Roughness and pH changes of enamel surface induced by soft drinks in vitro-applications of stylus profilometry, focus variation 3D scanning microscopy and micro pH sensor

Mie FUJII1, Yuichi KITASAKO1, Alireza SADR2 and Junji TAGAMI1,2

1Cariology and Operative Dentistry, Department of Restorative Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
2Global Center of Excellence Program; International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan

Corresponding author, Yuichi KITASAKO; E-mail: kitasako.ope@tmd.ac.jp

This study aimed to evaluate enamel surface roughness (Ra) and pH before and after erosion by soft drinks. Enamel was exposed to a soft drink (cola, orange juice or green tea) for 1, 5 or 60 min; Ra was measured using contact-stylus surface profilometry (SSP) and non-contact focus variation 3D microscope (FVM). Surface pH was measured using a micro pH sensor. Data were analyzed at significance level of alpha=0.05. There was a significant correlation in Ra between SSP and FVM. FVM images showed no changes in the surface morphology after various periods of exposure to green tea. Unlike cola and orange juice, exposure to green tea did not significantly affect Ra or pH. A significant correlation was observed between surface pH and Ra change after exposure to the drinks. Optical surface analysis and micro pH sensor may be useful tools for non-damaging, quantitative assessment of soft drinks erosion on enamel.

Keywords: Enamel erosion, Soft drink, Surface roughness, pH, 3D image

INTRODUCTION

Dental erosion is defined as the loss of tooth structure by acid dissolution. Tooth wear associated with the erosion by acidic foods and soft drinks is an increasing problem, frequently linked with individuals’ lifestyle and eating habits1-5. It has been suggested that for enamel demineralization to take place, the pH on the enamel surface must fall below 5.56; many available soft drinks have a pH value well below 5.57-8. Consumption of soft drinks is increasing world-wide, and it could become a potential problem. It has been suggested that the erosive potential of soft drinks is influenced by numerous factors such as the pH9, acidic species9,10, calcium and phosphate contents2,7 and exposure time8. Several studies have reported on pH values of soft drinks as an indicator of the erosive potential1-6,8; however, the actual changes in surface pH caused by exposure to soft drinks have not been previously studied. Recently, an experimental micro pH sensor using an ion-sensitive field-effect transistor (ISFET) pH sensor has been developed for laboratory and clinical trials. The new device has enabled assessment of the chemical characteristics and pH changes at the tooth surface9.

Most studies on dental erosion to date have focused on chemical aspects of erosivity, and it has been suggested that the erosive potential of soft drinks is influenced by numerous factors such as the pH10, acidic species2-5, calcium and phosphate contents2,7 and exposure time8. Several studies have reported on pH values of soft drinks as an indicator of the erosive potential1-6,8; however, the actual changes in surface pH caused by exposure to soft drinks have not been previously studied. Recently, an experimental micro pH sensor using an ion-sensitive field-effect transistor (ISFET) pH sensor has been developed for laboratory and clinical trials. The new device has enabled assessment of the chemical characteristics and pH changes at the tooth surface9.

Apart from chemical aspects of erosion, physical surface characteristics of enamel have been evaluated to indicate the loss of tissue due to exposure to soft drinks with low pH values. These characteristics have been conventionally evaluated by measurement of roughness parameters (most frequently Ra) in two dimensions and along a line by contact stylus surface profilometry (SSP)10. Such quantitative analysis has shown a high degree of resolution for investigation of enamel loss by soft drinks, but it might cause damage of the sample surface due to the contact stylus13.

Recently, a focus variation microscope (FVM) has been developed for 3D topographic surface analysis. FVM is a non-contact optical microscope based on the focus variation concept. This measurement is performed directly by optical imaging, causing no damage to the sample surface. Surface characteristics such as roughness can also be calculated from the image obtained. Ren et al12,13 performed morphological characterization on the enamel surface exposed to soft drinks using 3D measurements. They reported that the FVM was a powerful tool in evaluating surface topography associated with enamel erosion. Arithmetic average roughness (Ra) is the most frequently reported surface roughness parameter14. However, there has been no report on comparison between the FVM and conventional SSP for measurement of Ra on enamel surface.

Suitable tools must be developed and evaluated for non-damaging clinical diagnosis of enamel erosion, and appropriate prevention and treatment strategies. Moreover, investigating the relationship between surface roughness and surface pH of enamel after in vitro erosion is important to gain a deeper understanding about the erosion by soft drinks. Therefore, the aims of this study were to evaluate the surface roughness and surface pH on bovine enamel before and after exposure to three soft drinks using SSP, FVM and micro pH sensor. The
correlation between surface roughness parameter (Ra) and surface pH was also investigated. The null hypothesis was that there was no difference in enamel surface roughness and surface pH before and after exposure to the soft drinks.

**MATERIALS AND METHODS**

*Beverage selection and preparation of the enamel specimens*

Three soft drinks (cola, orange juice and green tea) were investigated in this study. Selected drinks and their contents are shown in Table 1. Freshly extracted, bovine incisors were used for preparation of enamel samples. The bovine teeth were used since it was reported that there was little variation in the structures and attributes of enamel between different bovine teeth. Enamel-dentin blocks (approximately 7×7×2 mm) were prepared using a 0.3 mm thick diamond disc (Isomet, Buehler, IL, USA) under permanent water-cooling. The surface of the block was ground flat using SiC papers up to 2000-grit and polished with diamond pastes (DP-paste, P Struers, Ballerup, Denmark) down to 0.25 µm particle size under running water. The polished enamel samples were cleaned ultrasonically (Micro cleaner, TOESCO, Kanagawa, Japan) in deionized water (pH 7.0) for 3 min to remove any traces of the polishing materials. The blocks were coated by an adhesive tape leaving approximately a 5×5 mm window on the enamel surface. A total of 51 teeth (12 samples for SSP and FVM, and 5 samples for surface pH in each drink) were used in this study.

*Erosive challenge*

Surface roughness was measured before exposure (control), and after the specimen was exposed to 20 mL of each drink for a total of 1, 5 or 60 min at the room temperature (23°C). For each measurement, the specimen was rinsed under running water and dried by a three-way air syringe. In order to measure the surface roughness, each enamel window was divided into two equal areas. SSP was conducted on one half, and FVM was performed on the other half as shown in Fig. 1. Following the roughness measurements at the baseline (control) and after 1, 5 or 60 min, 20 mL of fresh drink was used for the subsequent exposure period (1, 4 or 55 min, respectively) on the same specimen. Twelve samples were tested in for each drink (n=12).

![Fig. 1](image)

**Table 1** The selected beverages and ingredient as listed on the beverage bottles

<table>
<thead>
<tr>
<th>Name (trade name)</th>
<th>pH*</th>
<th>Ingredient List</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola (Coca-Cola)</td>
<td>2.2</td>
<td>Carbonated water, high fructose corn syrup, caramel color, phosphoric acid, natural flavors, caffeine</td>
</tr>
<tr>
<td>Coca-Cola Japan Co., Ltd., Tokyo, Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice (POM juice)</td>
<td>4.0</td>
<td>Orange juice, flavor</td>
</tr>
<tr>
<td>Ehime Beverage Inc., Ehime, Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green tea (Namacha)</td>
<td>6.3</td>
<td>Green tea, vitamin C, flavor</td>
</tr>
<tr>
<td>Kirin Beverage Co., Ltd., Tokyo, Japan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The pH values were measured by a hand-held electronic pH meter.
Surface roughness measurement

1. Contact Stylus Surface Profilometry (SSP)
   A profilometer (XP-200, Ambios Technology, CA, USA) with a stylus radius of 2.5 µm and the vertical resolution of 2 nm was used to measure the mean surface roughness (Ra). The designated half-side of each enamel window was divided into 4 areas for the SSP measurement. As shown in Fig. 1, the first one of the four areas was measured at the baseline as a control (A), the second one was measured after 1 min exposure (B), the third one was measured after 5 min exposure (C), and the fourth one was measured after 60 min exposure (D). The contact stylus traced a perpendicular straight line, 4 mm in length. Three tracings were performed for each of the four areas, and their average value was recorded as Ra for the specimen.

2. Focus variation 3D microscopy (FVM)
   An FVM (Infinite Focus G4 Microscope, Alicona Imaging, Grambach, Austria) was used to capture the three-dimensional topology of eroded enamel surfaces, before and after exposure to the soft drinks. The measurements were performed using the 100X objective at a vertical resolution of 20 nm on three areas (each 109×143 µm) on the designated half-side, and the average Ra was recorded for each specimen. The measurements were performed at the baseline (before erosive challenges, as a control), and after 1, 5 and 60 min exposure on the same three areas. FVM was also used to capture 3D images of the eroded enamel surfaces (80×80 µm in size) within the same area as where the surface roughness measurements were performed.

pH measurements

Surface pH values of enamel sample before and after erosive challenge were directly measured using an experimental micro pH sensor connected to a pH meter (F-53, Horiba Ltd., Kyoto, Japan). The sensor is covered with a layer of tantalum oxide (Ta₂O₅, Horiba Ltd., Kyoto, Japan). The sensor was calibrated using standard solutions of pH 4.0 and 7.0. For each measurement, the reference electrode (1 mm in diameter) and the micro pH sensor were in contact with a small quantity of ion-exchanged water (pH 6.8) placed on the tooth surface. A baseline pH value (control) was obtained on the polished enamel surface prior to the erosive challenge. The pH values after exposure of each sample to a soft drink for 1, 5 or 60 min were also recorded to one decimal place. The pH measurement for each specimen was repeated three times and the mean value was calculated, in the same manner as described by Shida et al.¹⁰.

Statistical analysis

Data obtained on Ra and surface pH were statistically analyzed by two-way ANOVA with drinks and exposure times as factors. Since the Levene test showed that the variances of groups were not homogeneous (p<0.05), the Games-Howell post-hoc was performed for multiple comparisons among the groups.

Pearson correlation and regression analyses were used to determine a relationship in Ra values measure on each specimen between SSP and FVM. In addition, the relationship between roughness increase and surface pH change was also examined by Pearson correlation between Ra increase (ΔRa) measured by each device (SSP or FVM) and pH, averaged for each treatment time with each drink evaluated. For each exposure time, ΔRa was calculated as the difference between mean enamel Ra after the exposure to drink compared to mean baseline Ra. For regression analysis of pH values in this study, molar H⁺ ion concentrations (10⁻pH) were calculated and subjected to the statistical tests. In all of the analyses, the significance level was set in advance at alpha=0.05. The statistical analysis was performed using the Statistical Package for the Medical Science (SPSS Ver.11 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

Ra results obtained from both SSP and FVM were shown in Table 2. Two-way ANOVA revealed that both factors

Table 2  Surface roughness (Ra) measured by SSP and FVM

<table>
<thead>
<tr>
<th>Immersion time</th>
<th>control (0 min)</th>
<th>1 min</th>
<th>5 min</th>
<th>60 min</th>
<th>control (0 min)</th>
<th>1 min</th>
<th>5 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola</td>
<td>4.7²⁺</td>
<td>11.6₃⁺</td>
<td>32.6₃⁺</td>
<td>106.0₄⁺</td>
<td>15.4₄⁺</td>
<td>22.7₃⁺</td>
<td>45.2₅⁺</td>
<td>84.7₆⁺</td>
</tr>
<tr>
<td></td>
<td>(±1.4)</td>
<td>(±1.6)</td>
<td>(±3.1)</td>
<td>(±18.9)</td>
<td>(±3.8)</td>
<td>(±2.6)</td>
<td>(±6.3)</td>
<td>(±8.9)</td>
</tr>
<tr>
<td>Orange juice</td>
<td>7.6²⁺</td>
<td>10.3₃⁺</td>
<td>20.8₄⁺</td>
<td>47.4₅⁺</td>
<td>17.3₄⁺</td>
<td>22.0₃⁺</td>
<td>30.1₅⁺</td>
<td>61.4₆⁺</td>
</tr>
<tr>
<td></td>
<td>(±2.4)</td>
<td>(±1.2)</td>
<td>(±5.6)</td>
<td>(±8.5)</td>
<td>(±3.2)</td>
<td>(±2.7)</td>
<td>(±2.7)</td>
<td>(±8.7)</td>
</tr>
<tr>
<td></td>
<td>(±1.0)</td>
<td>(±1.2)</td>
<td>(±1.8)</td>
<td>(±3.6)</td>
<td>(±3.4)</td>
<td>(±1.7)</td>
<td>(±4.0)</td>
<td>(±6.5)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Within each measurement, values with the same lowercase superscripts in each row show no statistically significantly difference; in each column, values with the same uppercase superscripts show no statistically significantly difference (p<0.05, ANOVA with Games-Howell post-hoc in both cases).

SSP: stylus surface profilometer, FVM: focus variation 3D microscope.

n=12
(drink and exposure time) had a significant effect on the roughness measured under both SSP and FVM, and the interaction of the two factors was significant \((p<0.001)\). Similar trends were observed in the Ra values measured by the two different devices. According to the multiple comparisons, significant differences in Ra were detected among various periods of exposure to cola or orange juice \((p<0.05)\), while there was no significant change in Ra when enamel was exposed to green tea \((p>0.05)\). Multiple comparisons among the drinks revealed that while there was no significant difference in Ra among the control specimens subjected to different drinks, there were significant differences among the specimens at 1, 5 and 60 min exposure to different drinks \((p<0.05)\).

Fig. 2 showed results of the regression analysis between SSP and FVM. There was a significant correlation on surface roughness between SSP and FVM \((p<0.001, r=0.937, R^2=0.878)\).

Surface pH analysis results are shown in Table 3. While the initial enamel pH was around 6.8 for all specimens, after 5 or 60 min, cola and orange juice showed a pH below the enamel erosion critical pH (5.5). After 60 min of exposure, enamel surface pH was the highest in green tea compared to other drinks. Two-way ANOVA showed that both factors of drink and exposure time had a significant effect on surface pH, and their interaction was significant \((p<0.01)\). When the results were compared between different times within the same drink, significant differences were found in surface pH for cola and orange juice \((p<0.05)\), while there was no significant change in surface pH for green tea \((p>0.05)\). Multiple comparisons among drinks at each time showed that at all times (except for the initial pH), there were significant differences in surface pH between green tea and the two other drinks \((p<0.05)\). Fig. 3 showed the results of the correlation between change in surface pH and Ra change \((\Delta \text{Ra})\). Pearson correlation showed a significant relationship between surface pH and Ra change for both devices \((p<0.001, r=0.963, R^2=0.927)\).
$r=0.963$ and $R^2=0.927$ for SSP and $p<0.001$, $r=0.981$ and $R^2=0.962$ for FVM).

The 3D images of enamel after exposure to cola, orange juice and green tea for 1, 5 and 60 min are shown in Fig. 4. Even after only 1 min, cola and orange juice showed morphological changes on the polished enamel surfaces. The changes were more pronounced at 5 min and dramatic morphological changes were observed at 60 min. On the contrary, no morphological changes were seen in any of the samples exposed to green tea.

**DISCUSSION**

Arithmetic average roughness (Ra) is the most frequently reported surface roughness parameter in the dental literature\(^1\). Ra value has been conventionally evaluated on enamel as an indicate of acid-erosion using the contact stylus surface profilometry (SSP)\(^1\)\(^7\)\(^,\)\(^8\). One can argue that the benefit of the profilometry by an advanced computerized SSP is the high vertical resolution (2 nm), especially for the measurement of low roughness values; however, mechanical scanning might cause damage to the sample surface due to the contact stylus\(^1\)\(^1\). Therefore, such damaging technique would be not adequate for long-term monitoring of the same area on a surface. Moreover, contact profilometry techniques are limited by the size of stylus tip, and by the difficulties measuring steep flanks and vertical edges on the surface.

Optical surface analysis techniques have the advantages of being non-damaging and performing scans within shorter times (a few seconds). The new focus variation 3D microscopy (FVM) used in this study could perform optical scans of the surface in three-dimensions, and calculate surface roughness quantitatively without any surface damages. In short, optical microscopic images are continuously captured as the distance between the object and objective is varied. Each position in depth has a different sharpness depending on the 3D structure of the specimen; the variation of sharpness is utilized for extracting depth information and roughness.
parameters. Also a dense 3D surface representation of the object in real colors is obtained.

No study to date has focused on the comparison between the two roughness measurement techniques utilized in the present work. According to a pilot study to compare the values of surface roughness measured by SSP and FVM, Ra of a standard roughness sample (roughness: 0.5 µm) measured by FVM showed a lower standard error compared to that of SSP (0.81 nm vs. 4.43 nm, respectively), while the average values were only slightly different (498.25 nm vs. 515.26 nm, respectively). No statistical differences were observed (25 measurements, unpublished data). In the current study, similar tendencies were observed in Ra results measured by the two devices despite the different values obtained. We speculate that the difference in nominal Ra values between the two devices was a due to the difference in the nature of measurements; i.e., SSP depends on the tactile probe tracing along a straight line, while FVM is based on optical analysis of an area. Nevertheless, a strong correlation was found between the two devices in Ra measurement, as seen in Fig. 2. The findings confirmed the utility of FVM for enamel surface roughness measurement. Moreover, the images obtained from FVM were effective in visual assessment of enamel surface changes with different exposure times to the drinks.

A visual comparison on the effects of different drinks and exposure times on enamel surfaces revealed that the eroded surfaces in cola and orange juice groups in this study were visibly roughened and had lost their luster as compared to the control surface. According to the FVM 3D images, a honeycomb-like morphology was observed on the surface of enamel after only 1 min of exposure and substance loss obviously continued with further exposure. On the other hand, no morphological changes to the surface were detected after various periods of exposure time to green tea, as confirmed by FVM observations. These observations were confirmed by the statistical analysis of Ra values among different groups.

Surface pH change on the enamel surface subjected to erosive challenge by acidic soft drinks was also evaluated in this study. Kitasako et al. suggested that surface pH measurements on enamel might reflect the pH of the enamel fluid, and that measuring the pH of the early enamel lesions could be used to assess changes in the lesion activity with time, providing an objective monitoring tool for clinical caries assessment. Results of the current study showed that after exposure of enamel to green tea (pH 6.3), the surface pH was above the enamel critical pH (pH 5.5), even after 60 min of exposure. On the other hand, cola and orange juice (pH<5.5) showed a remarkable decrease in surface pH values with exposure times as short as 1 min. Moreover, the significant differences in enamel surface roughness between cola and orange juice after 5 min of exposure confirmed a previous report stating that cola drinks were more erosive than orange juices within the first minutes after exposure.

The drop in surface pH indicated that exposure to the acidic drinks could alter the natural pH equilibrium at the surface in favor of acidity. While enamel surface pH could indicate the acidity of enamel fluid, it was also likely that the acidic surface pH was a result of acids in the drinks, remaining attached to the enamel surface even after rinsing under running water. In line with this speculation, it was suggested that the carboxylic groups of an organic acid (maleic acid) reacted with hydroxyapatite and formed a chemisorbed layer on the enamel surface, which was not readily removed by washing or ultrasonic treatment.

The results indicated that the risk of erosion increased as the duration of enamel exposure to the solutions with a low initial pH (pH 2.0–4.0) increased. This finding was in line with the previous reports. Cochrane et al. suggested that the greatest determinant of erosive potential identified was the pH of the beverage rather than other factors such as calcium, inorganic phosphate, fluoride content and titratable acidity. On the other hand, some of the studies reported that depending on their buffering capacity and ion content, some low-pH beverages may not cause enamel erosion.

It is noteworthy that in this in vitro study, a significant correlation was found between enamel surface roughness and surface pH, indicating that the extent of enamel surface dissolution by an acidic drink was reflected in the surface pH after the erosive challenge, regardless of the initial pH of the drink or exposure time. This finding is important, since in the clinical situation, measurement of enamel surface pH might serve not only as an indicator of the erosive potential of the consumed drink, but also as a sign of actual erosive damage exerted to the enamel surface by the drink.

Clinically, erosive lesions have higher caries risk and are susceptible to abrasive challenges. In addition, frequent consumption of acid-containing soft drinks may increase the erosive effects of a drink on enamel. Therefore, when evaluating enamel erosion, individual diet history in relation to acid drink consumption is also important. The type of soft drinks, the rate of intake, and the time of exposure in the oral environment must be considered thereby. Most of the marketed soft drinks, including pure fruit juices, have a high acidity (pH<4.0: 75%, pH<5.5: 79% in Japan [unpublished data]) and may soften and dissolve enamel and dentin. The softened dental structures are more susceptible to wear and abrasion by such challenges as tooth brushing, hence further accelerating surface tooth loss.

The most common clinical assessments for tooth wear have used a visual tooth wear index, which might depend on the operator’s subjective experiences. A non-damaging optical 3D imaging device could be used on the sites of clinical uncertainty as a diagnostic adjunct for determining an eroded lesion. The FVM sensor in its present form is too large for clinical application, but a smaller device would allow in vivo 3D imaging characterization of enamel eroded lesions. Moreover, surface pH analysis used in this study might be used in monitoring and assessment of enamel erosion, provided that in the future, in vivo studies would confirm the
relationship between enamel erosive loss and surface pH.

CONCLUSION

This study found that SSP, FVM and surface pH could be used in vitro to measure erosive changes on enamel surface. There was a good correlation on roughness between SSP and FVM. An association was found between enamel surface roughness change and surface pH, after erosive challenge by acidic soft drinks. Optical surface analysis and micro pH sensors may provide additional means of assessment of enamel erosion as non-damaging quantitative techniques.

ACKNOWLEDGMENTS

This work was supported by the grant #21592413 from the Japan Society for the Promotion of Science, and from the Japanese Ministry of Education, Global Center of Excellence (GCOE) Program, “International Research Center for Molecular Science in Tooth and Bone Diseases”. The authors would like to thank Dr. Satoshi Nomura, Dr. Masaomi Ikeda, Mr. Minoru Nakajima and Ms. Yuko Ohara for their helpful advice toward this manuscript.

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