Original

Comparison between a profilometer and a measuring microscope for measurement of enamel erosion

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Abstract: The aim of this study was to measure the difference in the erosion depth of enamel measured by profilometry (PM) and a measuring microscope (MM). Sixty enamel specimens were divided into ten groups. Each specimen group was exposed to 50 ml of a carbonated drink with pH 2.38 or orange juice with pH 3.67 for 15, 30, 60, 120, and 180 minutes. Depths of eroded areas were measured with a profilometer and a measuring microscope. Data of average enamel loss were measured by PM and MM for all erosion times and were scatter plotted on a graph with regression fit. Correlations between the enamel loss measured by PM and MM were analyzed with a paired sample t-test to compare the discriminatory abilities of the two methods of analysis for all erosion times. The regression fit in all study cases showed a high linear relationship ($R^2 = 0.90$) between measurements by PM and MM, but in cases where the erosion depth was lower than the depth of focus (DOF) of the MM objective lens, there were weak correlation coefficients (-0.007 – 0.303) for comparison between the two measurement methods. (J. Oral Sci. 50, 475-479, 2008)

Introduction

Numerous reports on evaluation of enamel erosion have been published since 1892 (1), and research on this subject

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has recently expanded and become more rigorous. *In vitro*, enamel erosion is measured by various methods including surface microhardness (2), loss of enamel weight (3), SEM or light microscope (4), microradiograph or image analysis (5), electron probe analysis (6), profilometry (7), and light induced fluorescence (8). However, each instrument has limitations. For example, some methods provide only qualitative information, some damage the surface or are very costly. Researchers are thus required to be familiar with the limitation of each methodology in order to obtain accurate and precise results. In a previous study (9), a simple, rapid and non-destructive method was introduced to measure the depth of the channel of a microfluidic chip by using a common microscope based on depth of focus and scales in which fine adjustment could be made. Using this concept, we employed a measuring microscope to measure the enamel erosion depth.

A three-direction measurement microscope is a non-contact optical microscope using light rays. It provides repeatable accuracy using automatic measurement in three axes (x, y, and z). The depth of the specimen surface in the z-axis was measured using the focusing method, allowing the measurement of enamel erosion. A few studies have used this microscope for measuring dental material dimensions (10), but there have not been any previous studies which employed a measuring microscope to evaluate enamel erosion. The aim of this study was to measure the difference in enamel erosion depths at different exposure times measured by a measuring microscope and pro-filometry, which was used as the gold standard (11).

Materials and Methods

Ten caries-free human third molars were used for the study. Teeth were extracted from patients, aged 25 to 45 years old, at the Dental Hospital, Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand. Longitudinal

enamel sections (n = 60) were prepared from 10 molars using a diamond saw (Isomet 4000, Buehler, IL, USA) under water irrigation. From each tooth, six sections were cut accordingly: one section from the distal, mesial and two sections each from both the lingual and buccal. Each specimen was embedded in acrylic resin and the outer enamel surface was ground flat using 320, 600 and 1200 grit silicon carbide paper (Wirtz Buehler, Düsseldorf, Germany). Specimens were assigned to one of the six groups. The results included five groups each for five different exposure times in orange juice (Tipco, Batch no.22:50A27, Tipco F&B Co., Ltd., Bangkok, Thailand) with pH 3.67 and another five groups each for five different exposure times in a carbonated drink (Coke, batch no. F42S1018, Thai Namthip Co. Ltd., Bangkok, Thailand) with pH 2.38. The pH values were measured by a pH meter (Precisa, pH900, Precisa Gravimetrics AG, Dietikon, Switzerland). The enamel specimens were covered with nail varnish leaving an area of approximately 1.5×1.5 mm^2 in the center area for exposure to the drink. This procedure ensured comparison between the eroded and uneroded area; the uneroded area was used as a reference for the erosion depth.

Ten specimens each were exposed to 50 ml of carbonated drink or orange juice for 15, 30, 60, 120, and 180 min. The specimen beakers were held and shaken in a continuously vibrating water bath (Memmert, WNB22, Memmert GmbH, Büchenbach, Germany) at 37°C for an assigned immersion time. Then, specimens were rinsed in tap water and dried naturally at room temperature for 15 min. Nail enamel remover was applied over nail varnish that covered the uneroded area until clean. A stereomicroscope (Nikon, SMZ1500, Nikon Corporation, Kanagawa, Japan) with a ×112.5 magnification was used to confirm that the nail varnish was completely removed from the enamel surface. Height levels of eroded and uneroded areas were measured with a profilometer and a measuring microscope.

After erosion, profilometry was performed using a contact profilometer (Surfcorder SE2300, Kosaka Laboratory, Tokyo, Japan) with a 5-μm radius diamond stylus tip under a 4-mN load. The stylus traveled across the eroded and uneroded areas at a velocity of 0.5 mm/min perpendicularly to the specimen surface for a tracing length of 2.5 mm. The vertical and horizontal magnification profiles were set at ×1,000 and ×50, respectively. The erosion depth is defined as the distance between the uneroded surface and the eroded bottom of the profile (Fig. 1), and erosion depth was measured for every 100 μm of tracing length on the eroded profile. The data was then averaged. Each specimen was traced three times for definition as the mean erosion depth.

Evaluation with the measuring microscope (Nikon, MM400, Nikon Corporation, Kanagawa, Japan) was done with a 500x magnification along the x, y, and z axes. A specimen block was mounted on a plane-leveling stage to ensure that the specimen surface under inspection was perfectly flat and then placed on the microscope x-y stage. For each measurement, two positions (R1 and R2) on the uneroded area were specified to be the reference height (Fig. 2). Depths of eroded areas were focused for every 100 μm along a distance of about 1.5 mm on the x-axis, and the data were averaged. The average value of three measurements was calculated for each specimen.

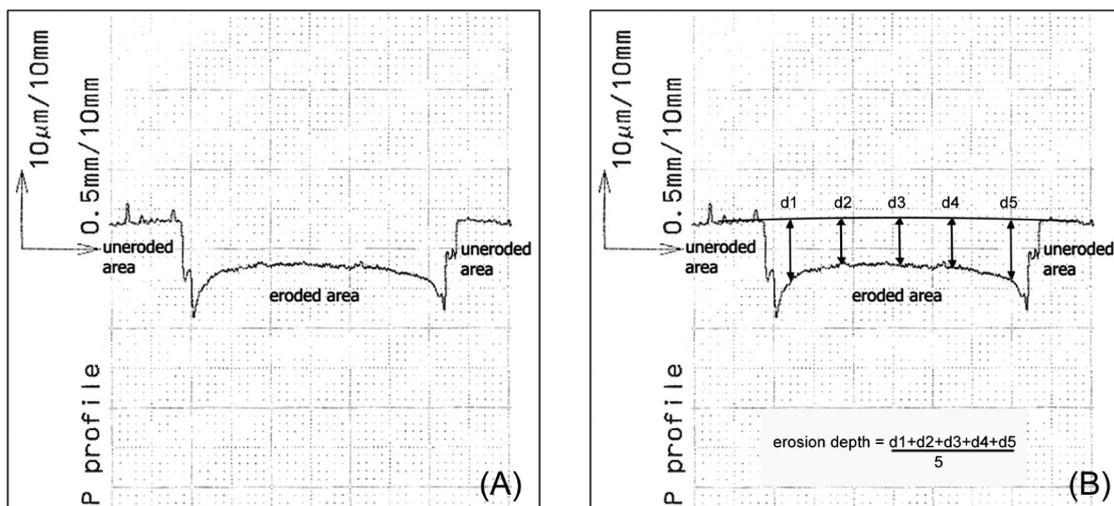Data of average enamel loss measured by MM and PM

Fig. 1 (A) Profile for the measurement of erosion depth by a profilometer. (B) Five positions on the profile were measured to find the depth between the uneroded and eroded line to calculate the average erosion depth.

on overall erosion times were scatter plotted on the graph with regression fit. For variable groups (beverage with exposure time), the paired *t*-test, with a level of confidence of 95%, was used to compare the results between PM and MM. Pearson's correlation coefficient was used to determine the relationship between the two methods.

Results

The scatter plot in Fig. 3 shows a highly linear relationship ($R^2 = 0.90$) between measurements by PM and MM for all cases. The range, mean, standard deviation and paired samples *t*-test were used to compare the erosive measuring abilities of the two methods for enamel in orange juice and Coke at different soaking times as shown in Table 1 and Fig. 4. Correlation coefficients and statistically significant correlations between PM and MM are presented in Table 2.

Orange juice showed lower erosive potential than Coke. Coke took 180 minutes to erode 8.29 µm of enamel, whereas for the same time period, orange juice eroded only 1.1 µm of enamel when measured with PM. Data from Table 2 showed a significant correlation between the two methods when enamel loss was approximately 1 µm. However, there was a low discriminatory ability for both MM and PM methods for measuring erosion in orange juice because enamel loss was less than 1 µm.
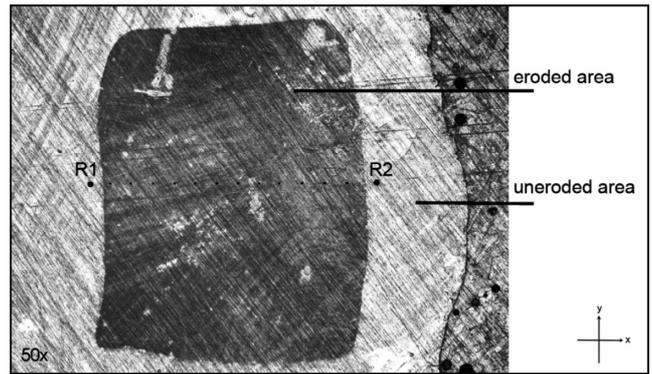

Fig. 2 Image of enamel surface for the measurement of erosion depth by measurement microscope.
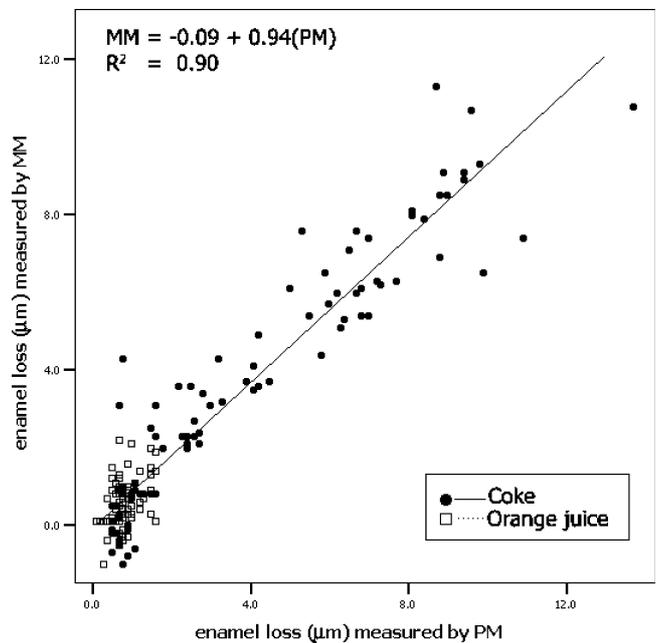
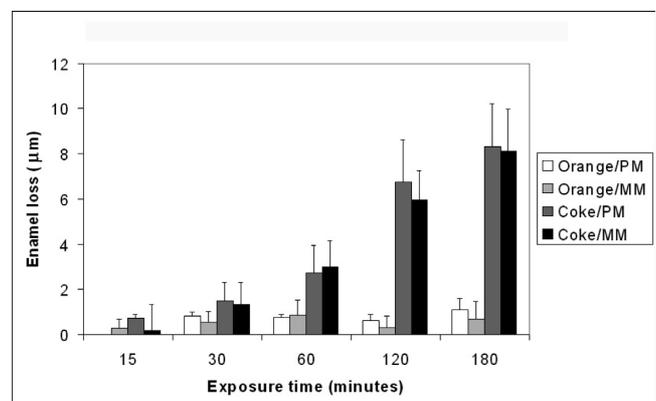Fig. 3 Correlation for erosion loss (µm) in orange juice and Coke, as measured by PM and MM.

Table 1 Distribution range and mean (S.D.) enamel erosive loss (µm) in orange juice and Coke at different exposure times by a profilometer (PM) and a measuring microscope (MM), and results of paired samples *t*-test to determine their relative effectiveness

Time (min)	Min to max (range)		Mean (S.D.)		*P*
	PM	MM	PM	MM	
Orange juice					
15	NA	-0.4 to 1.1 (1.5)	NA	0.25 (0.42)	NA
30	0.5 to 1.2 (0.7)	-0.1 to 1.5 (1.6)	0.80 (0.19)	0.56 (0.44)	0.044*
60	0.5 to 1.0 (0.5)	-0.3 to 2.2 (2.5)	0.75 (0.14)	0.85 (0.67)	0.548
120	0.1 to 1.3 (1.2)	-0.4 to 1.2 (1.6)	0.62 (0.28)	0.32 (0.48)	0.015*
180	0.2 to 1.6 (1.4)	-1.0 to 3.0 (4.0)	1.1 (0.49)	0.68 (0.79)	0.006*
Coke					
15	0.5 to 1.1 (0.6)	-1.0 to 4.3 (5.3)	0.71 (0.17)	0.18 (1.16)	0.072
30	0.6 to 2.7 (2.1)	-0.4 to 3.1 (3.5)	1.49 (0.82)	1.33 (0.97)	0.350
60	1.1 to 5.8 (4.7)	0.8 to 4.9 (4.1)	2.73 (1.23)	3.02 (1.12)	0.139
120	3.9 to 10.9 (7.0)	3.5 to 7.6 (4.1)	6.73 (1.88)	5.97 (1.29)	0.051
180	5.9 to 13.7 (7.8)	5.1 to 11.3 (6.2)	8.29 (1.93)	8.11 (1.87)	0.488

NA = Not applicable, *t*-test for paired sample, statistically significant at $P < 0.05$

Table 2 Correlations between a profilometer (PM) and a measuring microscope (MM) in determining enamel erosive loss (µm) in orange juice and Coke at different exposure times

	Exposure time (min)				
	15	30	60	120	180
Orange juice	NA	-0.007	-0.034	0.303	0.681*
Coke	0.016	0.697*	0.778*	0.644*	0.833*

* represents correlation significant at $P < 0.05$, NA = Not applicable

Fig. 4 Enamel loss in orange juice and Coke at different exposure times, as measured by PM and MM.

Discussion

With regard to the comparison between PM and MM at different erosive levels, carbonated drink and orange juice were chosen because they have different erosive potential (12,13). In this study, the erosive potential of orange juice and Coke on enamel was similar to those reported in a previous study (13,14). The erosive potential corresponded well to the pH of the beverage, thus Coke (pH 2.38) had a clearly higher erosive potential than orange juice (pH 3.67).

Based on the scatter plots of overall data and coefficients of determination (R^2) for the influence of beverages on enamel loss as measured by PM and MM, the R^2 was as high as 0.90 and the regression line nearly passed through the intersection point of the figure. These results indicate that the two methods had very good agreement. However, the overall R^2 was not applicable for each experimental group. Therefore, in the present study, Pearson's correlation coefficient and t-test statistical approaches were proposed to determine the concordance between PM and MM for each individual experimental group. Correlation coefficients measure the strength of a relationship between two variables, while the t-test determines whether there is a significant difference between the mean values of two groups (15).

In Table 1, the t-test for paired samples in the Coke group revealed that there was no significant difference between the two methods, whereas for the correlation test, Table 2 showed a high correlation (0.644 – 0.833) between PM and MM for erosive depth when enamel was immersed in orange juice for more than 180 min or in Coke for more than 30 min, where the average enamel loss was more than 1 μm. Conversely, when the average enamel loss was less than 1 μm, the correlation coefficient between PM and MM was weak. If the correlation between two measurement methods is weak and there is significant difference between the results obtained with the two methods, the two methods are not interchangeable (16). Thus, the difference in the results when the erosion depth is less than 1 μm is considered to be a limitation of the two measuring instruments.

Contact stylus profilometers are widely used for measurement of the contours of the enamel surface because they give highly repeatable and direct profiles of high resolution (17,18). However, a diamond stylus can scratch the enamel surface or a delicate surface such as dentin. Therefore, this method may not be appropriate for some specimens. Furthermore, the discrimination ability of PM varies based on its magnification. In this study, a vertical magnification of ×1,000 could not discriminate differences in height between uneroded and eroded profiles of enamel exposed for 15 min in orange juice. In practice, this effect may be overcome by using a higher vertical magnification but it may not appropriate for measurements of various depth levels such as those investigated in this study, because measurement of high erosion produced an overprofile and the results could not be reported.

For the measuring microscope used in this study, the ×50 magnification of the objective lens had a depth of focus (DOF) of 1.4 μm. The DOF is the distance from the nearest image plane in focus to that of the farthest plane which is also simultaneously in focus, and varies with numerical aperture and magnification of the objective. The enamel surface was then placed within this DOF range. The surface can be viewed with no loss of sharpness. Data of enamel loss of less than 1 μm showed a great standard deviation. In addition, there were numerous positions on the eroded enamel with a focused depth in the z-axis that was higher than the depth of the uneroded area (the negative value of enamel loss in Table 1). This information indicated that precision depends on DOF and focusing to obtain a sharp image. In theory, the error of measurement could not be greater than the DOF (9). In Table 1, enamel immersed in orange juice at 15 to 180 min lost less than 1 μm of tissue. Correlation indicated a low relation between the two methods. Although, measuring with a measuring microscope is a simple, rapid and non-destructive method, it may not be suitable for measuring enamel with very low erosion or initial erosion because the measurement accuracy is based upon the DOF of the objective lens. Thus, for decreasing erroneous measurement, an objective lens with a DOF of less than the erosive depth should be used.

Acknowledgments

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