorganisms. The possibility of a mouth wash developed from miswak for human use can be studied.

Conclusion

The silver care tooth brush with nano silver coating has shown maximum bristle protection compared with miswak or nylon bristles. Normal nylon bristles showed an increase in the growth of bacterium when stored in non-ventilated containers due to humid environment which favored the growth of the bacterium. Silver care nano silver coated bristles did not show any increase in growth in both ventilated and non-ventilated containers. Miswak natural tooth brush bristles maintained the antibacterial property in non-ventilated containers better than ventilated containers.

REFERENCES

Abo Al-Samh, D., and Al-Nazhan, S. 1997. In vitro study of the cytotoxicity of the miswak ethanolic extract: *Saudi Dental Journal,* 9(3):125-32

Al Bagieh, N., and Almas, K. 1997. In-vitro antibacterial effects of aqueous and alcohol extracts of Miswak (chewing sticks):, *Cairo Dental Journal*, 13(2):221-24.

Al-Bayaty, F.H., AI-Koubaisi, A.H., Ali,N., Abdulla, M.A. 2010. Effect of mouth wash extracted from Salvadora persica (Miswak) on dental plaque formation; A clinical trial:, *Journal of Medicinal Plants Research*, 4(14):1446-54.

Darmani, H., Nusayr, T., Al-Hiyasat, A.S. 2006. Effects of extracts of miswak and derum on proliferation of Balb/C 3T3 fibroblasts and viability of cariogenic bacteria. *International J of Dent Hygiene*, 4(2):62–65.

Darout, I.A., Christy, A.A., Skaug,N., Egeberg, P.K. 2000. Identification and quantification of some potentially antimicrobial anionic components in miswak extract Indian *J Pharmacology*, 32:11–4.

Edbatwa, M. 2006. The effectiveness of chewing Stick, 18(3):1-2.

Efstratiou, M. W., and Papaioannou, M., & Ktenas, E. 2007. Contamination of a Toothbrush with Antibacterial properties by oral microorganisms., *Journal of Dentistry*, 35(4):331–337.

Humberto Lara *et al*. 2011. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds, *Journal of Nano-biotechnology,* 9(30):1-8

Karibasappa, 2011. Assessment of microbial contamination of toothbrush head: An in vitro study, *Indian journal of dental research,* 22(1); 2-5.

Lara, H., Ayala, N., Ixtepan, L., Rodriguez, C. 2010. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology and Biotechnology,* (26):615-621.

Larsen, R. Toothbrush(online), 2010; available at: http://microbewiki.kenyon.edu/index.php/Toothbrush accessed on: 03/12/2012

Masoumeh, K., Solmaz, A., Mahdi, K., Fariborz, V. 2012. Comparison of the Antimicrobial Effects of Persica Mouthwash and 0.2% Chlorhexidine on Aggregatibacter Actinomycetemcomitans of Healthy Individuals and Patients with Chronic Periodontitis. *Journal of Medical Sciences,* 6(1):18-21.

Mehta, A. Sequeira, P.S. and Bhat, G. 2007. Bacterial contamination and decontamination of Toothbrushes after use. *The New York State Dental Journal*, 73(3):20–2.

Nowack, B., Harald, F. 2011. 120 Years of Nano-silver History: Implications for Policy Makers; *Environ Sci Technol,* 45(7):3189.

Padma, K.B., Kumar. A., Ksarkar. S. 2012. Assessment of Immediate Antimicrobial effect of Miswak Extract and Toothbrush on Cariogenic Bacteria; A clinical study, *Journal of advanced oral research*, 3(1):17-18.

Schrack, K. Dental Hygiene (online) available at 2013:http://ntperiohygiene.blogspot.ae/2013/04/worn-out.html accessed on: 01/12/2012.

Sun, R., Chen, R., Chung, N. 2005. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells, *Chem Commun (Camb)*, 40) 5059-61.

Svanberg, M. 2013. Statement on Toothbrush care. 2011 available at: http://www.ada.org/1887.aspxaccessed on: 05/02/2013.

Timothy J. Mitchell. 2003. Summary of the main virulence factors of four streptococcal species. *Nature Reviews Microbiology,* 1:219-230

Wikipedia, Streptococcus mutans (online) accessed on 08/03/2013.

****