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Research on the cleaning efficacy of micro-bubbles on dental plaque

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Abstract

Long-term bed-ridden patients do not usually have the same opportunity for proper dental hygiene as normal individuals, as they often have difficulty using toothbrushes to clean their teeth. Patients with periodontal disease are also at risk of increased bacterial infection due to the propensity for teeth brushing causing bleeding of the gums. Therefore, an alternative method of dental hygiene maintenance is required for these individuals. Our study proposed a method to clean dental plaque through a tooth tray with micro-bubbles and verified its cleaning efficacy through experiment. A cleaning device that produces micro bubbles (Braun MD20) was used in the study with five separately modified nozzle diameters as the independent variable: 0.16mm, 0.30mm, 0.63mm, 0.8mmand 1.2mm. The five different rotation speed settings of the device act as the other independent variable, with the resulting flow volume, velocity and the diameter of the micro-bubbles as the intermediate variables. The effects of these variables on cleaning dental plaque were investigated. Our results showed that an average of 45%~75% cleaning rate of dental plaque was achieved under all combinations of the variables. The best dental plaque removal variable combination was nozzle diameter 0.8mm with speed of 3527 rpm, in which 98% dental plaque removal was achieved. The dimension of the nozzle exerted greater influence on flow volume, flow velocity and bubble diameter than rotation speed. The effect of the control variables on plaque removal was also more significant than intermediate variables, with the nozzle dimension influencing plaque removal at 0.05 significance level.

Keywords: Micro-bubble; Dental plaque; Tooth tray; Periodontal disease

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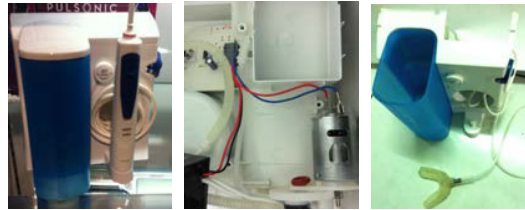 *E-mail address:* adobe33@hotmail.com

Fig. 1. Braun MD20 (left), internal structure (center) and modified device (right).

1. Introduction

For this study, we questioned many care centers and learned that many long-term bed-ridden chronic or hand paralysis patients have more difficulty in dental hygiene than normal individuals. Some patients require assistance from nursing staff; others rely on external help to clean their oral cavities. However, due to individual differences in dimensions of oral cavities, teeth size, shapes and arrangements, the brushing assistant may not always have a good grasp of the brushing feeling as experienced by the patient. This often resulted in increased difficulty of maintaining dental hygiene. Differences in personal hygiene and dietary habits also contributed to the many different bacteria strains in the oral cavity. Current information on dental plaque has listed more than 25,000 bacteria strains in the oral cavity; up to 10,000 different bacteria may exist in the mouth of just one individual [9]. As stated in the 《 Fundamentals of Oral Histology and Physiology》 by Hand and Frank (2014), teeth is the part of the mouth where most bacteria are found, as they easily form biomembranes over the surface of the teeth. When acidic materials began to erode the enamel, the teeth loses the ability of self-protection. The bacteria on the surface of the teeth and mucosa grow on the nutrients from the saliva; sufficient nutrients will result in elevated bacteria on the dental surface, forming dental plaques and hampers dental and gingival hygiene. Therefore, maintaining oral hygiene is essential, and the most common method to do so is through teeth brushing with toothbrush and toothpaste. However, brushing teeth with gingivitis may cause bleeding of the gum, which may lead to development of periodontal disease in diabetic patients whose wound healing ability is impaired, causing bacteria to growth on the gum. Studies have also shown that diabetic patients with periodontal diseases are more likely to develop destructive periodontitis [8,10] . Onaga et al (2006) have studied the use of ultrasound-induced micro-bubbles to clean dental plaque, and their results show that effective dental plaque removal can be achieved. However, this study has not yet been performed in human oral cavities. Various methods produce micro-bubbles. Onaga et al used ultrasound, and our study proposed a simpler method that produces micro-bubbles through mixing of water and air via a motor, and employed through a specially designed tooth tray to clean the dental plaques. Our study not only verifies that micro-bubbles produced from different devices have the same dental plaque removal capability, but also hopes to further investigate the relationship between plaque removal efficacy and different variables. There are currently many different types of air and water combination apparatus on the markets that produce micro-bubbles. For our study, we will be using the currently available Braun MD20 micro-bubble dental irrigator. The path of the micro-bubble will be modified to control the variables of bubble formation to suit the needs of our study (as shown in Fig. 1)

2. Literary reviews

2.1. Principles of micro-bubble formation and application in cleaning

Bubbles are formed in many of the natural processes. When gases and liquids are combined under pressure, bubbles of various sizes and shapes are formed. Bubbles can also be formed by emitting electricity, depressurization, increase temperature, ultrasound and electrolysis of various liquids. The bubble formation process, after multiple splitting, will result in very small dimensions, forming the so-called micro-bubbles. Based on the disintegration of air in the water, Fujikawa (2003) developed a device that produces micro-bubbles by channeling air through a compressor into a flat plate drilled with many pores, and controlled the rotation speed of the plate with a motor. This device uses shear force to slice through the bubbles, which decreases the bubble diameter with increasing rotation speed. Common applications of micro-bubbles are 1) cleaning action: detergent solution is

absorbed around the bubbles to increase the contact surface areas between the detergent and dirt, which improves the cleaning action. Miyamoto and Ueyama (2007) performed a study on using micro-bubbles with an average diameter of 70 micrometers to remove oil residues on surfaces, which had higher cleaning action than normal bubbles; 2) sterilization: the sterilization action of ozone can be improved by forming micro-bubbles and apply heat and high pressure to them. lkeura (2011) has used water with ozone micro-bubbles to remove pesticide residues and pests from vegetables and fruits. A study by Burns (1997) showed that the greater number of smaller diameter micro-bubbles there are, the more surface area there is. Higher contact surface area and longer retention time translated to better flotation effect. Hasegawa (2008) et al stated that micro-bubbles could be produced by mixing air and water through a series of thin slits inside a tube. In our study, air was mixed and dissolved in water to minimize bubbles. Hasegawa has also stated that the outlet diameter is a key factor influencing the dimensions of the micro-bubbles; we will also perform the study by modifying the diameter of the nozzles to control the size of the bubbles.

2.2. Oral diseases

In the "Fundamentals of Oral Histology and Physiology" by Hand and Frank (2014), common oral diseases include dental caries, tartar formation, gingivitis, periodontal disease and malocclusion. The purpose of brushing is to remove dental plaque (food residues on the teeth) and prevent the growth of bacteria. Plaque is the biomembrane on the surface of teeth; 2/3 of it is comprised of bacteria, which produces acidic products that can erode the enamel components of the teeth, causing caries. Haffajee and Socransky (2000) proposed the pathogenic criteria for periodontal disease: 1) association with periodontal disease – the quantity of bacteria at the site of disease must be multiplying; 2) reduction or elimination of bacteria after treatment; 3) must be able to induce host cellular or antibody immune responses; 4) must induce disease in animal models; 5) the bacteria must be able to produce virulence factors that may cause the destruction of periodontal tissues. Recent advancement in molecular biotechnology has also led to new discoveries of periodontal disease related oral bacteria strains, signifying the complexity and diversity of oral micro flora [4,18].

2.3. Periodontal disease and other related diseases

Morris (2001) and Oliver (1998) concluded that periodontal diseases occur frequently in adults; about 50% of adults are afflicted with periodontal disease. Endo (2007) et al investigated the inhibitory effects of mouthwash on dental plaques in 96 regularly monitored subjects, and found that regular cleaning improves oral hygiene and delays the onset of periodontal disease. However, while mouthwash is effective in decreasing dental plaques, the various components such as essential oil, triclosan, iodine and amine fluoride are extraneous burdens to users. Li (2000) and Emrich (1991) also mentioned that the oral cavity is one of the most easily infected body parts, especially for host with low immunity. Complications from periodontal diseases may also occur in patients with diabetes or rheumatoid arthritis. Failure to maintain oral hygiene may causes periodontal disease in diabetic patients and elevates the risk of destructive periodontal disease. Albandar & Rams (2002) suggested that maintenance of oral hygiene requires wide spread promotion and implementation of common awareness education by relevant authorities. Healthy individuals may maintain a balanced co-existence with oral bacteria with proper oral hygiene, as the saliva contains antibodies that keep the bacteria growth in check. However, individuals with poor immunity are at risk of deaths caused by infection of oral pathogens. There have been cases of unknown fevers in hospitalized patients, which were proven to be significantly related to oral pathogens. Therefore, it is essential that diabetic patients maintain good health with proper oral hygiene.

Fig. 2. Mega Speed HHC X2 (left), Contact Tachometer (right).

3. Method

In this study, we investigated the effects of dental plaque cleaning with micro-bubbles formed by a dental irrigator, with variables such as the rotation speed of the device motor, diameters of the nozzle, flow volume, flow velocity and diameters of the micro-bubbles in the water stream. The experiment was carried out on dentures fixed to a tooth tray. The cleaning action was measured by the amount of dental plaques removed and the procedures were listed in study by Lee (2011). Sterilized test samples were immersed in 10ml test tubes containing 6ml of Candida albicans solution (10^7cfu/ml), and were placed in an incubator for 2 hours at 37°C (180Hz). The samples were then cleaned with different methods and the amount of dental plaques left after cleaning formed the dependent variable of the cleansing effect. The following are descriptions of experiment tools, setup and procedures.

3.1. Micro-bubble formation and measurement

We modified the ejection path of a commercially available dental irrigator Braun MD20 (Fig.1) and used it as the micro-bubble generator, which operated on the same principles as Hasegawa's study (2008). A series of thin slits were placed in the water path and a mixture of air and water is flushed through these slits. Flow speed differential was produced by the difference in the specific density of air and water, which then expels both air and water and forms micro bubbles. However, our study differed from Hasegawa's in that only the pore diameter was changed instead of the tubular formation or internal angles. The original device has a 5-level speed control and its front nozzle can produce different ejection patterns (straight lines and spirals) by attaching different rotary parts. As the purpose of this study is to achieve dental cleaning effect with micro-bubbles through tooth tray, whether the spiraling ejection patterns expanded on the cleaning effect was not the scope of this study. However, as the effect of the ejection patterns on the dimensions of micro-bubbles were not certain, we used a high-speed camera (Fig.2 left) to investigate the dimensions of the micro-bubbles produced by the two ejection patterns under the five-level flow speed. Our results showed that there were no significant differences between both ejection flows on the diameters of micro-bubbles, therefore only the straight-line ejection pattern was used in this study to generate micro-bubbles.

A contact tachometer (Fig.2 right) was used for measuring the 5 level speed settings of MD20. 10 measurements were conducted per each setting and the mean was used as the representing value. The results were listed in the far left column of Table 1. Using an electronic caliper, the ejection nozzle diameter of MD20 was measured to be 0.63mm. To investigate the effect of different nozzle diameters on flow volume, velocity and dimensions of micro-bubbles, we fabricated stainless nozzles of different diameters with electrical discharge machining. Using 0.63mm as the basis, we produced nozzles that had about two-fold reduction (0.3mm) and four-fold reduction (0.16mm), as well as nozzles of 0.8mm (which is 0.2mm larger) and 1.2mm, respectively, with the latter having about two-fold magnification. A total of five different nozzles were produced (Fig. 3). We then used the tachometer to film the five nozzles at five speed settings, which equaled to a total of 25 experiment combinations, and measured the diameters of the generated micro-bubbles. High-speed photography (Fig. 4) was conducted by fixing the nozzle head of the bubble generator inside a square glass box with dimension of 200mmX200mmX200mm, and a thickness of 5mm. The box was then filled with high reverse osmosis pressure water at 23℃ to minimize impurities from impairing micro-bubble formation. The water was filled to a height of 10cm, which is higher than the nozzle head by 2cm. During measurement, the speed setting were adjusted first; to ensure the generator produces normal water ejection, the filming time was set at 3 sec after the device was switched on. A 3-minute interval was set between each photography to prevent residual micro-bubbles from the previous shot affecting the current shot. Each variable combination was shot for one second (1000 frames/sec). About 500~800 frames were then selected and played back in slow speed with Mega Speed AVI Player software. Ten frames with significant micro-bubbles were selected for measurement of bubble diameters and the mean value was taken as representative. The measurement of flow volume was done by measuring the total water volume for ten seconds with a flask, and the unit flow rate was then calculated. The intermediate variable of flow velocity was estimated by measuring the distances of a single micro-bubble between one and two seconds on the video, corresponding to the ratio of actual nozzle size. The measurements of the three intermediate variables are shown in columns A, B and C of Table 1, under each pore diameter.

Fig. 3. Metal fabrication: nozzle diameters (0.16mm, 0.3mm, 0.63mm, 0.8mm and 1.2mm).
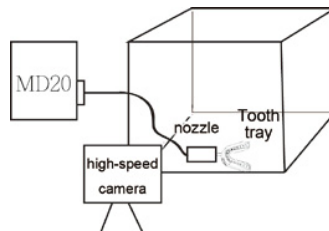

Fig. 4. High-speed photography setup.

Fig. 5. Tooth tray and experiment denture.

3.2. Cleaning samples and materials

To investigate the plaque-cleaning efficacy of micro-bubbles under various variable combinations, we used the micro-bubble generator to produce water stream with micro-bubbles and cleaned bacteria-loaded dentures on tooth trays. A colony counter was used to calculate the bacterial colonies on cleaned dentures (CFU). Tooth trays and bacteria-containing dentures were prepared before the experiment. The tooth tray was produced by a dental lab out of medical grade silicone with a hardness level of 40, as shown in left of Fig.5 (the tooth tray was drilled with small openings for the water streams; each opening is 1.6mm and a total of 14 openings were made). The experiment denture was the upper jaw configuration used in adults, with a total of 14 artificial tooth from GG Dental Co. of Taiwan (Fig.5 right). Bacterial culture procedure: bacterial strains were obtained from the oral cavities of patients with clinical periodontal diseases by rubbing the oral cavity with sterile cotton swabs dipped in sterile saline. The cotton swabs were then placed in sterile test tubes and cultured in Sabourand dextrose agar. Each bacterial strain was transfected to culture medium before each experiment. The medium was placed on an orbital shakers configured to shake at 180 rpm under 37°C, and cultured to maximum growth concentration in about 48 hours.

3.3. Procedures for cleaning dental bacteria

The cleaning experiment was carried out in a sterile laminar flow on adult dentures soaked in bacterial solution. The micro-bubble generator was coupled to the experiment nozzles and attached to the tooth tray. After setting the speed configurations, the cleaning of bacterial solution soaked dentures were carried under 25 sets of experiment combinations. First, the denture was sterilized and then soaked in 250ml of bacterial solution in a 500ml vessel, containing 8×10^{10} cfu/ml of bacterial count. After 30 minutes, the denture was removed and dried for 60 minutes, and then pressed onto a medium plate for 30 seconds. The colonies formed were counted and used as the control group. For the experiment group, the denture was washed for 3 minutes after soaking in bacterial solution for 30 minutes and dried for 60 minutes. The cleaned denture was placed in another sterilized glass plate, and was dried for 15 minutes with the tooth surface facing upward. The denture was then pressed onto a culture medium plate for 30 seconds and placed in room temperature for 24 hour, after which the colonies were then counted. The denture used

for each cycle of cleaning was identical, which was thoroughly sterilized after each cycles to ensure the starting denture was clean before the soaking and cleaning procedures were carried out.

Our study did not target a single bacterial strain when estimating the removal of bacteria, due to the wide variety of dental bacteria residing in the oral cavity; a single strain does not represent the entire oral flora. For calculation of colonies, a colony counter was used together with naked eye observation to count the colonies of all bacteria strains. Due to the horizontal placement of the culture medium plate, only the bacterial strains from the bottom part of the denture were calculated in our study. In addition, the colony counts that exceed the normal bacterial counts (30~300) were deemed as TNCT (too numerous to count), meaning there were too many colonies to be calculated accurately. However, for the ease of statistical analysis of the relationship between independent variables and dependent variables, all colonies that remained were counted and incorporated into the analysis.

4. Results and discussion

4.1. Correlation between the control and intermediate variables

We first investigated the relationship between the five different speed settings and nozzle diameters (0.16mm, 0.3mm, 0.63mm, 0.8mm, 1.2mm) of micro-bubble generator and the flow volume, velocity and bubble diameters of the ejected water stream. The results are should in columns A, B and C of Table 1. Flow volume increased with the increases in nozzle diameters and motor speed; linear regression analysis was then performed with the rotation speed and nozzle diameters as the independent variables, and the flow volume, velocity and bubble diameter as the dependent variable. The results are shown in A of Table 2. The regression coefficient were all positive for all three dependent variables, indicating that as the rotation speed and nozzle diameter increases, the volume of water stream increases, as well as the velocity and size of the micro-bubble. The level of significance for the three dependent variables (flow volume, speed and bubble diameter) influenced by the nozzle diameter was 0.00. There was a positive correlation between the dimensions of the nozzles and the dimensions of the micro-bubbles; the larger the nozzle diameter, the bigger the bubbles. Our results were similar to the theory proposed by Legner (1984) Merkle and Deutch (1992) on the relationship between nozzle and bubble dimensions. Rotor speed with flow volume and velocity also achieved 0.001 and 0.004 significance levels, respectively; there was no significance correlation between rotor speed and diameter of micro-bubble. Normalization coefficient (Beta) was used to determine the weight of the dimensions of nozzle diameter; the values were as follow: micro-bubble diameter (0.940) > flow volume (0.754)> flow velocity (0.671)

Table 1. Coefficient statistics of dependent and independent variables. A: Flow volume; B: Flow velocity; C: Micro-bubble diameter; D: Bacterial removal.

	0.16				0.30				0.63				0.8				1.2			
RPM	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
2580	0.8	0.18	0.08	1192	2	0.30	0.09	700	2.3	0.43	0.14	1420	2.4	0.65	0.22	1342	3	0.75	0.26	1412
3021	0.8	0.34	0.08	534	2.3	0.15	0.08	877	3	0.4	0.17	1405	3.4	0.67	0.14	1442	3.4	0.90	0.29	1085
3527	0.8	0.41	0.09	376	2.6	0.36	0.07	1287	3.3	0.48	0.19	1093	4	0.88	0.20	1467	3.9	0.86	0.28	1210
4210	0.8	0.71	0.11	1194	2.9	0.31	0.11	1400	3.9	0.5	0.15	979	4.3	0.9	0.22	1429	4.3	0.58	0.28	1179
5380	0.8	0.74	0.10	801	3.4	0.54	0.11	1373	4.1	0.68	0.15	1095	5	0.91	0.24	1357	5	0.89	0.23	972
mean				819.4				1127.4				1198.4				1407.4				1171.6

Table 2. Regression analysis of nozzle diameter and rotor speed on dependent variables.

			B	Std. Error	Beta	t	Sig.	R	R^2
	Rotor speed	Flow volume	.001	.000	.403	3.645	.001	.855	.731
	Nozzle diameter		2.651	.389	.754	6.815	.000		
	Rotor speed	Flow velocity	9.899E-05	.000	.422	3.245	.004	.792	.628
A	Nozzle diameter		.425	.082	.671	5.159	.000		

			B	Std. Error	Beta	t	Sig.	R	R^2
A	Rotor speed	Micro-bubble diameter	4.996E-06	.000	.071	.997	.330	.943	.889
	Nozzle diameter		.180	.014	.940	13.256	.000		
B	Rotor speed	Colonies	-.003	.057	-.011	-.054	.957	.405	.164
	Nozzle diameter		320.115	154.239	.405	2.075	.050		
C	Flow velocity	Colonies	32.651	358.530	.026	.091	.928	.496	.246
	Flow volume		94.662	58.792	.421	1.610	.122		
	Micro-bubble diameter		331.292	1349.418	.080	.246	.808		

4.2. Correlation between the control variables and plaque removal

In this study, we discovered that the effect of nozzle diameter dimensions on plaque removal was more significant than rotor speed (0.05 significance, Table 2. B). Fig.7 shows the relationship between rotor speed and nozzle diameter on colony counts. Our results showed that a nozzle diameter of 0.8mm had the most stable plaque removal under all 5 speed settings; the smallest nozzle diameter of 0.16mm had the least stable plaque removal under all 5 speed settings. Column D in Table 1 denoted colony removal after deducting the remaining colonies of experiment groups from the control group (Fig. 6 left). At 3rd speed setting (3527 rpm) nozzle diameter of 0.16mm had the lowest colony removal, indicating this variable combination was less effective at clearing plaques; the maximum removal was achieved with 0.8mm nozzle diameter at 3rd speed setting (3527 rpm), followed by 2nd speed setting of the same nozzle diameter. Mean overall dental plaque removal was better with nozzle diameter at 0.8mm, followed by the variables of 0.63mm; variables with 0.16mm nozzle diameter had the lowest mean plaque removal

4.3. Correlation between intermediate variables and plaque removal

We also performed linear regression analysis with colony removal as the dependent variable, and flow volume, velocity and micro-bubble diameters as the independent variables. The results were shown in Column C of Table 2. At $\alpha = 0.05$ significance level, the effect of flow volume, velocity and micro-bubble diameter on colonies were not significant, with only flow volume having a slight effect than flow velocity and micro-bubble dimensions. From Table 1 it can be discerned that for nozzle diameter of 0.16mm, the flow volume were at equal water level in all 5 rotor speed settings. This may be due to increased water resistance as the result of small nozzle diameter, and could be affecting the flow volume, velocity and micro-bubble dimensions, as well as hindering plaque removal.

Illustration	Control Group: Colonies without cleaning	Experiment Group: Colonies under 0.8mm nozzle and rotor speed 3527rpm
CFU	1590	123

Fig. 6. Colony formation of control and experiment groups.
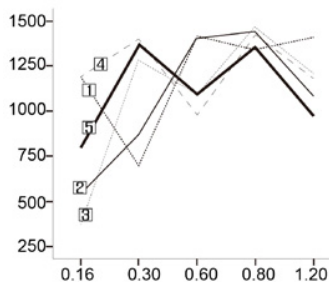

Fig. 7. Linear plot of the bacterial removal in the control variable.

5. Conclusions

In this study, we proposed a method to clean dental plaque on a denture by combining micro-bubbles with a tooth tray platform. The cleaning efficacy was determined by comparing the colony counts of the control group to the experiment group. There was a significant positive correlation between the micro-bubble water flow volume, velocity and rotor speed of the bubble generator and the dimensions of nozzle diameter; the dimensions of the micro-bubbles correlate positively with the nozzle diameter. An overall plaque-removing efficacy of 60% was achieved with the nozzle diameter having a slightly positive influence on cleaning efficacy, especially at nozzle diameter of 0.8mm where the cleaning effect was at its best. There were no significant effects of flow volume, velocity and micro-bubble diameter on cleaning efficacy, only with flow volume exerting a slight effect on plaque removal. This may be due to insufficient control variables and will be studied further in the next phase of the experiment. The study used denture to replace human oral cavity as the subject of experiment, and used bacterial colonies to verify cleaning efficacy. However, in this way, only the bacteria on the bottom of the teeth were approximated, while those on the inner and outer faces and adjacent surfaces were not assessed. For subsequent experiments, streak plate method may be used to conduct colony growth and counts. Our results showed that confining the micro-bubbles within a limited space in the mouth and using a tooth tray to circulate the bubbles may help to clean oral bacteria, and may be beneficial for long-term bed ridden patients in the future. While our results were limited to cleaning artificial dentures, it may be adapted in the future to cleaning teeth on humans, and will be the objective of the next phase of the study.

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