Effects of brushing timing after erosive challenge on enamel loss in situ: White light interferometer and nanoindentation study

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This in situ study aimed to evaluate effects of waiting periods after erosive challenge before toothbrushing on enamel abrasion and nanoindentation hardness. Ten subjects wore intraoral appliances each with a set of 4 bovine enamel blocks. The enamel blocks were subjected to 2 cycles a day for 3 days as follows: intraoral exposure to form acquired pellicle and extraoral erosion followed by either 0, 3, 30 or 60 min intraoral exposure and then brushing, which was performed using an automatic brushing machine. Abrasive loss was assessed by white light interferometry. Nanoindentation was performed to calculate relative hardness. Abrasion and relative hardness were statistically analyzed by ANOVA. Abrasive loss was significantly less in groups exposed to saliva compared with 0 min (p<0.05); there was no significant difference between 30 and 60 min (p>0.05). Relative hardness was statistically higher after intraoral exposure, but no differences existed among any intraoral exposure periods (p>0.05).

Keywords: Dental enamel, Nanoindentation, Tooth abrasion, Tooth erosion, White light interferometer

INTRODUCTION

The erosion-facilitated wear due to erosion in combination with abrasion and attrition, which results in accelerated loss of dental hard tissue¹. Erosive tooth wear has a multifactorial aetiology ranging from biological factors such as saliva to an individuals’ diet and their habits, e.g. excessive consumption of acidic foods and beverages, extreme oral hygiene². It has become a well-recognized and clinically important problem in dentistry². Several studies have analyzed the effect of different waiting time following an erosive challenge and before toothbrushing²,³. This idea of waiting times relies on the effect of saliva to allow enough time for the softened enamel surface to ‘reharden’ before applying abrasive forces with a toothbrush²,³. Using a laboratory-based erosion-abrasion cycling model, Hara et al. reported that softened (demineralized) enamel showed less surface loss when treated with artificial saliva compared to human saliva treatment³. Even though in situ results appeared to be less promising in terms of remineralizing and increasing enamel hardness, in other in situ studies the beneficial effects of saliva exposure could be clearly seen after 1 h²,³. This was in contrast to the results of an in situ study, reporting that prolonged salivary contact periods had limited efficacy for eroded-abraded enamel⁴. More recently, a laboratory study using stimulated saliva showed that 4 h exposure to this saliva was not able to achieve a full recovery of the surface microhardness recorded prior to acid challenge⁶. To date, the current research shows a complete recovery of the initial surface hardness after remineralization has not been reported⁶. Therefore, the effectiveness of the waiting time after acid exposure remains a matter of debate.

Apart from the role of salivary components compared to artificial saliva, the method of investigation appears to exert an influence on the outcomes achieved. Exposure of enamel to acid leads to a softening of the tissue and subsequent abrasion causes surface loss. Various techniques have been used to investigate these two aspects of erosive wear. Clinically, early enamel erosion is created leading to very small loss of mineral with erosive craters in a nanometer scale being formed⁷. It is therefore reasonable to assume that the structural changes occurring under clinical conditions are much less pronounced than those observed in laboratory reports⁸. The ability to detect such small alterations in the enamel surface would enable researchers to examine the effects after a brief erosive challenge instead of the long or repeated procedures used for laboratory or in situ experiments that possibly do not replicate the true condition⁹.

Previous studies have quantified the characteristics of enamel exposed to erosive wear using various methods. A white light interferometer (WLI) is a type of computerized optical interference microscopy that has been used to analyze substance loss of enamel after acid exposure in laboratory and in situ dental erosive studies⁸,¹⁰. It has been shown that WLI is able to operate with an accuracy down to 20 nm for measurements on polished enamel surfaces¹⁰. Moreover, nanoindentation is a useful method to detect very small changes in surface hardness at the early stages of in situ eroded enamel. In contrast to standard microhardness techniques such as Vickers and Knoop tests, nanoindentation enables a high-resolution measurement of hardness with loads smaller...
than 1 g and indentation depths at a nm scale\(^1\).

The aim of this in situ study was to evaluate the time after exposure of an erosive challenge to enamel with regard to the effects of toothbrushing on enamel abrasion using a white light interferometer and nanoindentation hardness measurements. The null hypothesis tested was that the time period after an erosive challenge has no effect on enamel substance loss or surface nanoindentation hardness.

MATERIALS AND METHODS

Study design
Ten subjects (5 males and 5 females, age 25–63 yrs) took part in the study that was performed according to the Declaration of Helsinki and was approved by Tokyo Medical and Dental University’s ethics committee (No. 889). The experiments were undertaken after explanation and written informed consent of each subject. Inclusion criteria were subjects that have physiological saliva flow rates (stimulated>1 mL/min; unstimulated>0.25 mL/min)\(^2\), good oral health (no frank cavities, no severe gingivitis, no chronic or aggressive periodontitis and no visible plaque deposits) and no removable prostheses or orthodontic appliances. Exclusion criteria also included presence of general/systemic illness, medications likely to interfere with saliva secretion, pregnancy, breastfeeding, and known allergy to components of soft drink products. Subjects exhibiting severe erosive tooth wear were also not included.

Sample preparation and intraoral appliances
Enamel blocks (5×5×2 mm) were prepared from the central part of the buccal surface of freshly extracted and defect-free bovine incisors using a water-cooled diamond saw (IsoMet, Buehler, Lake Bluff, IL, USA). The surface was ground with an automatic lapping machine (ML-160A, Maruto, Tokyo, Japan) using 600 up to 2000-grit SiC papers then polished down to 1 μm particle size diamond slurry under running water. The blocks were cleaned ultrasonically (Micro Cleaner, TOESCO, Kanagawa, Japan) to remove any traces of the polishing procedure. The samples were then sterilized using ethylene oxide gas. The polished surface of each sample block was divided into a testing (4×5 mm) and reference area (1×5 mm) using a thin layer of nail varnish (Kiss Nail Art Paint, KISS USA, NY, USA). A total of four samples were each placed in the buccal aspects of an intraoral appliance made from self-cured acrylic and retained by wire clasps on lower molars (Fig. 1a). The sample surface was flush with the surface of the appliance.

Erosive challenge
The 10 subjects were instructed to wear the intraoral appliances for 20 min to allow formation of an acquired pellicle\(^3\) before extraoral erosive challenge. Erosive challenges were performed twice a day (between 8:00 to 10:00 a.m. and between 4:00 to 6:00 p.m.). This was done by extraoral immersion of the appliance and enamel specimens in a carbonated beverage (Coca-Cola, Tokyo, Japan, pH 2.3) for 90 s\(^3\), then thoroughly rinsed with 3-way syringe for 30 s. A minimum of 4 h elapsed between the two extraoral immersions. The erosive challenge was performed at least 1 h after eating. A fresh bottle of Cola drink was assigned to each test subject every day.

Intraoral reinsertion
Before the reinsertion of the intraoral appliance, the subjects chewed an unflavored sugar-free gum designed for a saliva test (CheckBuf, Horiba, Tokyo, Japan) for 5 min to produce stimulated saliva, mimicking intraoral conditions that occur after eating or drinking. Enamel specimens were removed from the appliance and subjected to brushing at different times following the challenge. One of the four specimens was brushed immediately after the erosive challenge (0 min intraoral exposure group). The remaining samples were brushed after the intraoral appliances had been worn by subjects for 3, 30, and 60 min of intraoral exposure respectively. Specimens with erosion only (no brushing or reinsertion) were prepared as control sample for each individual. (Fig. 1b)
**Brushing procedures**

A commercially available toothbrush (Prospec toothbrush Young, GC, Tokyo, Japan) with medium hardness, flat trim and nylon filaments (diameter of 0.2 mm) was used for the abrasion of the enamel sample in each cycle. Brushing was performed extraorally in an automatic brushing machine (K236, Tokyo Giken, Tokyo, Japan). The toothbrush was fixed in the brushing machine with the angle parallel to the samples surfaces. Enamel specimens were brushed with 30 strokes in each cycle under a load of 250 g using a 1:3 dilution of a fluoride toothpaste slurry (CLINICA, Lion, Tokyo, Japan, 950 ppm F). After brushing, the samples were rinsed with tap water until all visible remnants of toothpaste were removed. The toothpaste slurry was replaced after each cycle, while the same toothbrush head was used for the same sample throughout the study. In order to fix the specimen in the automatic brushing machine a resin composite plate was used as a guide. In order to keep a constant and balanced load while brushing, an enamel block of the same thickness as the tested block was placed next to the tested sample to hold the toothbrush tufts.

For all subjects, the experimental cycle starting from the formation of an acquired pellicle to brushing procedure was repeated twice a day for 3 days. In total, 50 enamel specimens were processed in 5 groups (n=10). Samples were stored in a humid chamber at 4°C between each cycle.

**Surface analysis by WLI**

To evaluate the toothbrushing abrasion, the amounts of the enamel surface loss were measured in the nm order using the WLI (NanoMap-D, AEP Technology, Santa Clara, CA, USA). WLI is a computerized optical microscope operating in vertical scanning mode able to produce a three dimensional digital image shown as a topographic map, where various colors denote different heights for the picture elements. The image resolution was 1,024×1,024 pixels, scan range 1×1 mm, and vertical resolution 0.1 nm using ×10 objective lens. The points analyzed were 200 and 500 μm away from the reference (control) surface. After removing the nail varnish, both the tested and reference areas were scanned on a region of interest (ROI) of 1×1 mm. The mean surface loss was calculated throughout the ROI, as the average difference in axial location of the reference surface and challenged surface. The 3D characteristics/morphological changes were also measured using WLI (Fig. 2).

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![Fig. 2](image-url)  
(a) 3D topography images of white light interferometry. A is the reference surface and B is the abrasion surface. (b) The mean difference between (A–B) on 2D profile was reported as average surface loss.
Nanoindentation hardness
To analyze the hardness, a nanoindentation tester (ENT-1100a, Elionix, Tokyo, Japan) was used. The maximum force was 10 mN with a Berkovich diamond tip at a loading rate of 0.3 mN/s. The nanoindentation setup has been described in detail elsewhere. The indentation points were programmed under a 50× magnification lens of the charge coupled device (CCD) microscope connected to the nanoindentation system on two areas on each specimen; the unbrushed surface, remaining intact (with no scratches) between the toothbrush tuft bundles, and the reference area after the protective varnish was removed. Twenty points were programmed on a (5×4 matrix) with 10 μm spacing between each adjacent point. Relative hardness (%) was calculated as the mean hardness of the unbrushed surface/mean hardness of reference surface×100 (%).

Additional assessments
The secretion rates of resting and stimulated saliva were determined for each subject. Resting and stimulated whole saliva samples were collected from each subject once at between 9:00 to 10:00 am. Saliva was collected at least 2 h after meals and at least 1 h after brushing to minimize effects of the diurnal variability in saliva composition. Each subject sat quietly for at least 5 min, then had a sample of whole resting saliva collected by spitting (draining method) for 5 min into an ice-cooled container. For stimulated saliva collection, a 1 g piece of sugarless chewing gum was chewed for 30 s; after that, while the subject was chewing the gum, saliva was continuously collected into a container for 5 min. The volume of resting saliva was measured and the flow rate (mL/min) was calculated. Saliva pH change was measured directly using a hand-held pH meter (CheckBuf, Horiba). Immediately after collecting the resting and stimulated whole saliva, 0.25 mL of each saliva sample was placed onto the pH-sensitive electrode to measure the initial pH value. The saliva buffering capacity of resting and stimulated saliva were also measured using the hand-held pH meter. To determine buffering capacity of resting saliva, 0.25 mL of saliva and acid solution (lactic acid, pH 3.0) were mixed and then a further 0.25 mL of saliva was added. For resting saliva the pH was recorded twice; once after addition of acid solution (buffering capacity 1) and after the addition of 0.25 mL resting saliva (buffering capacity 2).

Statistical analysis
Data were initially analyzed to confirm a normal distribution. Enamel loss and hardness were analyzed using one-way ANOVA followed by post-hoc multiple comparisons with Bonferroni correction. Comparison of two groups was performed using the t-test. Enamel surface loss and relative hardness recovery trends were analyzed for each subject by calculating the slope of a regression line when the values of each variable were plotted against time. Relationships between the resulting trend slopes and salivary pH values were evaluated using Pearson correlation. All statistical analyses were performed with SPSS software and the significance level for all statistical tests was set at 0.05.

RESULTS
All subjects finished the study satisfactorily, and no sample was lost from the appliances.

Enamel loss data are presented in Fig. 3a. Toothbrush abrasion was significantly lower in intraoral exposure groups compared to the 0 min group (p<0.001).
There was a significant difference between 3 min and the other groups \((p<0.001)\), while there was no significant difference between 30 and 60 min \((p=0.036)\). Enamel loss from erosion only was 71.4±13 nm, which was significantly increased by immediate brushing \((466±95 \text{ nm}; \ p<0.001)\). Waiting for 60 min before brushing after erosive challenge decreased enamel loss by 71% compared to brushing immediately after the erosive challenge.

The results of the relative hardness are presented in Fig. 3b. Nanoindentation showed statistically significant, higher relative hardness after intraoral exposure \((3 \text{ min } p=0.008, 30 \text{ min } p=0.001, 60 \text{ min } p<0.001)\), but no differences were found among any intraoral exposure periods \((3 \text{ and } 30 \text{ min } p=0.101, 3 \text{ and } 60 \text{ min } p=0.032, 30 \text{ and } 60 \text{ min } p=0.858)\).

The results of the saliva test are shown in Table 1. The buffering capacity of the stimulated saliva for all 10 test subjects was evaluated as high; however, that of resting saliva was medium to high and differed among individuals.

The correlation coefficients between saliva pH and abrasion surface loss or hardness trend are shown in Table 2. The Pearson correlation revealed that abrasion trend was correlated with both buffering capacities (1 and 2) of resting saliva \((1: p=0.006, 2: p=0.02)\). On the other hand, the relative hardness trend was correlated with the initial pH and buffering capacity 1 of resting saliva and that of stimulated saliva \((p=0.042, p=0.02, p=0.041 \text{ respectively})\), but not with buffering capacity 2 of resting saliva \((p=0.316)\).

### Table 1  Saliva test results of 10 subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Flow rate (mL/min)</th>
<th>Initial pH</th>
<th>pH (Buffering capacity1)</th>
<th>pH (Buffering capacity 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td>7.3</td>
<td>6.6 H</td>
<td>7.3 H</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>7.2</td>
<td>5.7 M</td>
<td>6.9 H</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>7.3</td>
<td>5.6 M</td>
<td>6.9 H</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>7.1</td>
<td>4.9 M</td>
<td>6.5 H</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>7.2</td>
<td>4.8 M</td>
<td>6.4 H</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>6.9</td>
<td>4.6 L</td>
<td>6.2 H</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>7.0</td>
<td>5.3 M</td>
<td>6.0 H</td>
</tr>
<tr>
<td>8</td>
<td>0.6</td>
<td>7.0</td>
<td>4.3 L</td>
<td>5.8 H</td>
</tr>
<tr>
<td>9</td>
<td>1.6</td>
<td>6.9</td>
<td>4.2 L</td>
<td>5.8 H</td>
</tr>
<tr>
<td>10</td>
<td>1.3</td>
<td>7.7</td>
<td>5.1 M</td>
<td>5.5 M</td>
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</table>

### Stimulated saliva

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Flow rate (mL/min)</th>
<th>Initial pH</th>
<th>Buffering capacity pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>7.7</td>
<td>7.0 H</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>7.6</td>
<td>6.6 H</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>7.4</td>
<td>6.9 H</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>7.6</td>
<td>6.7 H</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>7.4</td>
<td>6.2 H</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>7.0</td>
<td>6.0 H</td>
</tr>
<tr>
<td>7</td>
<td>1.1</td>
<td>7.3</td>
<td>6.0 H</td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>7.4</td>
<td>6.6 H</td>
</tr>
<tr>
<td>9</td>
<td>3.2</td>
<td>7.2</td>
<td>6.4 H</td>
</tr>
<tr>
<td>10</td>
<td>2.7</td>
<td>7.7</td>
<td>6.9 H</td>
</tr>
</tbody>
</table>

To determine buffering capacity of resting saliva, first, 0.25 mL of saliva and acid solution were mixed (buffering capacity 1) and then 0.25 mL of saliva is added (buffering capacity 2).
DISCUSSION

This in situ study assessed the enamel surface loss and nanoindentation hardness to evaluate the effects of post-erosive challenge periods before toothbrushing. Since the availability of sound human enamel is limited, bovine enamel was chosen as the best alternative to obtain quantitative data at the early stages of in situ eroded enamel. Bovine enamel showed a slightly higher susceptibility to wear than human enamel in an in situ study. However, human and bovine enamel showed similar behavior after short-term acid exposures in an in vivo study.

Various laboratory and in situ assessment techniques have been applied to evaluate the loss of the demineralized enamel induced by erosive challenges. The WLI technique has the advantage of being non-damaging and able to perform scans within a few minutes. It is reported to be more accurate to assess surface loss or changes on enamel after acid exposure than other common instruments.

Apparently toothbrush wear can be influenced by various factors that include the frequency, duration and force of brushing. In this study, the toothbrush enamel abrasion test used a 250 g load, brushing time and frequency, as well as the erosive challenge period were considered to closely replicate the everyday condition. Since dietary intake usually occurs every 2–3 h and oral healthcare procedures (e.g. toothbrushing) are recommended at least twice daily, the duration of the recovery process for the softened enamel surface is thought to be limited to only about 1–3 h during the daytime. Therefore, waiting periods were chosen in the range of 1 h or less for all experimental groups in this study. This study design, which included acquired pellicle formation, was based on those protocols reported in previous studies.

The result of enamel loss supports previous reports, stating that immediate tooth brushing (0 min group) resulted in significantly greater enamel loss, while less substance loss was found with longer intra-oral exposure of the acid challenged surface. The WLI and nanoindentation results clearly indicate the efficacy of postponing brushing. However, even after waiting for 60 min, the substance loss was much greater than that of control or reference surface, and enamel recovery was not complete even after 60 min waiting period. This is reflected in the in situ study by Ganss et al., where the authors observed that even after waiting for 2 h, no protective effect against enamel abrasion was detected. Lussi et al. showed 4 h of waiting was not able to reduce abrasion in a laboratory study using stimulated saliva.

Nanoindentation suggested that a hardening of the previously softened enamel surface could be achieved; however not as a complete recovery. In this regard, it was inferred that the hardening is partly because porous inter-prismatic areas are simply filled with mineral precipitates rather than apatite crystallites. Stimulated saliva could significantly recover hardness in the initial 3 min after erosive challenge, but further hardness recovery was not statistically significant. Enamel rehardening has often been reported in laboratory studies where various calcifying solutions or artificial saliva have been used. However, it should be noted that compared to artificial saliva, an in situ model with fresh saliva is expected to have a different potential for remineralization, since it contains various proteins that are thought to inhibit mineral deposition on the tooth surface. The results of the current study are in line with those of microradiographic studies on the mineral content of enamel after erosion and subsequent immersion in cleared human saliva, or exposure to the oral cavity, where mineral gain and a 20 to 70% reduction in demineralization depth were reported. Nevertheless, this finding is in contrast to previous research work that suggested human saliva on its own did not possess the ability to remineralize the enamel surface in the oral environment. Differences between methodological approaches for evaluating the surface

Table 2 Correlation between saliva pH and abrasion or hardness trend

<table>
<thead>
<tr>
<th>Variables</th>
<th>Surface loss trend</th>
<th>Hardness trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial pH of resting saliva</td>
<td>.39</td>
<td>.65 *</td>
</tr>
<tr>
<td>Buffering capacity pH of resting saliva</td>
<td>.80 **</td>
<td>.72 *</td>
</tr>
<tr>
<td>Buffering capacity pH of resting saliva</td>
<td>.71 *</td>
<td>.35</td>
</tr>
<tr>
<td>Initial pH of stimulated saliva</td>
<td>.38</td>
<td>.68 *</td>
</tr>
<tr>
<td>Buffering capacity pH of stimulated saliva</td>
<td>.43</td>
<td>.65 *</td>
</tr>
</tbody>
</table>

(Correlation is significant at the 0.05 level with* and at the 0.01 level with **)
hardness changes of eroded enamel after human saliva exposure may result in different findings; for instance a microhardness test in a previous study was performed with a load of 50 g. While the nanoindentation test in the current study used a force of 10 mN (approximately 1 g). Apart from methodological differences among studies, the contrast in findings may also be related to the dynamic nature of remineralization and difference in remineralization ability and composition of saliva among different subjects. Nevertheless, the positive effects of the initial intraoral exposure on nanoindentation hardness are significant and remarkable.

The 0 min group with lower relative hardness values of enamel showed higher surface loss due to brushing. The toothbrushing challenge can remove the softened enamel layer which causes loss of surface mineral, but it has little effect on the underlying sound tooth surface. However, the statistical significance of recovery trends appeared to be different between the two variables; surface loss significantly decreased with intraoral exposure up to 30 min, but relative hardness did not show a difference beyond 3 min. This observation was supported by previous findings that suggested with prolonged exposure to erosive agents, abrasion increased proportionally compared to the decrease in hardness.

Some significant correlations were found between salivary initial and buffering pH values and the enamel recovery trends in the current study, particularly with resting saliva buffering capacity. The effects of human saliva have been discussed in other in situ studies; Jaeggi and Lussi observed less enamel abrasion after 60 min of intraoral exposure which was associated with the resting saliva secretion. While there appears to be an agreement on the general benefits of prolonged waiting periods among different studies, significant changes were observed as soon as 3 min intraoral exposure in the current study. This may be due to the effect of chewing gum used to stimulate secretion of saliva, providing higher and accelerated buffering effects compared to the previous studies which relied only on resting saliva. Lussi et al. reported that the buffering capacity of saliva of patients with erosion was lower compared to that of patients without erosion, and also a greater decrease in the pH at the tooth surface after acid attack. Resting saliva was expected to be involved with the stage of acquired pellicle formation, while stimulated saliva was mainly involved with the initial post-erosion intraoral exposure phase (3 min). However, it is difficult to clearly distinguish periods of the resting saliva and stimulated saliva exposure after acidic challenge; therefore, resting saliva may have also been largely involved during the prolonged intraoral exposure times (30 and 60 min group). Further clinical study is needed to understand the effects of resting saliva on eroded enamel.

The null hypothesis that the waiting periods after an erosive challenge had no effect on enamel substance loss or surface nanoindentation hardness was rejected. However, it is difficult to relate laboratory and in situ results to the clinical situation. An in vivo epidemiological study on 3,187 young adults (18–35 years) from seven European countries reported that waiting period after breakfast before toothbrushing was not associated with severe erosion. The present laboratory and in situ study was performed using bovine tooth samples and evaluated small surface changes using white light interferometer; however, such small differences between waiting periods might not be easily detected under an in vivo situation.

Consumption of sugary beverages such as Coca-Cola can be a cause of caries, and caries is one of the most frequent reasons for losing dental hard tissue. It should take into account that postponing toothbrushing can prevent hard tissue loss in nm or µm scale however the loss caused by caries can be more. To perform toothbrushing correctly is considered as an effective way to prevent caries and postponing toothbrushing is not recommended for patients from the aspect of caries prevention. Clinical advice on specific toothbrushing periods should not be generalized to all patients, but should be provided to specific groups of high-risk patients who already present erosive tooth wear.

The present in situ study was designed with the focus on imitating the clinical situation from an erosive agent to chewing gum to stimulate saliva. However, it was difficult to verify the extended waiting periods and periods between 0, 3, 30, 60 min and with extended pellicle formation period due to consideration of the subjects’ daily life. It must also be considered that the present study was carried out with 10 volunteers with good oral health and hygiene. Patients with erosive wear or impaired salivary conditions are the important group to study the effect of delayed brushing. Further investigations with greater subject numbers including those with erosive tooth wear or impaired salivary flow are necessary to understand fully the processes involved in the remineralization or rehardening of surface softened enamel in the oral environment.

CONCLUSIONS

Postponing toothbrushing after erosive challenge had significant effects on enamel surface loss and nanoindentation hardness under the conditions of this study. The short waiting period before toothbrushing is expected to be effective for patients with high-risk of erosion.

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