Relationship between fluorescence loss of QLF and depth of demineralization in an enamel erosion model
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The purpose of this study was to assess the relationship between quantitative light-induced fluorescence (QLF) values and demineralization depths in an enamel erosion model in vitro.

Flat labial enamel surfaces of bovine incisors were ground with 800-grit SiC and coated with nail varnish, but also leaving rectangular windows of enamel uncoated. Subsequently, they were immersed in a lactic acid gel (pH 5.0) for 0 to 7 weeks to make an enamel erosion model. Carious lesions thus induced were analyzed by QLF and the demineralization depths measured using SEM/EDS method at the end of each period. A wide range of erosive lesions were produced with a steady increase in both demineralizing depth and fluorescence loss (ΔF) over time. With this model, a good correlation was exhibited between each ΔF value and the demineralization depth. Results of this study indicated that QLF could detect and quantify mineral loss under the eroded surface of the enamel erosion model.

Keywords: Quantitative light-induced fluorescence, Erosion model, Energy-dispersive X-ray analysis

INTRODUCTION
Erosion of tooth surfaces occurs when the latter are frequently exposed to acid¹. A worrying trend emerges in that the prevalence of dental erosion is on the increase. Therefore, it is important to diagnose this condition as early as possible to initiate preventive measures so as to diminish further progression². In current dental care, both patients and dental clinicians are becoming increasingly aware of dental erosion. However, there is still a need to raise diagnostic competence through the use of a simple method that properly reflects the nature, extent and progression of the defects.

Quantitative light-induced fluorescence (QLF) is a powerful tool which makes it possible to diagnose the degree of demineralization in a non-destructive way³-⁵. When a sound tooth surface is irradiated by blue-green light, it emits fluorescence with a wavelength of 540 nm. However, in demineralized enamel prism, increase in the scattering and diffusion of fluorescence reflection causes enamel lesions to be visible as a dark spot in the fluorescence image. By means of this noticeable difference in fluorescence intensity between sound and demineralized enamel, QLF can detect early caries lesions sooner than optical diagnosis or radiographic diagnosis methods⁶. In particular for white spot lesions, a potentially useful application of QLF lies in its ability to assess the severity thereof. Apart from being able to detect the demineralization depth of an enamel lesion up to approximately 10 μm, QLF was also capable of quantitative analysis of the same lesion at different demineralization periods⁷.

In a bid to assess the ability of QLF in quantifying mineral loss in caries lesions, studies have been conducted to compare it with other equipment and existing ‘gold standard’ such as transverse microradiography (TMR), DIAGNOdent®, and Electronic Caries Monitor (ECM)⁸-¹⁰. Favorably, several studies⁷,⁸,¹¹-¹³ have reported on significant correlation between fluorescence loss measured by QLF and demineralization depths in enamel subsurface models. However, little attention has been paid to enamel erosion models. Therefore, the purpose of this study was to establish an enamel erosion model using bovine teeth and thereby investigate the relationship between fluorescence loss of QLF and demineralization depths of white spot lesions using this enamel erosion model.

MATERIALS AND METHODS
Sample preparation
Figure 1 illustrates the specimen preparation procedures for QLF measurement and scanning electron microscopy (SEM)/energy dispersive X-ray spectroscopy (EDS) analysis. Thirteen bovine incisors, which were stored frozen, were visually examined to confirm the absence of physical damage such as discoloration, surface texture, or cracks. The labial surfaces of the bovine incisors were sectioned (5 mm × 12 mm × 4 mm) from each tooth, and five pieces were...
embedded in a self-cured acrylic resin (Unifast Trad, GC Corp., Tokyo, Japan) per treatment group. After curing, all the specimens were ground with 400-, 600-, and 800-grit silicon carbide papers under running water to produce flat enamel surfaces. To achieve a uniform thickness for the enamel specimens, the time spent for polishing under the same hand pressure was stringently controlled. The specimens were then coated with a clear nail varnish (Top Coat, Aube Kao, Tokyo, Japan), leaving only enamel windows of approximately 1 mm × 3 mm exposed. A total of five groups of 10 windows were tested.

The specimens were demineralized for 0 (sound control), 1, 3, 5, or 7 weeks in 0.1 M lactic acid gel containing 6 wt% of carboxymethyl cellulose adjusted to pH 5.0 with KOH and maintained at 37°C.

QLF analysis
To standardize QLF measurements, the border of the resin block was fitted to the window of the QLF handpiece so that the focus was always constant. The tooth surfaces were washed in deionized water and dried for 15 minutes before measurement. All the QLF images were captured in an American Standards Association (ASA) Class I dark room at a room temperature of 22±1°C.

The QLF images were analyzed by a single examiner using a QLF software (version 2.0.0.38; Inspektor Research Systems BV, Amsterdam, The Netherlands). The control group without demineralization was analyzed to record the baseline, while the lesion models were analyzed using QLF at the end of each demineralization period. Figure 2 is a representative fluorescence image of a lesion from this study, comprising values such as ΔF (%): average fluorescence loss; White spot (WS) area (mm²): size of white spot lesion; ΔQ=ΔF×WS area (%•mm²): ΔF integrated by lesion area in mm². Further on ΔF, the following three values were used in the present study — namely, ΔF_{AVE.}: average loss of fluorescence intensity in the whole lesion; ΔF_{MAX.}: maximum loss of fluorescence intensity in the whole lesion; and ΔF_{CENTER}: loss of fluorescence intensity at the center part of the lesion. As for ΔQ and WS area values, they were not considered in this study although they are commonly used because the size of the artificial lesions was fixed.

SEM/EDS analysis
After QLF analysis, the specimens were sectioned vertically at the center of the demineralized area using a water-cooled diamond-edged blade in a sectioning machine (Isomet® Low Speed Saw, Buehler, IL, USA).
Each enamel specimen was embedded in an epoxy resin (Epoxicure™, Buehler), and the cross-sectioned surfaces were consecutively polished with 1000-, 1,200-, 1,500-, and 2,000-grit silicon carbide papers (Fuji Star, Sankyo Rikagaku Co. Ltd., Saitama, Japan), followed by consecutive polishing with 3,000-, 4,000-, 6,000-, 8,000-, 10,000-, and 15,000-grit aluminum oxide films (3M™ Lapping Film Sheets, 3M, MN, USA) under running water.

After drying, the specimens were sputter-coated with gold and examined using a SEM (S-4500, Hitachi Ltd., Hitachinaka, Japan). Following which, three out of 10 demineralized windows from each group were randomly selected for line scan analysis using an energy dispersive X-ray spectrometer (EMAX-7000, Horiba Ltd., Kyoto, Japan). For each demineralized area subjected to previous QLF measurement, 60 line scans were performed to measure its Ca and P concentrations. The acquisition time of the EDS spectrum was 100 seconds at an accelerating voltage of 15 kV and with a beam current of 0.1 nA. The EDS spectrum was quantitatively assessed according to the intensity of each spectrum.

Figure 3 is an illustrated cross-sectional view of the eroded enamel, where the following values were obtained from SEM observations and EDS analyses — namely, A: depth from the original surface to the bottom of eroded crater; B: depth from the original surface to the demineralization front; B–A: thickness of the mineral loss layer; C: enamel thickness. These values were measured at three points — namely at the center of the demineralized window (white arrow) and 100 µm from the center on the right and left sides. With these three values, the average was thereby calculated.

**Statistical analysis**

For each analysis method, mean values with standard deviation bars of ΔF and SEM/EDS data (n=50) were plotted against the demineralization time (0–7 weeks). The regression line for the prediction of ΔF value from the demineralization time was also included on the plots. Simple linear regression analyses were performed, with the demineralization time (week) as the dependent variables and the ΔF values as the independent variables. As no significant interaction was confirmed between demineralization time and ΔF at the three measuring points, analysis of covariance (ANCOVA) was done to compare the effects of the three measuring points of the demineralized areas on ΔF value (p=0.05). Where significant differences occurred in ΔF value among the three measuring points, pairwise comparisons with Bonferroni corrections were conducted to determine which ΔF value of the three measuring points differed from the others. Any value with p<0.017 was considered significant.

The same statistical procedure was used for the data obtained from SEM/EDS analysis (A, B, and B–A). Group mean comparisons of enamel thickness (C) were performed using the Kruskal–Wallis analysis.

**Fig. 2** QLF image of the enamel erosion model. ΔF_{AVE}:
average loss of fluorescence intensity in the whole demineralized area; ΔF_{MAX}:
maximum loss of fluorescence intensity in the whole demineralized area; and ΔF_{CENTER}:
loss of fluorescence intensity at the center part of the demineralized area.

**Fig. 3** Illustration of the cross-sectional view of the enamel erosion model.
A: Depth from the original surface to the bottom of the eroded crater
B: Depth from the original surface to the demineralization front
B–A: Thickness of the mineral loss layer
C: Enamel thickness
Pearson’s correlation coefficient was also calculated to investigate the relationship between ΔF values and (A), (B), or (B–A). All lesions were treated as individual samples. Statistical procedures were performed at a 95% level of confidence with the Statistical Package for Medical Science (Dr. SPSS II for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

QLF analysis
The relationship between ΔF values and demineralization time is shown in Fig. 4. Simple linear regression models were fitted to the ΔF values at three measuring points (ΔF_{AVE.}: \( R^2=0.78, \ p<0.0001 \); ΔF_{CENTER}: \( R^2=0.86, \ p<0.0001 \); ΔF_{MAX.}: \( R^2=0.82, \ p<0.0001 \)). As expected, specimens in all the groups showed a decrease in enamel fluorescence (ΔF values) with demineralization time. However, ANCOVA revealed that the slopes of the regression lines were not significantly different between ΔF_{MAX.} and ΔF_{CENTER} (\( F=0.06, \ p>0.05 \)), but were significantly different between ΔF_{AVE.} and ΔF_{MAX.} (\( F=15.65, \ p<0.05 \)) and between ΔF_{AVE.} and ΔF_{CENTER} (\( F=16.18, \ p<0.05 \)).

SEM/EDS analysis
A representative photograph acquired from SEM/EDS analysis is shown in Fig. 5. In the SEM image of the cross-sectioned enamel, a layer of mineral loss replaced the position of the surface originally exposed to lactic acid gel and which appeared brighter than the underlying sound enamel. In the EDS line scan from the surface toward the demineralization front, increasing concentrations of Ca and P were observed until sound enamel was reached, where the concentrations of Ca and P reached a plateau. The area that was observed as a bright area in the SEM image coincided with low Ca and P levels in the EDS profile. The demineralization front was determined by the inclination change of the Ca and P distributions in the EDS profile.

Based on the SEM/EDS analysis, Fig. 6 shows the effect of demineralization time on the depth parameter. For each value, the slope of increase with the demineralization period was linear (A: \( R^2=0.75, \ p<0.0001 \); B: \( R^2=0.98, \ p<0.0001 \); B–A: \( R^2=0.89, \ p<0.0001 \)). ANCOVA indicated that the slopes of the regression lines were significantly different between A and B (\( F=328.04, \ p<0.05 \)), A and B–A (\( F=38.48, \ p<0.05 \)), and B and B–A (\( F=91.36, \ p<0.05 \)).

As for enamel thickness (C), Fig. 7 shows that it did not differ significantly among the different demineralization periods (\( \chi^2=6.64, \ df=4, \ p>0.05 \)).
Fig. 6  Relationships between depth parameters of the erosion model and demineralization time. The lines are simple linear regressions.  
A: $y = 16.00x - 5.54$, $R^2 = 0.75$ ($p<0.0001$);  
B–A: $y = 28.32x + 17.80$, $R^2 = 0.89$ ($p<0.0001$);  
B: $y = 44.33x + 12.26$, $R^2 = 0.98$ ($p<0.0001$)

Table 1  Correlation coefficients between $\Delta F$ values and depths of demineralization in enamel ($n=50$)  

<table>
<thead>
<tr>
<th>$\Delta F$</th>
<th>$\Delta F_{\text{AVE.}}$</th>
<th>$\Delta F_{\text{MAX.}}$</th>
<th>$\Delta F_{\text{CENTER}}$</th>
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<tr>
<td>Depth</td>
<td></td>
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<tr>
<td>A</td>
<td>0.71**</td>
<td>0.73**</td>
<td>0.75**</td>
</tr>
<tr>
<td>B</td>
<td>0.91**</td>
<td>0.93**</td>
<td>0.94**</td>
</tr>
<tr>
<td>B–A</td>
<td>0.91**</td>
<td>0.92**</td>
<td>0.93**</td>
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**$p<0.01$.  

Correlation  
Table 1 shows the correlation coefficients between each $\Delta F$ value measured by QLF and the corresponding distance from the original surface to the measuring point. There were significant, strong and positive coefficients between each $\Delta F$ value and B ($0.91<|R|<0.94$, $p<0.01$), and between each $\Delta F$ value and B–A ($0.91<|R|<0.93$, $p<0.01$). There were also significant positive coefficients of more than 0.7 between each $\Delta F$ value and A ($0.70<|R|<0.75$, $p<0.01$).

DISCUSSION  
QLF is a visible light system that offers the opportunity to detect early caries and monitor its progression or regression longitudinally without destruction of the specimen in the clinical situation. The fluorescence of dental hard tissues has been known for a long time\textsuperscript{24}, and the source of autofluorescence is thought to be the dentinoenamel junction (DEJ). The excitation light passes through the transparent enamel and activates fluorophores contained within the DEJ\textsuperscript{25}. For QLF, its principle of mineral loss measurement is based on the increase in fluorescence scattering due to caries formation\textsuperscript{22}. This is because fluorescence radiance induced by exposure of the tooth to blue light scatters significantly more within demineralized enamel compared with sound enamel. As a result, demineralized areas appear as dark spots on the QLF image.

It is noteworthy that the fluorescence loss of QLF is influenced by several factors. On this ground, it is important to standardize the measurement conditions when conducting QLF measurements. In this study, the thickness of enamel (C) was controlled at a standardized value because of existing good correlation between fluorescence loss of QLF and enamel thickness\textsuperscript{26}. In addition, other factors that have been identified to affect QLF images when assessing enamel lesion severity include camera geometry\textsuperscript{18}, focal distance\textsuperscript{19}, environmental conditions\textsuperscript{21}, and degree of dehydration\textsuperscript{27,28}. In the present study, the measuring conditions of the specimens were standardized at each measurement to eliminate the influences of these factors.

The demineralizing gel used in the present study produced subsurface mineral loss followed by the development of enamel defects\textsuperscript{14,29}. In the current enamel erosion model, bovine enamel was used as a substituted material for human enamel. However, there are some morphological differences between
bovine and human enamel. It was reported that bovine enamel has a higher porosity compared to human enamel, resulting in higher rates of artificial caries lesion formation. Such a difference might contribute to disparities in ΔF values between bovine and human teeth.

SEM/EDS analysis was used to examine the demineralized enamel in this study. The EDS spectrometer is a powerful instrument for performing quantitative elemental analyses by measuring the characteristic of re-emanited X-rays. With a combined SEM-EDS analysis approach, the demineralization depths of the enamel lesions could be precisely measured. Indeed, even in the case of lesions with enamel defects, the lesion depth (mineral loss) and crater depth could be measured with the SEM/EDS analysis method.

In the SEM/EDS image (Fig. 5), some cracks were present in the underlying enamel where the mineral had preferentially dissolved. It was thought that partly dissolved crystal bundles were bound together as a result of porosity when the demineralized enamel was dehydrated during the preparation. Further, the appearance of this image was supported by the fact that the surface roughness of demineralized enamel apparently increases with drying time and demineralization time.

The ΔF value is the change in fluorescence emission intensity. In the present study, three ΔF values — namely, ΔFAVE, ΔFMAX, and ΔFCENTER — were used since the mineral concentration and lesion depth of the specimens were not uniform in the whole lesion. Results of this study showed that each ΔF value decreased with the demineralization period (Fig. 4). Nonetheless, the rate of reduction of ΔFAVE was the smallest among the three ΔF values as it was the average mineral loss in the whole lesion. With ΔFMAX and ΔFCENTER, similar rates of reduction were observed but these results did not coincide with each other. This meant that varied surface geometries existed in the current enamel erosion model.

The enamel erosion model included a crater and subsurface mineral loss. There was a linear increase in demineralization depth in the erosion model over the experimental period (Fig. 6). This linear pattern of structural loss has been noted with both bovine and human enamel, resulting in higher rates of artificial caries lesion formation. Nonetheless, the rate of reduction of ΔFAVE was the smallest among the three ΔF values as it was the average mineral loss in the whole lesion. With ΔFMAX and ΔFCENTER, similar rates of reduction were observed but these results did not coincide with each other. This meant that varied surface geometries existed in the current enamel erosion model.

ACKNOWLEDGMENTS

The authors wish to thank Daisuke Inaba of Iwate Medical University for the generous help and advice pertaining to the demineralizing gel, and Shizuko Ichinose of Tokyo Medical and Dental University for the kind help with the SEM/EDS analysis.

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