The effects of brushing on human enamel surface roughness after NaF gel and theobromine gel exposure

A Mahardhika, A Noerdin and Y K Eriwati*
Department of Dental Materials, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
*E-mail: yosiarianto@gmail.com

Abstract. This study aimed to determine the effects of brushing on human enamel surface roughness after different exposure times of 200 mg/L theobromine gel (8, 16, and 32 minutes) and 2% NaF gel (16 minutes). Twenty-four human upper premolars were used and divided into four groups. Group 1 was exposed to 2% NaF gel for 16 minutes. In contrast, groups 2, 3, and 4 were exposed to 200 mg/L theobromine gel for 8 minutes, 16 minutes, and 32 minutes, and each group was then brushed for 9 minutes and 20 seconds. After the treatment, samples were tested using a surface roughness tester (Mitutoyo SJ 301, Japan). The Wilcoxon test showed significant changes ($p < 0.05$) in roughness values after exposure to the theobromine gel or NaF gel and after brushing for 9 minutes and 20 seconds. It can be concluded that exposure to 200 mg/L theobromine gel or 2% NaF gel can soften the enamel surface and then increase roughness after brushing.

1. Introduction
Dental caries are a dental hard tissue disease with a high prevalence in Indonesia [1]. However, the disease can be prevented. One preventive measure can be performed by brushing regularly and using toothpaste that contains fluor or other topical fluoride, such as gel or varnish [2,3]. Fluor in toothpaste can strengthen dental enamel by remineralization the dental [4]. However, when used in excessive doses, fluor can endanger human health (e.g., dental fluorosis) [5]. As a result, some oral and dental products in the health industry do not contain fluor. Nonetheless toothpaste without fluor does not provide tangible benefits in addition to the cleaning [4]. Therefore, research using other alternative materials as an active agent, such as theobromine was conducted [4]. Theobromine is a methylated xanthine, and it can strengthen enamel [6].

Previous study stated that testing the effects of theobromine and sodium fluoride on dental enamel hardness shows that the theobromine solution with a concentration of 1 mg/L (0.006 mM) produces the hardness value of 0.25% (w/v) (59.5 mM). Sodium fluoride is usually found in commercial toothpaste. It was also revealed that the dramatic increase of dental enamel hardness occurs in the concentration of the theobromine solution between 100 mg/L and 500 mg/L [7]. In 2012, Sadeghpour et al. began introducing toothpaste based on chocolate as a replacement for the fluor in toothpaste [8]. The influence of theobromine in a different form is currently no well-known, such as in the form of gel. Gel can be attached at a specific time to the surface of the enamel, and be easily applied. In addition, research on the effect of a remineralization agent’s exposure to a gel containing 200 mg/L theobromine with three different exposure durations (8, 16, and 32 minutes) has scarcely reviewed the effect on enamel surface roughness. It is known that brushing every day has the effect of mechanical friction on the surface of the dental enamel, which can affect the surface roughness. In general, gel...
containing 2% sodium fluoride (NaF) was used for as long as 4 minutes for the one-time topical application for enamel remineralization [9]. The aim of this research is to understand the effects of brushing on human enamel surface roughness after exposure to 200 Mg/L theobromine gel and 2% NaF gel, which is considered to be one of the remineralization agents needed to maintain the condition of the enamel surface after mechanical friction from brushing.

2. Materials and Methods

This research was a laboratory experiment. Testing the roughness of the enamel surface (before, after gel exposure, and after brushing for 9 minutes 20 seconds) was done using a surface roughness tester (Mitutoyo SJ 301, Japan). The sample was 24 pieces of human premolar with smooth surface; these were divided into four groups. Group 1 (n = 6) was exposed to the 2% NaF gel for 16 minutes. Groups 2, 3, and 4 (n = 6), were given exposure to the 200 mg/L theobromine gel for 8, 16, and 32 minutes. The roughness value was then tested. The four groups were brushed for 9 minutes and 20 seconds to see the influence of the 200 mg/L theobromine gel compared to the 2% NaF gel on the enamel surface roughness, which had formed as a result of the mechanical friction from brushing.

The purpose of exposing the enamel with 2% NaF gel for 16 minutes was to simulate the effect of the gel being applied to teeth for one month once a week on individuals with a cavity with a frequency of once to two times each year (Mount & Hume, 2005). One-time gel application was done based on Liaison et al. (2008). The duration of the topical fluoride application (solution/gel/varnish) in most studies was 4 minutes, which showed more effective results than less than 4 minutes.  The three durations of applying the theobromine gel (8, 16, and 32 minutes) were meant to simulate applying the gel for one month with the different durations of application. The durations are twice a month, four times in one month, eight times in one month, and application duration of 4 minutes, mimicking the application procedure of 2% NaF gel.

The brushing duration is the conversion of brushing for one month (28 days) on one dental surface; it is and based on Ponduri (2005), where one dental surface was expected to receive 10 seconds of brushing in one cycle [11]. Therefore, when teeth are brushed two times a day and one brushing on one dental surface is ten seconds, 9 minutes and 20 seconds is equal to the brushing time for one month (28 days). The brushing load used was 200 grams. Based on Ranjitkar (2009), a 200-gram load is normal for a simulation when brushing dental enamel in vivo [12]. The load is still in the range of the load based on ISO/TS 14569-1:2007 (E), which is the technical specification for the wear test of tooth brushing (namely, that the pressure of the toothbrush against the specimen is between 0.5–2.5 N) [13]. The data was analyzed using the Kruskal-Wallis and Friedman tests. The Kruskal-Wallis test compared the average value of the four groups while the Friedman test was used on each of the groups to discover the difference in the roughness value before and after treatment, which was then followed by the post hoc test using the Wilcoxon test. The post hoc test was used on the data that had significantly different means (p<0.05) and to differentiate which measurements had significantly different means.

3. Results and Discussion

3.1 Results

Table 1 show the difference in the average value of enamel surface roughness (Ra) before and after exposure to the 2% NaF gel (16 minutes) and 200 mg/L theobromine gel (8, 16, and 32 minutes) followed by brushing (9 minutes and 20 seconds). The results of exposure to the NaF gel and the theobromine gel show that there is a decline in the average value the enamel surface roughness. The enamel becomes smoother, and there is an increase in the value of the average roughness after brushing enamel. Meanwhile, the average value of enamel roughness after brushing and exposure to the groups with NaF gel (16 minutes), theobromine gel (8 minutes), and theobromine gel (16 minutes) showed an increase in the average value of enamel roughness, which exceeded the average roughness of the group. However, for the group exposed to gel theobromine (32 minutes), there is an increase in the average value of enamel roughness that does not exceed the average roughness in the baseline group.
**Table 1.** The average roughness value of the enamel (in μm) before and after the exposure of the gel and brushing

| Surface Roughness (μm) | 2% NaF Gel (16’) | 200 mg/L Theobromine Gel (8’) | 200 mg/L Theobromine Gel (16’) | 200 mg/L Theobromine Gel (32’) |
|------------------------|------------------|---------------------------|-------------------------------|--------------------------------|
| Before Exposure        | 0.1611±0.0417    | 0.1611±0.0482             | 0.1611±0.0491                 | 0.1611±0.4598                  |
| After Exposure         | 0.1500±0.0420    | 0.1456±0.0449             | 0.1400±0.0506                 | 0.1367±0.0389                  |
| After Brushing         | 0.1950±0.0457    | 0.1867±0.0474             | 0.1628±0.0435                 | 0.1550±0.0424                  |

The data in Table 1 and Picture 2 above have been tested statistically using the Kruskal-Wallis and Friedman tests. The following table contains the test results and the Kruskal-Wallis and Friedman tests (Table 2).

**Table 2.** The results of the average value of roughness, the value of the significance of the Kruskal-Wallis test, and the value of the significance of the Friedman test

| | The baseline Ra (μm) | The Ra-exposed gel (μm) | The Ra-brushing (μm) | p-value |
|------------------------|-----------------------|------------------------|---------------------|---------|
| The 2% NaF Gel(16’)    | 0.1611±0.0417         | 0.1500±0.0420          | 0.1950±0.0457       | 0.002*  |
| The 200 mg/L Theobromine Gel (8’) | 0.1611±0.0482 | 0.1456±0.0449 | 0.1867±0.0474 | 0.002*  |
| The 200 mg/L Theobromine Gel (16’) | 0.1611±0.0491 | 0.1400±0.0506 | 0.1628±0.0435 | 0.009*  |
| The 200 mg/L Theobromine Gel (32’) | 0.1611±0.4598 | 0.1367±0.0389 | 0.1550±0.0424 | 0.006*  |
| p-value                | 1.000**              | 0.960**                | 0.361**             |         |

For the data in Table 2 on the Kruskal-Wallis test, the significance of the average roughness sound enamel before gel exposure, after gel exposure, and after brushing shows p>0.05. This can be interpreted as there being no difference between the value of the average roughness between the four groups, namely 2% NaF gel (16 minutes), theobromine gel (8 minutes), theobromine gel (16 minutes), and theobromine gel (32 minutes). In contrast, the data in Table 2 on the Friedman test showed the value of significance less than 0.05. This shows that there is no difference between the average enamel surface roughness values for the two measurements. Therefore, determining which measurements are different between the values of the average roughness is then continued with the Wilcoxon test (Table 3).

**Table 3.** The value of the significance of the Wilcoxon test

| | Significance value (after exposure in the baseline group) | Significance value (after brushing and gel exposure) | Significance value (after brushing in the baseline group) |
|------------------------|--------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------|
| 2% NaF Gel (16’)       | 0.027*                                                 | 0.028*                                               | 0.028*                                                   |
| 200 mg/L Theobromine Gel (8’) | 0.027*                                              | 0.028*                                               | 0.027*                                                   |
| 200 mg/L Theobromine Gel (16’) | 0.027*                                             | 0.027*                                               | 0.786**                                                   |
| 200 mg/L Theobromine Gel (32’) | 0.028*                                              | 0.028*                                               | 0.102**                                                   |

*: significantly different, **: not significantly different
In Table 3, it can be seen that almost all groups have significance values less than 0.05 ($p<0.05$) before and after treatment. This means that there is a difference between the means of the roughness values before and after treatment. This means there is no significant difference in the treatment means when comparing each group. The percentage difference of the average roughness value in the baseline group, roughness after applying the gel, and roughness after brushing the four groups can be seen in Tables 4, 5, and 6.

**Table 4.** The percentage difference in the value of the initial average enamel surface roughness and after exposure to the gel for the four test groups

|                        | The Ra - baseline (μm) | The Ra - after exposure to the gel (μm) | The percentage changes |
|------------------------|------------------------|----------------------------------------|------------------------|
| The 2% NaF Gel (16')   | 0.1611±0.0417          | 0.1500±0.0420                          | -6.9%                  |
| The 200 mg/L Theobromine Gel (8') | 0.1611±0.0482          | 0.1456±0.0449                          | -9.6%                  |
| The 200 mg/L Theobromine Gel (16') | 0.1611±0.0491          | 0.1400±0.0506                          | -13.1%                 |
| The 200 mg/L Theobromine Gel (32') | 0.1611±0.4598          | 0.1367±0.0389                          | -15.2%                 |

In Table 4, it can be seen that there is a decline in the value of roughness after exposure to NaF gel or theobromine gel in all groups. The samples that were given the theobromine gel for 32 minutes experienced the largest reduction in the value of enamel roughness (15.2%).

**Table 5.** The percentage difference in the average enamel roughness value after exposed to the gel and after brushing the four test groups

|                        | The Ra - after exposure of the Gel (μm) | The Ra - after brushing (μm) | The percentage changes |
|------------------------|----------------------------------------|-----------------------------|------------------------|
| The NaF Gel (16')      | 0.1500±0.0420                          | 0.1950±0.0457              | 30%                    |
| The 200 mg/L Theobromine Gel (8') | 0.1456±0.0449          | 0.1867±0.0474              | 28.2%                  |
| The 200 mg/L Theobromine Gel (16') | 0.1400±0.0506          | 0.1628±0.0435              | 16.3%                  |
| The 200 mg/L Theobromine Gel (32') | 0.1367±0.0389          | 0.1550±0.0424              | 13.4%                  |

From Table 5, it can be seen that there is an increase in roughness value after brushing all the samples. The group exposed to NaF gel for 16 minutes had the largest increase in enamel roughness (30%). The sample group that had lowest increase in roughness was the group given Theobromine gel for 32 minutes (3.4%).

**Table 6.** The percentage difference in the initial average enamel surface roughness value and after brushing the four test groups

|                        | The Ra - baseline (μm) | The Ra - after brushing (μm) | The percentage changes |
|------------------------|------------------------|-----------------------------|------------------------|
| The 2% NaF Gel (16')   | 0.1611±0.0417          | 0.1950±0.0457              | 21%                    |
| The 200 mg/L Theobromine Gel (8') | 0.1611±0.0482          | 0.1867±0.0474              | 15.9%                  |
| The 200 mg/L Theobromine Gel (16') | 0.1611±0.0491          | 0.1628±0.0435              | 1.1%                   |
| The 200 mg/L Theobromine Gel (32') | 0.1611±0.4598          | 0.1550±0.0424              | -3.8%                  |

From Table 6, it can be seen that the roughness value increases after brushing with NaF gel and theobromine gel (8 minutes and 16 minutes) compared to the original roughness value. However, there is a 3.8% decline in the value of roughness for the theobromine gel group (32 minutes).
3.2 Discussion
Based on the results, enamel surface roughness after exposure to the 2% NaF gel (16 minutes) and 200 mg/L theobromine gel with three different time durations (8, 16, and 32 minutes) is different (p < 0.05) in the average roughness of all samples after being exposed to the gel (compared to the original average roughness). The percentage difference of roughness shows that all group samples declined in roughness baseline after being exposed to gel. This shows that, after being exposed to the NaF gel or theobromine gel, the enamel became smoother. The 200 mg/L theobromine gel can reduce the enamel surface roughness more than the 2% NaF gel. The highest decreased roughness value is the enamel that was given exposure to the 200 mg/L theobromine gel for 32 minutes, which was 15.2%. Research by B.T. Amaechi et al. showed that an artificial saliva solution with theobromine can produce higher remineralization than saliva and toothpaste with NaF [14]. Therefore, for this research, samples were given longer exposure to the 200 mg/L theobromine gel (32 minutes); it was possible to boost the remineralization process, with the exposure to the 2% NaF gel for 16 minutes and 200 mg/L theobromine gel for 8 minutes and 16 minutes.

Based on the results (comparing the value of roughness after brushing to the value of roughness at the beginning for the theobromine gel group for 32 minutes), enamel roughness increases after brushing compared to the value of enamel roughness after exposure to the gel. However, the value was still lower than the earlier average value of roughness. This means that the value of the average roughness (for the theobromine gel treatment group for 32 minutes) after brushing was still more subtle of a value than the average roughness earlier. This is even though it is not significantly different. Increasing enamel roughness is in accordance with the research of Sukran et al., who stated that brushing with water and without toothpaste had an effect on increasing the enamel roughness [15]. Wiegand et al. stated that both manual toothbrushes and electric toothbrushes can cause abrasions on the surface of the enamel [16]. Therefore, the roughness that occurred in this research is suspected to be abrasion caused by the mechanical friction of the toothbrush [5].

The Kruskal-Wallis test was performed to compare the value of the average enamel roughness after exposure to the gel and after brushing the four groups—namely the 2% NaF gel (16 minutes) and the 200 mg/L theobromine gel (8, 16, and 32 minutes). It showed that there was no difference among the values of the average roughness means for the four groups. Based on the explanation above, it can be seen that the use of gel, which contains 2% NaF (16 minutes) and 200 mg/L theobromine with three different time durations (8, 16, and 32 minutes), can decrease the value of enamel surface roughness after being exposed to the gel. The largest decrease in enamel surface roughness is in the theobromine gel 32 minutes group. However, there are no significant differences between the four groups of samples after exposure to the gel.

Brushing was done after applying gel to each of the groups; there was an increase in enamel surface roughness. The largest increase in enamel surface roughness value is in the 2% NaF for 16 minutes group (Table 5). However, there is no significant difference in the means of the four groups after brushing. Based on the explanation above, it can be seen that the group with exposure to the theobromine gel for 32 minutes was still able to maintain enamel surface abrasion resistance/mechanical friction abrasion resistance due to brushing—even though it was not significantly different from sound enamel surface roughness. The existence of remineralization by theobromine and the theobromine effect can increase the size of the crystal hydroxyapatite. This allows enamel that has been exposed to theobromine to be more resistant to electric toothbrush abrasion [6,16]. The 200 mg/L theobromine gel used in this research is pure theobromine gel without any additional minerals like calcium and phosphorus. However, the 200 mg/L theobromine gel alone already can reduce the roughness of the enamel surface compared to the 2% NaF gel. Therefore, further research is needed on the addition of calcium and phosphorus to theobromine gel so that the remineralization effect can be optimal.
4. Conclusion
Giving gel containing 2% NaF (16 minutes) and gel containing 200 mg/L theobromine with different duration times (8, 16, and 32 minutes) can reduce the roughness of enamel surfaces. However, after brushing for 9 minutes and 20 seconds, there was an increase in enamel surface roughness.

References
[1] The research and development of Health Ministry of Health of the Republic of Indonesia. Basic Health Research (RISKESDAS) 2007 The National report 2007. December 2008 [cited 11 August 2012]. Available from: http://www.litbang.depkes.go.id/bl_riskesdas2007.
[2] Kidd E A M and Joyston-Bechal S 1992 The basics of Caries: disease and management. Over the Bible, Narlan Sumawinita, et al. (Jakarta: EGC).
[3] Darby M L, Walsh M M 2003 Dental Hygiene Theory and Practice. 2nd Ed. (St Louis Missouri: Saunders) p. 348-358, p 529-549
[4] Anderson K 2012 Theobromine for tooth decay Prevention. Cosmetics & Toiletries Magazine Available from: http://www.cosmeticsandtoiletries.com/research/techtransfer/145217445.html
[5] Wilkins E M 2009 Clinical Practice of the Dental Hygienist. 10th Ed. (Philadelphia: Lippincott Williams & Wilkins). p 408-411, 420, 425, 563
[6] Nakamoto T, Simmons W B and Falster A U 2001 Apatite-forming systems: Methods and Products. The patent number: US 6183711 B1.
[7] Arman S and Tetsuo N 2011 Methods and Compositions to receiving complain Mechanical Resistanceof Dental. The patent Cooperation Treaty. International Publication Number: WO2011/100671 A2.
[8] The dollar rose to Simon 2012 Chocolate-based Toothpaste Debuts. 6 January 2012. Tulane University. Available from: <http://tulane.edu/news/newwave/010612_toothpaste.cfm>
[9] Liaison, et al. 2008 Guideline on Fluoride Therapy. Reference Manual V 31/NO 6 09/10
[10] Mount G J and Hume W R 2005 Preservation and Restoration of tooth structure. 2nd Ed. (Queen sland: Knowledge Books and Software) p 3, 25-26, 43
[11] Ponduri S, Macdonald M and Addy M 2005 A study in vitro of the combined effects of soft drinks and tooth brushing with fluoride toothpaste on the wear of dentine. Int. J. Dent. Hygiene 3 7-12.
[12] Ranjitkar S 2009 Biology of Tooth Wear: Preventive Strategies [Thesis] (Adelaide: School of Dentistry University of Adelaide. [cited 11 Sept 2012]. Available from: <http://digital.library.adelaide.edu.au/dspace/handle/2440/60253>.
[13] International Organization for Standardization 2007 Technical Specification: Dental Materials-Guidance on Testing of wear. Part I: Wear by Tooth Brushing. International Organization for Standardization.
[14] Amaechi B T, et al. 2012 Remineralization of Early Caries Lesions by Theobromine. Abstracts: 59th ORCA Congres. #121. Caries. Res. 46 268-338
[15] Sukran B, et al. 2012 Effects of toothbrushing with fluoride abrasive and whitening dentrifices on both unbleached and bleached human enamels surface in terms of roughness and hardness: An in vitro Study. J. Contemp. Dent. Pract. 13 584-9.
[16] A. Wiegand, et al. 2012 Abrasion of sound and eroded and dentin enamels: effect of brushing force of the manual and sonic toothbrushes. Abstracts: 59 th ORCA Congres. #17. Caries. Res. 46 268-33