Original Article

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Effect of saliva exposure time on the abrasion resistance of enamel eroded by acidic chlorinated water

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Objectives: To evaluate saliva exposure time on the abrasion resistance of enamel eroded by acidic chlorinated water

Materials and Methods: This experimental study used forty human premolars. The specimens were exposed to acidic chlorinated water and randomly assigned into 4 groups (n=10): Group-1; 0-minute saliva immersion and brushed (0MinImmBr); Group-2; 15-minutes saliva immersion and brushed (15MinImmBr); Group-3; 30-minutes saliva immersion and brushed (30MinImmBr); and Group-4; 60-minutes saliva immersion and brushed (60MinImmBr). Each group was evaluated using a Knoop hardness tester at baseline, post-erosion, post-saliva immersion, and post-toothbrushing. The length of each indentation obtained from the Knoop hardness testing was used to calculate the depth of the indentation and to determine surface enamel loss. One-way repeated ANOVA, One-way ANOVA, and LSD multiple comparison tests were used and the significance level was set at 0.05.

Results: The 60MinImmBr group demonstrated the significantly highest Knoop microhardness values among the post-saliva immersion and post-tooth brushing procedures. Statistical analysis revealed no significant difference in surface microhardness after these two procedures ($p<0.05$) in the 60MinImmBr group. In contrast, in the other 3 groups, the surface microhardness before and after toothbrushing was significantly different. The mean surface enamel loss was significantly lowest in the 60MinImmBr group compared with the other groups. The mean surface enamel loss between the 0MinImmBr and 15MinImmBr groups was not significantly different ($p<0.05$), however, it was significantly higher compared with the 30MinImmBr and 60MinImmBr groups.

Conclusions: The abrasion resistance of eroded enamel caused by acidic chlorinated water increased over time and at least 60 min should elapse before tooth brushing.

Keywords: chlorinated water, erosion, toothbrushing

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Introduction

Dental erosion is the loss of dental hard tissue caused by acid without bacterial involvement [1-4]. The causes of erosion are both intrinsic, e.g. gastroesophageal reflux, and extrinsic sources, e.g. consuming acidic food or drinks. Moreover, swimming pool water was reported as a cause of dental erosion [5, 6].

Dental abrasion is defined as the wearing away of the dental hard tissues through physical means other than teeth [1]. One cause of aggravating dental erosion is the brushing force. Several studies have reported that brushing the already eroded teeth immediately resulted in significant surface enamel loss, as the softened demineralized enamel layer is susceptible to abrasion [7-9].

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Dental erosion in competitive swimmers was reported in many studies [10, 11] and 61.5% of dental hypersensitivity was found to be due to erosion in swimmers [11]. Two factors that are involved in tooth erosion in swimmers are the duration of the exposure and the pH value of the water. As the duration of the teeth in contact with swimming pool water increases or the pH of chlorinated water decreases, the amount of erosion increases [12]. Dental erosion was found in swimmers who spent at least 5 hours per week in swimming pools [13]. Furthermore, a mean duration of 2 h per day in a swimming pool for 4 weeks was sufficient for dental erosion to occur [14].

The recommended pH for chlorinated swimming pools ranges from 7.2 to 7.8. Exposure to water with a pH below 5.5, the critical pH for enamel, can cause enamel demineralization that can lead to enamel loss [15]. Approximately 31% of 139 swimming pools in Thailand were found to have a pH level lower than 5.5 [14]. A survey conducted by Ongthiemsak *et al.* found that 58% of 12 swimming pools in Hatyai, Thailand had a pH level below 5.5 [15]. However, even in well-maintained swimming pools with a pH of 6.8–8.0, dental erosion was found in more than 26% of competitive swimmers and 10% of recreational swimmers [16].

Based on the current evidence, swimmers are considered at high risk for enamel loss and dental hypersensitivity. Brushing teeth immediately after swimming should be avoided because it could lead to additional enamel loss. Patients have been advised to avoid toothbrushing for at least 1 h after their teeth contact with an acidic beverage [7, 9, 17]. However, there are no reports about how long swimmers should wait after their swimming session before brushing their teeth.

The purpose of this study was to saliva exposure time on the abrasion resistance of enamel eroded by acidic chlorinated water

Materials and Methods

The sample size was calculated using the nQuery Advisor program, using values based on the study “Use of Variable Remineralization Periods to Improve the Abrasion Resistance of Previously Eroded Enamel” [17]. Based on the calculation, 10 samples per group were randomly used in this study. The specimens were prepared from 40 sound human premolars extracted for orthodontic reasons. Teeth with sound enamel were collected and teeth with enamel surface abnormalities or cracks were excluded. This study was approved by the Ethics Committee of Mahidol University (MU-DT/PY-IRB 2020/DT038).

Specimen preparation

The specimens were embedded in PVC pipe (15 mm long, 18 mm diameter) using self-cured acrylic resin. The teeth were positioned to have the buccal surfaces of the teeth above the acrylic resin. The buccal surfaces were ground with 600, 800, 1,000, 1,500, 2,000, 3,000, 4,000, and 5,000 grit silicon carbide paper (Buehler, Lake Bluff, IL, USA) using a rotating polishing machine (RotoPol-2, Struers, Copenhagen, Denmark) until smooth and flat surfaces ~3 mm in diameter at the incisal to middle one-third of crowns were obtained. The specimens were kept in 0.9% normal saline at room temperature until used.

Experimental design

Forty specimens were randomly assigned into 4 groups (n=10): Group-1; 0-minute saliva immersion and brushed (0MinImmBr), Group-2; 15-minutes saliva immersion and brushed (15MinImmBr), Group-3; 30-minutes saliva immersion and brushed (30MinImmBr), and Group-4; 60-minutes saliva immersion and brushed (60MinImmBr). The baseline surface

microhardness was obtained from all the specimens before being placed in acidic chlorinated water prepared by mixing Trichloroisocyanuric Acid (TCCA) with tap water and adjusted to a pH of 3.5 modified from the study of Chuenarrom *et al.* [4]. The specimens were left in the acid for 1 h at room temperature. Post-erosion, the samples were rinsed with deionized water and blotted dry.

The 15MinImmBr, 30MinImmBr, and 60MinImmBr group specimens were placed in artificial saliva prepared. The artificial saliva contained: methyl-p-hydroxybenzoate, 2.00 g/l; sodium carboxymethyl cellulose, 10.0 g/l; KCl, 8.38 mM; MgCl2.6H2O, 0.29 mM; CaCl2.2H2O, 1.13 mM; K2HPO4, 4.62 mM; KH2PO4, 2.40 mM; fluoride, 0.022 ppm; the pH was adjusted to 7.2 using KOH and no precipitation was observed in the solution during the experimental period [18] for 15, 30, and 60 minutes respectively. Subsequently, the samples were rinsed and blotted dry.

The specimens in each group were brushed with an electric toothbrush (Oral-B Vitality Precision Clean) with artificial saliva. The toothbrush head was loaded with a 1.5N force for 30 sec [19] and the bristles were changed every 2 specimens. The toothbrush was fixed in a customized holder to standardize the brushing force and movement and was charged for 5 minutes before each use. Experiment flow is shown in Figure 1.

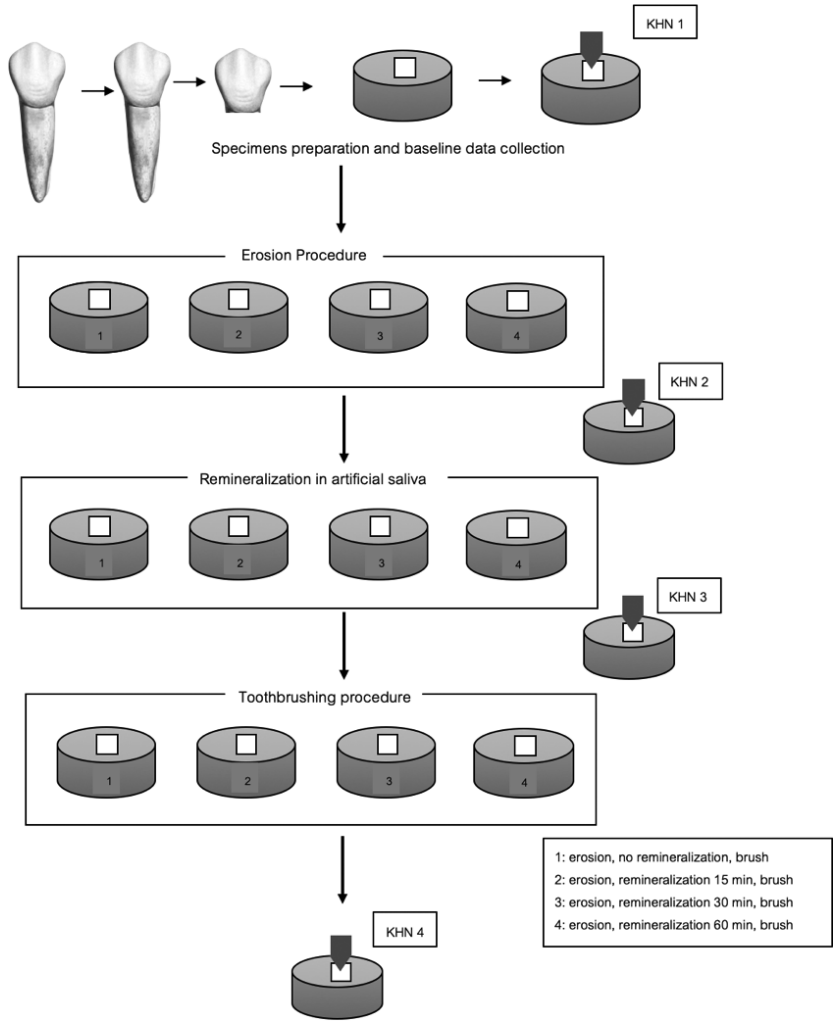

Figure 1 Experiment flow

Surface microhardness and Enamel loss measurements

The specimens' surface microhardness (SMH) was measured with a Knoop indenter tester (FM-ARS 9000, Future-Tech Corp., Kanagawa, Japan) using a 50 g force for 15 sec [20]. Four indentations were created at 100 µm intervals and 250 µm from each step [2]. and the mean of the measurements was calculated. The SMH was measured at each experimental step: baseline, post-erosion, post-saliva immersion, and post-toothbrushing.

The surface enamel loss was measured after 30 sec of brushing using a modified surface microhardness measurement [21]. The length(L) of each indentation before and after abrasion was obtained from the microhardness tester. The depth of each indentation was calculated using the geometrical formula: d = L/2*(tan 3.75°) [16] as shown in Figure 2.

The difference in the depth of each indentation before and after brushing was calculated and these values represented the surface enamel loss of that site.

Statistical analysis

The data was statistically analyzed using IBM SPSS Statistics version 26.0. The Kolmogorov-Smirnov test indicated that the data were normally distributed. One-way repeated-measures analysis of variance (ANOVA) was used to compare the Knoop hardness values and depth within each group. One-way ANOVA and LSD multiple comparison tests were used to compare the Knoop hardness values, depth, and enamel loss between groups. The significance level for all statistical tests was set at 0.05.

Results

The mean baseline microhardness value of the specimens was 345.07±7.86 KHN and the mean enamel microhardness value post-erosion was 266.37±7.81 KHN, which was a 22.81% reduction from baseline. The statistical analysis found no significant differences between the four groups at baseline or post-erosion. However, the mean microhardness in the groups was significantly different between baseline and post-erosion (Table 1).

After saliva immersion and toothbrushing, the 60MinImmBr group had the significantly highest surface microhardness of all 4 groups (Table 1). There was no significant difference between the surface microhardness values in the 0MinImmBr and 15MinImmBr groups ($p<0.05$).

For the intragroup comparison, the surface microhardness in the 60MinImmBr group was not significantly different before and after toothbrushing ($p<0.05$) (Table 1). In contrast, the other 3 groups demonstrated significant differences in surface microhardness before and after toothbrushing.

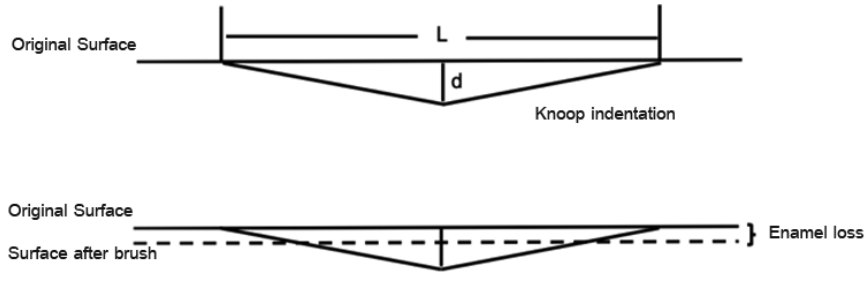

Figure 2 Modified surface microhardness illustration

Table 1 Mean (SD) Microhardness at baseline, post-erosion, post-saliva immersion, and post-brushing

Groups	Mean (SD) Microhardness at Baseline (KHN)	Mean (SD) Microhardness Post-Erosion (KHN)	Mean (SD) Microhardness Post-Saliva Immersion (KHN)	Mean (SD) Microhardness Post-Brushing (KHN)
0MinImmBr	345.50±8.64^{aA}	268.30±7.05^{aB}	268.30±7.05^{aB}	265.46±6.57^{aC}
15MinImmBr	345.51±8.87^{aA}	265.12±8.09^{aB}	269.71±6.45^{aC}	264.93±8.09^{aB}
30MinImmBr	342.30±8.84^{aA}	267.72±6.40^{aB}	303.13±5.18^{bC}	295.40±4.79^{bD}
60MinImmBr	346.98±4.79^{aA}	264.34±9.80^{aB}	337.36±6.81^{cC}	336.40±4.87^{cC}

Groups in each row with the same uppercase letters were not significantly different (multiple comparison test at $\alpha > 0.05$)
Groups in each column with the same lowercase letters were not significantly different (multiple comparison test at $\alpha > 0.05$)

The mean baseline depth of the specimens was 1.488±0.018 µm and the mean depth post-erosion was 1.692±0.025 µm. Statistical analysis showed no significant difference between the four groups at baseline and post-erosion (Table 2). In contrast, the mean depth of the specimens was significantly different between baseline and post-erosion.

The mean enamel loss was significantly lowest in the 60MinImmBr group compared with the other groups (Table 2). The mean enamel loss between the 0MinImmBr and 15MinImmBr groups was not significantly different ($p<0.05$), however, it was significantly higher compared with the 30MinImmBr and 60MinImmBr groups.

Discussion

The present study evaluated the abrasion resistance to toothbrushing of teeth exposed to acidic chlorinated water and then immersed in artificial saliva for different durations. The teeth in the 60MinImmBr group had the least enamel loss and demonstrated the greatest surface hardness. The mean baseline enamel microhardness and depth of enamel surface loss in this study were similar to a previous study [16]. All groups' mean microhardness values and depth at baseline and post-erosion were not significantly different. This indicated that the changes in enamel in the specimens would be due to the immersion time in artificial saliva and could be compared directly.

Table 2 Mean (SD) depth at baseline, post-erosion, post-immersion, post-brushing, and Mean (SD) enamel loss after toothbrushing

Group	Mean (SD) Depth at Baseline (µm)	Mean (SD) Depth Post-Erosion (µm)	Mean (SD) Enamel loss Post-Toothbrushing (µm)
0MinImmBr	1.488±0.021^{aA}	1.685±0.022^{aB}	0.182±0.06^{x}
15MinImmBr	1.485±0.019^{aA}	1.697±0.027^{aB}	0.162±0.63^{x}
30MinImmBr	1.492±0.019^{aA}	1.688±0.021^{aB}	0.065±0.02^{y}
60MinImmBr	1.481±0.010^{aA}	1.698±0.032^{aB}	0.033±0.14^{z}

Groups in each row with the same capital letters were not significantly different (multiple comparison test at $\alpha > 0.05$)
Groups in each column with the same small letters were not significantly different (multiple comparison test at $\alpha > 0.05$)

In the present study, artificial saliva was used as a remineralizing agent. Enamel remineralization can be indirectly demonstrated by an increase in its hardness. Several studies have demonstrated that artificial saliva effectively reharden eroded enamel [7, 22, 23]. Artificial saliva contains calcium and phosphate in amounts equal to or higher compared with that in human saliva [24]. However, the effectiveness of artificial saliva in rehardening eroded enamel was less than that of fluoride products [21]. The results of this study were similar to those of previous studies that showed after 60 minutes of immersion, the samples' surfaces were the hardest and had the significantly lowest enamel loss [7, 16].

We used chlorinated water at pH 3.5 modified from the study of Chuenarrom, *et al* (2014) which used chlorinated water at pH 2.91 and 3.75 [25]. We chose to use chlorinated water at pH 3.5 due to the limited duration study because of the erosion enamel of swimmers occurs when they swim in the low pH pool for a year. Moreover, the study of Visavakul *et al.* reported the 3.1-3.3 pH value of some swimming pools in Khon Kaen and Maha Sarakham province in Thailand [11].

Many *in vitro* and *in situ* studies have demonstrated that brushing with fluoridated toothpaste caused less abrasion on the enamel and dentin of the eroded teeth compared with brushing with non-fluoridated toothpaste [26-28]. However, no fluoride was used in the present study to determine the amount of enamel loss after brushing without the influence of fluoride products.

Hardness indentation can used to quantify the amount of surface loss of enamel by placing indentations and measuring their lengths before and after any experimental treatment [29]. Additionally, microhardness is a well-established technique that can be combined with abrasive surface loss measurements [30]. Polished enamel surfaces provide standardized starting conditions, reducing variability and enhancing measurement accuracy by eliminating natural irregularities. This control allows for a more precise investigation of specific variables. Anyway, polished enamel can change the natural properties of enamel, reducing relevance and potentially leading to the overestimation of erosion [31]. However, the method of this study not only measured KHN values but also calculated the loss of surface enamel value by using the geometrical formula: d = L/2*(tan 3.75°) [21]. Gyurkovics *et al*. revealed a strong general correlation between surface enamel loss measured using a contact stylus profilometer, focus variation 3D microscopy, and modified surface microhardness [21]. However, the direct measurement of microhardness was claimed only to represent the loss of hardness in the erosion model, not the amount of enamel loss in the erosion-abrasion model [32]. The results from the present study demonstrated that the KHN values after brushing were lower and corresponded with the enamel loss in all groups. These results suggest that the direct measurement of surface microhardness can also be used as a method to measure changes in the enamel surface in the erosion-abrasion model. Attin *et al*. also found a significant negative linear correlation between microhardness and enamel loss after erosion and abrasion [33]. However, the enamel surface loss measure using the modified surface microhardness is better than the direct measurement of microhardness because it measured the loss of enamel at the same spot before and after brushing, therefore, it directly represented the enamel loss of that site [20]. In contrast, the direct measurement of microhardness measured the microhardness loss at different sites.

The loss of enamel in this study was less than was found in a previous study [7]. This difference might be because of the different types of acid and pH used [34, 35], differences in the force applied to the specimens during toothbrushing[36] , the method used to determine enamel loss [23, 37], and different types of teeth used [32] between studies. A review article found that most of the studies used excessive force or duration of brushing compared with the clinical situation [21]. They also suggested that the force of an electric toothbrush should be 1.5–2 N, thus, the present study used a 1.5 N brushing force [19]. Moreover, in the present study, brushing was performed for 30 sec to mimic the clinical behavior of patients concerned with oral hygiene [16].

The results of the present study agreed with the results of other studies that the softened enamel caused by acid erosion is more susceptible to toothbrushing abrasion, especially when toothbrushing is performed immediately after erosion occurs [7, 8]. Thus, in these *in vitro* conditions, the enamel resistance to toothbrushing abrasion immersion time-dependently increased. However, after 60 min of saliva immersion, enamel loss after toothbrushing still occurred. However, it should be noted that the results of this study were obtained from tooth brushing alone without the fluoride products. Previous studies showed the importance of the use of toothpaste to restore the original tooth surface roughness, which, in the long term, can help in preventing more serious tooth erosion [38, 39].

Although there is no information about the pH values of swimming pools in other countries in the literature, dental erosion among swimmers has been consistently reported in both high and low-income countries. Therefore, the present study indicates that competitive swimmers and recreational swimmers should not brush their teeth right after swimming in a chlorinated pool, and waiting 15 min is insufficient to re-harden the enamel. They should wait at least 60 min after their teeth contact acidic pool water to prevent dental enamel loss and hypersensitivity. More than 60 min of saliva immersion or fluoride products should be considered in future investigations.

However, the study's results tend to overestimate the amount of enamel lost over 1 hour period and very low pH value, they cannot be interpreted as practical. These results could not accurately indicate the enamel loss due to chlorinated water because teeth were bathed in pool water for 1 hour without pH cycling between chlorinated water and saliva. During swimming, remineralization of teeth can occur by continually being bathed in natural saliva, which may reduce the erosive potential of chlorinated water. However, there is also a way to compare the erosive potential of pool water to previous studies that examined the effects of acidic liquids on enamel over a one-hour period. According to that research, cola beverages with a pH of 2.38 eroded 3.0 μm of enamel, and immersion in infant juices with a pH range of 3.5–4.0 resulted in an orderly loss of 1–5 μm of permanent enamel [25].

The limitation of this study was the *in vitro* design study. Some of the designs were still lacking in this laboratory study such as pH cycling to simulate the situation similar to clinical conditions. Moreover, some clinical conditions such as the mechanism of saliva to buffer acid or acquired pellicles might not be produced effectively in the laboratory setting.

Further study should focus on the designs of the study to mimic clinical conditions as closely as possible, an in situ study was suggested as it can represent real oral clinical conditions in swimmers.

This present study was designed to study the effect of brushing on swimmer's teeth. Some of the designs should be added to the laboratory study such as the use of fluoride products. More than 60 minutes of salivary immersion time should also be studied. To obtain a suitable waiting period of waiting time. pH cycling to simulate the situation similar to clinical conditions or even in situ study was suggested as it can represent real oral clinical conditions in swimmers.

Conclusion

The enamel resistance of eroded enamel caused by acidic chlorinated water increased over time. Based on the results of this study, at least 60 min should elapse before brushing after the teeth are exposed to chlorinated water.

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