Diagnosis of early caries with dye-enhanced laser fluorescence

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국문초록

초기 우식병소와 건전한 치질은 광학적 특성이 다르다는 사실에 근거하여 치과 치료용으로 개발된 가시광선 영역의 레이저의 형광현상과 광활성재를 이용하여 우식 치질과 건전한 치질간의 광학적 특성의 차이를 색조의 차이로 유도 · 전환함으로써 초기 우식 병소를 조기에 시각적으로 탐지하여 진단하는 방법을 개발하는데 본 연구의 목적이 있다.

소의 치아를 이용하여 여러 단계 깊이의 인공우식병소를 유발하고 우식병소의 표면에서 아르곤 레이저를 조사하여 건전 치질과 우식 치질 사이의 반사된 형광강도의 차이를 컴퓨터 영상분석프로그램을 통해 측정하였다. 또한 광활성재를 병소의 표면에 도포하여 형광현상의 색조대비 증진효과를 평가하였다. 실제 조직학적 병소와 레이저 형광법 사이의 상관관계 분석 및 회귀 분석을 통하여 다음과 같은 결과를 얻었다.

레이저 형광법에 의한 우식 치질은 건전 치질보다 어둡게, 그리고 광활성재를 도포한 후 레이저 형광법으로 관찰한 우식 병소는 건전 치질 보다 밝게 관찰 되었으며 광활성재를 이용한 레이저 형광법이 탈회의 초기단계에서는 레이저 형광법에 비해 진단학적 민감도가 컸다.

주요어 : 초기우식병소, 레이저 형광법, 광활성재

Ⅰ. Introduction

Early diagnosis of the dental caries is important, since early carious lesion can be recovered completely to original healthy state. But early carious enamel can be hardly distinguished from a healthy one in a wetted condition with saliva in the mouth[1]. Furthermore, early carious enamel is not easily found even with other clinical methods for caries detection, such as visual inspection, proving, and radiographs.

Numerous methods have been developed to aid the early diagnosis of the dental caries. Of the traditional methods, proving, visual inspection and radiographs are widely used and fiber-optic transillumination[2-8], endoscopic method[9], and electronic caries monitor[10,11] have been added to this list. Dyes have been used for many years to enhance the visual inspection for the caries detection[12-16].

Recently, a new diagnostic method for early detection of caries was presented. It is based on the fluorescent characteristic of tooth structure, which occurs when the teeth are illuminated with a broad beam of blue green light. Laser fluorescence for the caries detection was first reported in 1982 by Bjelkhagen et al[17]. Angmar-Mänsson and ten Bosch[18,19] detected the early enamel caries by irradiation of argon laser with 488nm wavelength. The fluorescence in the enamel occuring in the yellow region is observed through a yellow high-pass filter which exclude the tooth-scattered blue laser light. Dark regions characterize the demineralization of tooth structure[20-22].

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Many techniques for detection and quantification of mineral loss from early carious lesions are destructive and cannot be used clinically. Laser fluorescence may offer a solution to some of these problems but much research need to be conducted on whether laser fluorescence can be applied to assess the mineral changes and on whether such changes can be expressed visually or numerically[23-25].

The use of fluorescent dyes may further enhanced the detection of early caries in conjunction with laser fluorescence method[26]. Sodium fluorescence, one of the fluorescent dyes, has been used in ophthalmology and also in dentistry as a plaque disclosing agent because it is relatively nontoxic and pharmacologically inactive[27,28].

The purpose of this study was to evaluate the quantitative nature of laser fluorescence(LF) and dye-enhanced laser fluorescence(DELF) on early enamel caries.

Ⅱ. Materials and Methods

Specimen preparation

One hundred and forty 6×6×4 mm bovine enamel specimens were cut from the labial surface of bovine incisor teeth using slow-speed diamond wheel saw(Model #650, South Bay Technology Inc. San Clemente, CA, USA). Specimens were mounted individually in polymethyl methacrylate resin plate for easy handling(Fig. 1).

Each specimen was covered with nail varnish, leaving a half of the surface of exposed window. Specimens were numbered randomly, divided into 5 groups and placed in a demineralizing solution (STPP; 0.1 M lactic acid, 0.24 mM sodium-tripolyphosphate, pH at 4.2 with sodium hydroxide) at 37℃ for differing periods of time. The demineralizing solution contained 1 mol lactic acid and 1% Carbopol, 50% saturated with hydroxyapatite, and adjusted to pH 5 by 50% NaOH. Groups of 15 specimens were each placed in demineralizing solution at 37℃ for 12, 24, 48, 72, and 96 h, respectively to induce artificial carious lesions of various depths.

After demineralization, the nail varnish was removed using acetone and specimens were analyzed using laser fluorescence and polarizing microscopy.

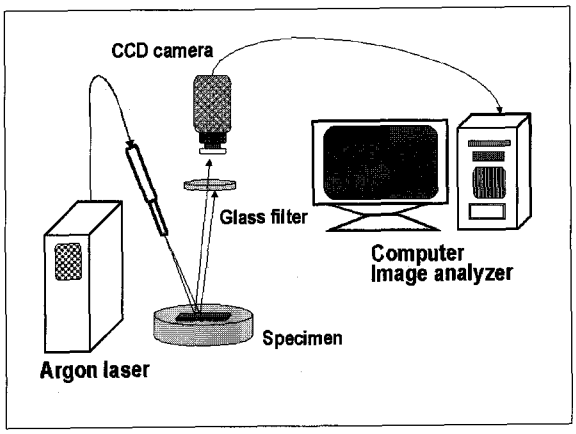

Fig. 1. Diagram of measuring system for fluorescence radiance on artificial caries.

Laser fluorescence(LF) examination

Specimens were exposed to a 488 nm argon laser of continuous-wave (SPECTRUM™, HGM Inc. Salt Lake City, Utah, USA). Laser power at the specimen surface was 0.6 W. The diameter of the optical fiber was 600㎛. A yellow high-pass filter(Kodak Wratten 16) was used to cut off the scattered light with a lower wavelength than 520 nm from the tooth and to observe only the pure fluorescent light. Fluorescent images were captured using a CCD camera and stored on computer as a visual files. These images were analyzed with computer image analyzing program(Image pro plus™, Media cybernetics Co, USA) by measuring the difference of fluorescence radiance between the carious enamel and sound enamel in each specimen(Fig. 1).

Dye-enhanced Laser fluorescence(DELF) examination

The specimens were rinsed and gently dried after LF examination. Then 0.075 % sodium fluorescein (Aldrich Chemical Co., U.S.A.) was applied to the lesion surfaces with small plastic applicator and allowing for a second. Specimens were rinsed for 20 sec with water spray and dried with compressed air. The examination procedure was same as LF.

Polarizing microscopic examination

Polarizing microscopic examination was done as the method for validating the presence of lesions and

their depth. After measuring the fluorescence radiance, specimens were sectioned at the middle of demineralization area by means of low-speed diamond wheel saw(Model 660, South Bay Technology Inc., CA, USA). These sections, cut 1mm-thick, were polished by high polisher (OMNILAP 2000™, South Bay Technology Inc. CA, USA) with 240.600 grit SiC polishing sheet. The lesion depth of each specimen was measured using a polarizing microscope(×100).

Statistical analysis

Mean values of fluorescence radiance of lesion surfaces and histologic lesion depths were calculated for each demineralization time. The one-way ANOVA test with an overall 95% confidence level was used to determine significant differences between the measurements. The correlation between optical densities and lesion depths was found using Pearson correlation coefficients. Regression analysis were done to extract the regression line for prediction of lesion depth from fluorescence radiance of lesion surface.

Ⅲ. Results

The fluorescence radiance of lesion surfaces measured with LF, DELF and the depths of lesions measured with the polarizing microscope according to the demineralization times are presented in Table 1. The radiance of fluorescence and the lesion depths were increased with increasing demineralization time(Fig. 2).

Lesions could be visually discriminated by darkness in LF examination and brightness in DELF examination.

Table 1. Mean lesion depth and fluorescence radiance of lesion surface according to the demineralization time

Deminteralization time(hours)	Lesion depth(㎛)	Difference of fluorescence radiance(DFR)	
		LF	DELF
12	93.67±24.46	0.27±0.11	0.73±0.28
24	172.24±53.10	0.51±0.17	1.51±0.47
48	411.33±72.05	0.91±0.22	2.47±0.85
72	553.17±86.24	1.87±0.74	2.76±1.78
96	849.01±102.38	3.54±1.91	3.49±1.02
F ratio	18.34	5.54	4.24
P value	0.004*	0.025*	0.029*

* : Statistically significant LF : Laser fluorescence DELF : Dye-enhanced laser fluorescence

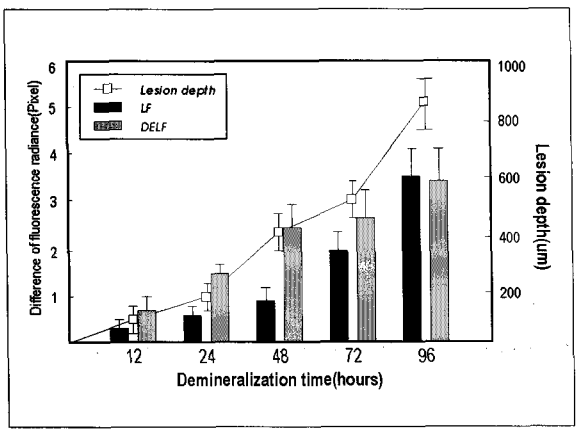

Fig. 2. Comparison of the lesion depths and the difference of fluorescence radiance(DFR) of the LF(laser fluorescence) and the DELF(dye-enhanced laser fluorescence) in accordance with demineralization time.

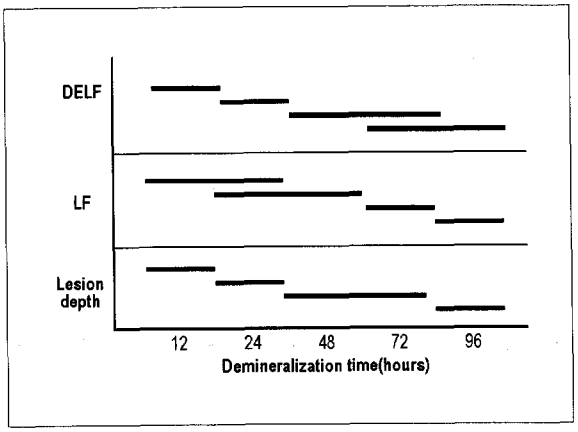

Fig. 3. The least square differentiation of ANOVA test for the lesion depth and difference of fluorescence radiance(DFR) of lesion surface in LF(laser fluorescence) and the DELF(dye-enhanced laser fluorescence) examinations according to demineralization time. Times joined by horizontal line are not significantly different.

Table 2. Pearson's correlation coefficients among variables related with carious lesions

	DFR in LF	DFR in DELF	Lesion depth
DFR in LF			
DFR in DELF	0.500*		
Lesion depth	0.755*	0.692*	

* : P<0.05

DFR : Difference of fluorescence radiance
LF : Laser fluorescence
DELF : Dye-enhanced laser fluorescence

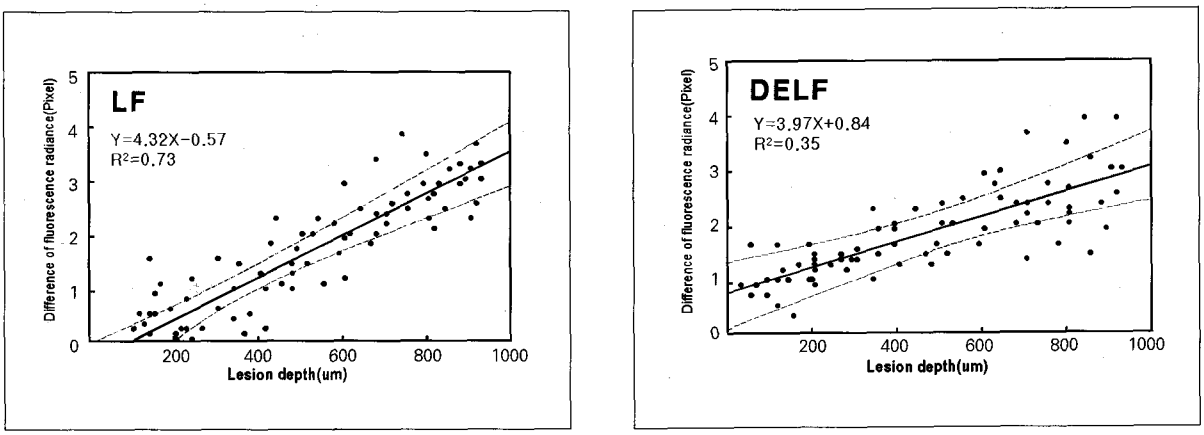

Fig. 4. The regression line for the prediction of lesion depth from fluorescence radiance(FR) of lesion surface in LF(laser fluorescence) and the DELF(dye-enhanced laser fluorescence) examinations.

Fig. 3. shows the ability of each technique to rank groups of lesions according to demineralization time and the results from the ANOVA test to determine differences between groups based on demineralization time.

The correlation coefficient(r) between the fluorescence radiance of lesion surfaces(LF and DELF) and the depth of lesions were showed in Table 2, which indicated the strong relationship between the two.

Furthermore, regression analysis revealed R-square of 0.73(LF) and 0.35(DELF). The linear equations are as follows and their graphs are shown in Fig. 4.

Ⅵ. Discussion

The white spot can be considered the minimum change of tooth surface which must occur just before a carious lesion can be visibly diagnosed[29,30]. Several visible detection methods have been used for diagnosis of incipient caries. The fiber optic transillumination method, which uses the phenomenon that a carious surface scatters more light and transmits less light than a normal surface, can be used to observe the amount of light transmitted through a tooth[2,31]. Although this method is easy and superior one, it is limited in the detection of proximal carious lesion[4,5,32]. Another method for detection of incipient caries is the light scattering which uses the fact that an incipient carious lesion appears more white than the color of its surrounding normal enamel because it scatters more light when irradiated with bright white light[33]. There have also been attempts to diagnose carious lesions upon the basis that demineralized regions irradiated with U-V are not fluorescent and therefor are seen as dark spots[3,34]. But U-V ray is known to be harmful to eyes and skin.

The quantified visible method using laser is one of the methods being studied to be used to overcome

these problems.

Our study attempted to assess the detection sensitivity of the laser fluorescence according to the depth of the carious lesion and whether this sensitivity can be quantified according to depth. In our study, the fluorescence emitted from the tooth was captured by CCD camera and the data were sent to a computer imaging analysis program, which interpreted the data and converted them into gray shades to calculate the optical density.

The results showed the increasing optical density as well as increase in depth of the histological carious lesion in accordance with the demineralization time. While the depths of the histological lesions increased steadily with demineralization time, the DFR of lesion surfaces, induced by LF, from the 12 h to 24 h and from 24 h to 48 h showed no significant difference. And DFR induced by DELF, from 24 h to 72 h and from 48 h to 96 h showed no significant difference.

This fact is revealing that the sensitivity of laser fluorescence for measuring a carious progression was less sensitive than that of histologic method by cross section of teeth(Fig. 3). Although our tests tried to standardize the images for minimizing test errors by fixing the CCD camera and the irradiation distance and angle of laser, the surface refraction of the light on the tooth may caused errors with the direction of the light. But when laser fluorescence is used clinically, relative comparison between the sound region and carious region of patient's teeth would be better than an absolute comparison of numerical values.

Laser fluorescence method provides easy, simple, visible detection of incipient caries and shows the increase in DFR of the carious lesion with increasing depth of the lesion(Table 1). The Pearson coefficient of 0.755(LF) and 0.692(DELF) ($p<0.05$) showed a high relationship between DFR of lesion surface and depth of the lesion. We have extracted a linear equation between the lesion depth and DFL from regression analysis, which enable laser the fluorescence to diagnose the caries as a quantitative assessment method (Fig. 4).

The correlation coefficient was higher in DELF than in DF (Table 2). Furthermore ANOVA test for the lesion depth and DFR of lesion surface according to demineralization time showed that horizontal bars are less overlapping at earlier stage of lesions in DELF and at later stage of lesions in LF (Fig. 3). From above data we found that the use of fluorescein dye in laser fluorescence examination enhanced the contrast between artificial carious lesion and surrounding tooth structure in earlier stage of lesions. This results show promise as a means of improving the sensitivity of caries detection and diagnosis with laser fluorescence.

Radiographs[35,36], electrical resistance[37,38], and other examinations have been applied to detect the occlusal fissure caries. But occlusal caries detection remains a difficult problem[39]. In regard above result, laser fluorescence with fluorescein dye would be especially better to determine the fluorescence radiance for reading the caries on suspicious occlusal surface than laser fluorescence alone.

Not only can laser fluorescence be useful in detecting incipient caries in the future, it can also be useful in extracting the basis for whether the incipient caries should be remineralized or restored with restorative material after cavity preparation by estimation of lesion depth in vivo[40]. However, it is not yet clear as to how deep the enamel caries can be remineralized. More studies are needed in this area.

Current difficulties with this technique include assess to lesions on interproximal and occlusal surfaces, the difficult in discriminating between hypomineralization due to conditions such as fluorosis and other developmental disorder and demineralization due to dental caries[23,41,42].

Once absolute or relative values according to lesion depth can be provided by a quantified laser fluorescence test, incipient caries can be easily detected and diagnosed whether or not it can be treated.

The findings of laboratory studies, such as those outlined in this paper should be reproduced by in vivo clinical studies. Further work is also needed to determine the application of the technique to interproximal and occlusal caries detection and quantification.

Ⅴ. Conclusion

To evaluate the quantitative nature of laser fluorescence(LF) and dye-enhanced laser fluorescence

(DELF) on early enamel caries, artificial caries were induced on the bovine enamels and argon laser was irradiated on the surfaces of lesions. Fluorescence radiance from enamel specimen was recorded with CCD camera which was connected to PC image analyzing system. Yellow high-pass filter which exclude the tooth-scattered blue laser light was used to catch the fluorescent radiance from enamel specimens.

The difference of fluorescence of radiance(DFR) between the carious and the sound enamel in each sample of the LF and the DELF groups were measured by Image Pro Plus®. Polarizing microscopic examination was using as the method for validating the presence of lesions and their depth.

The DFR of lesion surfaces measured with laser fluorescence and lesion depths were evaluated and the results are as follows:

1. The caries lesions were discriminated from sound enamels by the darkness with LF and by the brightness with DELF.
2. The DFR of specimen, measured by the LF and the DEFL were increased with increasing demineralization time.
3. The DFR of the lesion surface examed by the LF and the DELF were highly related to the histological depth of the lesion.
4. The DELF was more sensitive than the LF at the earlier stage of demineralization.
5. Regression analysis showed a linear relationship between the DFR and the lesion depth; a linear equation from this relationship were as follows:
   LF : $Y = 3.97 X + 0.84$ ($r^2=0.35$)
   DELF : $Y = 4.32 X - 0.57$ ($r^2=0.73$)

From the results presented in this paper, it was concluded that laser fluorescence was a suitable technique for the detection and the quantification of early caries.

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Abstract

DIAGNOSIS OF EARLY CARIES WITH DYE-ENHANCED LASER FLUORESCENCE

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To evaluate the quantitative nature of laser fluorescence(LF) and dye-enhanced laser fluorescence(DELF) on early enamel caries, artificial caries were induced on the bovine enamels and argon laser was irradiated on the surfaces of lesions. Fluorescence radiance from enamel specimen was recorded with CCD camera which was connected to PC image analyzing system. The difference of fluorescence of radiance(DFR) between the carious and the sound enamel in each sample of the LF and the DELF groups were measured by image analyzing program.

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Key words : Initial caries, Laser fluorescence, Dye-enhancer