The efficacy of oral hygiene by micro-bubbles cleaning.

Pei-Ju Lin[1]*, Ming-Chuen Chuang[1], Szu-Chung Chang[2]

[1]Department of Industrial design and Institute of Applied Arts, National Chiao Tung University, Taiwan
[2]CM Dental Clinic, Taichung, Taiwan

Abstract

Objective: Patients with periodontitis often have difficulty in maintaining oral hygiene with toothbrushes during treatment periods, especially for patients with severe conditions where brushing may cause bleeding of the gingiva and increase the chance of bacterial infection. Patients who have oral cancer, or who are bedridden long-term due to chronic diseases, also experience similar inconveniences in being unable to maintain oral hygiene through brushing their teeth and must do so through other means.

Methods: We explored the method for dental washer, using the micro-bubble generator connect the ejection nozzle to an ergonomically designed soft teeth-tray that fits with the tooth configuration of a typical human oral cavity to clean oral plaque bacteria. Five levels of rotor speed and 4 type of ejection hole diameter, totally, were using 20 combinations to clean oral plaques bacteria.

Results: Our results showed that with various combinations of motor speed settings and pore diameters, a clearing rate of 56% or more (average 79.55%) could be achieved, while in some combinations a clearing rate of 91.64% was possible. The nozzles with smaller diameters coupled with low speed motor had higher cleaning efficiency; the larger the diameter, the higher the volume and the larger the bubble dimensions and better bacteria removal.

Conclusion: The experimental verification is effective by controlling ejection hole diameter and soft tooth-tray on better bacteria removal. For future, it is hoped that can definitely solve the dental hygiene issue for long-term bed-ridden patients who could not use toothbrush.

Keywords: Dental cleaning device, Dental plaque bacteria removal, The soft teeth-tray, Oral hygiene.

Accepted on October 31, 2017

Introduction

The most common way of maintaining oral hygiene is through brushing teeth. However, for patients with periodontitis, brushing teeth afflicted with gingivitis caused by bacterial infection often causes gum bleeding. Studies have shown that patients with both diabetes and periodontitis had higher risk of developing destructive periodontitis through tooth brushing [1,2]. Periodontal disease will also influence health; studies have shown that for patients with cardiovascular diseases and diabetes, the pathogens from the periodontal disease blood vessel walls, causing cardiovascular inflammation of the vessel wall, blockage or severe heart attack [3]. Burns et al. [4] and Rubio et al. [5] showed that micro-bubbles with smaller diameters tend to gather more in higher surface areas; more contact surface areas with contaminants meant longer contact time and better removal efficacy. The diameter of ejection hole of water is a key factor influencing the dimensions of micro-bubbles; thus, in our study we modified the diameters of the nozzle (water ejection port) to experiment on different bubble dimensions [6]. Van der et al. [7] used tooth brushes from Braun and Philips and conducted an experiment on dental plaque removal in 35 college students, using 6 positions on each tooth for evaluation of dental plaque index. Grossman & Proskin [8] conducted a comparative study on using manual and electrical tooth brushes in the removal dental plaques in children aged 8 to 12 years old and also used dental plaque index to evaluate the efficiency of dental plaque removal. It can be seen that the dental plaque index was widely used in the aforementioned studies on dental plaque removal, oral cavity hygiene and prevention of periodontal diseases. However, although the index can be used to subjectively interpret the degree of cleanliness, bacteria are invisible to the naked eye and the actual plaque removal could not be objectively measured. Therefore, in order to more thoroughly investigate the effect of dental plaque removal, the authors used microbial validation as the basis of quantifying cleaning efficacy.

Methods

Micro-bubble generator and control variables

Figure 1 shows the customized ejection path of the

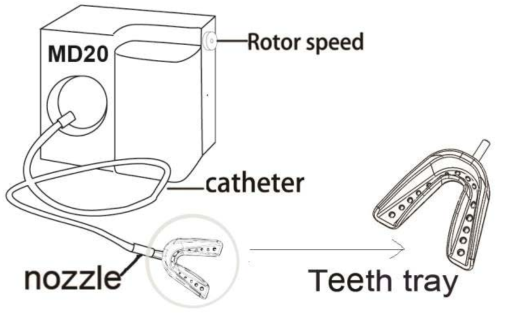

Figure 1. *The modified micro-bubble generator and the soft teeth-tray (made by medical silicone).*

commercially available dental irrigator Braun MD20 used as micro-bubble generator. There only the nozzle diameter and quantities in this device were changed, The MD20 dental irrigator has a five-step variable speed setting, which was preserved in our study and used as a dependent variable. The rotor speed was measured with a contact tachometer that measures by 10 times in each rotor speed, taking the mean as the representing value. The results were 2580 rpm, 3021 rpm, 3527 rpm, 4210 rpm and 5380 rpm. The hole of ejector nozzle, we built 4 stainless steel nozzle shapes with CNC (Computerized Numerical Control) fabrication, which measured 16mm in length, 6mm for the outer diameter and 5 mm for the inner diameter. The ejection hole diameter was fabricated with electric discharge machining, which were the 0.3 mm, 0.16 mm, 0.63 mm and 0.8 mm nozzles, respectively. The numbers of nozzles were three and arranged in a triangular configuration. A total of 20 experimental combinations were produced from five rotational speed and four diameters of the three-nozzle, which allowed generation of water streams with different flow volume, velocity and bubble diameter for carrying out dental plaque bacteria removal experiments.

Measurement of flow volume, flow velocity and micro-bubble diameters

In this study, we measured the flow volume, velocity and micro-bubble diameters produced by the micro-bubble generator, and set them as the under 20 different experimental conditions. The water flow volume was done by measuring the total water volume for 10 s with a measuring cup holding the water ejected from the micro-bubble generator connected the soft teeth-tray. The flow volume per second was then calculated, and the measurement was repeated 10 times for the average value. The dimension of the micro-bubbles was measured by photos taken with a high-speed camera. The micro-bubble generator was then connected to the fabricated nozzles and teeth-tray and was placed inside the box. The generator was turned on to eject water for 3 s. After the jet stream becomes stable, a high-speed camera (Mage Speed HHC X2) was used to photograph the jet stream for 1 s (1,000 frames/s). Of the 1,000 total photos taken, 500 (250^{th} to 750^{th}) were played back at slow speed with the Mega Speed AVI Player software. Of those, 10 clearer photographs are then selected to measure the bubble diameters, and the average value was taken as the representative value. From the filmed photos the position of a same bubble in 10 continuous photos was tracked, its distance measured to calculate the flow rate of the intermediate variable (M/S). The measurements of the statistics of control variables to flow volume, flow velocity, micro-bubble diameters are shown in Table 1.

Preparation of experiment and materials

The denture sample was created by a dental material company using adult tooth mold supplied and consisted of 14 false teeth (Good-Guys Dental Co., Ltd. Taiwan). The soft tooth-tray was made of medical silicone rubber with a hardness of 40 (Figure 1). The water ejection ports were fabricated corresponding to each tooth on the denture; holes were drilled on the bottom of the teeth-tray at an interval about 8mm between each tooth. There are 14 holes total with diameter of 1.6 mm.

Experiment steps in dental bacteria removal

To quantify the cleaning efficacy of micro-bubbles on dental plaques coated on denture, we collected bacterial strains from the clinical periodontal patients at a dental clinic. During sampling, a sterilized cotton swab was rubbed evenly around the oral cavity of the patient, and the collected samples were immediately placed inside a sterilized test tube. The samples were cultured using Sabouraud dextrose agar medium, and subsequently transfected to liquid medium.

Table 1. *Statistics of control variables to flow volume, flow velocity, micro-bubble diameters and bacteria removal.*

Ejection hole	Flow Volume						Flow Velocity					
	2580	3021	3527	4210	5380	Mean	2580	3021	3527	4210	5380	Mean
0.16	1.2	1.3	1.4	1.5	1.6	1.4	0.3	0.44	0.44	0.46	0.71	0.47
0.3	3.1	4	4.4	4.8	5.4	4.34	0.35	0.65	0.56	0.64	0.7	0.58
0.63	3.1	3.7	4.5	4.9	5.5	4.34	0.44	0.46	0.46	0.54	0.59	0.5
0.8	3.5	3.9	4.5	5	5.5	4.48	0.61	0.64	0.6	0.52	0.63	0.6
Mean	2.7	3.2	3.7	4.1	4.5	3.64	0.43	0.55	0.52	0.54	0.66	0.54

Ejection hole	Micro-bubble diameters						Bacteria removal					
	2580	3021	3527	4210	5380	Mean	2580	3021	3527	4210	5380	Mean
0.16	0.06	0.06	0.05	0.04	0.05	0.05	91.01	90.19	89.81	88.68	88.37	89.61
0.3	0.04	0.04	0.06	0.08	0.09	0.06	91.13	91.64	73.4	80.69	79.94	83.36
0.63	0.08	0.08	0.09	0.07	0.09	0.08	81.76	84.84	80.5	89.37	90	85.29
0.8	0.08	0.07	0.1	0.12	0.11	0.1	56.23	56.86	59.43	62.39	64.84	59.95
Mean	0.07	0.06	0.08	0.08	0.09	0.07	80.03	80.88	75.79	80.28	80.79	79.55

The bacterial culture was then placed on an orbital shaker in the incubator, and cultured for 48 h in 37°C at a speed of 180 rpm. The dental plaque bacteria removal experiment was conducted in a sterilized laminar flow cabinet. The test denture was first sterilized and immersed in a 250 ml square container with solution containing 8×10^{10} cfu/ml (colony-forming unit) bacterial solution for 30 min and then dried for 60 min. During the removal experiment, the bubble generator was connected to the nozzle heads of specific pore diameters. The nozzle was then connected to the water inlet of the teeth-tray and then placed in a sterilized 500 ml container, which had a 6.8 cm platform to prevent the wastewater during the cleaning process from overflowing the denture and affecting the results. The teeth-tray then covers the upper jaw section of the denture with the teeth-surface facing downward. The micro-bubble generator was then set at the desired speed and turned on; the denture was then cleaned for 3 min. After cleaning was completed, the residual bacteria on the denture were then calculated based on the methods and steps from Lee et al. [9]. Briefly, the cleaned denture was placed in another sterilized glass dish, and dried for 30 minutes with the tooth surface facing upward. After drying, the occlusal surface of the teeth was printed onto agar for 30 s with desktop holder which could give the same pressure, and the dish was then set for 24 h at room temperature. The bacteria on medium dish were counted by colony counter.

The level of residual bacteria after cleaning was then compared with the bacterial level of an uncleaned denture. Therefore, we must first calculate the cfu on uncleaned dentures. The uncleaned dentures were pressed onto the medium dish for 30 s and then stored for 24 h at room temperature. The colony units were then counted with the colony counter. Cleaning efficacy was represented as percentage of colony removal; the method of calculation was as difference in cfu between uncleared and cleaned denture in percent. The estimation of bacterial removal in this study was not specific to a single strain, but rather to the total number of bacteria. Since there are many bacterial strains in the oral cavity, a single strain was not representative of the overall situation. In fact, due to the flat nature of the culture medium, only the occlusal surface of the teeth was estimated in this study. The denture was carefully sterilized after each test to make sure it is completely sterilized and then re-immersed in bacterial solution for the next test.

Results and Discussion

The effect of the micro-bubble generator control variable on flow volume of jet stream, water flow velocity and diameter of the micro-bubbles

The water volume of the 0.16 mm nozzle was significantly lower than the all other larger nozzle diameters, although there were little differences between the rest of the three diameter sizes. The difference with other speed settings was significant. On the effect of nozzle diameter, the volume from the 0.16mm nozzle was significantly lower than other three nozzle sizes; however, there were no significant differences between the three larger nozzle sizes.

Data on water flow velocity under two experimental conditions and the line plot on the effect of control variables on velocity on the right have shown that as motor speed increases, flow velocity increased slightly but was not significantly affected by nozzle diameters (Table 1). Results also showed that only at the highest motor setting the flow velocity would be significantly higher than at lowest motor setting, while the differences between the rest of the speed settings were not significant. With the maximum 0.8 mm nozzle diameter, the flow velocity did not increase correspondingly with motor speed

Table 1 shows the changes in micro-bubble diameters under five speed settings and four nozzle dimensions. The 0.16 mm and 0.3 mm nozzles produced bubbles with smaller diameters; except for the 0.16 mm nozzle, the diameters of micro-bubbles increased slightly with increasing motor speed. However, the results did not indicate significant effect from motor speed settings; only the nozzle pore diameter was significant. It also showed that the bubble diameters between each adjacent blocks of the same pore diameter did not differed significantly in dimensions, but were significant with other pore sizes. Overall the smaller the pore diameter, the smaller the diameter of the micro-bubbles; the larger the pore diameter, the larger the bubble dimensions. Our results were similar with the studies by Legner [10].

Correlation between the control variables and dental bacteria removal

On the effect of dental bacteria removal, except for the 0.8 mm nozzle diameter which had less removal efficiency, all other pore diameters have achieved an average bacteria removal efficiency of 83% and above (Table 1), with the smaller pore diameter (0.16 mm) having the best efficacy. The effect of different motor speeds on bacteria removal were not significant; ANOVA results of Table 2 show that only the influence of nozzle pore diameter has achieved 0.05 significance. Student-Newman-Keuls test (SNK) showed that except for the bacteria removal efficacy of the 0.8 mm group that differed significantly with other groups, the differences between the rest of the groups were not significant. Table 1 also shows that when the pore diameters were 0.16 mm and 0.3 mm and the motor speeds were 2580 rpm and 3021 rpm, respectively, the bacteria removal efficacy was optimal and achieved over 90%. It seemed that better cleaning efficiency could be achieved by combining lower motor speed with smaller diameter nozzles.

Correlation between flow volume, flow velocity, micro-bubble dimensions and dental bacteria removal

We then investigated the effect of the water flow volume, flow velocity and micro-bubble dimensions on bacteria removal (Figure 2). Left of Figure 2, show the plots revealed that there was no clear correlation between water flow

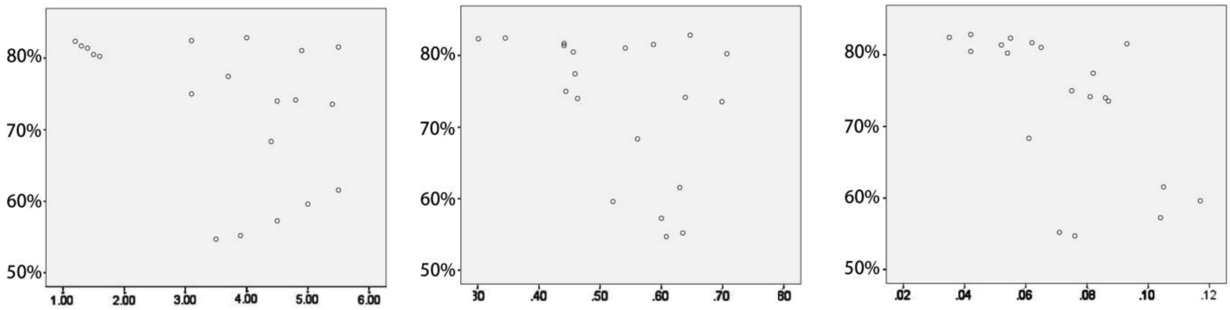

Figure 2. *Scatter plots on the relationship between variables and bacterial removal (Left: flow volume to bacteria removal efficacy; center: flow velocity to bacteria removal efficacy, right: micro-bubble dimension to bacteria removal efficacy).*

Analysis of variance on the effects of control variable to bacteria removal

	source	Type III of Squares	df	Mean Square	F	Sig.	R^2
Bacteria Removal	**Corrected Model**	2737.403[a]	7	391.06	15.3	0	0.9
	Intercept	126574.92	1	126574.92	4950.62	0	
	Rotor speed	72.94	4	18.23	0.71	0.6	
	Nozzle diameter	2664.47	3	888.16	34.74	0	
	Error	306.81	12	25.57			
	Total	129619.13	20				
	Corrected Total	3044.21	19				

SNK multiple comparison on homogeneous group of nozzle diameter to bacteria removal

	1	2
0.8	59.95	
0.3		83.36
0.63		85.3
0.16		89.61
Sig.	1	0.17

Regression analysis on the effect of intermediate variables and bacteria removal

Dependent		Independent	B	Std. Error	Beta	v	Sig.	R	R^2
Bacteria Removal	A	Flow velocity	-29.41	24.42	-0.26	-1.2	0.246	0.665[a]	0.443
		Flow volume	1.11	2.38	0.13	0.47	0.647		
		Micro-bubble diameter	-351.27	143.37	-0.63	-2.45	0.026		
	B	Flow velocity	-24.55	21.58	-0.22	-1.14	0.271	0.660[b]	0.435
		Micro-bubble diameter	-309.2	108.89	-0.55	-2.84	0.011		
	C	Micro-bubble diameter	-351.48	103.19	-0.63	-3.41	0.003	0.626[c]	0.392

Table 2. *ANOVA and SNK on effects of control variables to bacteria removal and regression analysis on the effect of intermediate variables and bacteria removal.*

volume and bacteria removal, and that it increased slightly with flow velocity and decreased with increasing bubble dimensions (center and right, Figure 2). Using flow volume, flow velocity and micro-bubble dimensions as independent variables and bacteria removal as dependent variable, we performed backward multiple regression analysis, and the results are shown in Table 2. At significance level of $\alpha=0.05$, only the micro-bubble diameter has significantly affected bacteria removal. The negative regression coefficient value showed that, as the bubble diameter decreases, the bacteria removal efficacy was increased.

Conclusion

In this study, we proposed using a micro-bubble generator outfitted with a three-nozzle head that is connected to a teeth-tray to clean dental plaque bacteria on denture. Our results showed that in all experimental combinations of motor speed settings and nozzle pore diameters, about 56% or more dental bacteria removal efficacy was achieved (average 79.55%) and in some combinations up to 91.64% removal efficacy was achieved, which validated the bacteria removal capability of the micro-bubbles. Generally, the three-nozzle with smaller pore diameters and lower motor speed settings resulted in better bacteria removal. The motor speed setting of the three-nozzle micro-bubble generator directly influenced the flow volume and velocity of the water stream; at higher motor setting, the flow volume and velocity increases; however, the micro-bubble dimensions were found to have no significant impact on bacteria removal. The nozzle diameters were found to significantly influence flow volume, bubble diameters and bacteria removal efficacy; the larger the diameter, the higher the volume and the larger the bubble dimensions and better bacteria removal. However, nozzle diameter had no significance influence on flow velocity. From these results we theorized that bacteria removal is influenced by the dimension of micro-bubbles; smaller bubble diameter led to improved bacteria removal efficacy, which was also confirmed by our regression analysis. Our conclusion was similar to the results by Burns et al. [4] and Rubio et al. [5].

In summary, our results showed that by restricting micro-bubbles in a confined space such as the teeth-tray, and allowing them to flow freely on the denture, the bubbles could help to clean dental bacteria. However, in the present study, a denture was substituted as the test subject instead of a real human oral cavity, and only the level of bacterial on the occlusal surface was used as cleaning indicator (residual bacterial level after cleaning), rather than taking into account also of the inner, outer and adjacent surfaces of the teeth. We hope to overcome this deficiency in the future by investigating with an actual human oral cavity, and to estimate the bacteria removal on all surfaces of the teeth, so that we may propose better solution on maintaining oral hygiene for long-term bed ridden patients.

References

1. Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. J Periodontol. 1991;62(2):123-31.
2. Beck J, Garcia R, Heiss G, et al. Periodontal disease and cardiovascular disease. J Periodontol. 1996;67:1123-37.
3. Horst ML, Buckley S. Oral care and dementia: Dispelling the myths. Can Nurs Home. 2008;19(4):22-4.
4. Burns SE, Yiacoumi S, Tsouris C. Microbubble generation for environmental and industrial separation. Sep Purif Technol. 1997;11(3):221-32.
5. Rubio J, Souza ML, Smith RW. Overview of flotation as a wastewater treatment technique. Miner Eng. 2002;15(3):139-55.
6. Hasegawa H, Nagasaka Y, Kataoka H. Electrical potential of micro-bubble generated by shear flow in pipe with slits. Fluid Dyn Res. 2008;40:554-64.
7. Van der WGA, Timmerman MF, Reijerse E, et al. Comparison of 2 electric toothbrushes in plaque-removing ability professional and supervised brushing. J Clin Periodontol. 1995;22(8):648-652.
8. Grossman E, Proskin H. A comparison of the efficacy and safety of an electric and a manual children's toothbrush. J Am Dent Assoc. 1997;128:469-74.
9. Lee HE, Li CY, Chang HW, et al. Effects of different denture cleaning methods to remove *Candida albicans* from acrylic resin denture based material. J Dent Sci. 2011;6(4):216-20.
10. Legner HH. A simple model for gas bubble drag reduction. Phys Fluids. 1984;27(12):2788-90.

***Correspondence to:**

Pei-Ju Lin
Department of Industrial design and Institute of Applied Arts
National Chiao Tung University
1001 University Road, 300 Hsinchu
Taiwan
Tel: +886953099188
E-mail: adobe33@hotmail.com