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Original Article

치주염 유발 동물모델에서 정량광형광검사 적용 가능성에 대한 예비 연구

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Pilot Study on the Usefulness of Quantitative Light-Induced Fluorescence-Digital for Dental Plaque Assessment in a Rat Model of Periodontitis

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Abstract

Objective: This study quantifies dental plaque in an animal periodontitis model using quantitative light-induced fluorescence-digital (QLF-D), providing fundamental data for establishing diagnostic criteria for periodontitis. **Methods:** Periodontitis was induced by ligating the mandibular first molars of Sprague–Dawley rats. After 18 days, QLF-D images showing the plaque on the ligatures were obtained intraorally before sacrifice and extraorally after sacrifice. The red and blue fluorescence values were measured using software, and the average red/blue (R/B) ratio was calculated for each image. Alveolar bone loss was evaluated histologically. **Results:** Ligation of the first molars resulted in an increase in the periodontal ligament area compared to that of the control group, demonstrating alveolar bone resorption. The extraoral lingual-side images obtained after sacrifice were reproducible and provided clear assessment criteria. The R/B ratio of the plaque accumulated on the ligatures was 1.9±0.3. **Conclusions:** Plaque formation, which is difficult to assess by visual inspection alone, can be evaluated using QLF-D. The animal model of periodontitis used in this study can be used to obtain preclinical data to develop future preventive and therapeutic strategies for periodontal diseases.

Keywords: Dental plaque, Experimental animal model, Periodontal disease, Quantitative light-induced fluorescence

색 인: 치면세균막, 동물모델, 치주질환, 정량광형광검사

1. Introduction

Periodontal disease encompasses a spectrum of inflammatory conditions that affect the supporting structures of the teeth. The main cause of periodontal disease is the formation of dental plaque, and chronic accumulation of dental plaque can lead to inflammation of the periodontal tissues(1, 2). Periodontitis, which is characterized by gingival inflammation, loss of periodontal attachment, and alveolar bone resorption, is prevalent worldwide and presents a significant public health burden.

Various animal models have been used to study the mechanisms underlying periodontal disease, its association with systemic conditions, and the therapeutic effects of substances(3, 4). Animal models of periodontitis can be obtained using various methods, including 1) induction of plaque formation via ligature placement, 2) topical application of lipopolysaccharides to periodontal tissues, and 3) oral inoculation of periodontal disease-causing bacteria or lipopolysaccharides. Among these, the ligature-induced periodontitis model has the advantage of facilitating the natural deposition of plaque around the molars or premolars, similar to dental plaque formation in humans. In addition, the ligature-induced periodontitis model exhibits alveolar bone loss and connective tissue inflammation during the initial period (within 7 days) of periodontitis induction(5). This enables the development of periodontal disease by promoting plaque accumulation along with normal chewing activities, thereby allowing the observation of seasonal variations in symptoms.

Detecting and predicting the pathogenic levels of dental plaque, which is the main cause of periodontitis, are important to prevent and manage periodontitis. In clinical practice, various plaque indices are used to assess the formation and removal of dental plaque(6, 7). However, observing dental plaque with the naked eye is difficult(8). Previous histopathological and micro-computed tomography studies have assessed connective-tissue and bone loss and cytokine levels in the serum and gingival tissues using animal models of periodontitis(9, 10). However, these methods primarily focus on periodontal tissue changes caused by dental plaque formation without confirming the presence of the plaque itself. Moreover, studies assessing plaque formation in animals are limited compared to those in humans because of the lack of effective, non-invasive diagnostic tools.

Recently, quantitative light-induced fluorescence (QLF) has been used as a diagnostic tool for dental caries and plaque. QLF uses visible light at 405 nm to detect and quantify early demineralized lesions by measuring the differences in the natural fluorescence of sound dental tissue and areas of mineral loss. Furthermore, it can detect the red fluorescence emitted by porphyrins, which are metabolic by-products secreted by the oral bacteria (11). This allows evaluation of dental plaque without using plaque-disclosing agents(12, 13). Older dental plaque tends to exhibit a stronger red fluorescence, and the fluorescence intensity can be quantified and measured to assess the severity of plaque buildup(14).

Observing the red fluorescence emitted from sites where dental plaque has been induced in animal models of periodontal disease could lay the foundation for developing assessment criteria for periodontal disease in animal models. Therefore, this study aimed to evaluate the usefulness of QLF-digital (QLF-D) for assessing plaque formation in an animal model of periodontal disease.

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2. Materials and Methods

2.1. Animals

Male Sprague–Dawley rats (7 weeks old) were purchased from Central Lab. Animal Inc. Rats were housed in specific pathogen-free conditions at constant temperature (22°C) and humidity of 40- 70%, and a 12 h light and dark cycle. Rats were fed standard chow and water ad libitum. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University (Approval Number: 2023-0138).

2.2. Induction of periodontitis

For the periodontitis experiments, rats randomly divided into two groups: control (n=5) and periodontitis (n=4). Periodontitis was induced by placing dental-floss (Essentialfloss OralB, The Procter & Gamble Company, OH, USA) ligatures around the mandibular first molars and was maintained for 18 days. To observe periodontitis induction after ligation, we measured the distance from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) distal to the first molar and the residual alveolar bone (AB) area in the first-molar furcation. The presence of dental floss and body weight were checked daily during the experimental period.

2.3 Assessment of red fluorescence intensity of plaque

Eighteen days after ligature placement, the first molar was photographed using QLF technology (QLF-C System, AIOBIO, Seoul, Korea) to evaluate plaque formation under different conditions using the following settings: shutter speed, 1/10 s; aperture value, 10.0; and ISO, 1600. 1) To detect dental plaque under intraoral conditions, the rats were positioned in a specially designed light-proof box and photographed after opening their mouths (Figure 1A) under inhalation anesthesia using 2% isoflurane in oxygen (2 L/min) for 2 min. 2) The mandible was extracted and photographed in the dark to detect dental plaque under extraoral conditions (Figure 1B). 3) To confirm whether the ligated dental floss exhibited red fluorescence outside the oral cavity, the floss was retrieved from the teeth, collected in a 96-well black plate, and photographed in dark (Figure 1C). Q-Ray Clinical Software (AIOBIO, Seoul, Korea) was used to automatically capture and store all digital images on a personal computer. The region of interest (ROI) for analyzing the red fluorescence of the plaques was in the cervical region of the tooth near the dental floss, excluding the knot. The red and blue fluorescence values for each pixel within the ROI were measured using ImageJ software (National Institute of Health, Bethesda, MD, USA), and the average red/blue (R/B) ratio was calculated for each image. Plaque fluorescence intensity was calculated by multiplying the R/B ratio by the fluorescent ligature area to represent the comprehensive fluorescence properties of the floss on each tooth. All the analyses were performed by a single trained examiner.

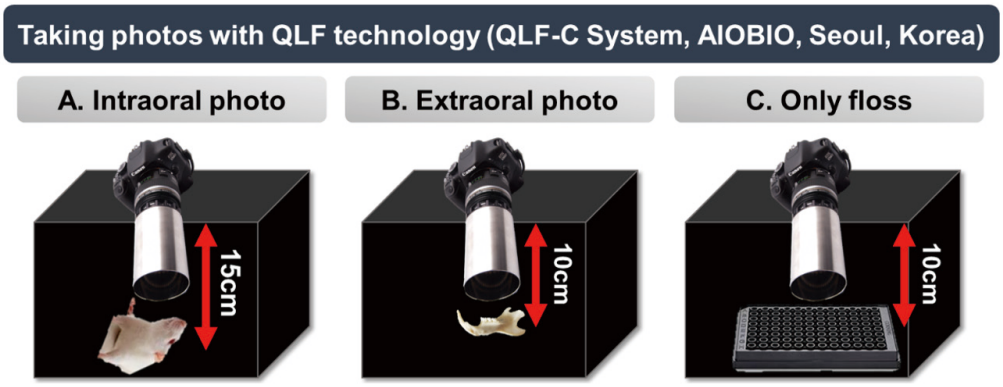

Figure 1. Experimental design for evaluating plaque formation. The procedure was conducted in a sequential manner. (A) Fluorescence emitted intraorally from a supine rat was captured in a light-proof box. (B) Fluorescence emitted from the mandible was captured immediately following extraction in the dark. (C) Fluorescence emitted by individual pieces of dental floss placed in a black 96-well plate was captured in the dark.
QLF-D: Quantitative light-induced fluorescence-digital.

2.4. Histological analyses

After euthanasia, the extracted mandibles were fixed with 10% neutral-buffered formalin for 1 day. The mandibles were decalcified using 10% ethylenediaminetetraacetic acid for 2 months, embedded in paraffin, and serial 4 μm thick sagittal sections were prepared. Sections were selected based on the clear appearance of the dental pulp of the mesial and distal roots of the first molars and stained with hematoxylin and eosin (H&E). To quantify alveolar bone loss, the distance from the CEJ to the ABC on the distal side and the percentage of periodontal ligament (PDL) area in the ROI, which was 0.8 mm below the top of the furcation were measured under X10 magnification(5,10). H&E stained slides were scanned using a ZEISS Axioscan 7 (Carl Zeiss, Jena, Germany) and analyzed using ZEN blue edition software, version 3.4. (Carl Zeiss, Jena, Germany).

2.5. Statistical analysis

Data were compared between the groups using the Mann Whiteny U -test. All values are expressed as the mean ± standard error. SPSS 26.0 statistical software (IBM Corp., Armonk, NY USA) was used for statistical evaluation. Statistical significance was set at $p<0.05$.

3. Results

3.1. Detection of dental plaque

To ensure reproducibility and consistency of imaging, we systematically tested various photographic conditions. Prior to euthanasia, photographs were captured within a specially designed lightproof box to verify the intraoral fluorescence expression of the biofilm. Although the ligatures surrounding the teeth exhibited red fluorescence, obtaining uniform images of the same area from individual animals was challenging because of technical constraints

(Figure 2A-D). To mitigate this issue, the extracted mandibles were positioned horizontally on a flat surface for photography (Figure 2E-H).

Red fluorescence was detected around the dental floss placed around the first molar, whereas the fur showed blue fluorescence (Figure 2B, 2D). Extraoral photographs revealed red fluorescence around the dental floss placed around the first molar (Figure 2F, 2H).

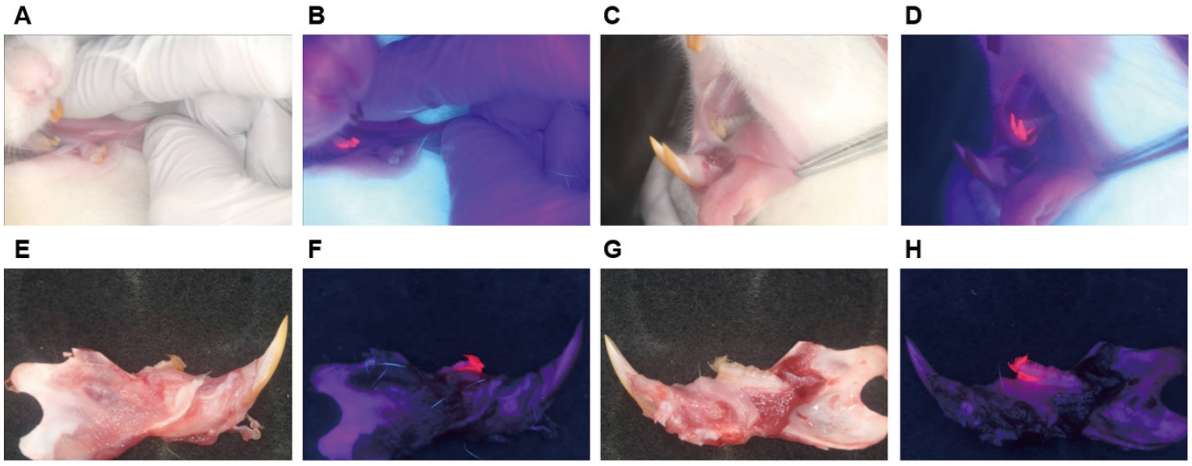

Figure 2. Detection of dental plaque using QLF-D. (A–D) Fluorescence emitted intraorally from a rat positioned supine was captured in the dark (Figure 1A). (A) Intraoral photo of the left first molar without QLF. (B) Intraoral photo of the left first molar with QLF. (C) Intraoral photo of the right first molar without QLF (D) Intraoral photo of the right first molar with QLF. (E–H) Fluorescence emitted from the mandible was captured immediately following extraction in the dark (Figure 1B). (E) Extraoral photo of the left first molar without QLF. (F) Extraoral photo of the left first molar with QLF. (G) Extraoral photo of the right first molar without QLF. (H) Extraoral photo of the right first molar with QLF.
QLF-D: Quantitative light-induced fluorescence-digital.

3.2. Alveolar bone loss

The distance from the CEJ to the ABC in the periodontitis group was 0.6 ± 0.1 mm while it was 0.2 ± 0.1 mm in the control group (Figure 3A-C). The AB area in the periodontitis group was 28.0 ± 9.9%, whereas that in the control group was 53.2±8.7% (Figure 3D-F).

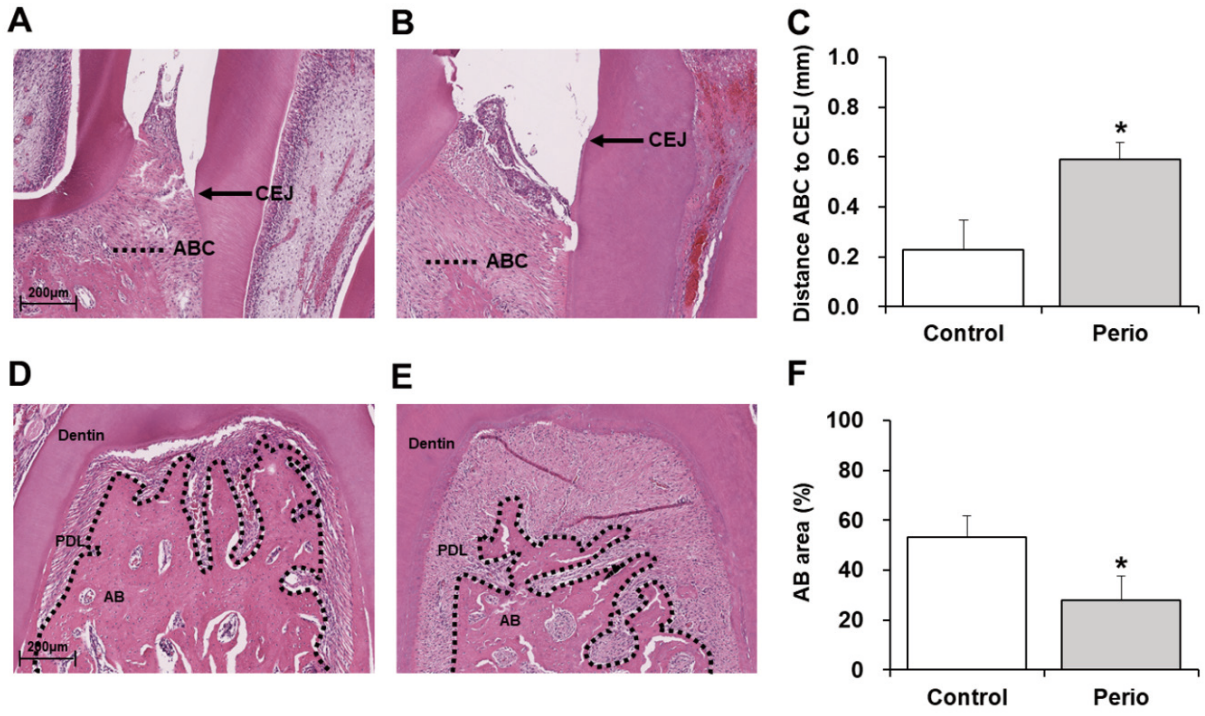

Figure 3. Alveolar bone loss. (A–C) Representative images and measurements of the distance from CEJ to ABC. The dotted line indicated ABC. The black arrow indicated CEJ. (A) Control group. (B) Periodontitis group. (D–F) Representative images and measurements of AB area in furcation. Excluding the bone marrow region, the dense area of deep pink within the black dotted line represents AB. (D) Control group. (E) Periodontitis group. Scale bars: 200 μm. Data are presented as mean±SD. *Significant difference compared to the Control group(p<0.05).
CEJ: Cementoenamel junction, ABC: Alveolar bone crest, AB: Alveolar bone

3.3. Fluorescence intensity of plaque

Owing to the anatomical structure, ligatures photographed from the buccal side showed only a partial view (Figure 2F). Therefore, lingual-side photographs that clearly depicted the ligature around the tooth were selected for analysis (Figure 2H).

The R/B ratio of plaque in the ROI in the periodontitis group was 1.9±0.3, and the plaque fluorescence intensity was 25.3 ± 5.6. QLF-D revealed that dental floss ligated to the teeth showed red fluorescence, whereas dental floss that was not ligated to the teeth did not show fluorescence (Figure 4C, 4F).

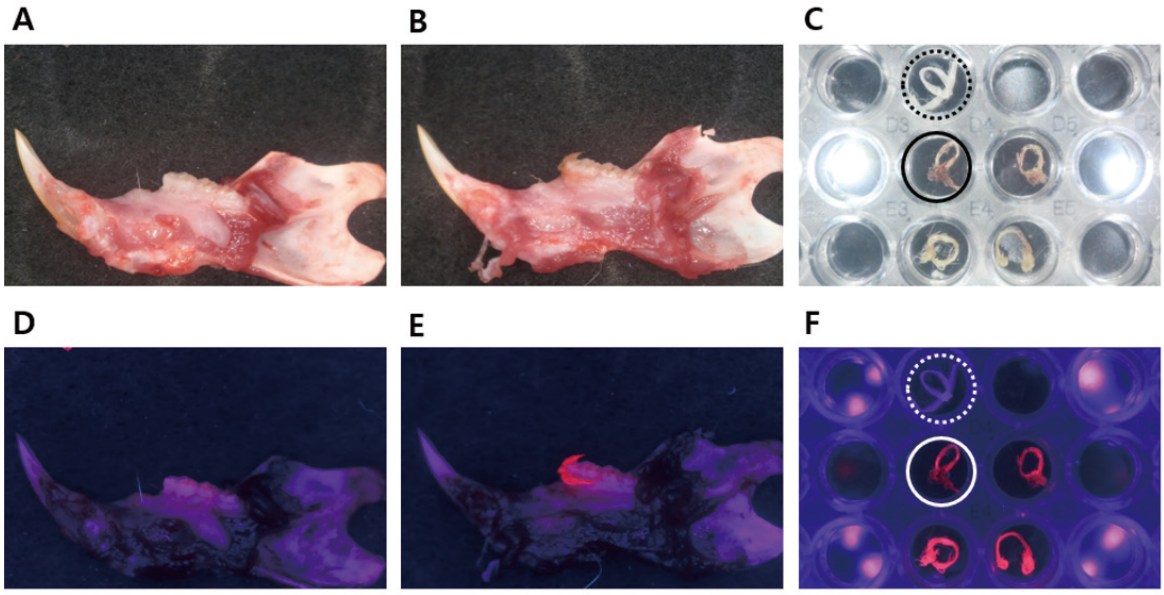

Figure 4. The fluorescence of plaque. (A) Left first molar without QLF in the Control group. (B) Left first molar without QLF in the Periodontitis group. (C) Only ligature without QLF. (D) Left first molar with QLF in the Control group. (E) Left first molar with QLF in the Periodontitis group. (F) Only ligature with QLF. The dotted circle indicates dental floss that is not ligatured in the oral cavity. A solid line circle indicates dental floss ligatured around the first molar.
QLF-D; Quantitative light-induced fluorescence-digital.

4. Discussion

This study assessed the presence of dental plaque in an animal model of periodontitis by evaluating the red fluorescence emitted from biofilms surrounding ligatures. The results confirmed the effectiveness of QLF for visualizing dental plaque *in vivo*. Because dental plaque is a major etiological factor in periodontal diseases, plaque autofluorescence imaging serves as an objective and convenient method for evaluating plaque in humans(11). The red fluorescence observed using QLF is primarily attributed to the presence of porphyrins. Certain bacteria involved in dental plaque formation and periodontal diseases, including the genera *Treponema, Prevotella,* and *Fusobacterium* detected in red fluorescent plaque(15). The intensity and distribution of red fluorescence can provide insights into the presence and extent of microbial activity, offering a noninvasive and effective method for assessing oral health and periodontal-disease progression(11, 12).

This study found that strong red fluorescence and alveolar bone loss were observed in the ligature-induced periodontitis model. The R/B value, which quantifies the fluorescence intensity of the biofilm, was 1.9 in plaque 18 days after ligature placement. Our results are consistent with those of Kim YS et al. and Lee ES et al.(14,16), which showed that biofilms matured for more than 3 days expressed fluorescence with an R/G value of 1.35 or higher. Lee ES et al.(15) has also indicated that not all biofilms emit red fluorescence, suggesting that those emitting red fluorescence are more mature and have been cultured for at least 3 days. The intensity values are correlated with the extent of microbial activity, further demonstrating the usefulness of QLF in periodontal research. The findings of this

study suggest that QLF is a valuable tool for detecting the etiological factors of periodontal disease in animal models.

During the induction of periodontitis in rats by ligation, plaque accumulates around the ligature, inducing inflammation that leads to alveolar bone loss(5). Kim et al.(4) have shown that infiltration of inflammatory cells and osteoclast formation occur 3 days after ligation, and that osteoclast formation peaks on day 7 and decreases thereafter. In contrast, alveolar bone loss begins 3 days after ligation and continues for 60 days(17). In this study, ligation was maintained for 18 days, resulting in observable alveolar bone loss. This observation is consistent with the findings of Lin P et al. and Khuda F et al.(3, 4) and highlights the validity of the model for mimicking human periodontal disease.

Several studies have examined the oral microbiota in ligature-induced periodontitis models(18, 19). In rats with periodontitis induced for 42 days, high proportions of human host-compatible species, such as *V. parvula*-, *Streptococcus*-, and *Actinomyces*-like species, were observed on the ligature(18). A whole-genome sequencing approach using 16S rDNA demonstrated that *Proteobacteria* and *Fusobacteria* were the most abundant in the orthodontic wire ligation model(19). The oral microbial communities in the ligation model were significantly different from those in the controls, indicating a shift from a symbiotic to dysbiotic microbial community structure.

This study had some limitations, including the small number of experimental animals and the lack of microbiome analysis. Future research should address these limitations by including a larger number of experimental animals and incorporating microbiome analyses to enhance the comprehensiveness and reliability of the results.

5. Conclusion

This study aimed to quantify dental plaque in an animal model of periodontitis using QLF-D and provide foundational data for establishing diagnostic criteria for periodontitis. The findings are as follows:

1. The results showed that ligation was maintained for 18 days, leading to alveolar bone loss. The distance from the CEJ to the ABC in the periodontitis group was significantly higher than in the control group(p<0.05), while the AB area in the periodontitis group was significantly lower than in the control group(p<0.05)

2. The extraoral lingual-side images obtained after sacrifice were reproducible and provided clear assessment criteria.

3. QLF-D detected that dental floss ligated to the teeth emitted red fluorescence and the R/B ratio of plaque in the periodontitis group was 1.9±0.3.

In summary, this study is meaningful, because it is the first to provide evidence for the use of QLF-D as a new detection device in an animal model of periodontitis. Therefore, this technology can be effectively used to obtain preclinical data for the development of future preventive and therapeutic strategies for periodontal diseases.

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Author contributions

Conceptualization: Kim JH, Bak EJ; Data curation: Kim MS; Formal analysis: Kim AR; Funding acquisition: Kim JH; Investigation: Kim MS, Kim AR, Kim JH; Methodology: Bak EJ, Kim JH; Project administration: Kim JH; Resources: Bak EJ, Yoo YJ; Software: Kim JH; Supervision: Yoo YJ; Validation: Bak EJ; Visualization: Kim MS; Writing - original draft: Kim JH, Kim AR; Writing - review & editing: Kim JH, Kim AR.

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