Original Article

pISSN, eISSN 0125-5614
M Dent J 2021; 41 (1) : 75-82

Evaluation of the optimum brushing force on dental plaque removal: an *in vitro* study

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Objective: To determine an optimum brushing force for dental plaque removal on enamel surface when brushing with manual toothbrush

Materials and Methods: This *in vitro* study used seventy-two extracted bovine incisal teeth to evaluate an efficacy of plaque removal by automatic brushing machine with toothbrush (divided into positive control group, negative control group and six testing groups, each group n=9). *Streptococcus mutans* was incubated for 24 hours to form bacterial plaque on the enamel surface. Samples were stained with erythrosine red. Positive group was a maturation plaque stained and negative group was a plaque free sample with staining. Brushing test was performed by an automatic brushing machine under a controlled condition. Loading force was applied from a minimum of 1 newton (N), with an increment of 1 N to a maximum of 6 N. Sample's focusing area photographs were used to interpret a remaining percentage of red color in the Red Green Blue system (RGB). Comparing mean difference by Welch's ANOVA and Games Howell post hoc test.

Results: After testing with various brushing force, less percentage of red color remained when more force was performed. The 3 N group showed significantly less remaining red color than the positive control group but not significantly different from the negative control group ($p<0.05$). Less brushing force (1 N and 2 N group) showed significantly lower of remaining red color than the positive control group and significantly higher than the negative control group.

Conclusion: Brushing with 3 N force was recommended for dental plaque removal from enamel surface by manual toothbrush.

Keywords: bovine teeth, brushing force, brushing machine, erythrosine dye, plaque removal, *S. mutans*

How to cite: Pujarern P, Rodanant P, Warinsiriruk E, Rattanasuwan K. Evaluation of the optimum brushing force on dental plaque removal: an *in vitro* study. M Dent J 2021; 41: 75-82.

Introduction

Bacterial plaque has been well accepted as an etiology of gingivitis [1]. An increasing accumulation of pathogenic bacteria in the plaque can implicate in the progression of gingivitis to periodontitis [2]. An adequate plaque control is the key procedure to prevent and treat gingivitis and periodontitis [3]. Tooth brushing is a preferable recommended method for plaque control [4]. However, excessive brushing force, too often brushing frequency, improper brushing technique and hard toothbrush bristle may cause damages to oral hard and soft tissue in the oral cavity. These are considered as co-factors of multifactorial etiology of non-carious cervical lesions (NCCL) [5-8].

NCCL is the loss of tooth structure at the cementoenamel junction (CEJ) level that is unrelated to dental caries [9]. Tooth sensitivity is

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Received : 18 November 2020 Revised: 6 January 2021 Accepted: 8 January 2021

a major complaint of patients who have NCCL [10]. The prevalence of NCCL is 2 - 90% [8]. The etiology of NCCL is multifactorial in origin [5, 11-13]. Abrasion, erosion and abfraction are three main contributing factors [14].

Cervical abrasion, a type of NCCL, is defined as a tooth wear resulting from excessive brushing or brushing with abrasive toothpaste [5, 15]. Abraded enamel and dentine lesions are frequently detected in the posterior maxillary teeth which are characterized by smooth and glazed appearance with less plaque accumulation [9, 16]. Initially, it occurs as a small horizontal groove at the buccal surface near CEJ. In later stage, surrounding walls of groove abrades exposing more dentine and turns into V-shape in characteristic causing gingival recession and root surface exposed [17, 18].

Considered as a contributing factor of cervical abrasion, brushing force can vary from 1 – 11 newton (N) (102 – 1121 g) with the average of 1.6 N when performing by manual toothbrush [19, 20]. *In vivo* and *in vitro* studies demonstrated direct correlation between oral hygiene practice (brushing force and frequency) and the development of surface tooth wear [7, 16, 21-23]. Moreover, evidences emphasized that force applying during brushing was responsible for the burden of tooth structure while a physical characteristic of toothbrush or bristle firmness were unrelated [22, 24].

An ideal brushing should have an effective plaque removal without harmful effect on hard and soft tissue. Most previous studies interested in the damage of oral soft and hard tissues due to tooth brushing while very few studies focused on the effect of manual brushing on plaque removal. It is the interest of present study to determine an optimum brushing force for dental plaque removal on enamel surface.

Materials and methods

Ethical approval (FTM-ACUC 005/2018) for bovine tooth collection was granted by the Faculty of Tropical Medicine-Animal Care and Use Committee, Mahidol University Bangkok, Thailand. Sample size calculation was based on the study of Wiegand *et al.* [20]. Given power level 80% and significance level at 0.05, thus, the total of 72 bovine teeth was collected and divided into eight groups (positive control group, negative control group and six testing groups, each group n=9).

Tooth sample preparation

Stocked caries free bovine incisors with no or less staining on insical half were recruited in this study. After cleaning the enamel surface with periodontal curette, tooth was cut with fissure diamond bur at CEJ leaving part of the tooth covered by enamel was collected as specimen. Each specimen was wrapped with transparent dressing film (Tegaderm™ Film, 3M, USA) exposing 5 mm of incisal area where designated as plaque formation area (Figure 1). The tooth samples were sterilized by steam-under-pressure (autoclave) and then kept at 4ºC until use.

Figure 1 A tooth sample was wrapped with transparent dressing film except 5 mm of incisal area where the plaque was formed.

Acquired pellicle formation

Saliva collected from the investigator (Putr Pujarern) was processed by centrifugation at 4000 x g for 15 minutes. The supernatant was diluted 1:10 in phosphate buffered saline (PBS), and sterilized by filtration using 0.2 µm millipore membrane. Test and positive control samples were incubated with the preparated saliva at 37°C for 24 hours letting acquired pellicle to form on enamel.

Plaque formation

Streptococcus mutans (ATCC 25175), kindly provided from Assoc. Prof. Dr. Ratchapin Srisatjaluk, Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, was maintained in Brain Heart Infusion (BHI) agar. Prior to the experiment, the 3-5 bacterial colonies were cultured in the BHI broth, incubated in a 5% CO_2 incubator at 37°C for 24 hours. Then the bacterial culture was transferred into another BHI broth containing 5% sucrose. Adjusted the turbidity of cultured broth to equal the standard McFarland 0.5 which achieved an estimating cell density of 1-5 x 10^8 CFU/ml.

The acquired pellicle formation samples were placed in 6-well polystyrene culture plate then immersed with 8 ml of bacterial suspension in each well. Incubated at 37°C in a 5% CO_2 incubator for 24 hours. After incubation, transparent dressing film was removed, the bacterial plaque at the incisal area of test and positive control samples were stained with 6% erythrosine red color solution (M-Dent®, Mahidol University, Thailand). Positive control samples were the stained tooth samples with plaque to demonstrate the plaque formation of *S. mutans*. Negative control samples were the stained tooth sample without plaque to demonstrate the baseline staining of non-plaque formation on the tooth surface. Both positive and negative control samples were also rinsed with tap water for 30 seconds to remove excess dye and did not test with a brushing machine.

Assessment of plaque removing force

Test samples were brushed with different vertical brushing forces with an automatic brushing machine which was designed and manufactured by the Faculty of engineering, Mahidol University (Figure 2). Each test sample was rinsed with tap water for 30 seconds to remove excess erythrosine dye before fixed on a brass mold attached to the automatic brushing machine. A soft and leveled bristle toothbrush (M-Dent®, Mahidol University, Thailand) was fixed in the holder of brushing machine and adjusted until a long axis of the bristle were perpendicular to the tooth surface. Brushing test was performed without toothpaste in linear mesio-distal motion with a frequency of 4 strokes per second for 4 seconds. Brushing time used for each sample was calculated from a total time of 2 minutes required to perform for full mouth brushing [4]. The toothbrush holder was adjusted allowing the toothbrush bristle to press onto the tooth surface until the loading monitor indicated the designated vertical loading force. Loading force was applied from a minimum of 1 N, with an increment of 1 N to a maximum of 6 N. Each sample received only a single test and new toothbrush was given in every new test. Nine samples were used in each testing force.

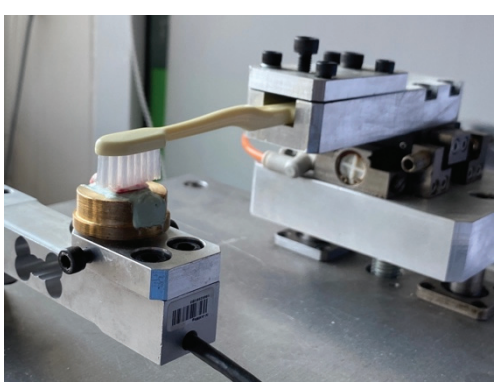

Figure 2 A toothbrush fixed with a holder attached to an automated brushing machine and tooth sample fixed in a brass mold.

Sample imaging

Photographs of the tooth samples were used to determine the red color of the stained plaque that remaining on the tooth surfaces after brushing compared to the other samples. Images of the tooth samples were taken using a camera (Canon® 100D, Japan) mounted in the same spot. A 4 x 4 mm focusing area was selected at the center of the testing area. The focusing area was gridded into 100 squares. A hex color code was assigned to each square to calculate the percentage of the red color in the RGB color system (Adobe Creative Cloud 2018). For each sample, the mean percentage of red color from 100 squares was calculated to represent the remaining plaque.

Statistical analysis

Statistical analysis was performed using a SPSS 18.0, (IBM. Chicago, USA). Welch's analysis of variance (ANOVA) followed by Games Howell post hoc test was used to evaluate if there are differences in mean percentage of red color between groups. The level of significance was considered at $p<0.05$.

Results

After brushing with various forces, the remaining red color which represents the remaining plaque on the samples were assessed and compared to the positive control group (plaque without brushing) and the negative control group (no plaque). Representative images from each group showing the red color of the stained *S. mutans* plaque on the tooth surfaces after testing with various brushing force are illustrated in Figure 2.

The significant difference of the red color obtained between the groups indicated the effectiveness of brushing force to plaque removal. Games Howell results of the mean difference in comparison of the percent red color by brushing forces between each group are shown in Table 1. The results indicated the statistically significant difference of the positive control from other groups ($p<0.05$). Negative control without plaque had the baseline of red color staining, of which was statistically significant difference from all groups ($p<0.05$). The significant difference of the red color was found between the group of 1 N and other groups ($0.05>p$). The group of 2 N also had a statistically significant difference from other groups ($p<0.05$) but not significantly different from the group of 3 N ($p<1$). The group of 4 N was statistically significant difference from other groups ($p<0.05$). The group of 5 N and 6 N were statistically significant difference from other groups ($p<0.05$) but were not statistically significant difference between each other ($p=1$)

The mean percentage of remaining red color and standard deviations value are shown in Table 2. Positive control group showed the mean percentage of red color at 84.37 ± 4.2, whereas the negative control group showed the mean percentage of red color at 47.42 ± 1.62. Positive control group was significantly different from the other groups. The Groups of 1 N, 2 N, 4 N, 5 N, and 6 N were significantly different from the negative control group. Only the 3 N group was not significantly different from the negative control. The groups of 4 N, 5 N and 6 N have less percentage of remaining red color than negative control. The results indicated that the brushing forces at 3 N – 6 N could significantly remove plaque.

Figure 2 The representative images of each study group. (N: Newton)

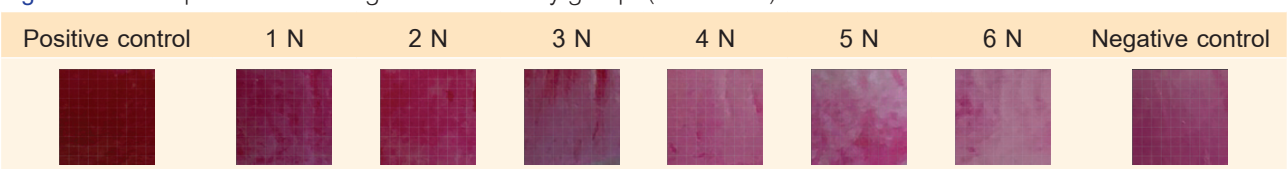

Table 1 Games Howell results of the mean difference in comparison of the percent red color by brushing forces.

Variable	Positive control	p-value	1 N	p-value	2 N	p-value	3 N	p-value	4 N	p-value	5 N	p-value	6 N	p-value	Negative control	p-value
Positive control (n = 9)	-	-	30.68	<.01*	33.06	<.01*	34.91	<.01*	39.28	<.01*	40.86	<.01*	40.75	<.01*	36.95	<.01*
1 N (n = 9)	-30.68	<.01*	-	-	2.38	.042*	4.23	.007*	8.60	<.01*	10.18	<.01*	10.07	<.01*	6.27	<.01*
2 N (n = 9)	-33.06	<.01*	-2.38	.042*	-	-	1.85	.527	6.21	<.01*	7.80	<.01*	7.68	<.01*	3.89	.002*
3 N (n = 9)	-34.91	<.01*	-4.23	.007*	-1.85	.527	-	-	4.36	.006*	5.95	<.01*	5.84	.001*	2.04	.428
4 N (n = 9)	-39.28	<.01*	-8.60	<.01*	-6.21	<.01*	-4.36	.006*	-	-	1.59	.013*	1.47	.025*	-2.32	.033*
5 N (n = 9)	-40.86	<.01*	-10.18	<.01*	-7.80	<.01*	-5.95	<.01*	-1.59	.013*	-	-	-0.11	1.00	-3.90	0.001*
6 N (n = 9)	-40.75	<.01*	-10.07	<.01*	-7.68	<.01*	-5.84	.001*	-1.47	.025*	0.11	1.00	-	-	-3.80	0.001*
Negative control (n = 9)	-36.95	<.01*	-6.27	<.01*	-3.89	.002*	-2.04	.428	2.32	.033*	3.90	0.001*	3.80	0.001*	-	-

*The level of significance was considered at $p<0.05$

Table 2 The mean percentage of remaining red color and standard deviations.

Group	Mean ± SD
Positive control	84.37 ± 4.2
1 N	53.69 ± 1.28
2 N	51.31 ± 1.55 *
3 N	49.46 ± 2.34 *,¶
4 N	45.09 ± 0.73
5 N	43.51 ± 0.86 †
6 N	43.62 ± 0.88 †
Negative control	47.42 ± 1.62 ¶

The level of significance was considered at $p<0.05$

*No significant difference between 2 N and 3 N ($p=0.527$)

¶No significant difference between 3 N and Negative control ($p=0.428$)

†No significant difference between 5 N and 6 N ($p=1$)

Discussion

Results from this study showed that after brushing with the 3 N, there was a significant difference of the intensity of red color from the positive control group but not significant difference from the negative control group. It suggests that brushing with 3 N was an optimal force for dental plaque removal from the enamel surface. Although less brushing force (1 - 2N) showed significantly less intensity of the red color from the positive controls, these forces magnitude could not be judged as optimal forces since the intensity of red color after brushing with these forces was significantly higher than the negative controls. However, more brushing force (4 N, 5 N and 6 N) showed a significantly lower remaining red color when compared to negative control. This suggests that brushing with 4 N, 5 N and 6 N were too much force to remove bacterial plaque. Previous studies demonstrated that too much brushing force exerted a harmful effect on the tooth structure. High brushing force (400g) could expose more dentinal tubules than less force applied [23]. It was also observed that there was direct correlation between the occurrence of NCCL and hard brushing force (Odds ratio = 2.43) [16, 22]. Thus, brushing forces more than 3 N might cause damage to tooth structure and we would not recommend using brushing force more than 3 N.

The brushing force and duration are factors that affect the outcomes of plaque removal. The success of manual tooth brushing on plaque removal was evidently reported. In a systematic review, it concluded that manual brushing could reduce plaque score from baseline score on an average of 42% [25]. In term of quantity measurement, a weight mean reduction of 30% was reported [26]. There was a significant relationship between brushing time/brushing force and plaque removal efficacy [27]. Moreover, there was a positive relation between brushing force and plaque removal up to a level of 4 N force [28]. These data were consistent with present study.

This *in vitro* study was designed to determine the optimal brushing force for dental plaque removal on enamel surface. For the convenience of sample recruitment and preparation, extracted bovine incisor was chosen for the experiment. No differences between human and bovine enamel were observed, so that the bovine enamel could be used as an alternative to human enamel in dental abrasion studies [29]. The success of the *in vitro* plaque formation on the conditioned (saliva-coated) enamel surfaces in this study can be explained by the ability of *S. mutans* to hydrolyze sucrose in the culture media and to produce extracellular polysaccharide glucans. Stained the tooth sample with erythrosine is a simple and effective method to make plaque deposition visible.

In order to standardize the experiment, this study performed the test under the controlled and repeatable conditions and process; brushing force, brushing frequency, brushing angle, and brushing duration using an automatic brushing

machine. The same brand-new toothbrush (M-Dent®) was used for brushing each tooth sample. Since previous studies reported that brushing force applied during manual tooth brushing was between 1 to 11 N with the mean force of 1.6 N [19, 20], this study, thus, chose to perform brush test using various brushing forces from 1 to 6 N. Brushing duration of 2 minutes conformed with the approximate time to perform adequate full-mouth brushing recommended by the American Dental Association [4].

In this study the RGB color system was used to evaluate the percentage of red color from sample images. RGB stands for "Red Green Blue" which refers to three hues of light that can be mixed together to create different colors. Combining red, green, and blue light is the standard method of producing color images on screens, such as TVs, computer and smartphone screens. The RGB color model is an "additive" model. When 100% of each color is mixed together, it creates white light. When 0% of each color is combined, no light is generated, creating black. From the theory of the RGB model, pure white color in the image consists of equal three light colors, so there is 33.33% of red in white image. In clinical practice, enamel color is an off-white shading and if there are no plaque left on the enamel surface, it is possibly at least 33.33% of red when analyzing with the RGB system.

From the result of this study may create a new daily life device such as a modern toothbrush with force detector to allow people to brush their teeth with an optimum brushing force. However, further studies are needed to make a conclusion in an abrasive effect on enamel surface in order to fabricate a new design toothbrush with an efficacy of plaque removal and do not have a harmful effect on tooth surface.

Conclusion

To the limitation of this *in vitro* study, the optimal brushing force to remove dental plaque from enamel surface using manual toothbrush was 3 N.

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