Evaluation of etched enamel using quantitative light-induced fluorescence

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ABSTRACT

The purpose of this study is to evaluate the effect of etching on the ground and unground enamel using quantitative light-induced fluorescence (QLF). Thirty extracted incisors were used in the study. One-half of the labial surface of each tooth was ground with #400-grit SiC paper. The other half was left intact. Ground and intact surfaces were coated with nail varnish leaving rectangular windows of enamel uncoated. A 32% phosphoric acid gel was applied for 15, 30, 60 seconds. The mineral loss in terms of percentage of fluorescence (ΔF) was assessed by QLF at baseline and after etching. Effect of etching time and mechanical pretreatment were analyzed with independent t-test and one-way ANOVA. Grinding the enamel before etching for 15 seconds and 30 seconds had a significant effect on demineralization. However, no significant difference in demineralization was observed between the ground and unground enamel with 60 seconds of etching. The ΔF values decreased with increased etching time. But there was no significant difference in the ΔF values between 15 seconds and 30 seconds, and 30 seconds and 60 seconds in both ground and unground enamel. Demineralization after acid-etching can be quantified using QLF. The result indicated that the degree of demineralization increases with etching time and mechanical pretreatment.

KEY WORDS: Etched enamel, Ground enamel, Quantitative light-induced fluorescence (QLF)

Introduction

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

Numerous studies have investigated the effect of acid etching on enamel with regard to 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 non-destructive quantification of demineralization [7-9]. When a sound tooth surface is illuminated by blue-green light, it emits fluorescence with a wavelength of 540 nm. However, in demineralized areas, enamel lesions appear as dark spots on the fluorescence image due to increases in the scattering and diffusion of the light. By evaluating this noticeable difference in fluorescence intensity between sound and demineralized enamel, QLF has gained acceptance as a quantification system for assessing early demineralization or remineralization of enamel following various treatments [10,11].

The objective of the present study was to quantify the effect of etching on bovine enamel using QLF. The null hypothesis was that there would be no differences in fluorescence loss between ground and unground enamel, and

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that would be differences in fluorescence loss according to etching time.

Materials and Methods

Specimen preparation

Thirty extracted bovine incisors without cracks or white spots were used for the present study. The teeth were cleaned and frozen. The root was removed using a low-speed diamond disk. The teeth were embedded into the plastic caps of 1000 ml 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 enamel (3 mm × 3 mm) 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 s. The acid was rinsed off with distilled water for 20 s, following which the surfaces were air dried for 20 s.

QLF analysis

QLF-Digital (QLF-D Biluminator™, Inspektor Research Systems BV, Amsterdam, Netherlands) was utilized in the present study. Fluorescence images of all specimens were captured with a full-sensor, live-view-enabled digital SLR camera (model 550D, Canon, Tokyo, Japan) using the following settings: shutter speed: 1/45 s, aperture value: 3.2, and ISO speed: 1600. Measurement height was 15 cm. Proprietary software (C3 v1.18, Inspektor Research Systems BV) was used to automatically capture and store all digital images on a PC. All fluo-

rescence images were analyzed using QA2 Version 1.18 software (Inspektor Research Systems BV) by a single examiner (Fig. 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 fluorescence loss) were calculated at the 5% threshold level.

FE-SEM analysis

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

Statistical analysis

The influence of various etching times on ΔF values was analyzed via a one-way analysis of variance (ANOVA). Post hoc multiple comparisons were performed using Dunnett's T3 and Tukey's tests. An independent t-test was used to assess differences between unground and ground enamel.

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

Results

QLF analysis

The ΔF values for the six groups are shown in Table 1 and Fig. 2. Grinding the enamel before etching for 15 s and 30 s had a significant effect on enamel fluorescence. However, no significant differences were noted between ground and unground enamel when etching was performed for 60 s.

There was no significant difference between ground and

Fig. 1. The $\Delta A2$ analysis is shown in a representative image of the QA2 analysis.

Table 1. Values of ΔF for etched enamel

Mean $\pm$ SD	15s	30s	60s
Unground	$-5.385 \pm 0.19^{A,a}$	$-5.485 \pm 0.1^{A,a,b}$	$-5.695 \pm 0.42^{A,b,c}$
Ground	$-5.46 \pm 0.36^{B,a}$	$-5.68 \pm 0.19^{B,a,b}$	$-5.885 \pm 0.38^{A,b,c}$

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 ($p<0.05$).

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

unground enamel that etching time had a significant effect on ΔF values only between 15 s and 60 s. There was no significant difference between the values at 15 s and 30 s,

and 30 s and 60 s in both ground and unground enamel.

FE-SEM analysis

FE-SEM images revealed morphological differences between ground and unground enamel (Fig. 3). In ground enamel, micro-irregular etch patterns exposing individual enamel crystals were clearly observed across the whole surface (Fig. 3B, 3D, and 3F), while a large number of porous enamel crystallites were observed in unground enamel. Analysis of etching quality associated with 32% phosphoric acid was time-specific: etching durations of 15 s were less effective than durations of 30 or 60 s, although no difference in efficacy was noted between durations of 30 s and 60 s (Fig. 3A, 3C, and 3E).

Fig. 3. FE-SEM images of the etched enamel surface. A, unground enamel etched for 15 S; B, ground enamel etched for 15 S; C, unground enamel etched for 30 s; D, ground enamel etched for 30 s; E, unground enamel etched for 60 s; F, ground enamel etched for 60 s.

Discussion

Adhesion to enamel is achieved via acid etching of this highly mineralized substrate, which substantially enlarges the surface area for bonding [12-14]. Numerous studies have investigated methods for improving the adhesion procedure and minimizing unnecessary mineral loss. Previous research on enamel etching has utilized FE-SEM for qualitative analysis of the tooth surface in order to compare shear bond strength [1-3]. Thus, we aimed to quantitatively evaluate the demineralization of etched enamel using QLF.

The present study revealed that grinding the enamel prior to etching for 15 s and 30 s had a significant effect on demineralization, leading to the rejection of the first null hypothesis. These findings may be due to the structural difference between the two types of enamel, as subsurface enamel is more soluble than surface enamel. Previous studies have also demonstrated that the surface of intact enamel is composed of a dense layer of hydroxyapatite crystals without any intercrystallite spaces [15,16]. In the 60-s etching group, there were no significant differences between ground and unground enamel. Such a result may be due to the use of 32% phosphoric acid, which has a high acidic capacity ($\text{pH} < 1$) and clearly causes enough mineral dissolution to permit the formation of macro- and micro-retentive resin tags between and within enamel prisms, regardless of grinding [17,18].

Etching time had a significant effect on demineralization between 15 s and 60 s for both ground and unground enamel, leading to the rejection of the second null hypothesis. Our results indicate that the extent of demineralization produced by phosphoric acid at 32% was time specific, with 15 s being significantly less effective than 60 s. However, no significant improvements in demineralization of the etched enamel were noted between etching times of 30 and 60 s.

FE-SEM analysis was used to examine the surface morphology of the etched enamel. The present results revealed that a longer etching time resulted in increased dissolution and removal of the enamel mineral phase, although no morphological differences were observed in the ground enamel surface. However, structural differences were observed between ground and unground enamel, in accordance with the findings of a previous study [19]. When the prismless layer of enamel is removed, the typical prism patterns are

obtained from the underlying enamel following etching.

The traditional visual methods of detecting etched enamel involve confirming the presence of a “chalky white” surface. In contrast, the QLF-D system allows for the immediate visualization of these effects as well as quantitative comparison of current and past images from the same patient. QLF-D has been used to detect early caries and monitor progression or regression longitudinally in a clinical setting, without destruction of the specimens [20]. The principle of measuring mineral loss is based on the increase in fluorescence scattering due to the formation of caries [21]. When the teeth are illuminated with high-intensity blue light, green fluorescence is induced from the dentino-enamel junction (DEJ). The light scatters significantly more within demineralized enamel than within sound enamel. As a result, the demineralized area appears darker than sound areas on the QLF image. Therefore, the QLF-D system can be used to quantitatively assess the degree of demineralization after etching on enamel.

Since the enamel window was identical in size for all specimens, only average ΔF values were recorded at the 5% threshold level between sound and etched enamel. The enamel window on unground and ground enamel was made adjacent to sound enamel, thus allowing us to compare the effect of demineralization between etched areas and sound enamel. It should be noted that several factors affect the fluorescence loss observed using the QLF-D system. Therefore, we standardized the measurement conditions when conducting QLF measurements by fixing camera geometry, focal distance, and environmental conditions. Furthermore, bovine enamel was used as a substitute for human enamel, which exhibits some structural differences from its human counterpart [22]. For example, previous studies have reported that bovine enamel is more porous than human enamel and thus less resistant to acid diffusion. Such a difference may produce disparities in acid diffusion. Also, ΔF are also affected by the site at which the enamel window is created, as cervical areas exhibit increased resistance to acid due to the aprismatic nature of the enamel in these regions.

The results of the present *in vitro* study indicate that the QLF-D system is capable of detecting and monitoring mineral loss in etched enamel, and that the extent of fluorescence loss measured by QLF-D also reflects the degree of mineral loss. These findings suggest that the non-destructive QLF-D system may be useful for clinical purposes as well as *in vitro* research. However, further

studies are required in order 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 methods may be used to evaluate the prognosis of enamel bonding in clinical settings.

Conclusion

The present in vitro study demonstrated that QLF may be used to quantitatively analyze the demineralization of enamel with regard to varying etching times and surface treatments. Despite the limitations of the study, our findings suggest that QLF may be used as a conservative method for evaluating the demineralization of etched enamel.

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Conflict of Interest

The authors declare that they have no competing interests.

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References

- Swift EJ Jr, Perdigao J, Heymann HO. Bonding to enamel and dentin: a brief history and state of the art, 1995. *Quintessence Int* 1995;26:95-110.
- Shimada Y, Tagami J. Effects of regional enamel and prism orientation on resin bonding. *Oper Dent* 2003;28:20-27.
- Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. *Caries Res* 1975;9:373-387. doi: 10.1159/000260179.
- Silverstone LM. Fissure sealants. Laboratory studies. *Caries Res* 1974;8:2-26. doi: 10.1159/000260090.
- Gwinnett AJ. Structure and composition of enamel. *Oper Dent* 1992;suppl 5:10-17.
- Tagami J, Hosoda H, Fusayama T. Optimal technique of etching enamel. *Oper Dent* 1988;13:181-184
- Hafström-Björkman U, Sundström F, de Josselin de Jong E, Oliveby A, Angmar-Månsson B. Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of in vitro enamel caries. *Caries Res* 1992;26:241-247. doi: 10.1159/000261446.
- Al-Khateeb S, ten Cate JM, Angmar-Månsson B, de Josselin de Jong E, Sundström G, Exterkate RA, Oliveby A. Quantification of formation and remineralization of artificial enamel lesions with a new portable fluorescence device. *Adv Dent Res* 1997;11:502-506. doi: 10.1177/08959374970110041801.
- de Josselin de Jong E, Sundström F, Westerling H, Traanaeus S, ten Bosch JJ, Angmar-Månsson B. A new method for in vivo quantification of changes in initial enamel caries with laser fluorescence. *Caries Res* 1995;29:2-7. doi: 10.1159/000262032.
- Pretty IA, Edgar WM, Higham SM. The validation of quantitative light induced fluorescence to quantify acid erosion of human enamel. *Arch Oral Biol* 2004;49:285-294. doi: 10.1016/j.archoralbio.2003.11.008.
- Wu J, Donly ZR, Donly KJ, Hackmyer S. Demineralization depth using QLF and a novel image processing software. *Int J Dent* 2010;1-7. doi: 10.1155/2010/958264.
- Gwinnett AJ, Buonocore MG. Adhesion and caries prevention. A preliminary report. *Br Dent J* 1965;119:77-80.
- Gwinnett AJ, Kanca J. Micromorphology of the bonded dentin interface and its relationship to bond strength. *Am J Dent* 1992;5:73-77.
- Inoue M, Finger WJ, Mueller M. Effect of filler content of restorative resins on retentive strength to acid-conditioned enamel. *Am J Dent* 1994;7:161-166.
- Speirs RL. The nature of surface enamel in human teeth. *Calcif Tissue Res* 1971;8:1-16.
- Habelitz S, Marshall SJ, Marshall GW, Jr., Balooch M. Mechanical properties of human dental enamel on the nanometre scale. *Arch Oral Biol* 2001;46:173-183. doi: 10.1016/S0003-9969(00)00089-3.
- Meola MT, Papaccio G. A scanning electron microscope study of the effect of etching time and mechanical pretreatment on the pattern of acid etching on the enamel of primary teeth. *Int Dent J* 1986;36:49-53.

18. Shichi MJ, Soma K, Nakabayashi N. The effect of phosphoric acid concentration on resin tag length and bond strength of a photo-cured resin to acid-etched enamel. *Dent Mater* 2000;16:324-329. doi: 10.1016/S0109-5641(00) 00024-5.
19. Godoy F, Gwinnett AJ. Effect of etching times and mechanical pretreatment on the enamel of primary teeth: An SEM study. *Am J Dent* 1991;4;115-118.
20. Nakata K, Nikaido T, Ikeada M, Foxton R, Tagami J. *Dent Mater J* 2009;28:523-529. doi: 10.4012/dmj.28.523.
21. Pretty IA, Edgar WM, Higham SM. The effect of ambient light on QLF analyses. *J Oral Rehabil* 2002;29:369-373. doi: 10.1046/j.1365-2842.2002.00914.x.
22. Arends J, Christoffersen J, Ruben J, Jongebloed WL. Remineralisation of bovine dentine in vitro. The influence of the F content in solution on mineral distribution. *Caries Research* 1989;23:309-314. doi: 10.1159/000261198.