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Detection of subgingival calculus and dentine caries by laser fluorescence

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Objectives: Detection of subgingival calculus and dentine caries in the bottom of deep periodontal pockets is often difficult without visual observation. We thus examined the possibility of its detection using autofluorescence induced by laser irritation.

Methods: Autofluorescence was measured at various excitation and emission wavelength settings in five specimens each of sound dentine and enamel, subgingival calculus, and root caries. Periodontopathic model teeth with bacterial cells and blood clots were also irritated by laser to obtain autofluorescent images.

Results: Subgingival calculus and dentine caries showed a characteristic 700 nm emission when excited at 635 nm or a 720 nm emission when excited at 655 nm; sound dentine or enamel, however, did not. The calculus differentiation power, however, was higher with excitation at 635 nm than at 655 nm. The autofluorescent images photographed at an excitation of 633 nm provided clear calculus identification in periodontopathic model teeth when a 700 nm band-pass filter or a 700 nm high-pass filter was used. However, fluorescence intensity was masked when the calculus surface was covered by bacterial cells or blood clots. For clinical use, it would be important to remove subgingival plaque and debris from root surfaces before attempting to detect subgingival calculus and root caries with this manner.

Conclusion: The autofluorescence method employing excitation of 633–635 nm was found to be a powerful tool for detecting subgingival calculus and root caries.

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Autofluorescence is an immediate electromagnetic reaction caused by the absorption of radiation from a specific source of excitation, thus the results of emission intensity are dependent upon absorption of the excitation spectra (1). Several reports have described the autofluorescence of hard dental tissues (2), which has been found to vary according to pathological changes resulting from alterations in the structure of calcium crystals and mineral concentration, as well as from the presence of certain kinds of proteins and drugs (3). Foreman reported that pure dentine powders which were excited by a wavelength of 350 nm showed an emission peak of 400 nm (3). Van der Veen *et al*. also noted that sound dentine excited in the range of 360–470 nm showed an emission peak of 520 nm (4, 5). Kvaal and Solheim reported > 590 nm emission due to the 546 nm excitation (6).

The demineralized dentine and enamel samples have been reported to show several additional or lost peaks when compared to sound hard tissues. As observed with a confocal scanning laser microscope, 488 nm excitation by an argon laser has been found to induce 520 nm emission in sound dentine (4, 7–10), and 540 and 610 nm emissions in sound enamel (11). Decreases of the autofluorescence intensity (FI) of emission from dentine and enamel have been reported in parallel with the degree of mineral loss when excited at 488 nm (11–15), or

when excited with ultraviolet light (16). This phenomenon can facilitate the detection of dental caries lesions and is the basis of a method employing laser irritation, i.e. the quantitative light-induced fluorescence method, which is useful in the diagnosis of enamel caries lesions on accessible smooth surfaces (17–20).

On the other hand, the laser fluorescence method has been reported to be useful in the diagnosis of the diseased condition of dental hard tissues. With this method, autofluorescence from diseased lesions rather than from sound hard tissues was induced. This laser fluorescence method has been reported to enable the objective diagnosis of the initial stage of caries lesions (21–23) or the detection of subgingival calculus (24) without disturbing the lesions by hand instrumentation. However, the power of detection of disease sites by this method is still controversial.

The aim of this study was to determine the autofluorescence profiles of each component of periodontal hard tissues and diseased lesions and to pursue the possibility of detecting subgingival periodontopathic conditions, including subgingival calculus and dentine caries, on the root surfaces. The results will hopefully contribute to the creation of a diagnostic tool for unvisualized root surfaces in gingival pockets.

Materials and methods

Clinical hard tissue specimens

Extracted teeth were pooled and enrolled in the study after receiving permission from all patients, who had no history of long-term antibiotic administration. Blocks of enamel and dentine were sectioned from five submerged wisdom teeth, and then these blocks were ground to form particles less than 0.5 mm in size using a mechanical homogenizer that was cooled with liquid nitrogen (Coolmill, Toyobo Biochemicals, Osaka, Japan). Five teeth with an adequate amount of subgingival calculus were cleaned thoroughly using a toothbrush. The calculus was collected carefully using a hand scaler so as not to damage the cementum. Another five teeth with root caries which showed colored and softened surfaces with cavities were washed to remove the soft debris. The softened dentine caries were collected using an excavator with scraping by hand. The calculus and dentine caries specimens were ground under pressure in a Pyrex glass dish. The amount of each specimen was greater than 20 mg. The samples were stored at −30°C until autofluorescence measurement.

Bacterial strains and culture

Four microorganisms harbored in subgingival plaque and *Escherichia coli* cells were cultured in Brain Heart Infusion broth (Becton Dickinson and Co., Cockeysville, MD, USA) containing 0.5% yeast extract (Difco Laboratories, Detroit, MI, USA) and 20% hemolyzed human blood. *Actinomyces viscosus* ATCC19246 and *E. coli* O111 were cultured aerobically, and *Actinobacillus actinomycetemcomitans* Y4 was cultured with 5% CO_2 in air. *Fusobacterum nuclatum* ATCC25586 and *Porphyromonas gingivalis* FDC381 were cultured anaerobically using Anaeropack Kenki (Mitsubishi Gas Chemicals, Osaka, Japan).

Determination of autofluorescence properties

All hard tissue specimens were fixed on non-fluorescent glass plates with non-fluorescent petroleum gel, then placed into a Front Surface Accessory unit of a Luminescence Spectrometer LB50B (Perkin Elmer Inc., Wellesley, MA, USA). The background FI reading of the glass plate was subtracted from each result. The excitation scanning pattern was determined with an emission monochromator set at 700 nm, whereas the emission scanning pattern was determined with an excitation monochromator set at 635 or 655 nm. After measurements of autofluorescence were taken, the samples were photographed and the percentage area of hard tissue particles was calculated to normalize the FI.

Autofluorescence measurements of suspended bacteria and heparinized blood were performed using a Spectrofluorophotometer RF-1500 (Shimazu Co., Kyoto, Japan). After cultivation, the bacterial cells were washed with phosphate-buffered saline and suspended in phosphate-buffered saline at 0.3 OD_{700}. Heparinized blood samples were obtained from three volunteers after receiving informed consent and suspended in phosphate-buffered saline at 0.2 OD_{700}. The samples were immediately transferred to A-208 acrylate disposable cells (Spectrocell, Oreland, PA, USA) and autofluorescences were measured to obtain excitation and emission scanning patterns, as described above.

Autofluorescence images of periodontopathic tooth models

Teeth in which the crevicular areas of root surfaces were covered with calculus were selected as models of periodontopathic root surfaces. In some teeth, several hemispheric pits with a depth of 1 mm were prepared on the calculus with a carbide bar, these pits then being filled with blood clots and cultured bacterial cells. These model teeth were then set in front of a CCD video camera (CS8580, Tokyo Denshi Inc, Hino, Tokyo, Japan) and illuminated with defocused light from a He-Ne laser (633 nm, 10 mW; Model 1135P, JDS Uniphase, Manteca, CA, USA). Video images were then recorded using a 700 nm sharp-cut high-pass filter or a 700 $\pm$10 nm interference band-pass filter (Sigma Koki, Hidaka, Saitama, Japan). Using Photoshop 5.0 software (Adobe Systems Inc., San Jose, CA, USA), autofluorescent images were determined by subtracting background FI.

Results

Excitation and emission scanning pattern of periodontopathic components

When the emission was set at 700 nm, subgingival calculus and dentine caries showed several excitation peaks, although sound enamel and sound dentine did not (Fig. 1A). Subgingival calculus showed peaks at 402, 412, 506, 577, and 628 nm, and dentine caries samples showed peaks at 394, 527, and

629 nm. The heparinized blood samples showed small peaks at 620 and 654 nm, whereas cultured periodontopathic bacteria showed no excitation peaks (Fig. 1B). From these results, we selected 635 and 655 nm as appropriate excitation wavelengths to detect periodontopathic components in subgingival pockets for the following experiments, because those excitation wavelengths elicited autofluorescence under conditions of periodontopathy and semiconductor laser diodes were available for the development of clinical equipment.

As shown in Fig. 2(A and B), the emission scanning pattern of calculus and dentine caries recorded an emission peak at 783 nm when excited at 635 nm, or a peak at 810 nm when excited at 655 nm. However, broad peaks of sound hard tissues overlapped in the same wavelength. Subgingival calculus and dentine caries showed other broad emission peaks overlaid with excitation light at around 700 or 720 nm when excited at 635 or 655 nm, respectively. On the other hand, cultured bacteria and blood samples showed no autofluorescence from 705 to 900 nm when they excited at 635 or 655 nm (data not shown).

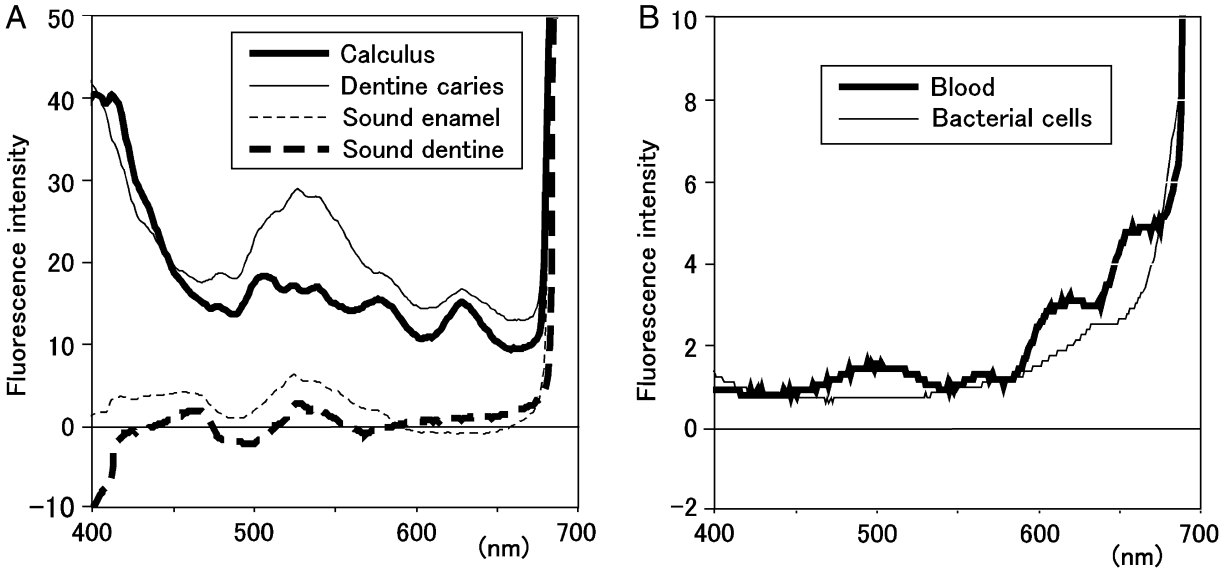

Fig. 1. Excitation scanning profiles of periodontal components in subgingival pockets. (A) Excitation scanning profile of calculus, dentine caries, and sound enamel and dentine. (B) Excitation scanning profile of heparinized blood and bacterial cells. All bacterial cells or blood showed similar excitation profiles. The excitation scanning profiles are the averages of the autofluorescences of the tested samples.

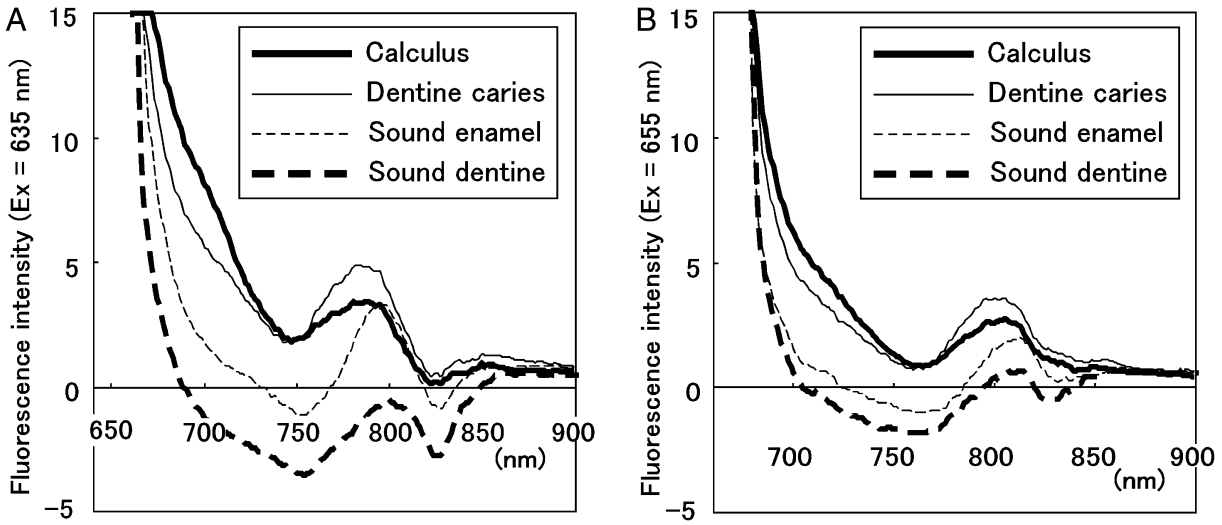

Fig. 2. Emission scanning profile of periodontal components in subgingival pockets. Emission scanning profile of calculus, dentine caries, and sound enamel and dentine were obtained with 635 nm excitation (A) or 655 nm excitation (B). Each excitation curve was calculated as the average of the tested samples.

After several repeated experiments sweeping the excitation and emission wavelength settings, we found the setting of 700 nm emission with 635 nm excitation or that of 720 nm emission with 655 nm excitation to be useful in characterizing the periodontopathic conditions of root surfaces and distinguishing from sound hard tissues.

Detection of root surface deposits by autofluorescence

The subgingival pathological components showed characteristic autofluorescence when excited at 635 or 655 nm. To determine the differentiation power for those two excitation wavelengths, we calculated the differentiation ratio ($FI_{calculus}/FI_{dentine}$) of the subgingival calculus. In Fig. 3, the excitation setting at 635 nm showed a higher capability of differentiation of subgingival calculus compared with excitation at 655 nm.

To distinguish subgingival components, we compared the integrated FIs of various wavelength ranges. In Fig. 4(A), it is shown that the integrated FI from 690 to 710 nm with 635 nm excitation resulted in good detection between subgingival calculus or dentine caries and sound dentine. Integrated FI results from 700 to 900 nm at an excitation of 635 nm (Fig. 4B) and that from 720 to 900 nm at 655 nm (Fig. 4D) showed a higher detection power for dentine caries than for subgingival calculus. Thus, the integrated FI from 690 to 710 nm with 635 nm excitation seemed to be effective in the detection of subgingival calculus in periodontal pockets.

Detection of subgingival calculus in model teeth

We next examined a periodontopathic tooth model by simulating periodontal pockets in a clinical situation. Eight extracted teeth with subgingival calculus were irritated with 633 nm of He-Ne laser. All subgingival calculus were clearly visualized by using a 700 nm sharp-cut high-pass optical filter (Figs 5C, F, and I) or by using a 700 ± 10 nm band-pass optical filter (data not shown). However, some parts of calculus which adhered as a thin layer showed faint fluorescence for visualization in this experiment. When the calculus was overlaid by cultured bacterial cells or blood clots, such fluorescence was masked and reduced (Fig. 5I).

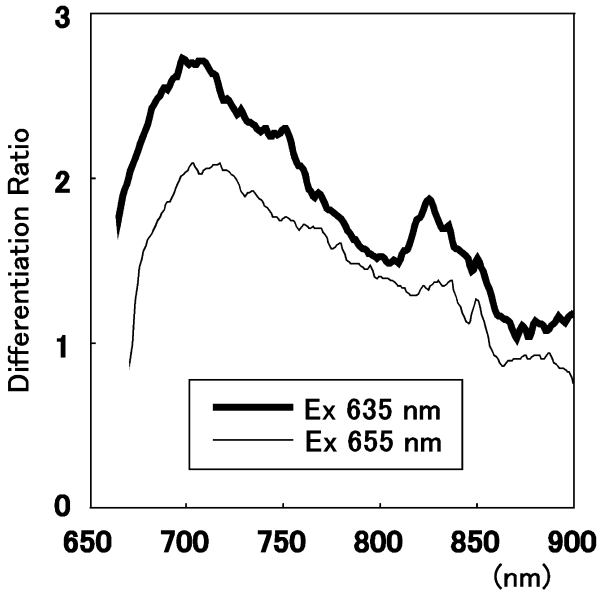

Fig. 3. Differentiation ratios of subgingival calculus. The average emission FI of subgingival calculus was divided by the emission FI of sound dentine, yielding the indicated Differentiation Ratio. Differentiation ratios were obtained with 635 nm or 655 nm excitation.

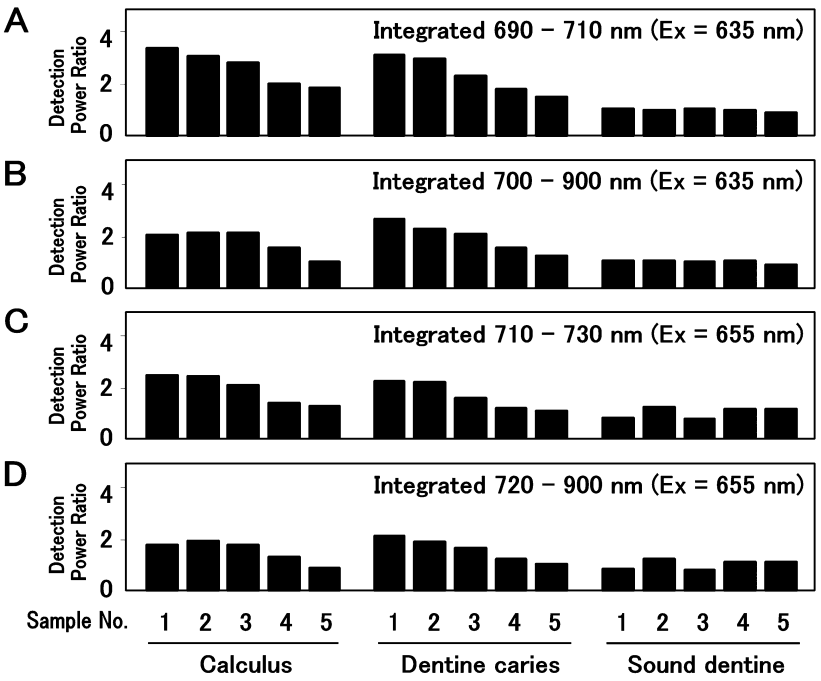

Fig. 4. Detection power ratio of subgingival components. The value of emission FI integrated from 690 to 710 nm (A) and that from 700 to 900 nm (B) were calculated when samples were excited at 635 nm. Also, the FI integrated from 710 to 730 nm (C) and that from 720 to 900 nm (D) were calculated when samples were excited at 655 nm. The Detection Power Ratio was calculated as follows: all integrated FIs were divided by the average of the integrated FI of sound dentine.

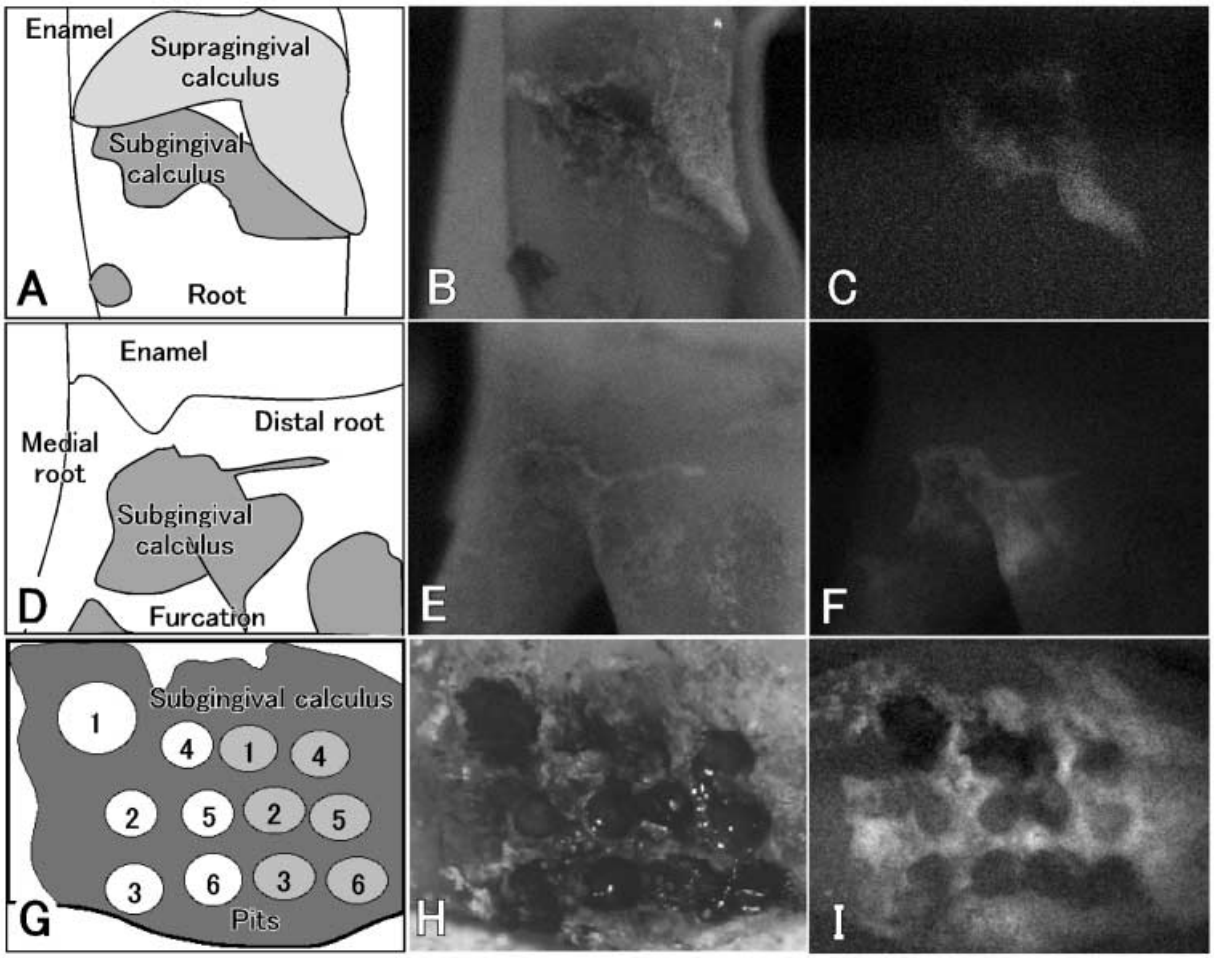

Fig. 5. Autofluorescence images of subgingival calculus at an excitation of 633 nm. Three teeth (A, B, C, or D, E, F, or G, H, I) with subgingival calculus (illustrated A, D, and G; photographs B,E,and H) were illuminated with a defocused 633 nm He-Ne laser. Autofluorescence images were photographed by using a 700 nm high-path optical filter (C, F, and I)To simulate clinical situations, we made 1 mm deep pits on subgingival calculus, the bottoms of which were within the calculus,(G, gray circle) or reached into the sound dentine, (G, white circle). All pits indicated as gray circles were confirmed to show fluorescence equal to that of intact calculus when excited by laser. Numbers indicate the contents of pits filled with cells or blood clots: 1: *Porphyromonas gingivalis*; 2: *Escherichia coli*; 3: *Actinobacillus actinomycetemcomitans*; 4: *Actinomyces viscosus*; 5: blood clots; 6: *Fusobacterium nucleatum*.

Discussion

A few reports have described the excitation scanning profiles of subgingival calculus. Clarke noted that calculus samples from 28 domestic cats were excited by infrared light (25). When comparing the excitation scanning profiles of sound dentine and subgingival calculus, we found that the 635 nm excitation enabled the unique detection of subgingival calculus while sound dentine was not excited. Furthermore, the excitation scanning profiles of dentine caries were similar to those of subgingival calculus. With regard to this result, subgingival calculus and dentine caries had the same excitation peaks at around 629 nm, whereas sound dentine and enamel did not show any peaks near that excitation wavelength. For the construction of subgingival calculus detection devices for clinical application, we have to select a light source whose wavelength is around 629 nm. As the light sources for such a device, the He-Ne laser (633 nm) and the AlGaInP system laser diode (630–680 nm) are available. Considering ease of operation and commercial availability, a 635 nm or 655 nm laser diode should be selected for the excitation light sources, since they are commonly used in digital optical disk systems. Considering all these factors, our study was carried out with 633–635, and 655 nm excitation as the light source.

Our results also showed that subgingival calculus and dentine caries demonstrated characteristic broad 700 and 785 nm peaks in response to 635 nm excitation. When compared with the emission scanning profile of sound dentine, the peak at 700 nm was considered to be a good marker for both types of subgingival pathogenic structures. Taubinsky *et al.* also reported the same FI profile of around 700 nm for subgingival calculus and dentine caries specimens when excitation of 633 nm was used (26). On the

other hand, there was no detectable fluorescence excited at 635 nm from cultured bacteria, blood, or blood clots. From the autofluorescence images of periodontopathic teeth, we demonstrated that the bacterial cells and blood clots masked the characteristic 700 nm emission light of subgingival calculus. From this result, we considered that application of washing systems would be required to remove bacterial soft deposits or blood before attempting to examine root surfaces in periodontal pockets *in situ*.

The fluorophore content of subgingival calculus and caries lesions might be different, since calculus is a mineralized calcium deposit and not the result of demineralization. Dolowy *et al*. reported that metal-free porphyrins in dog, cat, and human calculus specimens seemed to be the major source of emissions when excited in the range of 405–430 nm (27). Furthermore, Foreman reported that a fluorophore of dentine excited by ultraviolet light was tryptophan (3). In the present study, we grained the dentine samples at an ultra-low temperature and kept them wet while excluding any fixation reagents, as we considered that this preparation method would have no effects on the dentine crystal structure or organic dentine components. Further study is needed to determine which fluorophore in periodontopathic root surface deposits elicits autofluorescence at 635 and 655 nm when this sample preparation is used.

In clinical situations, hard laser radiation devices are used for root surface debridement (28–30). Our study also indicated the possibility of detecting diseased sites in periodontal pockets by laser devices (31). Folwaczny *et al*. reported use of a fiber optic device with a 655 nm laser diode to detect subgingival calculus (24). However, our results indicated improved detection of calculus by use of a wavelength of 633–635 nm. Thus, the emission intensity at 635 nm with a 700 nm band-pass filter is appropriate for subgingival calculus detection, whereas that at 635 nm with a 700 nm high-pass filter is apparently suitable for dentine caries detection. Application of plastic optical fiber would facilitate the construction of visible-light oriented equipment for this purpose. Moreover, new technology has become available with the introduction of a 405 nm blue GaN laser diode for detection of subgingival calculus and dentine caries. Thus, a 635 and 405 nm combination laser diode system may be able to provide a more accurate diagnosis of periodontopathic deposits on invisible root surfaces in periodontal pockets.

In conclusion, we obtained scanning profiles of autofluorescence of each periodontal component and confirmed the feasibility of using a 633–635 nm light source and a 700 nm fluorescence detector for the clinical detection of subgingival periodontopathic condition. However, additional study and clinical trials are required to confirm if this detection system is able to perform well in all clinical situations.

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