Lesion Activity Assessment of Early Enamel Caries using Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF)

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Lesion Activity Assessment of Early Enamel Caries using Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF)

Directed by Professor Baek Il Kim

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교실에서 보낸 지난 6년은 값지고도 귀한 시간이었습니다. 이를 마무리하면서 본 지면을 통해 논문 완성에 도움을 주신 분들께 감사의 인사를 드리고자 합니다.

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ABSTRACT

Lesion Activity Assessment of Early Enamel Caries using Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF)

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(Directed by Professor Baek Il Kim)

Early caries lesions have the potential to progress to cavitated lesions depending on their lesion activity. Therefore, it is important to assess caries lesion activity and to provide preventive treatments. Since the characteristics of the lesion surface determine the caries activity, the International Caries Detection and Assessment System in conjunction with Lesion Activity Assessment (ICDAS-LAA) and the Nyvad system have been mainly used. However, these methods are difficult to evaluate lesion activity objectively because they depend on human senses in evaluating the characteristics of the lesion surface.

In previous studies, to objectively measure the characteristics of the lesion surface using optical technology, the water present in the porous structure of the lesion surface was

dehydrated with compressed air. Then, the Quantitative Light-induced Fluorescence (QLF) system was used to observe the change in fluorescence of the caries lesion during the dehydration process. In this study, this whole process is called the QLF method. However, the previous studies that evaluated the lesion activity of natural caries with this method showed no fluorescence changes in caries lesions because there is only a minute difference in the porous structure of the surface layer of active and inactive lesions. Therefore, the QLF method was not suitable for assessing the lesion activity of natural caries.

Previous studies have introduced a method of observing the changes in the fluorescence of the caries lesion with the QLF system after infiltrating a fluorescent dye into the porous structure of the lesion surface layer. In this study, this whole procedure is called the Dye-Enhanced QLF (DEQLF) method. In previous studies, early caries lesions could be detected by strong autofluorescence of the fluorescent dyes used in this method. Nevertheless, there is no study which has evaluated the various surface porosities of early caries using the DEQLF method or to assess the lesion activity of early caries. Therefore, this study aimed to evaluate the usability of DEQLF as a method for assessing the lesion activity of early caries.

The first study aimed to perform the DEQLF method on artificial caries in bovine enamel to compare the fluorescence parameters between active and inactive lesions. Moreover, it confirmed the usefulness of the DEQLF method in distinguishing active and inactive lesions compared to the QLF method for the various surface porosities of early caries. The second study aimed to classify the active state of caries lesions by performing the DEQLF method on natural caries and comparing the fluorescence changes in the caries lesions between active and inactive lesions. Subsequently, the QLF method and the DEQLF method were performed on natural caries to compare the usefulness of the two methods for

differentiating lesion activity of early caries. Furthermore, the validity of changes in fluorescence of caries lesions assessed by the DEQLF method was evaluated in distinguishing the active state of natural caries.

In the bovine tooth study, demineralized lesions were artificially formed and assigned to the active groups (A*x*, *x* indicates the demineralization period). Then, the demineralized lesions were remineralized and assigned to the inactive group (I*x*, *x* indicates the demineralization period). In addition, caries lesions with various surface porosities were formed through 3, 5, and 10 days of demineralization. White and fluorescence images were obtained using the QLF system while assessing caries lesions with the DEQLF method. From the fluorescence images, the rate of increase in the fluorescence intensity of the caries lesion related to sound enamel (ΔG, %) was calculated using image analysis software. Moreover, the fluorescence change (ΔΔG, %) between the two assessments (QLF method and DEQLF method) was calculated as the difference in ΔG between before and after the assessment. The absolute value of the fluorescence change (|ΔΔG|) was used to compare the fluorescence change between the two assessments. As a result, in artificial caries assessed by the DEQLF method, positive ΔG in active groups and negative ΔG in inactive groups was shown. Meanwhile, as a result of comparing |ΔΔG| between the active caries lesions assessed by the two assessments, the DEQLF method showed higher |ΔΔG| by 30.2~64.6% than the QLF method ($P < 0.001$). On the other hand, in group I3 and group I5, the DEQLF method showed statistically significantly lower |ΔΔG| by 2.9% and 2.1%, respectively, compared to the QLF method ($P < 0.001$ and $P = 0.016$, respectively). In group I10, there was no statistically significant difference between the two assessments.

Teeth having a natural carious lesion on the smooth surface were used in the human tooth study. The active state of natural caries lesions was evaluated using the conventional visual-

tactile method-based caries lesion activity assessment (Nyvad system; Active $_{Nyvad}$ and Inactive $_{Nyvad}$) and a new fluorescence-based caries lesion activity assessment (DEQLF method; Active $_{DEQLF}$ and Inactive $_{DEQLF}$). As a result of evaluating the distribution of the caries lesions classified by the two assessments, the percent agreement was 58.6%. As a result of comparing ΔΔG between Active $_{DEQLF}$ group and Inactive $_{DEQLF}$ group, there was no statistically significant difference between the two groups in natural caries lesions assessed by the QLF method. However, Active $_{DEQLF}$ group showed higher value by 2.9% than Inactive $_{DEQLF}$ group in these natural caries lesions assessed by the DEQLF method (P = 0.029). In the distinction between Active $_{DEQLF}$ group and Inactive $_{DEQLF}$, ΔΔG showed an AUROC of 0.68, a sensitivity of 0.58, and a specificity of 0.80.

In summarizing the results of this study, when the DEQLF method was assessed for artificial and natural caries, the active lesions showed a higher fluorescence parameter than inactive lesions. By confirming good validity for differentiating lesion activity with the fluorescence parameter, it was possible to confirm the usability of DEQLF as a lesion activity assessment for early caries. In addition, compared to the QLF method, it was confirmed that the DEQLF method could more clearly visualize the porosity of the lesion surface layer between active and inactive lesions with the quantitative parameters.

Therefore, the DEQLF method could provide clinicians with a method to assess the lesion activity of early caries objectively and could be used as evidence to help decision-making. In addition, it could be used to monitor the progression of the caries lesion by using the image and quantified parameters.

Keywords: Caries lesion activity assessment, Dye-Enhanced Quantitative Light-induced Fluorescence, Early enamel caries lesion, Fluorescein, Fluorescent dye

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I. INTRODUCTION

When demineralization predominates in the dynamic balance between demineralization and remineralization, the caries process will be initiated (Featherstone 2004; Pitts et al. 2017; Selwitz, Ismail, and Pitts 2007; Takahashi and Nyvad 2008). Early caries lesions with an intact surface layer are formed in the early stages of the caries process (Arends, Dijkman, and Christoffersen 1987). Furthermore, as continuous demineralization occurs, the disruption of the lesion surface of early caries leads to the development of cavitated caries lesions. Since remineralization treatment can increase the mineral content of the

lesion surface as well as inside the lesion, early caries lesions can be prevented from progressing to cavity caries lesions and recovered reversibly. Therefore, early detection of caries lesions is crucial in providing appropriate preventive treatment such as remineralization treatment.

The intact surface layer of early caries lesions consists of a porous structure (Arends, Dijkman, and Christoffersen 1987; Hariri et al. 2013). Surface porosity, which is the degree of porosity of the surface layer of early caries lesions, increases in the case of ‘net mineral loss' where demineralization predominates as a result of a dynamic balance, while it decreases in the case of ‘net mineral gain' where remineralization predominates (Artun and Thylstrup 1986; Featherstone 2004; Holmen, Thylstrup, and Artun 1987; Takahashi and Nyvad 2008). In the consensus report by ORCA (the European Organisation for Dental Research) and cariology research group of IADR (International Association for Dental Research), caries lesion activity is a concept that reflects the mineral balance, in terms of net mineral loss, net mineral gain, or stasis over time (Machiulskiene et al. 2020). In terms of the caries lesion activity, previous studies have referred to the lesions where net mineral loss persists as 'active', and the lesions where demineralization is obstructed or stopped due to net mineral gain as 'inactive' (Ismail et al. 1992; Machiulskiene et al. 2020; Manji et al. 1991; Pitts 2009). Therefore, the surface porosity might be possible method to evaluate the lesion activity of early caries because it has shown a causality with caries activity.

To measure the surface porosity of early caries lesions to assess the activity of the caries lesions requires various quantitative methods. Among these methods, micro-computed tomography (micro-CT) and transverse microradiography (TMR) were used to measure the mineral contents, therefore, the surface porosity of the cares lesion can be evaluated. According to previous studies where micro-CT and TMR were used, the surface layer of

the active lesion was less porous than that of the inactive lesion (Cochrane et al. 2012). However, in evaluating the caries lesions, micro-CT and TMR require extracted teeth and sectioned teeth, respectively, so neither method can be used in clinics. Therefore, it is necessary to develop a new method for evaluating surface porosity so that the lesion activity of early caries can be non-invasively and easily evaluated in the clinical environment.

Clinicians mainly use visual-tactile methods for evaluating the activity status of early caries. Representative caries lesion activity assessments based on the visual-tactile method include the International Caries Detection and Assessment System-Lesion Activity Assessment (ICDAS-LAA) and the Nyvad system (Braga, Mendes, and Ekstrand 2010; Ekstrand et al. 2007; Nyvad, Machiulskiene, and Baelum 1999). ICDAS-LAA is used to distinguish the active state of the caries lesion by evaluating the color, the presence of dental plaque, and the surface texture. In addition, the Nyvad system is used to distinguish the active state of the caries lesion by evaluating color, translucency, and the surface texture. However, these methods are not objective because they diagnose the active state by evaluating the clinical characteristics of the lesion with human senses (Nyvad and Baelum 2018; Tikhonova et al. 2014). In addition, damage to the weakened lesion surface can be caused by evaluating the texture of the lesion surface. Therefore, to overcome these shortcomings, an objective and non-invasive method of evaluating caries lesion activity is needed.

Quantitative light-induced fluorescence (QLF) is an optical technology that uses blue visible light of 405 nm, and is mainly used to detect diseases on dental hard tissue (e.g., dental caries, tooth abrasion, tooth fissure, etc.) (Jun et al. 2016; Jung et al. 2018; Kim, Jung, and Kim 2020; Lee et al. 2018; Park et al. 2019b). Under the blue visible light of

QLF technology, while sound enamel emits autofluorescence, caries lesions emit less autofluorescence relative to sound enamel (Amaechi and Higham 2002; de Josselin de Jong et al. 1995; Tranaeus et al. 2001). This is because caries lesions are more porous than sound enamel due to the lower mineral content, which shortens the average free photon path length and increases scattering (Angmar-Månsson and Ten Bosch 1987; Mujat et al. 2004). When water present in the porous structure of the caries lesion is dehydrated with compressed air, photons scatter more, and the intensity of the autofluorescence in the caries lesion decreases. The process of dehydrating teeth with compressed air and observing them with QLF technology is referred to as the QLF method in this study.

A previous study has evaluated artificial caries lesions of various surface porosity by the QLF method (Ando, Shaikh, and Eckert 2018). As a result, it is useful in distinguishing between the less porous surface layer and the more porous surface layer of caries lesions. However, when natural caries were evaluated by the QLF method, no difference was detected in the fluorescence parameter between the active and inactive lesions, so the active state of the natural caries lesion could not be distinguished by the QLF method (Ando et al. 2017). This is because the porous structure due to demineralization and remineralization is mixed on the lesion surface of natural caries, whereas the porous structure due to demineralization and remineralization is clearly distinguished on the lesion surface of artificial caries. Therefore, there is a need for a method capable of distinguishing subtle differences in the porosity of demineralized and remineralized surfaces.

The method of applying a fluorescent dye to the tooth and observing it with a QLF system can make the autofluorescence of a caries lesion brighter. This method has been mainly used to detect caries lesions earlier (Eggertsson et al. 1999; Zandoná et al. 1998). In this study, this method is named Dye-Enhanced QLF (DEQLF). The fluorescent dye for

the DEQLF method, fluorescein sodium, has been approved by the Food and Drug Administration (D&C Yellow No.8), is used as a fluorescent dye for detecting corneal defects in ophthalmology, and as a fluorescent contrast agent for angiography (Qaiser et al. 2020; Tabery 1992). In addition, fluorescein sodium emits strong and bright yellow-green fluorescence under blue visible light (Romanchuk 1982). In a previous study that evaluated artificial root caries using this DEQLF method, demineralized lesions were found to emit stronger autofluorescence than remineralized lesions (Pretty et al. 2003). Because the demineralized lesions were more porous than the remineralized lesions, the fluorescent dye could penetrate more. The DEQLF method can therefore be used to evaluate the porous structure of caries lesions and distinguish between demineralized and remineralized lesions. However, no studies have evaluated early caries lesions with various surface porosities and the lesion activity of natural caries using the DEQLF method.

Therefore, this dissertation aimed to evaluate the usability of the DEQLF method as a lesion activity assessment for early caries. This involves studies of artificial caries lesions and natural caries lesions, and the detailed objectives of the study are as follows.

First, we performed the DEQLF method on artificial caries lesions to compare the fluorescence parameters between active and inactive lesions. In addition, we confirmed the usefulness of the DEQLF method for various surface porosities of caries lesions compared to the QLF method.

Second, the QLF method and the DEQLF method were performed on natural carious lesions to compare the fluorescence indicators between active and inactive lesions. In addition, the validity of the DEQLF method was evaluated in distinguishing between active and inactive lesions.

II. MATERIALS AND METHODS

2.1. Materials

2.1.1. Bovine tooth study model

2.1.1.1. Preparation of bovine tooth specimen

One-hundred twenty-six bovine teeth having no dental cracks, dental caries, dental erosion, dental fluorosis, and enamel hypoplasia were prepared. The teeth were cut with a diamond disk and a low-speed saw (NTI-KAHLA GmbH, Kahla, Germany) in order to produce a specimen having a size of 4 × 3 × 3 mm around the maximum height of the contour of the buccal surface (Figure 1A). All specimens were embedded in an acrylic mold (20 × 12 × 7 mm) with 9 mm diameter holes using self-curing resin (Ortho-Jet, Lang Dental Manufacturing, Illinois, USA) (Figure 1B). The surface of each specimen was flattened with cool-water and 800, 1200, and 2000 grit abrasive paper (SiC Sandpaper, R&B Inc., Daejeon, Republic of Korea).

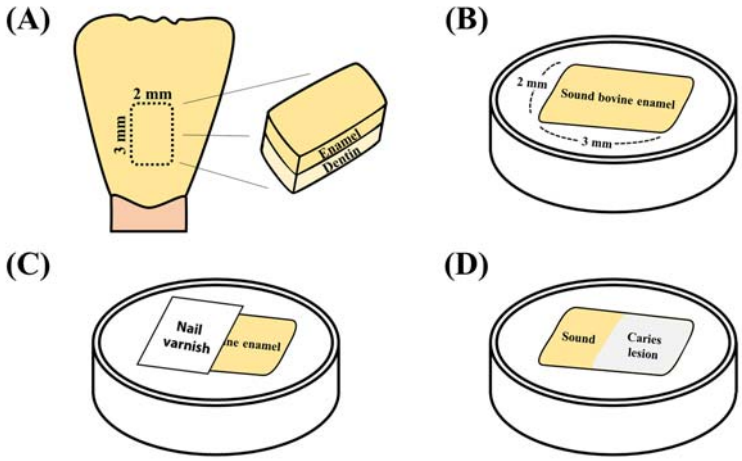


Figure 1. Schematic image of preparing the bovine tooth specimens.
(A) sectioning, (B) embedding into acrylic mold, (C) sound enamel protection with transparent nail varnish, (D) varnish removal after formation of the caries lesions.

2.1.1.2. Formation of active caries lesions

Active lesions were simulated in this study by artificially forming demineralized lesions. 126 specimens were equally allocated to the three active groups (A*x*, *x* means the demineralization period) (Table 1). In addition, to form caries lesions with various surface porosities, the demineralization period was set to 3 days (A3), 5 days (A5), and 10 days (A10), respectively. An acid-resistant varnish was applied to the surface of each specimen of the dimensions 2 × 3 mm to protect the sound enamel before the caries lesion was formed (Figure 1C).

First, a demineralization solution (0.1 M lactic acid gel containing 1% Carbopol ETD 2050 polymer) with a pH of 4.8 saturated with 50% hydroxyapatite (calcium phosphate tribasic, Sigma, USA) was prepared (White 1987). Next, 40 ml of the demineralization solution was put in a conical tube, and the specimen was immersed and stored in an incubator set at 37°C. Demineralization was performed for the set period for each group. The specimens were taken out from the demineralized solution after the demineralization process was completed, and the demineralized solution remaining on the surface of the specimens was washed with distilled water.

Table 1. Procedure of all experimental groups used in bovine tooth study (n=126, equally distributed among 6 groups).

Group		Procedure
Active (A*x*)	A3 (n=21)	de-mineralized for 3 days
	A5 (n=21)	de-mineralized for 5 days
	A10 (n=21)	de-mineralized for 10 days
Inactive (I*x*)	I3 (n=21)	de-mineralized for 3 days and then re-mineralized for 10 days
	I5 (n=21)	de-mineralized for 5 days and then re-mineralized for 10 days
	I10 (n=21)	de-mineralized for 10 days and then re-mineralized for 10 days

x means demineralization period.

2.1.1.3. Formation of inactive caries lesions

This study simulated inactive lesions by artificially forming remineralized lesions. Thus, half of the specimens in the active group were reallocated to the inactive group (I*x*, *x* is the demineralization period). The inactive groups were named I3, I5, and I10, respectively (Table 1).

First, the specimens were immersed in 3 ml of 2% sodium fluoride solution (sodium fluoride, 99+%, Sigma-Aldrich, Missouri, USA; 9,050 ppm F, pH 7) for 4 minutes (Kim, Kwon, and Kim 2013). After taking the specimens out of the solution, the remaining solution on the surface of the sample was removed with paper and the specimens were stored in artificial saliva (0.22% mucin, 30mM KCl, 10 mM KH_2PO_4, 13 mM NaCl, 3 mM CaCl) of pH 6.8 for 24 hours. This process was repeated for 10 days, and the fluoride solution and the artificial saliva were replaced every day. After all caries lesions were formed, the acid-resistant varnish was removed with acetone (Extra Pure GRADE, Duksan Pure Chemicals, Ansan, Republic of Korea) (Figure 1D). Thereafter, the specimens were stored in a refrigerator at 4 °C and 100 % relative humidity.

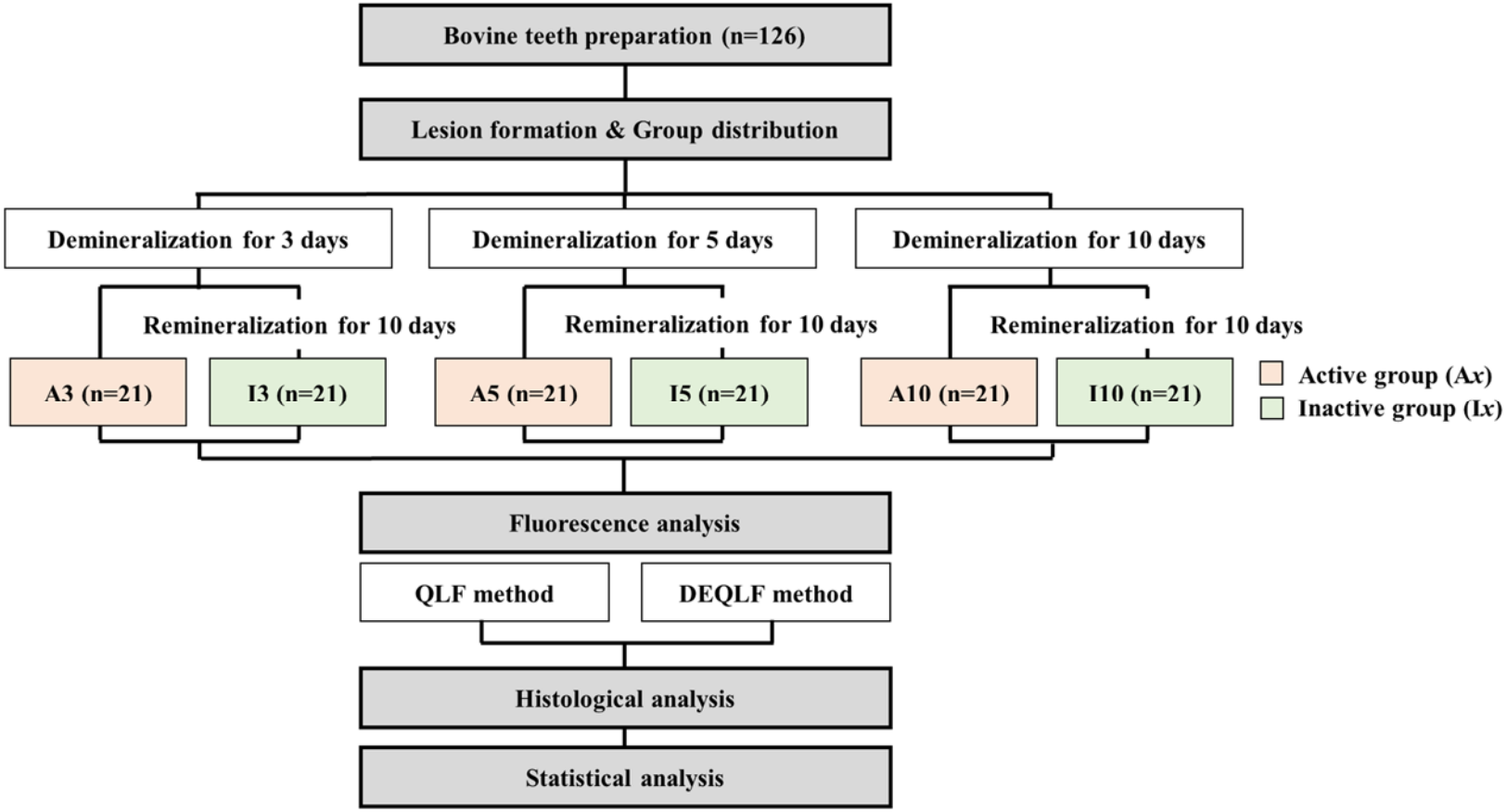


Figure 2. Flow chart of bovine tooth study model.

2.1.2. Human tooth study model

2.1.2.1. Preparation of human tooth specimen

The ethics committee of Yonsei University Dental Hospital approved this study (IRB 2-2017-0044). Informed consent was obtained from participants without systemic disease (mean age ± standard deviation, 33.7 ± 9.7). Human teeth were extracted from the participants for periodontal, orthodontic, and prosthetic purposes. As a result, 120 permanent human teeth without dental fluorosis and enamel dysplasia were collected. The teeth with early enamel caries lesions corresponding to international caries detection and assessment system (ICDAS) code 1 or code 2 on the smooth surface were included. Immediately after tooth extraction, the periodontal tissue and dental calculus on the tooth surface were removed with a dental scaler (Figure 3A), and soft attachments such as dental biofilm were removed using tap water and a toothbrush (Figure 3B). Although the above procedure was performed, the attachments on the caries lesion on 6 teeth were unable to be removed and so these teeth were excluded from the study. The specimens were then embedded vertically in an acrylic mold (20 × 12 × 7 mm) with a 9 mm diameter hole using a self-cured resin (Ortho-Jet, Lang Dental Manufacturing, Illinois) (Figure 3C). All specimens were stored in a dark box and stored frozen (-20°C) until they were used in the experiment.

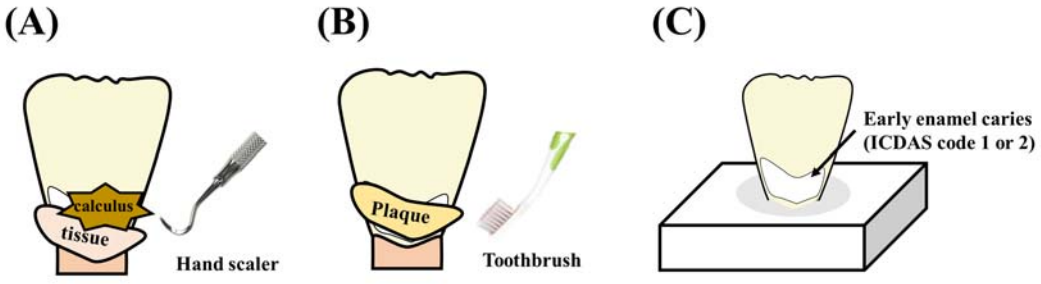


Figure 3. Schematic image of human tooth specimen preparation.

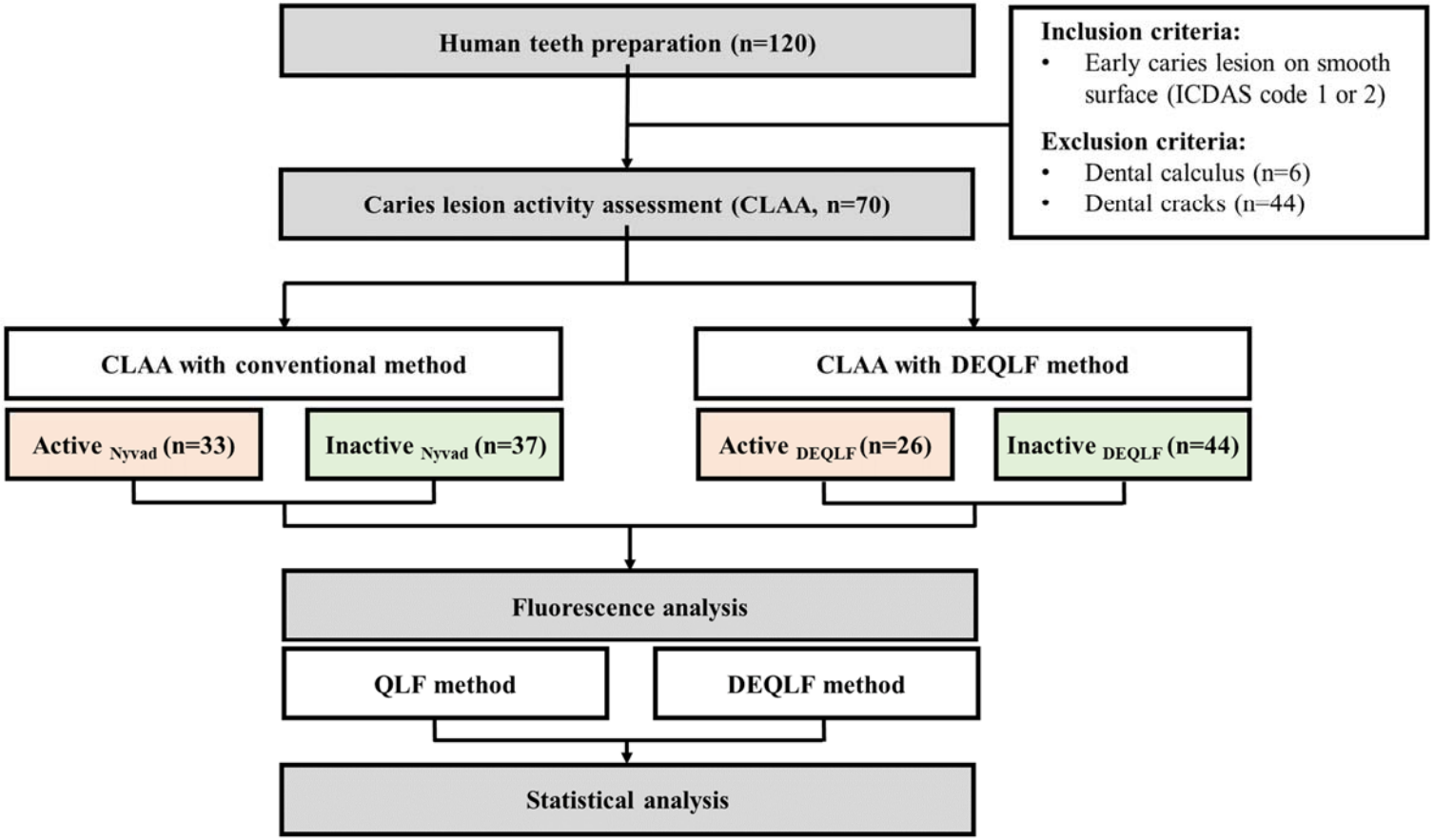


Figure 4. Flow chart of human tooth study model.

2.2. Fluorescent analysis

2.2.1. QLF method

The QLF method largely consists of dehydrating a specimen with compressed air and observing the specimen with a QLF system during dehydration (Ando et al. 2018). The detailed process is as follows. First, cotton pellets containing distilled water were placed on the surface of the specimen for 60 seconds to hydrate the specimen sufficiently. Thereafter, the hydrated specimen was dehydrated for 10 seconds with compressed air from a 3-way syringe (Figure 5). After dehydration and hydration, white and fluorescence images of all specimens were captured using QLF-D (Quantitative Light-induced Fluorescence-Digital 2+ Billuminator, Inspektor Research System BV, Amsterdam, The Netherlands) and proprietary imaging software (C3 1.26, Inspektor Research System BV). The imaging conditions for the white and fluorescence images are presented in Table 2. Except for 44 specimens in which tooth cracks were observed in the lesion in the fluorescence image, 70 teeth were used until the end of this study.

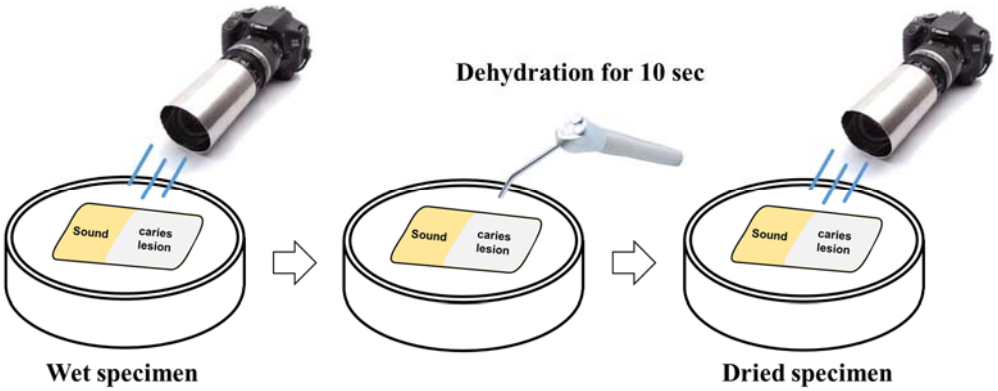


Figure 5. Schematic diagram of the procedure of the Quantitative Light-induced Fluorescence (QLF) method.

Table 2. Imaging condition of white and fluorescence image.

	White image	Fluorescence image
Shutter speed	**1/90s**	**1/30s**
Aperture value	**13.0**	**10.0**
ISO speed	**1600**	**1600**
Pixel size	**2592 x 1728**	**2592 x 1728**

2.2.2. DEQLF method

The DEQLF method consists of penetrating a fluorescent dye into a dehydrated specimen and observing the specimen with QLF during penetration. The detailed process is as follows. Fluorescence dye was prepared by adding 0.0376 mg fluorescein sodium (Sigma-Aldrich, Missouri, USA) to 100 ml of a 50% EtOH solution (DUKSAN, Seoul, Republic of Korea). Cotton pellets containing the fluorescent dye were placed on the surface of the specimen for 10 seconds (Figure 6). Subsequently, the remaining fluorescent dye present on the surface of the specimen was washed with distilled water. Before and after performing the DEQLF method, white and fluorescence images of the specimens were also captured in the same procedure as the QLF method (Table 2).

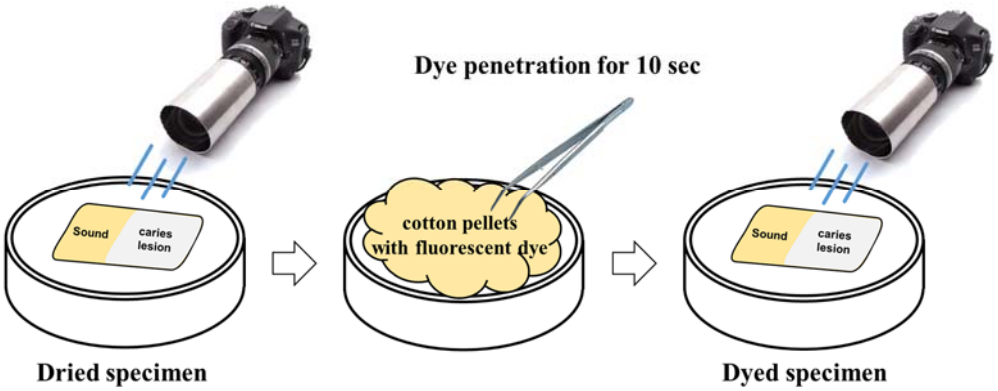


Figure 6. Schematic diagram of the procedure of the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

2.2.3. Image analysis

Image analysis was performed using Image-Pro PLUS 6.0 (Media Cybernetics, Maryland, USA).

2.2.3.1. Bovine tooth study model

Before performing the assessments, the areas of interest, which were the sound enamel (AOIs) and caries lesions (AOIc), were set in the fluorescence images of the specimens (Figure 7A).

To measure the fluorescence intensity of sound enamel and caries lesions by brightness, all fluorescence images were converted into 8-bit grayscale images (Figure 7B). In the 8-bit grayscale images, the mean gray level of sound enamel (MGLs) and mean gray level of caries lesion (MGLc) were measured. To evaluate how bright or dark the autofluorescence of the artificial caries lesions was compared to that of sound enamel, the rate of increase of the mean gray intensity of the caries lesions against sound enamel (ΔG, %) was calculated (Figure 7C).

If ΔG was positive, it means that the autofluorescence of the caries lesions was brighter than that of the sound enamel. Conversely, if ΔG was negative, it means that the autofluorescence of the caries lesions was darker than that of sound enamel.

Then, the fluorescence change (ΔΔG, %) of the caries lesion by the assessments (QLF and DEQLF) was calculated using ΔG (Figure 6C). ΔΔG is the difference between ΔG before and after assessment (ΔG after assessment – ΔG before assessment).

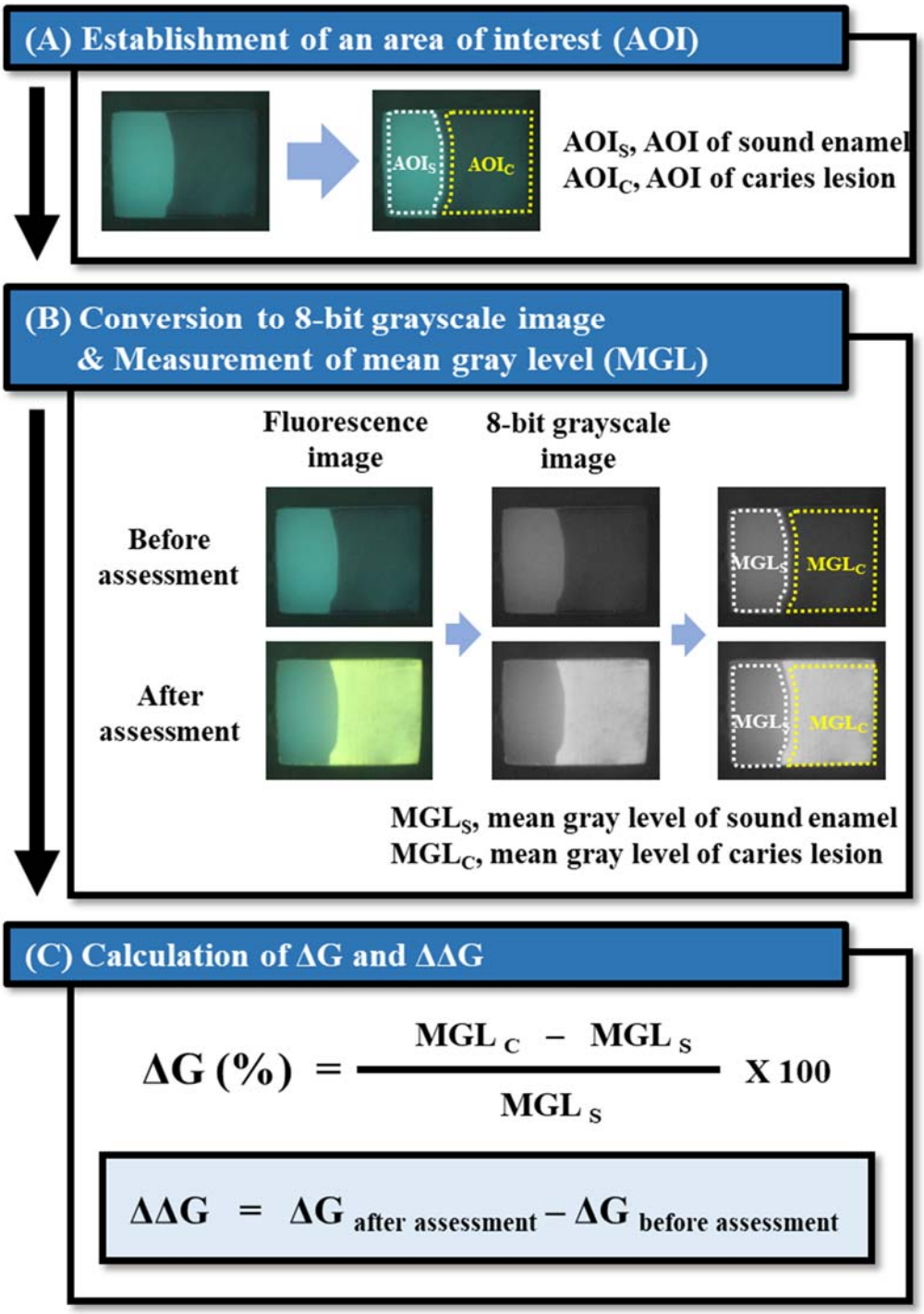


Figure 7. Schematic diagram of the procedure of calculating the fluorescence parameters (ΔG and ΔΔG) using image analysis software.

2.2.3.2. Human tooth study model

In the fluorescence image of the specimen before evaluation, the fluorescence loss area was set as the AOI of the caries lesion (AOIc), and the area 2 mm the outside of the fluorescence loss area was set as the AOI of sound enamel (AOIs) (Figure 8A).

The process of calculating ΔΔG was performed in the same procedure as in the bovine tooth study model (Figures 8B and 8C).

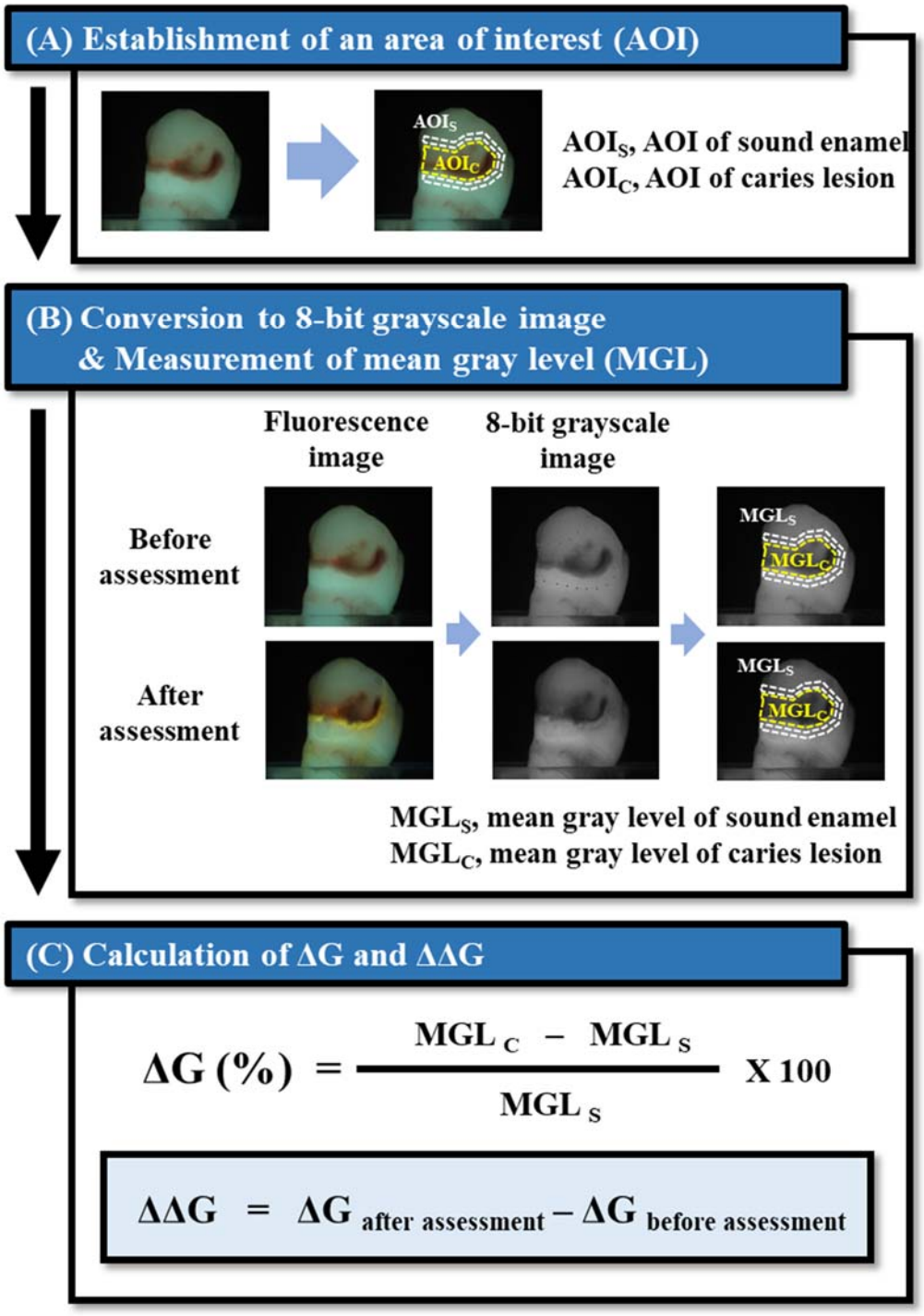


Figure 8. Schematic diagram of the procedure for calculating the fluorescence parameters (ΔG and ΔΔG) using image analysis software.

2.3 Histological analysis (bovine tooth study model)

Histological analysis was performed to evaluate the histological characteristics (lesion depth and the presence of remineralized surface layers) of the artificial caries. The specimens were cut to a thickness of 300 um using a microcutter (TechCut 4™, Allied High Tech Products, California, USA). Until the thickness of the slice became 100 μm, the cut surface was polished with 800 grit abrasive paper (SiC sandpaper, R&B Inc., Daejeon, Republic of Korea). The sectioned surface was captured using a polarized light microscope (magnification 100x and 400x, CX31-P, Olympus, Tokyo, Japan).

The lesion depth (Ld) was measured using Image-Pro Plus (ver. 6.0, Media Cybernetics Inc., Maryland, USA). The lesioin depth was measured at 3 locations of the caries lesion, and the average value of the measured depth was used as a representative value.

2.4. Caries lesion activity assessment (human tooth study model)

2.4.1. Conventional visual-tactile method (Nyvad system)

Two dentists (J.-W. Lee and M.-J. Jung), who have clinical experience of over 10 years, evaluated the lesion activity of early caries with dental probes under the light condition of 980 LUX.

The Nyvad system, a visual-tactile method, was used as the conventional caries lesion activity assessment. According to this system, the enamel surface of the active lesion is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved gently across the surface. In contrast, the enamel surface of an inactive lesion is whitish, brownish or black; the enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface (Nyvad and Baelum 2018). According to previous studies, in the case of a caries lesion in which the characteristics of active and inactive lesions were mixed, the lesion was considered to be an active lesion (Nyvad, Machiulskiene, and Baelum 1999; Tikhonova et al. 2014). If the results were not consistent between the two examiners, the results were discussed and an agreement was made by the two examiners.

2.4.2. Fluorescence-based method (DEQLF method)

The caries lesion activity assessment using the DEQLF method was performed by two trained examiners (S.-M. Kang and S.-W. Park). If yellow-green fluorescence of the fluorescent dye was observed in the caries lesion, the status of the caries lesion was defined as Active $_{DEQLF}$ (Figure 9A). On the other hand, if yellow-green fluorescence of the fluorescent dye was not observed in the caries lesion, the status of the caries lesion was defined as Inactive $_{DEQLF}$ (Figure 9B). If the results were not consistent between the two examiners, the results were discussed and an agreement was made by the two examiners.

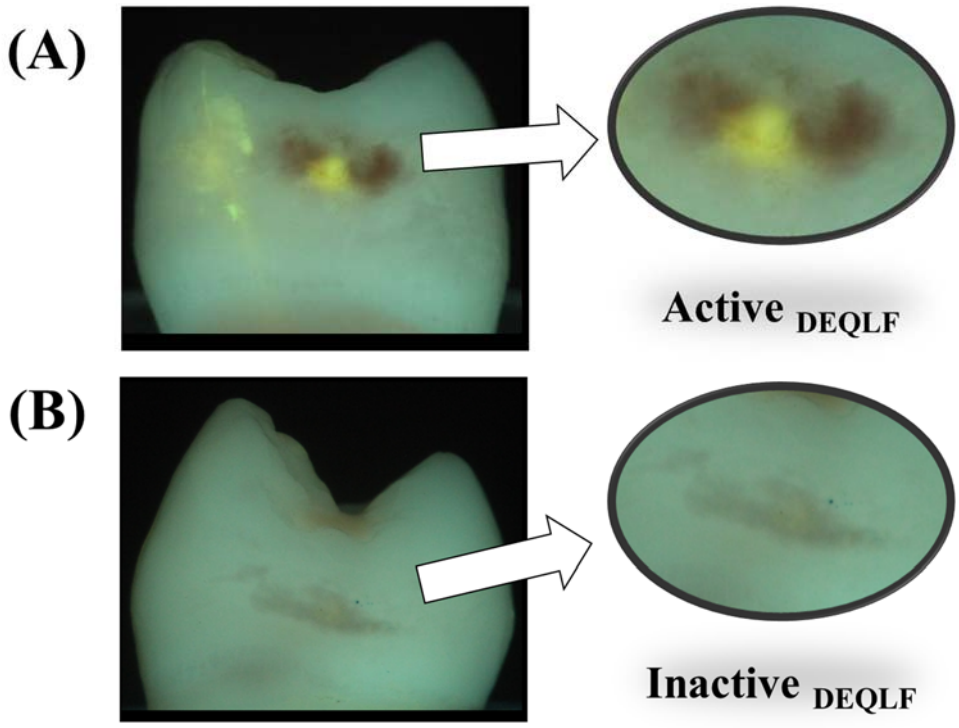


Figure 9. Operational definition of Active $_{DEQLF}$ and Inactive $_{DEQLF}$ lesions of natural caries.

2.5. Statistical analysis

All statistical analyzes were performed using SPSS 25.0 (IBM, Illinois, USA) and MedCalC® (MedCalC Software Ltd, Ostend, Belgium).

2.5.1. Bovine tooth study model

The difference in the following variables was performed using independent *t*-test (α=0.05).

- Difference in Ld between active and inactive lesions
- Difference in ΔG between active and inactive groups in artificial caries lesions that performed the QLF method or the DEQLF method
- Difference in ΔΔG between active and inactive groups in artificial caries lesions that performed the QLF method or the DEQLF method
- Difference in |ΔΔG| between QLF and DEQLF methods in artificial caries lesions

2.5.2. Human tooth study model

The difference in the following variables was performed using independent *t*-test (α=0.05).

- Difference in ΔΔG between Active $_{DEQLF}$ and Inactive $_{DEQLF}$ in natural caries lesions that assessed by the QLF method or the DEQLF method
- Difference in ΔΔG between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ in natural caries lesions that assessed by the QLF method or the DEQLF method

Finally, the receiver operator characteristics (ROC) curve was calculated to determine the validity of ΔΔG in distinguishing between Active $_{DEQLF}$ and Inactive $_{DEQLF}$ or between Active $_{Nyvad}$ and Inactive $_{Nyvad.}$ The area under the ROC curve (AUROC), sensitivity, and specificity was measured in the ROC curve. When the sum of sensitivity and specificity was the highest, the ΔΔG was the optimal cut-off value.

III. RESULTS

3.1. Bovine tooth study model

3.1.1. Comparison of fluorescence between active and inactive caries lesions

3.1.1.1. Caries lesions assessed by QLF method

As a result of performing the QLF method on artificial caries lesions and comparing ΔG (fluorescence increase rate of caries lesions to sound enamel) between the active group and the inactive group, the active group showed a statistically significantly lower ΔG than the inactive group ($P < 0.001$, Table 3). ΔG in the active groups was lower by 14.2%, 18.4%, and 20.2% than those in the inactive groups in the caries lesions demineralized for 3, 5, and 10 days, respectively ($P < 0.001$, Table 3).

Similarly, as a result of comparing ΔΔG (fluorescence change of the caries lesions by the QLF method) between the active group and the inactive group in the caries lesions demineralized for 3, 5, and 10 days, ΔΔG in the active groups was lower by 8.7%, 16.3%, and 13.4%, respectively, than that in the inactive group ($P < 0.001$, Table 4). In addition, as a result of visually evaluating the change in fluorescence of the caries lesion in the fluorescence images of the specimens taken before and after assessment with the QLF method, it was observed that the fluorescence of the caries lesions after assessment with the QLF method became darker than those assessed before the QLF method (Figure 10). Among them, it was found that the fluorescence of the caries lesions in the active group became darker than those in the inactive group.

Table 3. Comparison of ΔG between the active and inactive groups analyzed by the Quantitative Light-induced Fluorescence (QLF) method.

Demineralization period (day)	Active	Inactive	*P*-value
3	-24.1 (6.6)	-9.9 (5.0)	< 0.001
5	-27.4 (5.3)	-9.0 (6.8)	< 0.001
10	-38.9 (7.3)	-18.7 (8.7)	< 0.001

Mean (standard deviation).

ΔG means percentage of fluorescence increase of caries lesion with respect to that of sound enamel (unit: %).

P-value was evaluated by independent *t*-test (α=0.05).

Table 4. Comparison of ΔΔG between the active and inactive groups analyzed by the Quantitative Light-induced Fluorescence (QLF) method.

Demineralization period (day)	Active	Inactive	*P*-value
3	-14.2 (3.4)	-5.5 (2.5)	< 0.001
5	-21.3 (3.2)	-5.0 (3.0)	< 0.001
10	-24.4 (4.1)	-11.0 (3.7)	< 0.001

Mean (standard deviation).

ΔΔG means the difference in ΔG between before and after performing QLF method (unit: %).

P-value was evaluated by independent *t*-test (α=0.05).

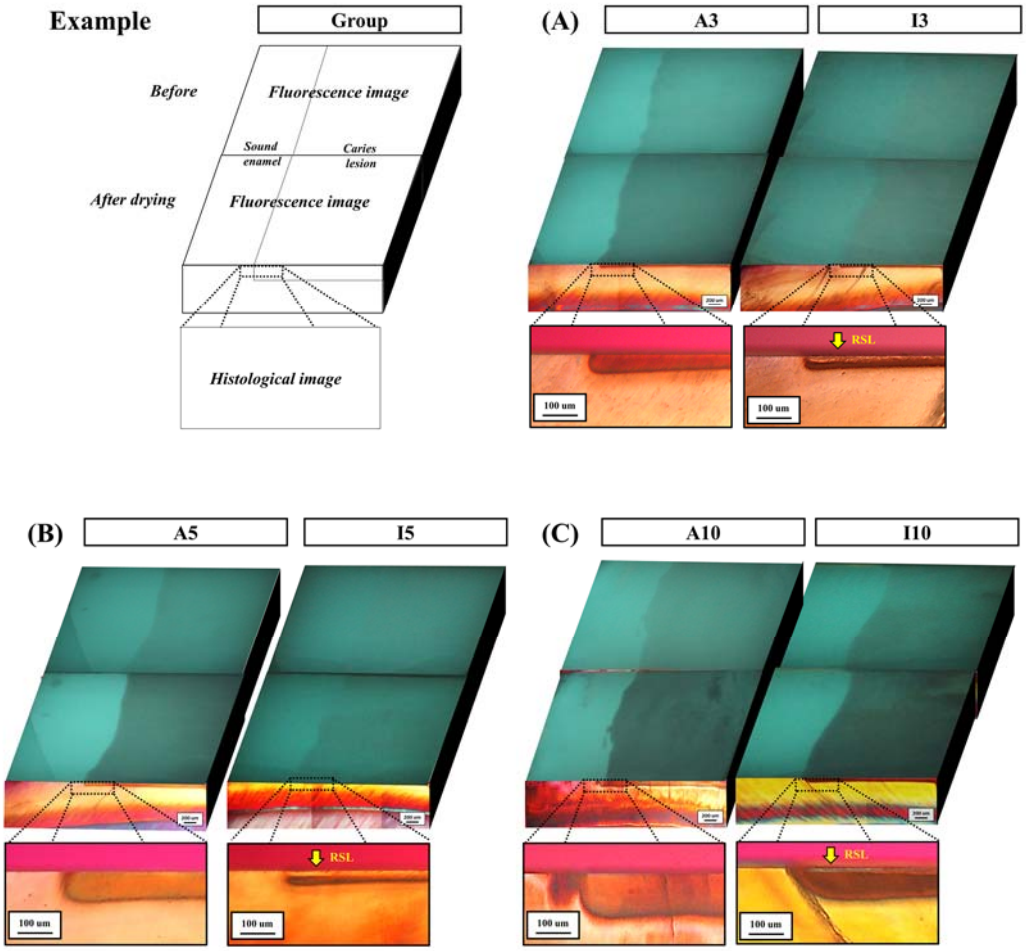


Figure 10. Representative fluorescence and histological images of the specimens analyzed by the Quantitative light-induced fluorescence (QLF) method.

A*x* refers to the caries lesions that were demineralized lesion for *x* days; I*x* refers to the caries lesions that were remineralized for 10 days after demineralization for *x* days.

RSL refers to the remineralized surface layer of a caries lesion.

3.1.1.2. Caries lesions assessed by DEQLF method

When the DEQLF method was performed on artificial caries lesions and ΔG was compared between the active and the inactive groups, the active groups showed statistically significantly higher ΔG values than the inactive groups ($P < 0.001$, Table 5). In particular, ΔG values of the active groups were positive values (24.0 to 46.4 %), whereas ΔG values of the inactive groups were negative values (-12.6 to -9.7 %).

Similarly, as a result of comparing ΔΔG between the active groups and the inactive groups, ΔΔG values in the active groups were higher by 33.7 %, 49.1 %, and 59.0 % than the inactive groups in the caries lesions demineralized for 3, 5, and 10 days, respectively ($P < 0.001$, Table 6). In addition, as a result of visually evaluating the change in fluorescence of the lesions in the fluorescence images of the specimens taken before and after assessing with the DEQLF method, the fluorescence of the caries lesions in A3, A5, A10, and I10 after assessing with the DEQLF method was brighter than before assessing with the DEQLF method. (Figure 11). Among them, the fluorescence of the caries lesion was brightened overall in A3, A5, and A10, whereas the fluorescence of the caries lesion was brightened only in partial areas in I10. In contrast, in the caries lesions of I3 and I5, there was no change in fluorescence due to the assessment of the DEQLF method.

Table 5. Comparison of ΔG between the active and inactive groups analyzed by the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

Demineralization period (day)	Active	Inactive	*P*-value
3	24.0 (12.6)	-9.7 (4.8)	< 0.001
5	38.9 (11.3)	-10.2 (5.5)	< 0.001
10	46.4 (16.2)	-12.6 (9.8)	< 0.001

Mean (standard deviation).

ΔG value means percentage of fluorescence increase of caries lesion with respect to that of sound enamel (unit: %).

P-value was evaluated by independent *t*-test (α=0.05).

Table 6. Comparison of ΔΔG between the active and inactive groups analyzed by the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

Demineralization period (day)	Active	Inactive	*P*-value
3	44.5 (14.5)	2.6 (2.0)	< 0.001
5	66.1 (13.6)	2.9 (2.4)	< 0.001
10	89.0 (16.4)	9.1 (8.5)	< 0.001

Mean (standard deviation).

ΔΔG means the difference in ΔG of the specimens between before and after performing DEQLF method (unit: %).

P-value was evaluated by independent *t*-test (α=0.05).

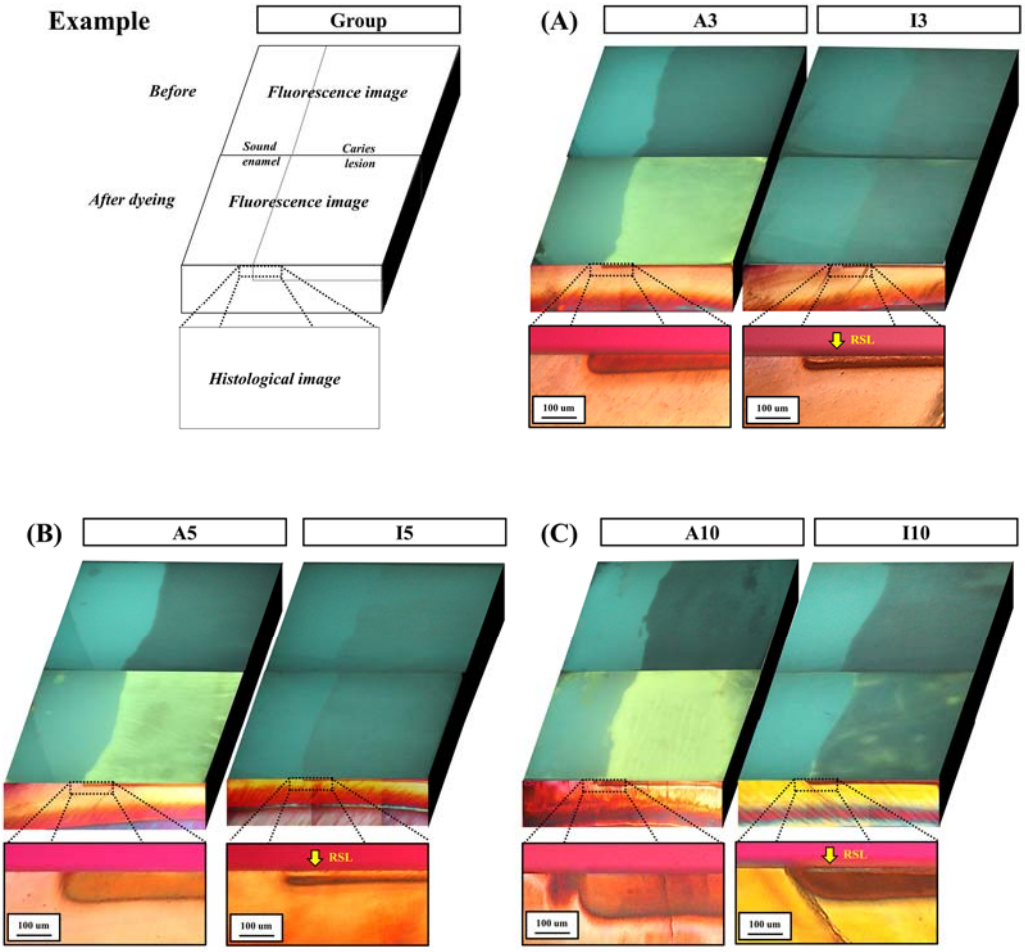


Figure 11. Representative fluorescence and histological images of the specimens analyzed by the Dye-enhanced Quantitative light-induced fluorescence (DEQLF) method.

A*x* refers to the caries lesions that were demineralized for *x* days; I*x* refers to the caries lesion that were remineralized for 10 days after demineralization for *x* days.

RSL refers to the remineralized surface layer of a caries lesion.

3.1.2. Comparison of the fluorescence between caries lesions assessed by QLF method and by DEQLF method

As a result of comparing the fluorescence change of the caries lesions (|ΔΔG|) between the QLF method and the DEQLF method in A3, A5, and A10, the DEQLF method showed a higher |ΔΔG| than the QLF method by 30.2 %, 44.9 %, and 64.6 %, respectively ($P < 0.001$, Figure 12). In addition, in I3 and I5, the DEQLF method showed lower |ΔΔG| values by 2.9 % and 2.1 % compared to the |ΔΔG| values from the QLF method ($P = 0.016$). On the other hand, in I10, the DEQLF method showed lower |ΔΔG| by 1.9 % than the QLF method, but there was no statistically significant difference.

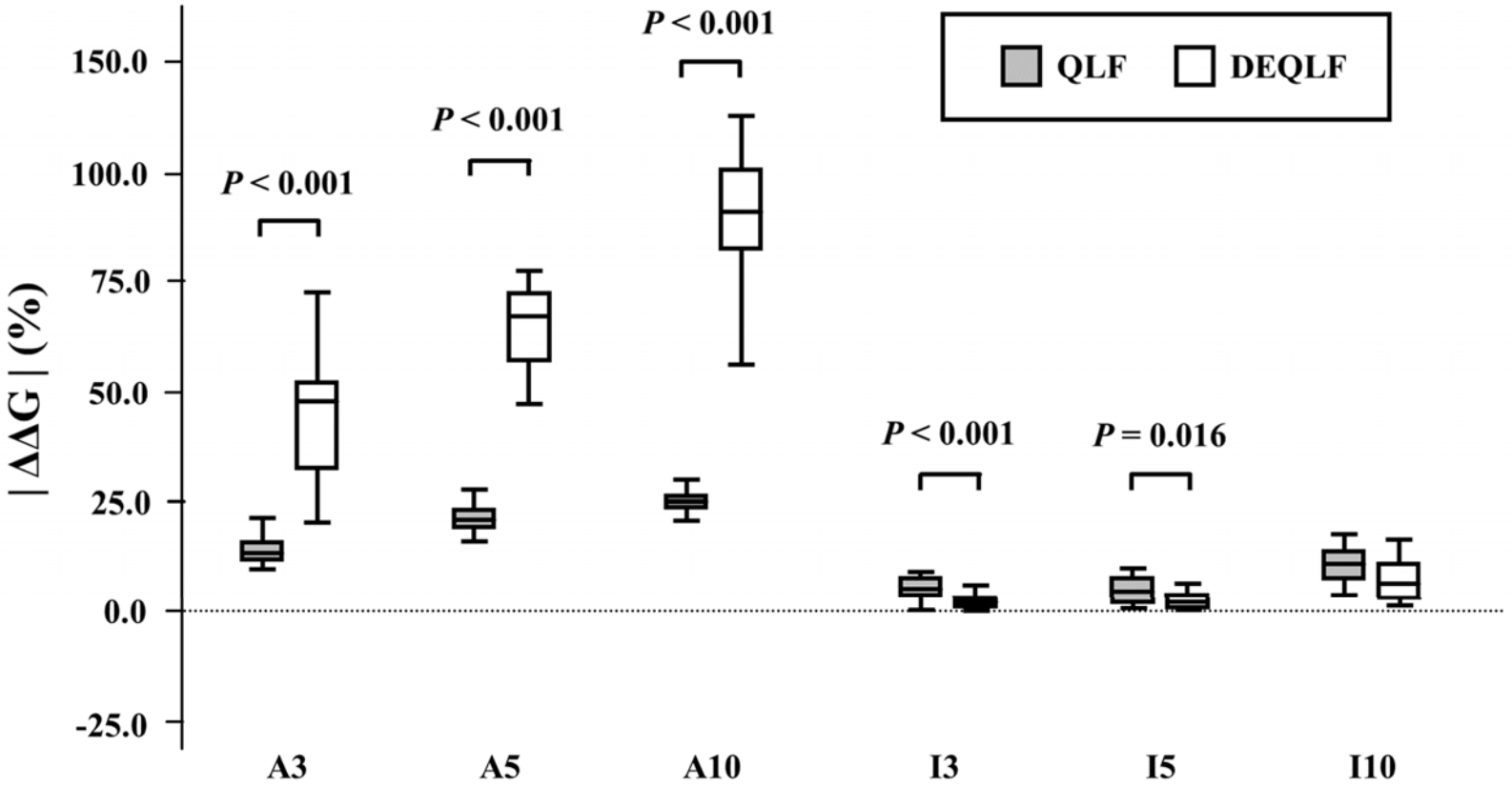


Figure 12. Comparison of the changes in autofluorescence (|ΔΔG|) between caries lesions analyzed by Quantitative Light-induced Fluorescence (QLF) method and Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

D*x* means the demineralized lesion for *x* days; RD*x* means the remineralized lesion for 10 days after demineralization for *x* days.

3.1.3. Comparison of lesion depth between active and inactive groups

As a result of comparing the Ld (mean ± standard deviation, unit: μm) between the active group and the inactive group in the caries lesions demineralized for 3 days, there was no statistically significant difference between the active group (A3, 44.5 ± 8.9) and the inactive group (I3, 45.9 ± 8.7). On the other hand, in the caries lesions demineralized for 5 days, the active group (A5, 59.0 ± 11.3) had a statistically significantly higher Ld than the inactive group (I5, 36.7 ± 6.8, $P < 0.001$). In the caries lesions demineralized for 10 days, the active group (A10, 122.4 ± 12.8) showed a statistically significantly higher Ld than that of the inactive group (I10, 86.9 ± 25.7) ($P < 0.001$).

3.2. Human tooth study model

3.2.1. Distribution of lesion activity between caries lesions assessed by Nyvad system and by DEQLF system

In Active $_{Nyvad}$ lesions (n=33, 100.0%), the number of Active $_{DEQLF}$ lesions (n=15, 45.5%) was less than that of Inactive $_{DEQLF}$ lesions (n=18, 54.5%) (Table 7). Conversely, in Inactive $_{Nyvad}$ lesions (n=37, 100.0%), the number of Inactive $_{DEQLF}$ lesions (n=26, 70.3%) was more than that of Active $_{DEQLF}$ lesions (n=11, 29.7%). Additionally, the percent agreement on the active status between the two methods was 58.6%.

Table 7. Distribution of Active $_{DEQLF}$ and Inactive $_{DEQLF}$ lesions according to the activity status of the caries lesions that were evaluated by conventional caries lesion activity assessment (Nyvad system).

	Active $_{DEQLF}$	Inactive $_{DEQLF}$	Total
Active $_{Nyvad}$	15 (57.7)	18 (40.1)	33 (47.1)
Inactive $_{Nyvad}$	11 (42.3)	26 (59.9)	37 (52.9)
Total	26 (100.0)	44 (100.0)	70 (100.0)

N (%).

Active $_{Nyvad}$ and inactive $_{Nyvad}$ mean activity status of caries lesions distinguished with conventional visual-tactile based lesion activity assessment (Nyvad system).

Active $_{DEQLF}$ and inactive $_{DEQLF}$ mean activity status of caries lesions distinguished with Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

3.2.2. Comparison of changes in fluorescence between caries lesions assessed by QLF method and by DEQLF method

3.2.2.1. Caries lesions assessed by Nyvad system

As a result of comparing ΔΔG (mean ± standard deviation) between Active $_{Nyvad}$ and Inactive $_{Nyvad}$, there was no statistically significant difference in ΔΔG by the QLF method between Active $_{Nyvad}$ (-1.1 ± 1.7) and Inactive $_{Nyvad}$ (-1.3 ± 1.7). In contrast, analysis of the ΔΔG by the DEQLF method between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ found that Active $_{Nyvad}$ (3.8 ± 5.6) showed a higher ΔΔG by 2.8 % than Inactive $_{Nyvad}$ (1.0 ± 2.5) (P = 0.012, Figure 13).

3.2.2.2. Caries lesions assessed by DEQLF method

As a result of comparing ΔΔG between Active $_{DEQLF}$ and Inactive $_{DEQLF}$, there was no statistically significant difference in ΔΔG by the QLF method between Active $_{DEQLF}$ (-1.1 ± 1.7) and Inactive $_{DEQLF}$ (-1.3 ± 1.8). In contrast, analysis of the ΔΔG by the DEQLF method between Active $_{DEQLF}$ and Inactive $_{DEQLF}$ found that Active $_{DEQLF}$ (4.1 ± 6.3) ΔΔG was higher by 2.9 % than Inactive $_{DEQLF}$ (1.2 ± 2.5) (P = 0.029, Figure 14).

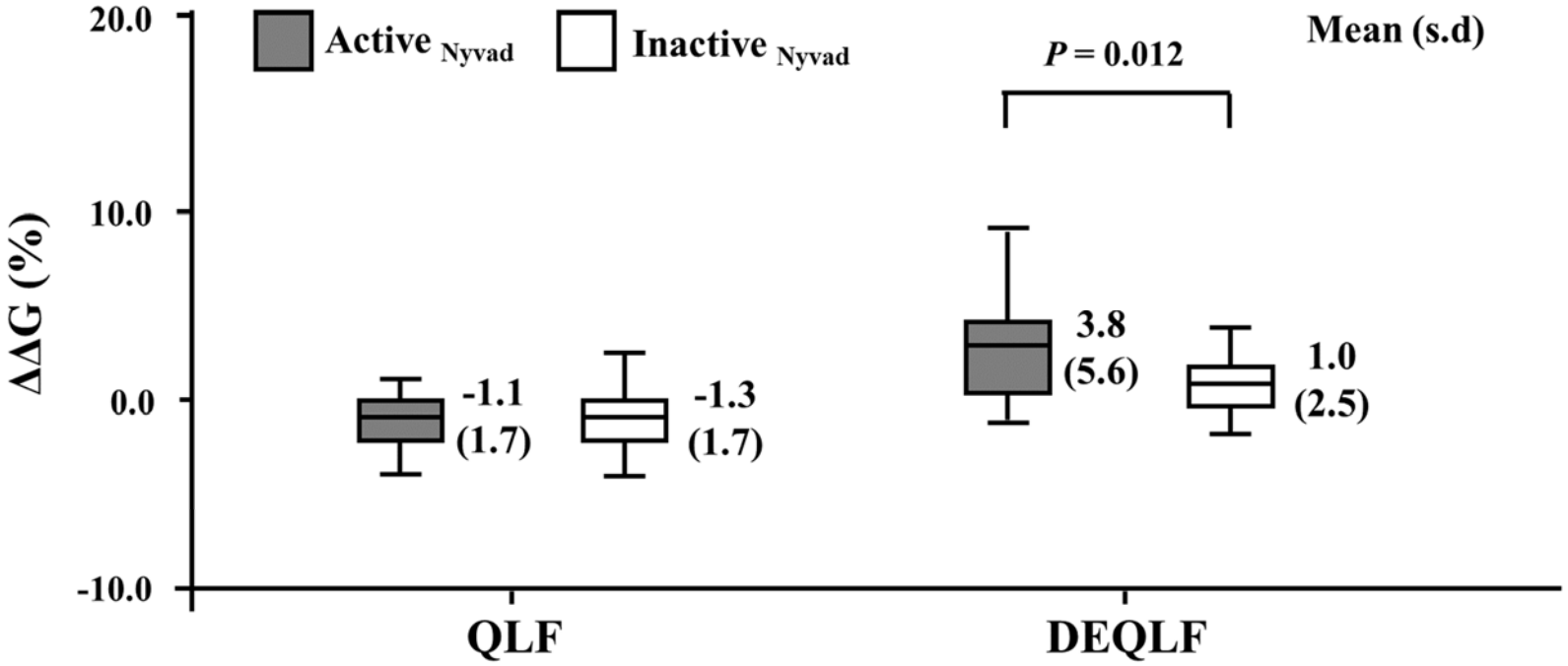


Figure 13. Comparison of the changes in autofluorescence (ΔΔG) of the caries lesions analyzed by the Quantitative Light-induced Fluorescence (QLF) or Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ lesions.

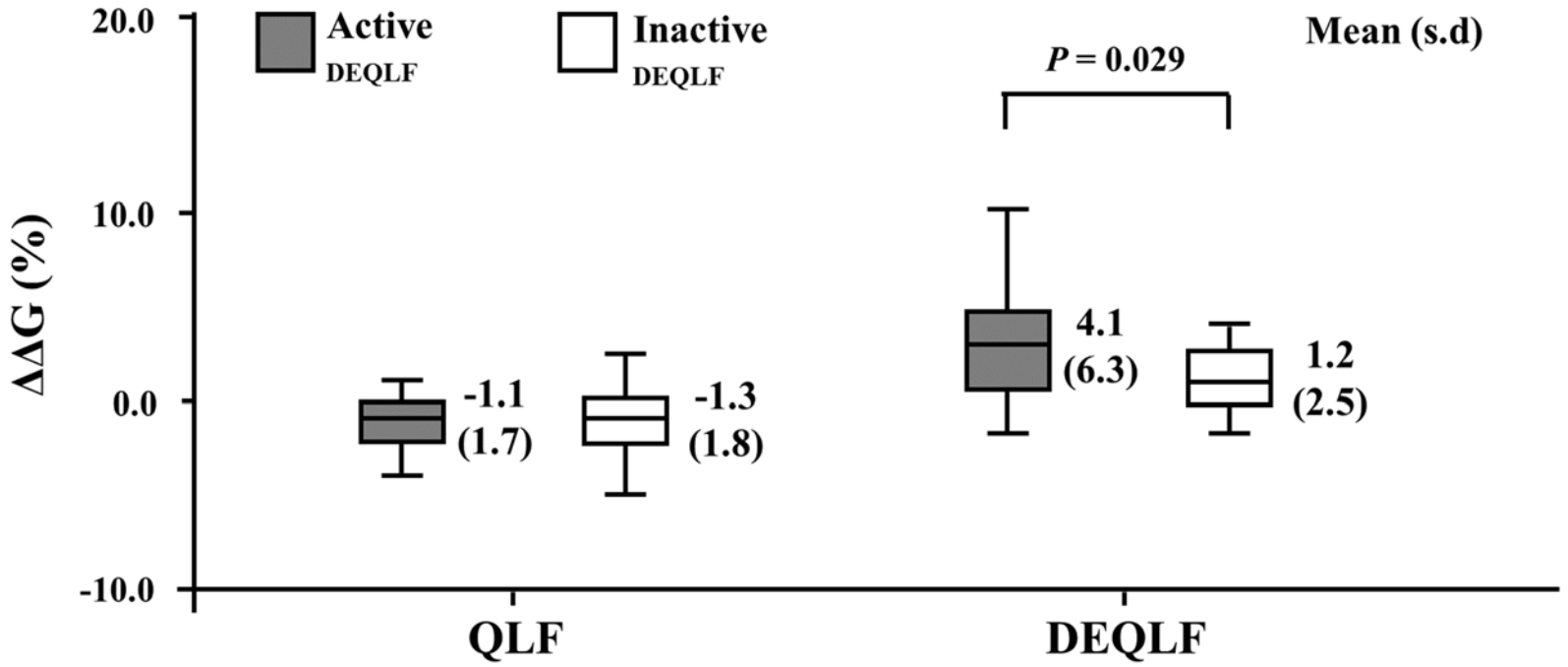


Figure 14. Comparison of the changes in autofluorescence (ΔΔG) of caries lesions analyzed by the Quantitative Light-induced Fluorescence (QLF) method or Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ lesions.

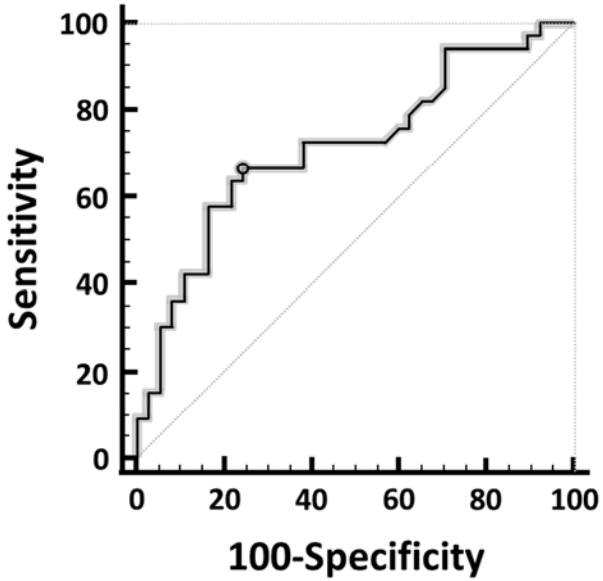
3.2.3. Validity

3.2.3.1. Caries lesions assessed by Nyvad system

Evaluating the validity of ΔΔG by the DEQLF method in distinguishing between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ showed good validity with an AUROC value of 0.72 (Figure 15). In addition, ΔΔG showed a sensitivity of 0.67 and a specificity of 0.76.

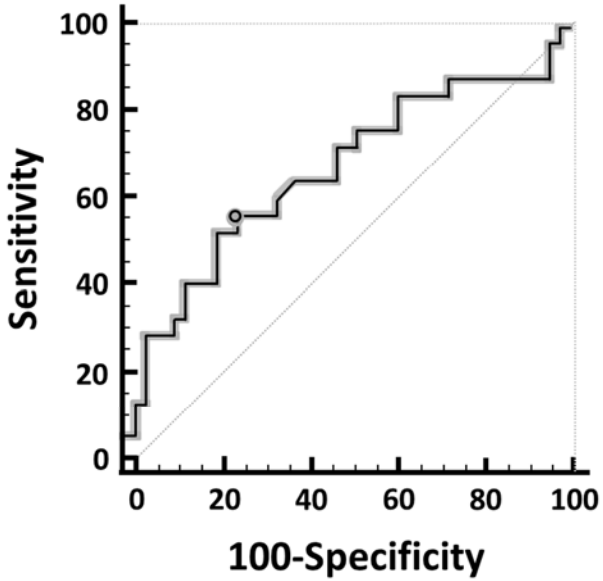
3.2.3.2. Caries lesions assessed by DEQLF method

Evaluating the validity of ΔΔG by the DEQLF method in distinguishing between Active $_{DEQLF}$ and Inactive $_{DEQLF}$ showed a good validity with an AUROC value of 0.68 (Figure 16). In addition, ΔΔG showed a sensitivity of 0.58 and a specificity of 0.80.



	ΔΔG
Cut-off	1.47
AUROC (95% CI)	0.72 (0.60-0.82)
Sensitivity	0.67
Specificity	0.76

Figure 15. Validity of changes in fluorescence (ΔΔG) of caries lesions analyzed by the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method to differentiate between Active $_{Nyvad}$ and Inactive $_{Nyvad}$.



	ΔΔG
Cut-off	2.51
AUROC (95% CI)	0.68 (0.54-0.81)
Sensitivity	0.58
Specificity	0.80

Figure 16. Validity of changes in fluorescence (ΔΔG) of caries lesions analyzed by the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method to differentiate between Active $_{DEQLF}$ and Inactive $_{DEQLF}$.

IV. DISCUSSION

This study confirmed that the DEQLF method, in which a fluorescent dye is penetrated into caries lesions, can be used to objectively distinguish the active state of caries lesions by evaluating the surface porosity of the caries lesions. As a result of performing the DEQLF method on caries lesions, active lesions showed brighter fluorescence than inactive lesions and significantly higher fluorescence indicators. In addition, the change in fluorescence of lesions as a result of the DEQLF method showed excellent validity in distinguishing the active and inactive lesions classified by the conventional caries lesion activity evaluation method or the active and inactive lesions classified by the DEQLF method.

In the bovine tooth study, the DEQLF method was performed on artificial caries lesions. The fluorescence of the caries lesions was contrarily observed according to the active state. As a result of comparing ΔG (mean ± standard deviation), the rate of increase in fluorescence of caries lesions to sound enamel, between the active and the inactive groups, the active groups showed positive ΔG (24.0 ± 12.6 to 46.4 ± 16.2), and the fluorescence of caries lesions was brighter than that of sound enamel. However, the inactive group showed negative ΔG values (-9.7 ± 4.8 to -12.6 ± 9.8), and the fluorescence of caries lesions was darker than that of sound enamel (Table 5). This means that while the fluorescent dye could penetrate the lesion through the porous surface layer due to demineralization, it could not penetrate the lesion through the less porous surface due to remineralization (Figure 17). In the histological evaluation results of this study, it was confirmed that the caries lesions in

the inactive groups had a remineralized surface structure similar to the mineral content of sound enamel, unlike the caries lesions in the active groups. This difference was confirmed to affect the penetration of the fluorescent dye (yellow arrows, Figure 11). In addition, when the QLF method was performed on artificial caries lesions, in general, both demineralized lesions and remineralized lesions emitted darker fluorescence (negative ΔG) compared to sound enamel. Only penetrating the fluorescent dye into the caries lesion can observe brighter fluorescence (positive ΔG) compared to sound enamel (Kim, Kwon, and Kim 2013; van der Veen et al. 1996). By confirming that the DEQLF method is a method showing the penetration pattern of the fluorescent dye according to the structural characteristics of the caries lesion, it will be possible to objectively and conveniently evaluate lesion activity.

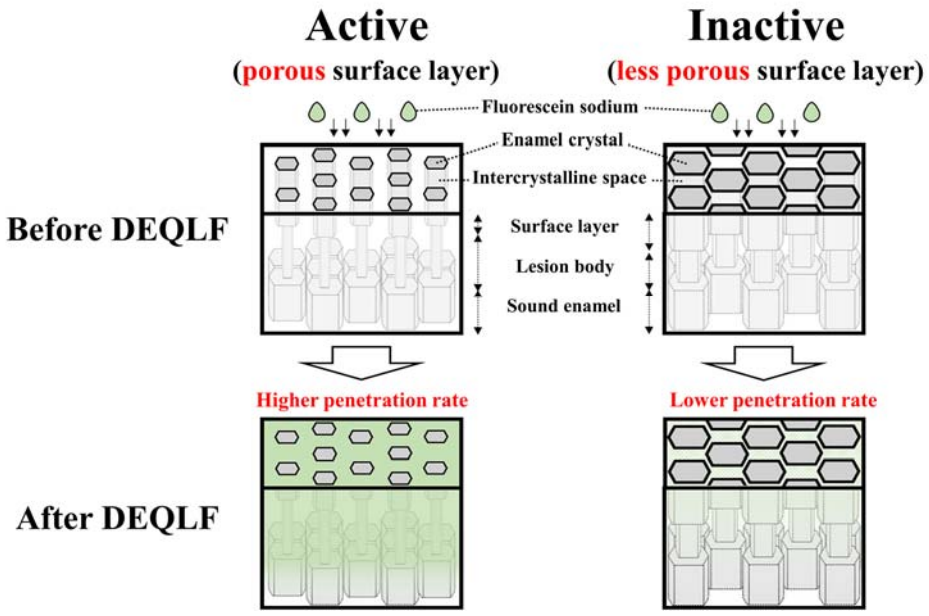


Figure 17. Schematic diagram of the fluorescence changes in caries lesions analyzed by the Dye-Enhanced Quantitative Light-induced Fluorescence (DEQLF) method.

On the other hand, when the artificial caries lesions were evaluated by the QLF method, it was difficult to distinguish the active state of the caries lesions because both active and inactive groups showed darker fluorescence than sound enamel. As a result of comparing ΔG (mean ± standard deviation) between the active groups and the inactive groups, both the active groups (-38.9 ± 7.3 to -24.1 ± 6.6) and the inactive groups (-18.7 ± 8.7 to -9.0 ± 6.8) showed negative ΔG values and showed darker fluorescence than sound enamel (Table 3). In addition, as a result of comparing the A3 group and the I10 group in fluorescence images, a relatively low porosity surface layer was shown, but the caries lesions of the I10 group with relatively deep lesion depth (86.9 ± 25.7㎛) and the caries lesions of the A3 group with shallow lesion depth (44.5 ± 8.9㎛) were not quantitatively and visually identified (Figure 10). This is related to the fundamental principle of the QLF system. This is because, in the process of dehydration of the caries lesion by the QLF method, the fluorescence of the lesion darkens not only when the surface porosity increases but also when the lesion depth increases (Mujat et al. 2004). Therefore, it was confirmed that the fluorescence change of the QLF method alone is not suitable for distinguishing the active state of caries lesions due to the differences in surface porosity and lesion depths of caries lesions.

The DEQLF method can show the porous structure of a caries lesion more clearly by showing more change in fluorescence in the same caries lesion compared to the QLF method. In this study, the QLF method and the DEQLF method were performed on the

same caries lesions, and the fluorescence change (|ΔΔG|, mean ± standard deviation) of the caries lesions was compared between the two methods. As a result, the DEQLF method in the active groups (44.5 ± 14.5 to 89.0 ± 16.4) showed statistically significantly larger |ΔΔG| values, to be larger by 30.3 to 64.4 %, than the QLF method (14.2 ± 3.4 to 24.4 ± 4.1) ($P <$ 0.001). Whereas |ΔΔG| of the caries lesions in the I10 group, the QLF method (11.0 ± 3.7) and the DEQLF method (9.1 ± 5.5) showed no statistically significant difference (Figure 11). In the I3 group, |ΔΔG| by the DEQLF method (2.6 ± 2.0) was statistically significantly lower than |ΔΔG| by the QLF method (5.5 ± 2.5) by 2.9 % ($P < 0.001$, Figure 11). Similarly, in the I5 group, |ΔΔG| by the DEQLF method (2.9 ± 2.4) was statistically significantly lower than |ΔΔG| by the QLF method (5.0 ± 3.0) by 2.1 % ($P = 0.016$, Figure 12). The principle of the QLF method is to dehydrate the water present in the porous structure of the surface layer of the caries lesions and thereby show a change in fluorescence due to the difference in refractive index. While the fluorescent dye used in the DEQLF method penetrates the porous structure of the dehydrated surface layer, the fluorescence of the caries lesions become brighter (Pretty, Edgar, and Higham 2004). Therefore, the DEQLF method will more sensitively deliver information on the porous structure of the surface layer to the clinician.

In addition, the DEQLF method makes it possible to evaluate the degree of remineralization of caries lesions through the fluorescence of the fluorescent dye that has penetrated early caries. In this study, unlike the I3 and I5 groups, the caries lesions in the I10 group emitted bright fluorescence in the caries lesion partially (Figure 11). As a result

of observing the fluorescence of the caries lesions according to the histological characteristics, the caries lesions in the I3 and the I5 groups had a completely remineralized surface layer, whereas the caries lesion in the I10 group had a partially remineralized surface layer (yellow arrows, Figure 11). From this result, it is predicted that the fluorescent dye did not penetrate into the completely remineralized surface layer, whereas it could penetrate into the partially remineralized surface layer through the gap, so that the fluorescence of the fluorescent dye was observed in the corresponding area. As such, depending on the degree of surface porosity, there may be a difference in the degree of remineralization, which can be confirmed by evaluating the penetration of the fluorescent dye using the DEQLF method. According to a previous study, when fluoride treatment was performed on demineralized lesions with various surface porosities, a fluoride response differential occurred according to the surface porosity (Lippert and Juthani 2015). In another previous study, 2% sodium fluoride solution (9000 ppm) was used to treat early caries of various surface porosities, and the QLF system evaluated how much the caries lesions were remineralized (Kim, Kwon, and Kim 2013). As a result, shallow lesions (lesion depth of 40-60 um, the caries lesions corresponding to I3 and I5 in this study) showed a remineralization rate of 48.0-62.9% with a fully remineralized surface layer, whereas a relatively deep lesion (lesion depth of 120 um, the caries lesion corresponding to I10 in this study) showed a remineralization rate of 27.4% with a less remineralized surface layer. Therefore, if the DEQLF method is used, it will be possible to quantitatively predict and evaluate the degree of remineralization as well as the surface porosity of the

caries lesion.

In this study, artificial caries lesions were formed using an acidified polymer gel partially saturated with hydroxyapatite to evaluate the surface porosity. These caries lesions are classified as 'low-R' lesions by the R ratio, which is the ratio of mineral loss (ΔZ) to the lesion depth (Lippert et al. 2011; Lynch, Mony, and Ten Cate 2007). Low-R lesions show a relatively high level of mineralization in the surface layer compared to high-R lesions, because carbopol, a surface-protecting polymer containing calcium and phosphate ions, protects the surface layer during lesion formation (White 1987). Because of the low surface porosity of the low-R lesions, fluoride ions cannot penetrate deeply, resulting in more mineral reprecipitation on the surface than on the body (Cochrane et al. 2010; Lippert and Juthani 2015). As a result, the artificial demineralized lesion formed of the polymer gel has an intact but porous surface layer, and the artificial remineralized lesion can have a sufficiently mineralized surface layer. Therefore, the artificial demineralized lesions were able to reproduce a completely open (more porous) surface layer, and the artificial remineralized lesions were able to reproduce completely closed (less porous) surface layers only in the I3 and the I5 groups. This was useful in evaluating the QLF method and the DEQLF method using surface porosity (Lynch, Mony, and Ten Cate 2007).

In the human tooth study, after performing the DEQLF method on natural caries lesions, a new method for evaluating the activity of caries lesions was proposed, which classified lesions as 'Active $_{DEQLF}$' when fluorescence of the fluorescent dye was observed in the caries lesion, and 'Inactive $_{DEQLF}$' when fluorescence was not observed. As a result of confirming

the distribution between the caries lesions classified by the DEQLF method (Active $_{DEQLF}$ and Inactive $_{DEQLF}$) and the caries lesions classified by the conventional method (Nyvad system; Active $_{Nyvad}$ and Inactive $_{Nyvad}$), a slightly low percent agreement (58.6 %, Table 7) was found. This may be due to the difference between the two methods in evaluating the surface characteristics of natural caries. For example, when there is a difference in surface porosity in part of the whole area of a caries lesion, the lesion is difficult to detect because the existing method uses human senses. However, the new caries activity evaluation method using DEQLF can easily detect whether or not the fluorescent dye has penetrated through bright fluorescence. In fact, in the case of natural lesions in this study, in the caries lesions classified as Active $_{DEQLF}$, the fluorescence of the fluorescent dye was mostly observed in part of the caries lesion, not the whole area. This can be supported by the distribution of 42.3% of Inactive $_{Nyvad}$ lesions in Active $_{DEQLF}$ lesions. Therefore, the DEQLF method can be used as a method that overcomes the difficulties in evaluating the surface characteristics of natural caries lesions since even demineralized areas located in part of whole lesions can be visualized through the fluorescence of the fluorescent dye.

The DEQLF method could be used to distinguish the active state of natural caries lesions due to differences in surface porosity. When the DEQLF method was performed on natural caries lesions, the fluorescence change in the active lesions was larger by 2.8 to 2.9 % than that of the inactive lesions ($P = 0.012$, Figure 13; $P = 0.029$, Figure 14). However, the $\Delta\Delta G$ values of the active and inactive groups in the human tooth study were lower when compared to the results in the bovine tooth study. These results indicate that natural caries

had less porous surface layers than artificial caries (Cochrane et al. 2012; Magalhães et al. 2009). In spite of the evaluation of natural caries showing a difference in surface porosity between the active groups and the inactive groups, the DEQLF method was able to objectively distinguish the active state of the lesions.

On the other hand, the QLF method was not suitable for determining the active state of natural caries lesions. In this study, when ΔΔG was evaluated in natural caries with the QLF method, there was no statistically significant difference between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ groups (Figure 13). These results were similar to the results of an *in vivo* study in which the QLF method was performed on natural caries lesions in the oral cavity, and it was not possible to distinguish between Active $_{Nyvad}$ and Inactive $_{Nyvad}$ (Ando et al. 2017). In addition, there was no statistically significant difference in ΔΔG between Active $_{DEQLF}$ and Inactive $_{DEQLF}$ groups (Figure 14). This may be due to the surface characteristics of natural caries. Natural caries may have an incompletely remineralized surface because the surface of remineralized caries is a mixture of demineralized and mineralized areas (Cochrane et al. 2010; Pitts et al. 2017). Therefore, the QLF method was able to distinguish between active and inactive lesions of artificial caries with a clear difference in surface porosity, but could not distinguish between active and inactive lesions of natural caries with a relatively small difference in surface porosity.

The change in fluorescence (ΔΔG) by the DEQLF method showed good validity (AUROC of 0.68) in distinguishing Active $_{DEQLF}$ and Inactive $_{DEQLF}$ lesions (Figure 15). In addition, a sensitivity and a specificity showed 0.58 and 0.80, respectively. Among these,

the relatively low sensitivity can be explained in two ways. First, it may be influenced by the characteristics of the natural caries lesions used in this study. The active caries used in this study contained a mixture of remineralized regions, not whole demineralized lesions. This is because the teeth used in this study were mainly obtained from adults and may have been affected by saliva and remineralized substances. In addition, there is a limitation of the fluorescence parameters of the DEQLF method in evaluating such lesions. In the active state classified with the DEQLF method, caries lesions are regarded as Active $_{DEQLF}$ by the fluorescence observed in the 'partial area' of the caries lesion, while the fluorescence variable ($\Delta\Delta G$) quantifies the fluorescence of the lesion in the 'whole area' of the caries lesion. Therefore, it was confirmed that the sensitivity was somewhat low due to the difference between the two regions. Therefore, further *in vitro* study of caries lesion that have a whole demineralized region or an *in vivo* study to evaluate lesion activity need to be performed in order to quantify the fluorescence of 'partial areas' in children who have many teeth with caries lesions. Therefore, it is necessary to check the diagnostic accuracy of the DEQLF method through further studies.

Using the DEQLF method, it is possible to distinguish the active state by observing the bright fluorescence in the caries lesion regardless of the lesion depth of the early caries. Some previous studies attempted to determine the active state by the red fluorescence observed in the natural caries lesion, and as a result, the active lesions showed statistically significantly higher red fluorescence parameters (intensity and area) than the inactive lesions (Kim et al. 2019; Lee and Kim 2020). However, the red fluorescence parameters

may also change depending on the depth of the lesion (Park et al. 2019b). For this reason, after correcting for the lesion depth, it was necessary to confirm whether the active state of the lesion could be distinguished by the red fluorescence parameters through clinical studies (Cochrane et al. 2012). Therefore, the specimens in this study were stored in a dark box capable of blocking external light to prevent photobleaching of the red fluorescence of the caries lesion. Nevertheless, since only 28 of the 70 caries lesions had red fluorescence, the specimens used in this study were not suitable for discriminating the active state using red fluorescence parameters. However, the red fluorescence in the caries lesion may change the color of the fluorescence of the fluorescent dye that has penetrated into the lesion, and thus, there may be a difference in the quantitative fluorescence parameters compared to caries lesions without red fluorescence. Therefore, it is necessary to confirm the effect of red fluorescence present in the caries lesions through a further study calculating the fluorescence parameters of the caries lesions by the DEQLF method.

It was also important to select an appropriate fluorescent dye for the DEQLF method. Fluorescein sodium was selected due to the following advantages. First, it is the fluorescent dye (D&C Yellow no. 8) approved by the U.S. Food and Drug Association (FDA). It is non-toxic and does not have any chemical bonding in the body, so it is easy to discharge to the outside of the body. Due to these advantages, it has been used as the contrast agent for angiography and as the fluorescent dye for the detection of corneal defects in the medical field (Shinoda et al. 2003; Tabery 1992). Second, fluorescein sodium emits strong yellow-green fluorescence under the blue visible light of the QLF system, and as its concentration

increases, the fluorescence becomes stronger (Romanchuk 1982). At this time, it was important to find an appropriate concentration of the fluorescent dye, because the fluorescence emitted from the fluorescent dye, natural teeth, dental plaque and caries lesions must be distinguished. Previous studies used various concentrations of fluorescein sodium such as 13.5 g/L (enamel caries detection), 10 g/L (corneal defect detection), and 0.2 g/L (root caries detection) (Hamamcıoğlu et al. 2016; Pretty et al. 2003; Shern, Kennedy, and Roberts 1990; Shinoda et al. 2003; Tabery 1992; van der Veen and ten Bosch 1993; van der Veen et al. 1996). Therefore, in this study, fluorescein sodium at a concentration of 0.376 g/L was used to set an appropriate concentration that could be distinguished from the fluorescence of enamel, but was not harmful to the human body. Finally, it can easily penetrate into the porous structure of microscopic mineral defects (caries lesions or cracks) or dental plaque (Pretty et al. 2003). Therefore, based on the above advantages, fluorescein sodium was selected as the fluorescent dye for use in the DEQLF method to evaluate the lesion activity state of early caries.

The penetration time of the fluorescent dye is also an important factor in the DEQLF method. Previous studies have reported penetration times of 2 to 5 minutes for a fluorescent dye to detect a caries lesion early under visible light with a wavelength of 430 nm or 515 nm (van der Veen and ten Bosch 1993; van der Veen et al. 1996). However, in this study, it was necessary to intensively observe the phenomenon occurring in the porous structure of the lesion surface layer rather than the porous structure of the whole caries lesion by reducing the penetration time of the fluorescent dye. For this reason, the pilot study with

the QLF system evaluated the active and inactive lesions of natural caries with the fluorescent dye for 3 minutes at 10-second intervals (Park et al. 2019a). As a result, when the fluorescent dye had penetrated into the caries lesion for only 10 seconds, the fluorescent dye penetrated only into the surface layer, not the inside of the lesion body of the caries lesions, thereby widening the difference in the amount of dye penetration in the two lesions with different surface porosity. The DEQLF method described in this study can distinguish the active status of early caries lesions with only a short penetration time of 10 seconds, so it will be possible to evaluate the active status of lesions time-efficiently in dental clinics.

Considering that the DEQLF method used in this study is a technique that can objectively evaluate the activity of early caries lesions, the results of this study may have clinical significance. The optical technology used in this study minimizes subjectivity, which is a major limitation of clinical evaluation methods, and makes it possible to visualize things that are difficult to observe with the naked eye. In addition, the variable quantified by the DEQLF method can be used as an objective indicator to provide a preventive treatment suitable for this by evaluating the activity state of early caries lesions. Since the surface layer of the caries lesions classified as Active $_{DEQLF}$ have an open porous structure, non-operative treatment, such as fluoride treatment, or microinvasive treatment, such as resin infiltration, should be performed (Meyer-Lueckel and Paris 2010; Paula et al. 2017). On the other hand, the caries lesions classified as Inactive $_{DEQLF}$ should be monitored periodically rather than performing treatment due to the surface layer having a mineral content similar to that of sound enamel.

V. CONCLUSION

In this study, the fluorescence parameters of active and inactive lesions were compared in artificial and natural caries with the DEQLF method. Based on this, the DEQLF method was proposed as a new lesion activity assessment of early enamel caries, and the validity of the fluorescent parameter by the DEQLF method was evaluated in determining the active state. Finally, the fluorescence parameters of the QLF method and the DEQLF method were compared in evaluating caries lesions. The results of the study were as follows:

1. In artificial caries analyzed with QLF method or DEQLF method, active and inactive lesions could be distinguished through the fluorescence change of the caries lesion.

2. In natural caries in which the DEQLF method was performed, active and inactive lesions could be distinguished through the fluorescence change of the caries lesion, and the fluorescence change of the caries lesion ($\Delta\Delta G$) showed good validity in distinguishing the active state of the caries lesion.

3. Unlike the QLF method, the fluorescence change of the natural caries lesions by the DEQLF method showed a significant difference between active and inactive lesions.

In summarizing the above results, the fluorescence change of early caries lesions as a result of performing the DEQLF method can be used to evaluate their activity state. These results can help make decisions about caries lesion activity by using optical technology to determine the fluorescence change of caries lesions when it is difficult to secure the reliability of a lesion activity evaluation in a clinical environment.

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ABSTRACT (IN KOREAN)

Dye-Enhanced Quantitative Light-induced Fluorescence(DEQLF)를 이용한 초기 법랑질우식의 병소활성평가

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박석우

초기우식병소는 활성 상태에 따라 와동형 병소로 진행될 가능성이 있다. 따라서, 병소의 활성 상태를 평가하고 이에 적합한 예방적 처치를 수행하는 것이 중요하다. 병소의 활성 상태는 병소 표층 특성에 의해 결정되기 때문에, 이를 시각과 촉각으로 평가하는 Nyvad system과 International Caries Detection and Assessment System in conjugation with lesion activity assessment (ICDAS-LAA)가 임상에서 사용되어 왔다. 하지만 이러한 방법들은 병소 표층 특성을 감각으로 평가하여 활성 상태를 판정하기 때문에 객관성을 확보하기에 어려움이 있다.

선행연구들에서는 광학 기술을 이용하여 병소 표층 특성을 객관적으로 측정

하기 위해, 병소 표층의 다공성 구조에 존재하는 수분을 압축공기로 탈수하고, Quantitative Light-induced Fluorescence (QLF) system을 이용하여 탈수에 의한 병소의 형광 변화를 관찰하였다. 본 연구에서는 이 과정을 QLF 방법으로 명명하였다. 그러나, 선행연구에서 이 방법을 이용하였음에도 불구하고 자연우식에서 활성 병소와 비활성 병소 간 표층 다공성 구조의 미세한 차이를 구분할 수 없었기 때문에, 자연우식의 병소활성평가법으로써 사용하기에 적합하지 않았다.

선행연구는 병소 표층의 다공성 구조에 형광 염색제를 침투시켜, QLF로 침투에 의한 병소의 형광 변화를 관찰하는 방법을 소개하였다. 본 연구에서는 이 방법을 Dye-Enhanced QLF (DEQLF)으로 명명하였다. 선행연구는 이 방법에 의한 형광 염색제의 강한 자가형광으로 초기우식병소를 조기에 발견할 수 있었다. 그럼에도 불구하고, DEQLF 방법을 이용하여 다양한 표층 다공성의 초기우식을 평가하거나, 병소의 활성 상태를 구분하기 위한 연구는 현재까지 수행되지 않았다. 따라서 본 연구의 목적은 초기우식의 병소활성평가법으로써 DEQLF의 활용 가능성을 평가하고자 하였다.

첫 번째 연구의 목적은 인공우식병소를 대상으로 DEQLF 방법을 수행하여 활성 병소와 비활성 병소 사이의 형광지표를 비교하는 것이었다. 그리고, 그리고 다양한 표층 다공성의 우식병소들에서 QLF 방법과 비교하여 DEQLF 방법의 활성 병소와 비활성 병소 구분에 대한 유용성을 확인하는 것이었다. 두 번째 연구의 목적은 자연우식에 DEQLF 방법을 수행하여 병소의 활성 상태를 분류하고, 두 활성 상태 간 병소의 형광 변화를 비교하는 것이었다. 이어서 자연우

식에 QLF 방법과 DEQLF 방법을 수행하여 두 방법간 활성 상태 구분에 대한 유용성을 비교하였다. 나아가, 자연우식의 활성 상태를 구분함에 DEQLF 방법이 수행된 병소의 형광 변화에 대한 타당도를 평가하였다.

첫 번째 연구에서는 인공적으로 탈회 병소를 형성하여 활성군(Ax, x는 탈회 기간)에, 재광화 병소를 형성하여 비활성군(Ix, x는 탈회 기간)에 배정하였다. 그리고, 3일, 5일, 10일의 각기 다른 탈회 기간을 통해 다양한 표층 다공성을 가진 우식병소들을 형성하였다. 각 병소에 DEQLF 방법을 수행하여 병소를 염색한 후 QLF system으로 백색 이미지와 형광 이미지를 각각 채득하였고, 이미지 분석 프로그램을 이용하여 형광 이미지에서 정상 법랑질에 대한 병소의 형광증가율(ΔG, %)를 산출하였다. 그리고, 두 방법들(QLF 또는 DEQLF)에 의한 병소의 형광변화(ΔΔG, %)를 수행 전과 후 ΔG의 차이로 산출하였고, 두 방법들 간 병소의 형광변화량을 비교하기 위해 절대값으로 사용하였다. 그 결과, DEQLF 방법이 수행된 인공우식에서 활성군들에서는 양의 ΔG를, 비활성군들에서는 음의 ΔG를 보였다. 그리고, 두 방법들 간 |ΔΔG|를 비교한 결과, 활성군들에서 DEQLF 방법은 QLF 방법에 비해 30.2~64.6만큼 더 높은 |ΔΔG|를 보였다($P < 0.001$). 그리고, I3군과 I5군에서 DEQLF 방법은 QLF 방법에 비해 각각 2.9와 2.1만큼 통계적으로 유의하게 낮은 |ΔΔG|를 보였던 반면(각각, $P < 0.001$와 $P = 0.016$), I10군에서 두 방법 간에 통계적으로 유의한 차이를 볼 수 없었다.

두 번째 연구에서는 평활면에 자연우식병소가 있는 사람의 치아를 사용하였다. 자연우식병소의 활성 상태는 기존에 널리 사용되던 시각-촉각법 기반 우

식병소활성평가법(Nyvad system; 활성$_{Nyvad}$와 비활성$_{Nyvad}$)과 형광 기반 새로운 우식병소활성평가법(DEQLF; 활성$_{DEQLF}$와 비활성$_{DEQLF}$)을 이용하여 평가하였다. 두 방법으로 분류된 병소들의 분포를 확인한 결과, 58.6%의 일치도를 보았다. 그리고 방법들(QLF 또는 DEQLF)이 수행된 자연우식에서 활성$_{DEQLF}$군과 비활성$_{DEQLF}$군 간 ΔΔG를 비교한 결과, QLF 방법이 수행된 자연우식에서는 두 군 간에 통계적으로 유의한 차이가 없었던 반면, DEQLF 방법이 수행된 자연우식에서는 활성$_{DEQLF}$군은 비활성$_{DEQLF}$군과 비교하여 2.9만큼 더 높은 값을 보였다(P = 0.029). 그리고, 활성$_{DEQLF}$군과 비활성$_{DEQLF}$를 구분함에 ΔΔG는 0.68의 AUROC, 0.58의 민감도 그리고 0.80의 특이도를 보였다.

결론적으로 본 연구 결과를 종합해보면, 인공 및 자연우식에 DEQLF 방법을 수행하였을 때 비활성 병소와 비교하여 활성 병소에서 더 높은 형광 지표가 산출되었으며, DEQLF 방법을 이용하여 병소 활성을 구분하는데 있어 우수한 타당도를 확인함으로써, 초기우식의 병소활성평가법으로써 DEQLF의 활용 가능성을 확인할 수 있었다. 또한, QLF 방법과 비교하여 DEQLF 방법은 활성과 비활성 간 병소 표층의 미세한 다공성 차이를 더 극명하게 가시화하고 정량적 차이로 보여줄 수 있음을 확인하였다. 따라서, 임상가는 DEQLF 방법을 이용하여 객관적으로 병소의 활성 상태를 평가할 수 있으며, 이를 근거자료로 사용하여 의사결정에 도움을 줄 수 있을 것이다.

핵심되는 말: 우식병소활성평가, 초기우식병소, 플루오레세인, 형광-강화 정량광형광유도법, 형광염색제