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ORIGINAL ARTICLE

Erosive potential of soft drinks on human enamel: An *in vitro* study

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Background/Purpose: Most soft drinks are acidic in nature. Regular consumption of these drinks may result in dental erosion. The aim of this *in vitro* study was to evaluate the erosive potential of different soft drinks in Taiwan by a novel multiple erosive method.
Methods: Four commercially available soft drinks in Taiwan were selected for this study. The properties of each product were analyzed to measure their pH, titratable acidity, and ion contents. The erosive potential of the soft drinks was measured based on the amount of loss of human enamel surface following its exposure to the soft drinks tested for different periods (20 minutes, 60 minutes, and 180 minutes). The enamel loss was measured using a confocal laser scanning microscope.
Results: The pH values of the soft drinks were below the critical pH value (5.5) for enamel demineralization, and ranged from 2.42 to 3.46. The drink with ingredients of citric acid and ascorbic acid had the highest titratable acidity (33.96 mmol OH^-/L to pH 5.5 and 71.9 mmol OH^-/L to pH 7). Exposure to all the soft drinks resulted in loss of human enamel surface (7.28—34.07 μm for 180-minute exposure). The beverage with the highest calcium content had the lowest erosive potential.
Conclusion: All tested soft drinks were found to be erosive. Soft drinks with high calcium contents have significantly lower erosive potential. Low pH value and high citrate content may cause more surface enamel loss. As the erosive time increased, the titratable acidity to pH

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7 may be a predictor of the erosive potential for acidic soft drinks. The erosive potential of the soft drinks may be predicted based on the types of acid content, pH value, titratable acidity, and ion concentration.

Introduction

Consumption of soft drinks has become very popular in Taiwan recently.[1] Previous studies have suggested that consumption of sugar-sweetened beverages leads to higher calorie intake, which subsequently leads to the problem of obesity.[2] Results of various studies have shown that nonalcoholic beverages are mostly acidic in nature.[3] Therefore, prolonged contact with such a solution may destroy the hard tissues of the teeth by erosion.[4] Dental erosion is the chemical loss of tooth structure due to the action of acids that are not produced by bacteria.[5] Dental erosion is an important clinical problem in Taiwan. Previous studies have shown that the prevalence of dental erosion is on the rise. Deery et al[6] examined 11—13-year-old adolescents from the United States and the United Kingdom, and reported dental erosion in 41% and 37% of adolescents, respectively.

Among children, dental erosions are mainly caused by excessive consumption of erosive soft drinks.[7] Growing evidence suggests a considerable increase in consumption of potentially erosive drinks.[8] Reports have also been published regarding significant associations between soft drink consumption and dental erosion. A report by the Health Promotion Administration, Ministry of Health and Welfare Studies (Taipei, Taiwan) showed that the prevalence of dental caries in children and adolescents has declined over the past 20 years in Taiwan. However, the survey also reported that there is a rapid increase in the consumption of sugar-containing beverages among children and adolescents.[9] This practice may lead to other health-related problems. To analyze various chemical properties of soft drinks and their effects on human enamel, the erosive potential of four commercially available soft drinks in Taiwan was investigated in this *in vitro* study by a novel multiple erosive method. The null hypothesis tested was that there was no difference in the erosive potential of any of the soft drinks.

Table 1 Ingredients of soft drinks selected for analyses.

Brand	Ingredients (as listed by the manufacturers)	Volume (mL)
A	Carbonated purified water, high-fructose syrup, sucrose, cane sugar, phosphoric acid, nature flavorings, caffeine	600
B	Carbonated purified water, high-fructose syrup, granulated sugar, citric acid, cane sugar, flavor	600
C	Water, diluted fermented milk, skimmed milk powder, citric acid, flavor, soybean polysaccharides, sodium citrate, sucralose, acesulfame potassium	500
D	Water, granulated sugar, lemon juice, citric acid, flavor, sodium citrate, ascorbic acid, safflor yellow, calcium pantothenate, vitamin B_6, carotene	500

Materials and methods

Soft drink preparation and analyses

Four commercially popular bottle drinks were selected and purchased for analyses (Table 1). Freshly opened beverages were analyzed in triplicate to determine their pH values and titratable acidity at room temperature. The pH values of the experimental drinks were measured with a previously calibrated pH meter (Suntex Instruments Co., Ltd, Taipei, Taiwan). Values for titratable acidity were determined as the volumes of 0.1 M NaOH required to increase the pH of 50 mL of the beverages to 5.5 and 7.0. The values were then converted to mmol of OH^- per liter to the pH of soft drinks. The calcium, inorganic phosphate, and fluoride contents of the beverages were determined using an automatic ion chromatography system (861 Advanced Compact IC; Metrohm Ltd, Herisau, Switzerland). The concentrations of ions were used in an iterative computational procedure with the modified Debye—Hückel equation to determine ion activity product (Iap) for hydroxyapatite.[10] The solubility product (Ksp) for hydroxyapatite was based on the research of McDowell et al.[11] The degree of saturation with respect to hydroxyapatite (DSHA) was determined using the following equation:

$$DSHA = (Iap/Ksp)^{1/n} \quad [1]$$

where n equals the number of ions in a unit cell.[10]

Preparation of enamel samples and erosive challenge

Human molar teeth used in the study experiment were collected from the dental clinic of the National Taiwan University Hospital (NTUH; Taipei, Taiwan). This study was approved by the Institutional Review Board of NTUH (Approval Number: 200806002R). The teeth were carefully examined for evidence of caries, enamel hypoplasia, and other defects. Only sound teeth were included for analysis.

Enamel sections from the buccal and lingual surfaces of the crowns were obtained by cutting the tooth with a diamond blade on a low-speed sectioning machine. Each individual section was mounted in cold cure acrylic resin (size: 8 mm $\times$ 6 mm $\times$ 3 mm). The samples were polished sequentially with silicone carbide paper of 600, 1200, 2500, and 4000 grit on a rotating polishing machine under constant water irrigation, which exposed approximately 4 mm $\times$ 3 mm of the enamel area. Samples were cleaned by ultrasonication in distilled water for 3 minutes before and after polishing. The enamel samples were randomly allocated to one of the four experimental groups. Each group contained 10 enamel samples. For the erosive challenge experiment, the samples were divided into three groups based on their exposure time to the soft drink samples (20 minutes, 60 minutes, and 180 minutes, respectively). The exposed enamel surface was divided into three parts, one each for different periods of erosion. After exposing the samples to the soft drinks for the aforementioned periods, the eroded areas in each sample were observed. Initially, two thirds of the polished surface was covered with polyvinyl chloride (PVC) tape (Scotch 810; 3M, St. Paul, MN, USA) to preserve a reference area unaffected by erosion. The PVC tape was removed sequentially according to the design of the experiment. Residual glue on the samples after tearing the tape was removed with a cotton swab and ethanol.

For the erosive challenge, the enamel samples were immersed into 100 mL of freshly opened beverages for the aforementioned periods. The exposure experiment was conducted in an incubator shaker at room temperature. Once the erosive process was complete, the enamel samples were washed with deionized water, and the PVC tape was removed from the samples. The enamel samples were then stored in deionized water until measurement of enamel loss.

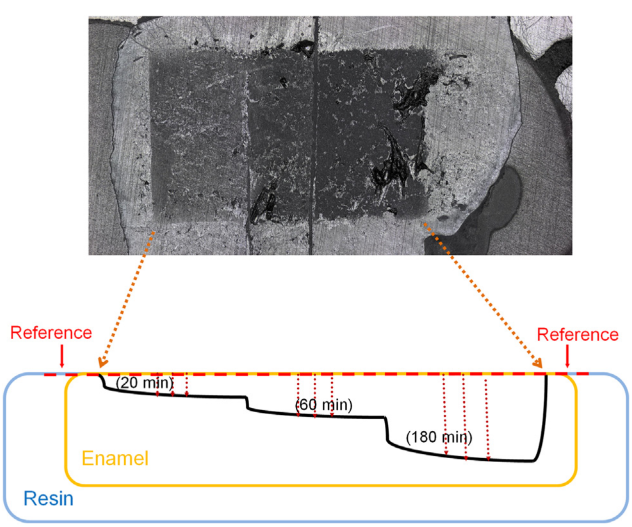

Figure 1 Schematic representation of the confocal laser scanning microscopic measurements. For each erosive area, the enamel loss was calculated from the base to the reference (non-erosive) surface.

Measurements of enamel loss

Enamel loss was measured using a confocal laser scanning microscope (CLSM; Keyence VK9710, violet laser; Keyence Corporation, Osaka, Japan). Using this microscope, both real color image (using optical light) and surface scan image (using a laser with a wavelength of 408 nm) can be obtained. A single image was captured from the surface of each sample using a 20 $\times$ objective lens (field of view, 675 μm $\times$ 506 μm). The image containing the three erosive areas on each sample could be constructed precisely using the VK assembler software (Keyence Corporation, Osaka, Japan) (Fig. 1). After image construction, enamel loss was calculated using two methods, namely, the two- and three-dimensional methods (2D and 3D, respectively). In the 2D method, three trace lines were placed across the erosive surface reference area, with each line being 50 μm wide. For each trace line, four points were selected, one from each reference area and the other three from the lowest erosion in each area. The software calculated the difference in erosion depth between the eroded enamel surface and the reference area, and then the values for loss of enamel surface in each area were obtained. For calculation using the 3D method, the operator outlined the visibly eroded portion on the 2D image and the software was then used to calculate the area as well as the volume of enamel loss in this area up to the height of the reference. The volume was divided by the area to obtain the enamel loss height.

The 2D calculation was considered by the operator in cases where there was a maximum amount of surface loss. A pre-defined algorithm was used to calculate the 3D range in all regions. Because the eroded enamel surface was not evenly smooth, the 3D measurement might be better suited than the 2D measurement.

Statistical analysis

Differences in enamel loss produced upon exposure to different soft drinks were determined using the Kruskal—Wallis test, and comparisons between two soft drinks were conducted using the Wilcoxon rank sum test with a Bonferroni correction. For all statistical analysis, the significance level was set at $\alpha = 0.05$. Statistical analyses were performed using SPSS version 12 (SPSS Inc., Chicago, IL, USA).

Results

The ingredients of soft drinks tested are shown in Table 1. Of the four soft drinks selected, two were carbonated drinks. The other two were juice-containing and skimmed milk-containing drinks. The pH value, titratable acidity, and ion contents for each soft drink are shown in Table 2. The soft drink A had the lowest pH value (2.42), whereas the soft drink C had the highest pH value (3.46). The soft drink D had the highest titratable acidity. The soft drink C contained the highest level of calcium than the other three beverages. All soft drinks only contained a low level of fluoride (0.1—0.18 ppm). The apparent degree of saturation with respect to hydroxyapatite was less than 0.01.

Table 2 pH values, titratable acidity, and ion contents of the four soft drinks.

Soft drink	pH	Titratable acidity		Calcium (ppm)	Phosphate (ppm)	Fluoride (ppm)	DSHA[a]
		mmol OH^-/L to pH 5.5	mmol OH^-/L to pH 7.0				
A	2.42 ± 0.09	11.84 ± 0.57	41.98 ± 1.49	11.40 ± 1.46	522.47 ± 13.42	0.1 ± 0.06	0.0035
B	3.33 ± 0.03	12.26 ± 0.16	40.12 ± 2.21	5.69 ± 1.09	2.88 ± 0.01	0.17 ± 0.07	0.0005
C	3.46 ± 0.02	18.72 ± 0.13	27.76 ± 0.73	75.94 ± 4.34	141.24 ± 3.18	0.18 ± 0.04	0.0085
D	3.03 ± 0.02	33.96 ± 0.37	71.90 ± 1.20	11.72 ± 1.86	6.50 ± 0.63	0.15 ± 0.04	0.001

Values are presented as mean ± standard deviation.

[a] Degree of saturation with respect to hydroxyapatite (DSHA) is calculated using the following equation: DSHA = $(Iap/Ksp)^{1/n}$, where Iap is the ion activity product for hydroxyapatite and Ksp is the solubility product for hydroxyapatite.

The 3D images of enamel surfaces eroded by soft drinks are shown in Fig. 2. Color changes in different areas represent different heights of the surface eroded. As the immersion time increased, the enamel loss increased gradually. The 2D images (both laser scanning and color images) captured by CLSM are shown in Fig. 3. The structure of enamel rods can be seen in the image captured by CLSM (magnification, 1000 ×). Destruction of enamel was found in the body of the rods initially. The honeycomb appearance was prominent when the erosive process was continued (Fig. 3). The erosive potential of the soft drinks is shown in Fig. 4 (results of the 2D method). The soft drink C produced the lowest amount of enamel loss at the three periods of immersion. The soft drinks A and D produced more enamel loss than the other two at the 180-minute immersion. The amount of volume loss of enamel induced by soft drinks is shown in Fig. 5A. The enamel loss (height) due to exposure to soft drinks was calculated by volume loss divided by each selected surface area (Fig. 5B). The results obtained using the 3D method showed that enamel loss produced by soft drink C (at 60-minute immersion time) was significantly less than those of the other three soft drinks. The enamel loss due to soft drink A was slightly higher than that due to soft drink D at 180-minute immersion time; however, the difference was not statistically significant. The correlation between the erosive potential and DSHA of the soft drinks was low. Interestingly, when the etching time increased, the amount of enamel loss was found to be associated with the titratable acidity of the soft drinks (to pH 7).

Discussion

In this study, all soft drinks were found to be acidic in nature and substantially undersaturated with respect to hydroxyapatite. The pH values of the soft drinks tested (2.42–3.46) were below the critical value of 5.5 for enamel demineralization. The null hypothesis was rejected as differences

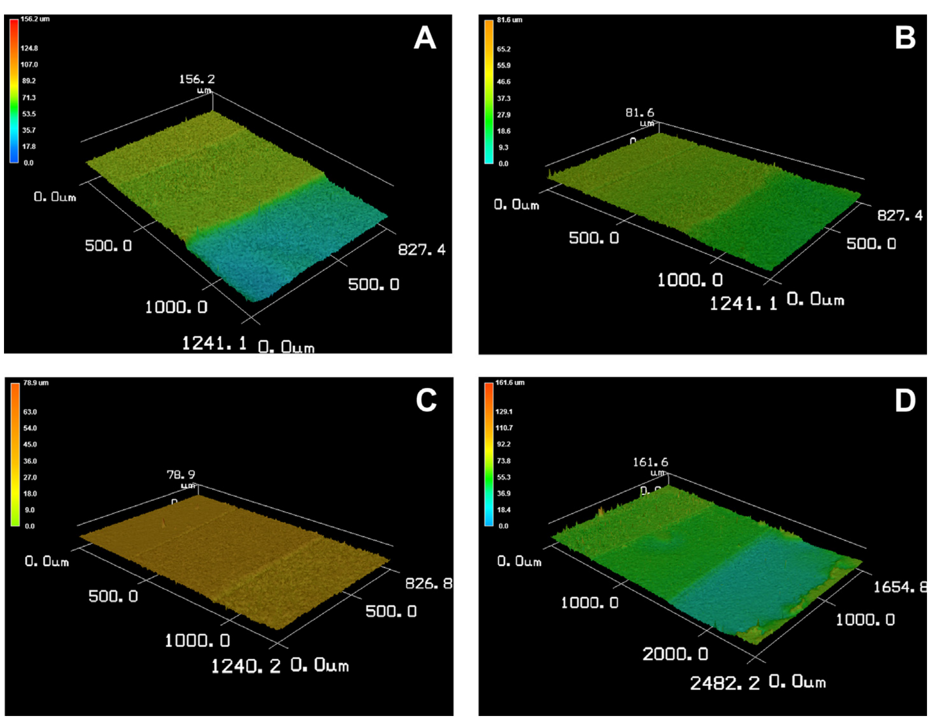

Figure 2 Three-dimensional images of erosive enamel surfaces produced by soft drinks A–D; three regions for the different immersion periods (20 minutes, 60 minutes, and 180 minutes, respectively) are shown.

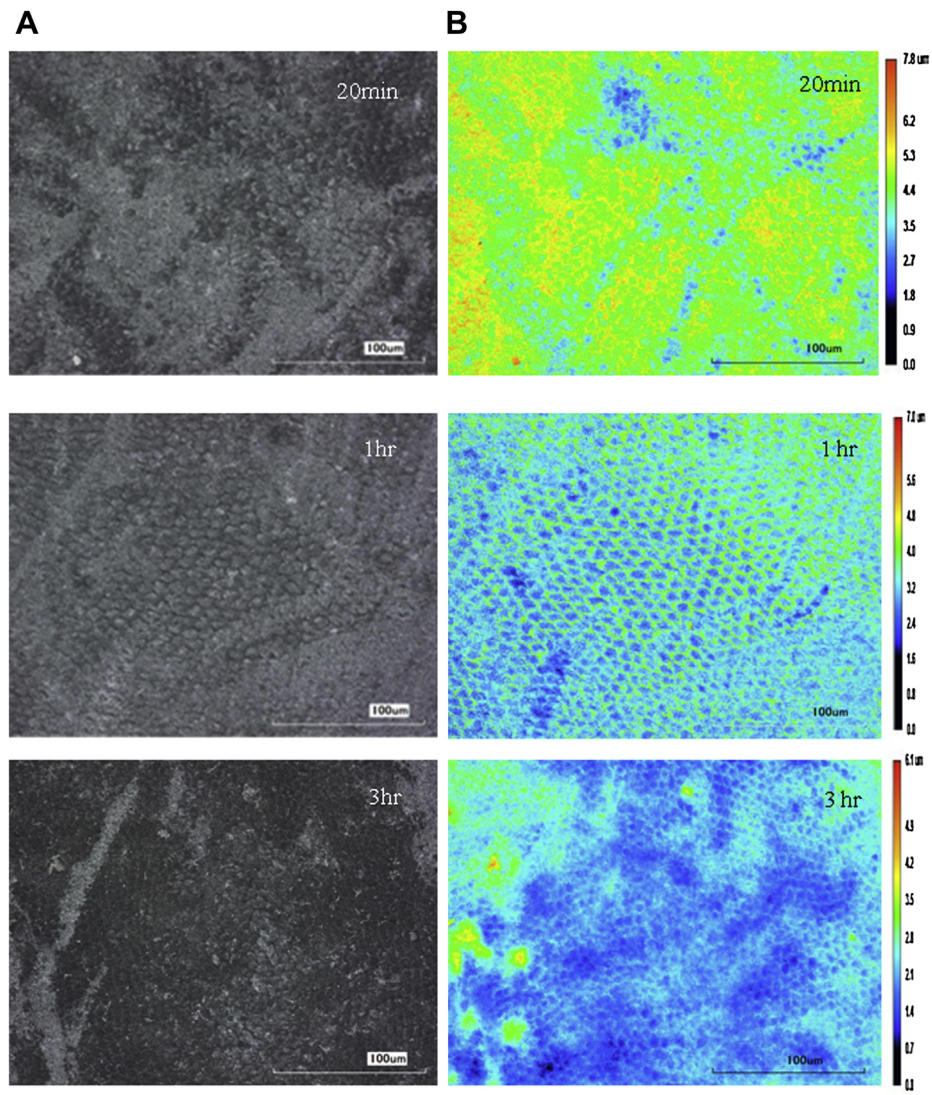

Figure 3 Eroded enamel surfaces (two-dimensional image) produced by soft drink A at different immersion periods captured using a confocal laser scanning microscope. (A) Laser scanning images. (B) Images showing height difference with reference color bars.

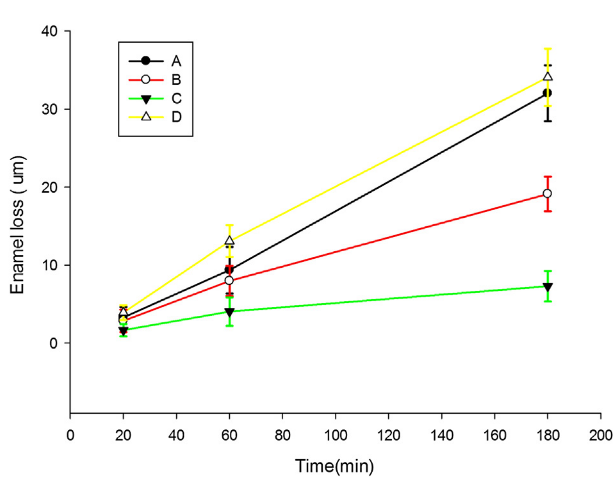

Figure 4 Surface loss of enamel produced by soft drinks A—D calculated using the two-dimensional method.

existed between the erosive potential of the different soft drinks. The soft drinks with low pH value produced more enamel loss. The enamel loss produced by soft drinks A and D at 180 minutes was higher than that produced by soft drinks B and C ($p < 0.05$). The high titratable acidity of the soft drink D may be attributed to the high content of citric acid. Citric acid is particularly harmful to teeth, as the citrate anion is capable of chelating calcium in addition to the erosive effect of the protons released.[12] Although soft drink D had a high pH value, the enamel loss produced by it was only slightly greater than that of soft drink A (results of the 2D method; $p > 0.05$). Our experimental results showed that the erosive pattern caused by the soft drinks was closely related to the titratable acidity to pH 7 when the etching time increased. Although the critical pH value for enamel demineralization was 5.5, the soft drink with higher values of titratable acidity to pH 5 indeed resulted in greater enamel loss (soft drink D). As the etching time was prolonged, the experimental system approached toward a neutral condition. The titratable acidity to pH 7 was the amount of hydroxide ion needed for

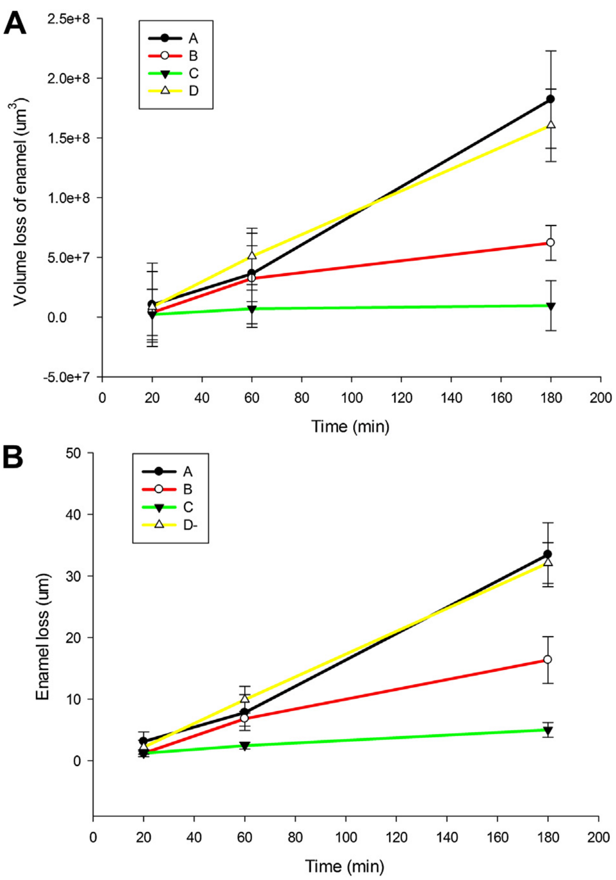

Figure 5 Representation of the results calculated by the three-dimensional method. (A) Volume loss of enamel samples produced by soft drinks A—D. (B) The enamel loss (height) of soft drinks was calculated by volume loss divided by each selected surface area.

the solution to reach the neutral condition. In the present erosive system, the source of hydroxide ion was possibly hydroxyapatite. The pH of the system was below the critical pH value of enamel. Therefore, the titratable acidity to pH 7 may be used to estimate the erosive potential of soft drinks, when the etching time was increased. Barbour et al[13] pointed out that the addition of calcium and phosphate may be a practical means of reducing the erosive potential of soft drinks. The soft drink C had the highest pH value, calcium content, and DSHA compared with the other drinks tested. It had the lowest erosive potential in this study. However, the high phosphate content of the soft drink A did not lead to a low erosive potential.

For measurements of enamel surface loss, many instruments have been used, such as stylus profilometry, atomic force microscopy (AFM), and CLSM.[14] Traditional profilometry produces scratches on the enamel surface, which may affect the measurement results. AFM uses the attractive and repulsive forces between the surface and a tip to detect different surface characteristics. It has a high resolution and the ability to measure the surface hardness by nanoindentation. CLSM can produce images of a sample surface by scanning the surface with a laser and using the principle of confocal imaging. Thus, it is possible to measure the difference in height and volume between an eroded enamel surface and a given reference area. The results could be calculated as described previously.[15] The two methods can produce similar results in some cases. The CLSM can have the resolution to 0.001 μm. In the present study, the honeycomb picture of the enamel surface could be seen in the CLSM image. Initial demineralization was shown in the body of the enamel rod due to its high mineralization consistency compared with the peripheral area (Fig. 3). As the erosion progressed, destruction at the enamel rod sheath, in the form of a less mineralized area, might be found. Upon completing the erosion challenge experiments, three eroded surfaces could be observed in one enamel sample. The 3D image of the eroded surface can be obtained with the software. The CLSM is a very useful instrument for analyzing enamel erosion.

The 2D measurement showed little difference in the results at the immersion time of 20 minutes and 60 minutes. This difference was very clear when the 3D measurement was used. The 2D calculation was considered by the operator in cases where there was a maximum amount of surface loss. By contrast, 3D measurement included the whole surface area and might be more representative of the erosive effect. Because the eroded area of each enamel sample was not the same, the amount of volume loss could not be used to compare the erosive potential of the soft drinks. It should be translated into height difference for comparison, and the process was thus more complicated.

It is very important to understand the erosive potential of soft drinks on human enamel not only for the general population but also for patients with schizophrenia, disability, or oral mucosal disease on or near the gingiva. Patients with mental or physical disorder have high caries experience because of neglect of oral hygiene or lack of ability to maintain good oral hygiene.[16] Moreover, patients who have oral mucosal disease on or near the gingiva (such as lichen planus) have difficulty in keeping the lesion—adjacent teeth clean due to contact pain.[17,18] For the aforementioned patients, drinking too much soft drink may induce severe destruction of tooth enamel.

In conclusion, all of the four soft drinks tested can produce enamel loss. Soft drinks with high calcium contents have significantly lower erosive potential. Low pH value and high citric acid content may cause more surface loss. As the erosive time elongates, the titratable acidity to pH 7 may be a predictor of erosive potential for acidic soft drinks. The erosive potential of the soft drinks may be predicted based on the types of acid content, pH value, titratable acidity, and ion concentration.

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