2015년 2월 석사학위 논문 Evaluation of etched enamel using Quantitative light-induced fluorescence (QLF) 이 아 름

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석사학위 논문

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QLF를 이용한 법랑질 산부식 평가

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이 논문을 치의학 석사학위신청 논문으로 제출함.

2014년 12월

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이아름의 석사학위논문을 인준함.

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2014년 12월

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QLF를 이용한 법랑질 산부식 효과 평가

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본 연구의 목적은 우상치에서 법랑질의 산부식 효과를 Quantitative light-induced fluorescence (QLF)를 사용하여 정량적으로 평가하는 것이다.

30개의 발거된 소의 절치를 사용하였다. 치근을 잘라낸 치관부 치아만을 1000ml 생리식염수 병뚜껑에 매몰하였다. 치관의 절반을 #400-Grit SiC paper로 삭제하고. 나머지 절반은 삭제하지 않았다. 각 표면에 투명한 네일바니쉬를 도포하여 3×3 mm 크기의 enamel window를 형성하였다. 총 120개의 window를 형성하여 무작위로 20개씩 6개 그룹으로 나누어 15, 30, 60초간 32% 인산을 적용한 후 수세, 건조하여 QLF와 FE-SEM분석을 시행하였다.

QLF 분석결과, 15초, 30초간 산부식한 그룹에서, 법랑질 표면을 삭제한 그룹이 삭제하지 않은 그룹보다 유의적으로 높은 형광소실도를 보였다. 60초간 산부식한 그룹에서는, 표면삭제유무에 따른 유의적인 차이는 존재하지 않았다. 법랑질 표면을 삭제한 그룹과 삭제하지 않은 그룹 모두에서, 15초 산부식은 60초 산부식보다 유의적으로 낮은 형광소실도를 나타내었다. 그러나 15초와 30초, 30초와 60초 산부식 그룹간에 유의적인 차이는 존재하지 않았다. FE-SEM 분석결과, 산부식시간이 증가할수록 법랑소주의 노출이 증가하였으며, 표면삭제한 군은 삭제하지 않은군보다 더 균일한 표면을 보였다.

본 연구에서 다양한 산부식 시간과 삭제유무에 따른 법랑질 산부식 효과를 QLF를 이용해서 정량적으로 평가할 수 있었다.

I. Introduction

Since acid-etching was introduced in 1955, phosphoric acid etching has been used as a standard and predictable procedure in enamel bonding,[1] , which enables the micromechanical interlocking between composite resins and the enamel surface.[2,3] Acid conditioning removes a few microns of enamel exposing porous prismatic structure and roughening the surface.[4]

Numerous studies have been conducted on the effect of acid etching on enamel regarding several parameters such as acid concentration, etching time and enamel grinding.[5,6] However, Quantitative information on demineralization after etching with respect to enamel bonding is lacking.

Quantitative light-induced fluorescence (QLF) is a diagnostic tool for the quantification of demineralization in a non-destructive way.[7,8,9] When a sound tooth surface is illuminated by blue-green light, it emits fluorescence with a wavelength of 540 nm. However, in demineralized area, 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 has been widely used as a quantification system for assessing early demineralization or remineralization of enamel by thoroughly investigating the correlation under various treatments.[10,11]

The objective of this study was to quantify the effect of etching to bovine enamel using QLF. The null hypothesis was that there are no differences in fluorescence loss between ground and unground enamel and that there are differences in fluorescence loss with various etching time.

II. Materials and Methods

1. Specimen preparation

Thirty extracted bovine incisors without cracks or white spots were used for this study. The teeth were cleaned and stored frozen. The root was removed using low-speed diamond disk. The teeth were embedded into plastic caps of 1000ml bottles with utility wax. One-half of the labial surface of each tooth was ground with #400-grit silicon carbide paper. The other half was left intact. Ground and intact surfaces were coated with a clear nail varnish, leaving rectangular windows of 3 mm × 3 mm enamel exposed. A total of six groups of 120 windows were tested. A 32% phosphoric acid (Uni-Etch®, Bisco, Schaumburg, IL, USA) was applied to the enamel window for 15, 30, or 60 seconds. The acid was rinsed off with distilled water for 20s and the surfaces were air dried for 20s.

2. QLF analysis

QLF-D (QLF-D biluminator^{TM}, Inspektor Research systems BV, Amsterdam, Netherlands) was used in this study. Fluorscence image if all specimens were captured with a 'live view' - enabled digital full-sensor SLR camera (model 550D, Canon, Tokyo, Japan) at the following setting : shutter speed of 1/45 s, aperture value of 3.2, and ISO speed of 1600. Measurement height was 15 cm. Proprietary software (C3 v1.18, Inspektor Research systems BV) was used to capture and store all digital images on a PC automatically. All fluorscence images were analyzed using a software (QA2 v1.18, Inspektor Research Systems BV) by a single examiner (Figure 1).

Demineralization quantity was calculated before and after etching. A

region of interest was defined by manually outlining the surface using an interface within the capture software. Delta F values (defined as a percentage of fluorscence loss) was used at the 5% threshold level.

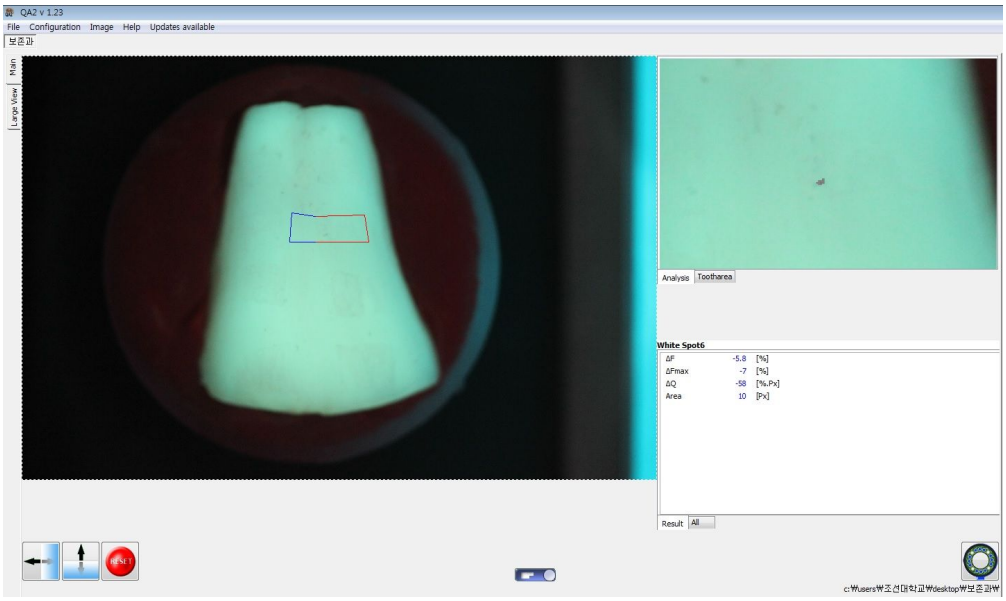

Figure 1. QA2 analysis image of the etched enamel. Image showed the ΔF values.

3. FE-SEM analysis

After QLF analysis, the specimens were mounted on aluminum stubs, sputter-coated with gold-palladium and examined under the FE-SEM(S-4800, Hitachi, Tokyo, Japan). Photographs of the most expressive regions were take at ×2,500 magnification.

4. Statistical analysis

The influence of different etching time on ΔF values were analyzed by one-way ANOVA. Post hoc multiple comparisons were performed using Dunnett T3 and Tukey's test. The independent t-test was used to assess differences between unground and ground enamel.

All statistical procedures were performed using the SPSS 12.0 for windows (IBM corp., Armonk, NY, USA). The significance level was set at p=0.05

III. Results

1. QLF analysis

The ΔF values for the six groups are shown in Table 1 and Figure 2. Grinding the enamel before etching for 15s, 30s had a significant effect on enamel fluorescence (values as ΔF : average fluorescene loss). But there was no singinificant difference between ground and unground enamel for 60s etching.

ΔF values decreased with increased etching time. The ANOVA results showed that etching time had a significant effect on ΔF values only between 15s and 60s. There was no significant difference between 15s and 30s, 30s and 60s in both ground and unground enamel.

Table 1 ΔF values for etched enamel

Mean ± SD	15s	30s	60s
Unground	$-5.385\pm0.19^{A,a}$	$-5.485\pm0.1^{A,a,b}$	$-5.695\pm0.42^{A,b,c}$
Ground	$-5.46\pm0.36^{B,a}$	$-5.68\pm0.19^{B,a,b}$	$-5.885\pm0.38^{A,b,c}$

Within a column, significantly different values are followed by different uppercase letters ($p<0.05$). Within a row, significantly different values are followed by different lowercase letters ($p<0.05$).

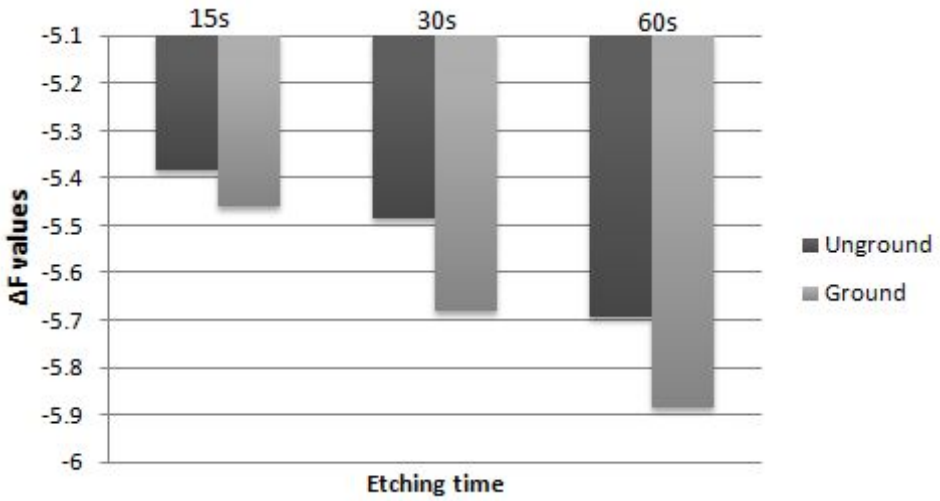

Figure 2. Bar diagram showing mean values for fluorescence loss of etched enamel.

2. FE-SEM analysis

FE-SEM images showed that there were morphological differences between ground and unground enamel (Figure 3). In ground enamel, micro-irregular etch patterns exposing individual enamel crystals were clearly observed in the whole surface (Figure 3B, D, F) In unground enamel, porous and numerous enamel crystallites could be observed. The quantity of good-quality etch produced by phosphoric acid at 37% was time specific, with 15 s being less effective than 30 or 60 seconds. However, 60 s was not better than 30 s (Figure 3A, C, E).

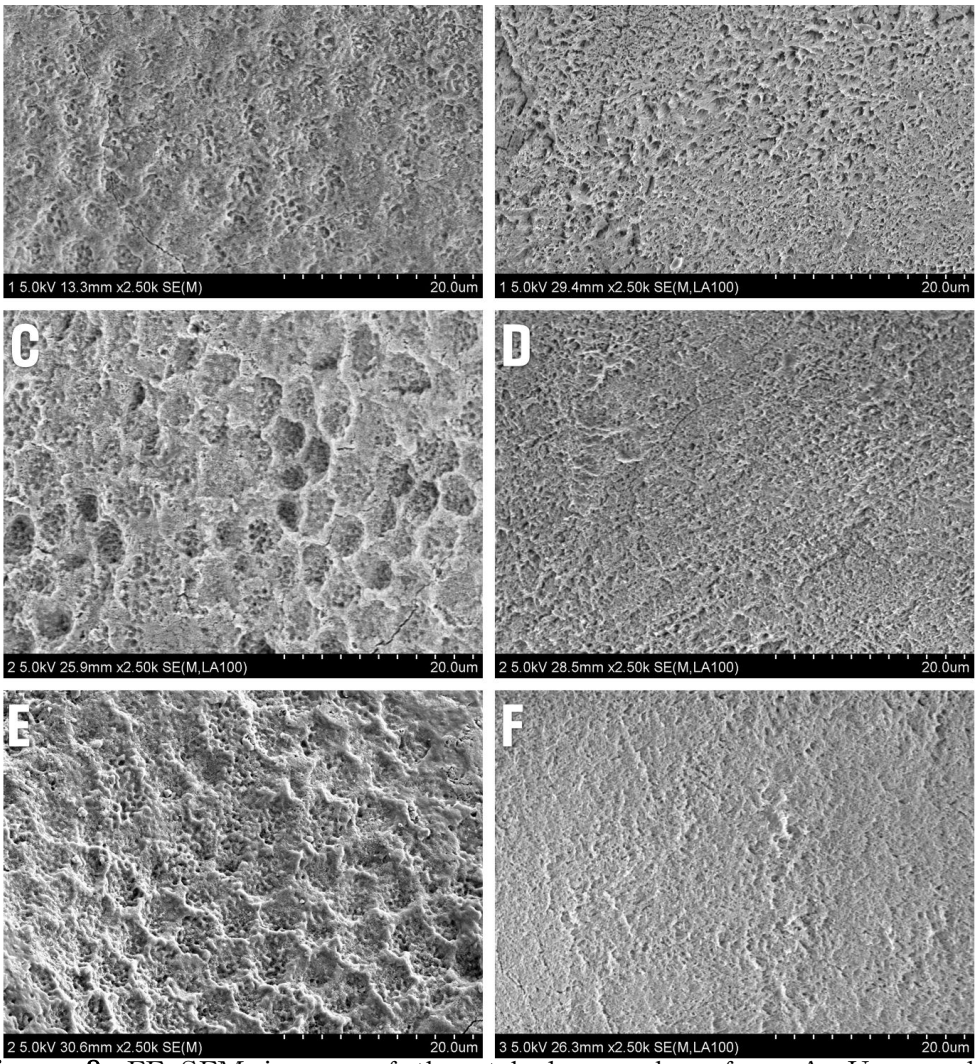

Figure 3. FE-SEM images of the etched enamel surface. A, Unground enamel etched for 15s; B, Ground enamel etched for 15s; C. Unground enamel etched for 30s; D, Ground enamel etched for 30s; E. Unground enamel etched for 60s; F. Ground enamel etched for 60s. ×2,500 magnification.

IV. Discussion

Adhesion to enamel is achieved through acid etching of this highly mineralized substrate, which substantially enlarges its surface area for bonding.[12-14] There had been many attempts to improve the adhesion procedure for successful bonding and also minimizing unnecessary mineral loss. Previous research on the topic of etched enamel was performed for qualitative surface analysis using FE-SEM or comparing the shear bond strength.[1-3] Thus, our study tried to quantitatively evaluate the deminearalization of etched enamel using QLF.

The present study revealed that grinding the enamel before etching for 15s and 30s had a significant effect on demineralization. This led to rejecting the first null hypothesis. These findings could be due to the structural difference between two enamel types. It can be interpreted that subsurface enamel is more soluble than surface enamel. Previous studies also demonstrated that the surface of intact enamel is composed of a dense layer of hydroxyapatite crystals without any intercrystallite spaces.[15,16] In 60s etching group, there was no significant difference between ground and unground enamel. This might have resulted from using 32% phosphoric acid. This acid has high acidic capacity (pH<1), and clearly causes enough mineral dissolution to permit the formation of macro- and microretentive resin tags between and within enamel prisms regard- less of grinding.[17,18]

Etching time had a significant effect on demineralization between 15s and 60 in both ground and unground enamel. This led to reject the second null hypothesis. The results indicate that the quantity of demineralization produced by phosphoric acid at 32% was time specific, with 15 seconds being significantly less effective than 60 seconds. However,

demineralization of etched enamel for 60 seconds was not significantly better than that of 30 seconds.

FE-SEM analysis was used to exmaine the surface morphology of the etched enamel. The present results showed that a longer etching time result in a more dissolution and removal of the enamel mineral phase, but there was no morphological difference in the ground enamel surface. There was structural difference beteween ground and unground enamel. These findings agree with previous study.[19] which reported structural difference between two enamel type. When the prismless layer of enamel is removed, the typical prism patterns are obtained from underlying enamel when etched.

The traditional visual methods of detecting etched enamel is confirming the "white chalky" surface. But the QLF-D system allows immediate visual effect and offers quantitative comparison of current images with past images from the same patient. It has been developed to detect early caries and monitor its progression or regression longitudinally without destruction of the specimen in the clinical situation.[20] Principle of measuring mineral loss is based on the increase in fluorescence scattering due to caries formation.[21] When the teeth are illuminated with high intensity blue light, green fluorescence is induced from DEJ. The light scatters significantly more within demineralized enamel compared with sound enamel. As a result, demineralized area appears darker than sound area on t,he QLF image. Therefore, the QLF-D system can be used as a quantification system for assessing the degree of demineralization after etching on enamel.

ΔF value was analyzed to evaluate mineral loss in this study. Since the enamel window was identical in size for all specimens, only average ΔF values were recorded at 5% threshold level between sound and etched enamel.

The enamel window on unground and ground enamel was made adjacent to sound enamel, thus making comparsion the effect of demineralization between etched area and sound enamel.

It is noteworthy that several factors affect the fluorescence loss of QLF-D system. To standardize the measurement conditions when conducting QLF measurements, camera geometry, focal distance, environmental conditions was fixed.

Bovine enamel was used as a substitute for human enamel. However, there are some structural differences between bovine and human enamel.[22] It was reported that bovine enamel is more porous than those of human enamel, thus less resistant to acid diffusion. Such a difference might affect to disparities in ΔF values between bovine and human enamel.

Also, ΔF values is affected by different site of enamel window. For example, enamel window on cervical area is more acid-resistant because of aprismatic enamel.

This in vitro study showed that the QLF-D system was capable of detecting and monitoring mineral loss in the etched enamel. The fluorescence loss measure by QLF-D system reflected degree of mineral loss. It is suggested that the non-destructive QLF-D system is considered to be useful not only for clinical but also in vitro research. Futher studies should be conducted to demonstrate the bond strength of composite resin to enamel according to the QLF value of demineralization. If there is a significant relationship between bond strength and QLF value, QLF method might be used to evaluate the prognosis of enamel bonding in clinical situation.

V. Conclusion

This in vitro study demonstrated the demineralization of enamel with regard to varying etching times and surface treatment is quantitatively analyzed by QLF. Within the limits of this study, QLF method may be used to evaluate the demineralization of etched enamel conservatively.

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