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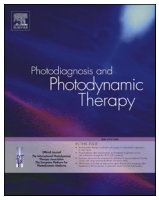

Research Paper

Efficient gingival health screening using biofluorescence of anterior dental biofilms

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ABSTRACT

Purpose: In study, we aimed to evaluate the biofluorescence of anterior dental biofilms using Quantitative Light-induced Fluorescence (QLF) technology to screen for gingival health.
Methods: Fifty-five ($n = 55$) adult participants aged ≥ 20 years with gingivitis were included in this study. Fluorescence images of the upper and lower anterior teeth were obtained using Qraycam Pro, a device based on the QLF technology. To evaluate the biofilm fluorescence deposition level, the percentage of the dental biofilm area detected using red biofluorescence was calculated relative to the total surface area of the anterior teeth. To evaluate the gingival health status, the Silness-Löe plaque index (PI), Löe -Silness gingival index (GI), and bleeding-on-probing index (BOP) were assessed. Pearson's correlation and logistic regression analyses were performed to examine the relationship between biofilm biofluorescence and the gingival health status.
Results: The group with the larger fluorescent biofilm area exhibited significantly higher GI and BOP values ($p < 0.05$) than the group with the smaller fluorescent biofilm area. Furthermore, significant correlations were found between the fluorescent biofilm area and GI, BOP, and PI ($r = 0.422$, $r = 0.376$, and $r = 0.499$, respectively; $p < 0.05$). Logistic regression analysis adjusted for sociodemographic variables, smoking, and alcohol consumption showed that the odds ratio for moderate gingivitis in the group with a larger fluorescent biofilm area was 6.07 compared with that in the group with a smaller area ($p < 0.05$).
Conclusion: Evaluating the biofluorescence of anterior dental biofilms using QLF technology is an effective indicator for screening gingival health and identifying individuals at a high risk for gingivitis.

1. Introduction

Teledentistry is no longer an unfamiliar concept in the dental field. Historically, the adoption of remote care in dentistry has progressed slower than in other areas of healthcare. However, the global experience of the pandemic has led to a surge in research on the application of teledentistry in dental practice [1,2]. Beyond its utility during lockdowns or pandemics, teledentistry is increasingly recognized for its potential to reduce healthcare disparities and enhance equitable access to oral health services [3,4].

Teledentistry can be conducted as real-time consultation (synchronous) or store-and-forward (asynchronous) [1]. In the store-and-forward approach, patients provide information such as photographs or videos, which clinicians then evaluate to determine subsequent actions. An essential part of remote-care protocols is the screening process to identify patients with potential issues. Recently, an artificial inteligence-based smartphone application was reported to analyze facial actions for the early detection of symptoms in stroke patients, demonstrating its potential utility [5]. Similarly, mobile application technology that analyzes images of skin lesions captured using smartphones is considered a simple and inexpensive solution for the early detection and treatment of skin cancers [6]. These screening technologies operate effectively in environments with limited information and resources, and the development of methods for rapid and accurate patient screening is important in teledentistry. However, previous studies have identified that the primary challenge faced by dental professionals in teledentistry is the inability to perform essential diagnostic procedures such as percussion or palpation in remote settings [1]. To overcome these problems, it is essential to provide useful information for screening patients with pathological conditions in limited situations.

There are two broad categories of tests used for disease detection: diagnostic and screening tests. The primary purpose of screening tests is

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to detect the disease before the appearance of clinical signs. Screening tests are particularly useful when the early detection of a disorder can either prevent its progression to a more severe clinical form or enable effective treatment after identification [7]. Gingivitis, a representative oral disease, is an effective candidate for early diagnosis through screening tests as it satisfies both of these conditions. Various methods, including surveys and saliva-based biomarkers, have been used to screen for periodontal diseases [8,9]. Recent technological advancements have spurred research on screening methods using photographic analysis and machine learning [10–12]. Studies employing deep learning approaches for gingivitis screening have shown promising results [11,12]. However, in this study, we focused on exploring a screening method that relies on a single photograph to assess the gingival health status, prioritizing accessibility and cost-effectiveness. Specifically, we aimed to determine whether a single image of the anterior region alone could sufficiently reflect the overall oral health status, emphasizing its simplicity and adaptability for remote applications.

Previous studies investigating the concordance between clinical examinations and photographic assessments have reported that, in the case of gingival examinations, the level of agreement tends to be lower than that in other assessments [13]. This is because the symptoms of gingivitis, such as changes in gingival color and gingival bleeding [14], are challenging to accurately assess based solely on images. One potential approach to overcoming this limitation is the use of biofluorescence. In gingivitis, dental biofilm formation is a critical factor in the onset and progression of the disease [14]. Using visible light at a wavelength of 405 nm, red biofluorescence caused by porphyrins, which are metabolites secreted by oral bacteria, can be detected. Red biofluorescence can be evaluated using quantitative light-induced fluorescence (QLF) technology to assess the pathogenicity of dental biofilms. Red biofluorescence detected in dental biofilms is closely associated with dental caries and gingivitis and can be utilized to evaluate the pathogenicity of dental biofilm [15–18].

Therefore, we aimed to screen gingival health by evaluating the biofluorescence of dental biofilms on anterior teeth using QLF technology and investigating its relationship with clinical parameters (PI, GI, and BOP) of the entire dentition, including posterior teeth, in patients with gingivitis. The null hypothesis of this study was that the red biofluorescence of dental biofilms on the anterior teeth was not associated with the severity of gingivitis in the entire oral cavity.

2. Materials and methods

2.1. Subjects

This study was approved by the Institutional Review Board (IRB) of Yonsei University Dental Hospital to ensure the ethical protection of participants (IRB No. 2–2020–0108). The participants were healthy individuals aged 20 years or older who voluntarily expressed willingness to participate. All participants received both written and verbal explanations about the study's purpose, methods, confidentiality, and their right to withdraw from participation, and they provided written informed consent. The following inclusion criteria were applied to recruit participants with gingivitis: at least 20 remaining natural teeth and those with gingivitis. In addition, the following exclusion criteria were applied: pregnant or breastfeeding individuals, individuals with severe pathological conditions in the oral tissues (e.g., oral cancer or oral inflammation), individuals with severe periodontitis or multiple dental caries, and individuals with five or more teeth requiring immediate dental caries treatment.

2.2. Biofluorescence imaging

2.2.1. Image acquisition

To evaluate the biofluorescence characteristics of dental biofilms, images were captured using a digital camera equipped with QLF technology (Qraycam Pro, Aiobio, Seoul, Republic of Korea). For all the participants, images of the anterior region were obtained using edge-to-edge occlusion (Fig. 1).

2.2.2. Image analysis

Biofluorescence analysis of the anterior dental biofilms was performed using fluorescence images of the anterior region (Fig. 2). All images were saved as JPEG files and analyzed using an image analysis program (Image-Pro Plus® v6.0, Media Cybernetics Inc., Bethesda, MD, USA). From all the teeth captured in the anterior region, areas of interest (AOI) were defined around the anterior teeth (maxillary and mandibular central incisors, lateral incisors, and canines). Areas involving restorations were excluded from the AOI to avoid potential interference caused by differences in natural fluorescence and fluorescence emission between natural teeth and restorative materials. Subsequently, within the defined AOI, red biofluorescence was detected using the eye-dropper tool built into the analyzer to identify all pixels of the same color. Finally, the red biofluorescence area (RF area) was calculated as the percentage of the red biofluorescence detected dental biofilm area relative to the total AOI area. To compare the oral health status (plaque index, gingival index and bleeding on probing) based on the distribution of red fluorescence (RF area), participants were categorized into two groups according to the median RF area value (3.5): a low red biofluorescence (low-RF) group and a high red biofluorescence (high-RF) group.

2.3. Examination of oral health status

2.3.1. Plaque index

The plaque index (PI) was assessed for all teeth excluding the restorations. Each tooth was divided into six sites for evaluation (disto-, mid-, mesiobuccal, and disto-, mid-, and mesiolingual). After drying the teeth, a dental mirror and periodontal probe were used to score plaque accumulation according to the criteria of Silness and Löe [19] on a scale of 0 to 3: 0= no plaque; 1= a film of plaque along the gingival margin; 2= moderate accumulation of soft deposits within the gingival pocket; and 3= an abundance of soft matter along the gingival margin and within the gingival pocket. The mean PI score of the assessed sites was calculated and used as a representative value for each participant.

2.3.2. Gingival index

The gingival index (GI) was scored on a scale of 0 to 3 according to the criteria of Löe and Silness [19]: 0= normal gingiva; 1= mild inflammation (slight change in color and edema but no bleeding on probing); 2= moderate inflammation (redness, edema, glazing, and bleeding on probing); and 3= severe inflammation (marked redness, edema, ulceration, and tendency for spontaneous bleeding). The evaluation targeted the index teeth (#12, # 16, # 24, # 32, # 36, and # 44), implants, and rearmost molars. Each tooth was divided into six sites (disto-, mid-, mesiobuccal, and disto-, mid-, and mesiolingual) for assessment. The mean GI score for all the assessed sites was calculated and used as a representative value for each participant. To examine differences based on gingivitis severity, participants were categorized into mild and moderate gingivitis groups based on a clinical threshold of 1.0, according to the established evaluation criteria [20].

2.3.3. Bleeding on probing

Bleeding on probing (BOP) was assessed using a periodontal probe to evaluate the presence or absence of bleeding within 10–30 s after probing. A score of 0 was assigned if no bleeding occurred, and 1 was assigned if bleeding was present. The evaluation sites were the same as those used for the GI. The mean BOP score for all the assessed sites was calculated and used as a representative value for each participant.

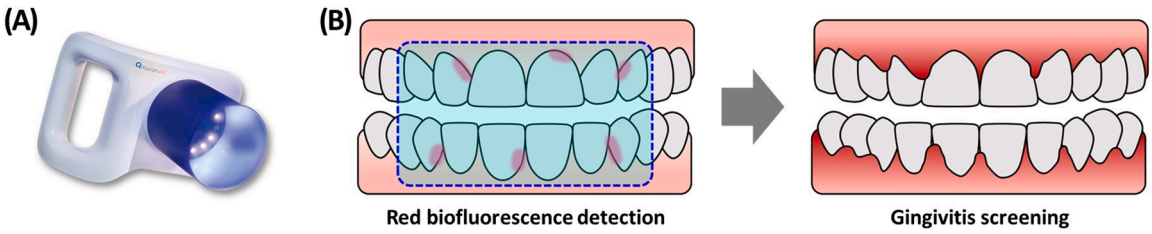

Fig. 1. Workflow of red biofluorescence detection for gingivitis screening. (A) Quantitative light-induced fluorescence (QLF) device (Qraycam Pro) was used for imaging. (B) Schematic representation of gingivitis risk screening for the entire oral cavity based on red biofluorescence detection in anterior teeth.

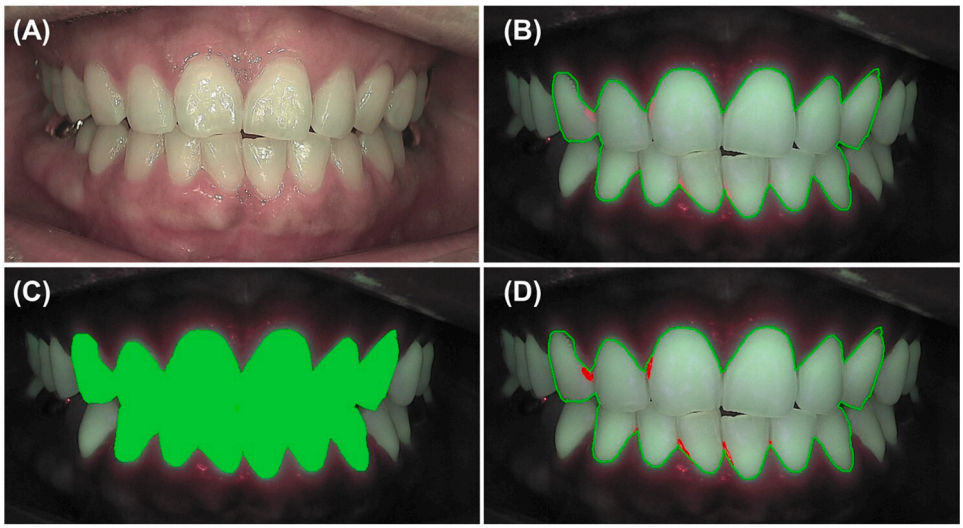

Fig. 2. Quantitative light-induced fluorescence (QLF) image analysis process. (A) White-light image. (B) An area of interest around the anterior teeth. (C) Total area of anterior teeth. (D) Red biofluorescence area of anterior teeth.

2.4. Questionnaires

Self-administered questionnaires were used to collect sociodemographic information from all participants. The questionnaire items included educational level, household income, current systemic diseases, and smoking and alcohol consumption.

2.5. Statistical analysis

The sociodemographic characteristics of the participants were analyzed as categorical variables and are presented as n (%). Comparisons of oral health status (PI, GI, and BOP) between the RF groups were conducted using independent *t*-tests, and the correlations between oral health variables and the RF area were evaluated using Pearson's correlation analysis. Additionally, to assess the impact of RF on gingivitis severity, logistic regression analysis was performed. Model 1 was a crude model without adjustments, whereas Model 2 was adjusted for age, sex, educational level, household income, and the number of systemic diseases. Finally, Model 3 was further adjusted to include smoking and alcohol consumption in addition to the variables in Model 2.

3. Results

3.1. Participant characteristics

The characteristics of the 55 participants are presented in Table 1. Among the participants, those in middle adulthood accounted for the largest proportion (56.4 %) and the majority were female (67.3 %). Regarding monthly household income, 36.4 % of the participants reported a monthly income of 5 million KRW or more, which is above the

Table 1
Participant characteristics.

Variables		n (%)
Age		
	<40	13 (23.6)
	40–64	31 (56.4)
	>64	11 (20.0)
Sex		
	Male	18 (32.7)
	Female	37 (67.3)
Education		
	<Middle school	6 (10.9)
	High school	11 (20.0)
	University	29 (52.7)
	>University	9 (16.4)
Monthly household income (10,000 KRW)		
	<300	16 (29.1)
	300–500	19 (34.5)
	>500	20 (36.4)
No. systemic disease		
	0	35 (63.6)
	1	12 (21.8)
	>2	8 (14.5)
Smoking		
	Never	48 (87.3)
	Former	2 (3.6)
	Current	5 (9.1)
Alcohol consumption		
	No	37 (67.3)
	Yes	18 (32.7)

national average. Additionally, most participants (87.3 %) had no history of smoking and 67.3 % reported not consuming alcohol.

3.2. Oral health status according to RF

The GI was approximately 21.6 % higher in the high-RF group than in the low-RF group, showing a statistically significant difference ($p = 0.049$, Table 2). Similarly, BOP was 48.0 % higher in the high-RF group than in the low-RF group, with a statistically significant difference ($p = 0.029$). For the PI, the high-RF group showed a mean value 0.15 higher than that of the low-RF group; however, the difference was not statistically significant.

3.3. Correlation between oral health status and RF

Significant correlations were observed between the RF area in the anterior teeth and all oral health-related variables (PI, GI, and BOP) ($p < 0.01$, Table 3). Among these, the RF area showed the strongest correlation with PI ($r = 0.499, p < 0.001$). The correlation coefficient between GI and RF areas was 0.422 ($p = 0.001$), which was higher than that between BOP and RF areas ($r = 0.376$, $p = 0.005$).

3.4. Effect of RF group on gingivitis severity

In Model 1 (crude model), which did not adjust for any sociodemographic variables, the RF group did not show a significant effect on gingivitis severity ($p = 0.134$, Table 4). In model 2, which adjusted for sociodemographic characteristics (age, sex, educational level, household income, and number of systemic diseases), the odds ratio (OR) for moderate gingivitis in the High RF group compared to the Low RF group was 4.35 ($p = 0.049$). In Model 3, which was additionally adjusted for smoking and alcohol consumption, the OR for moderate gingivitis in the High RF group increased to 6.07 ($p = 0.036$).

4. Discussion

The findings of this study indicated that the red biofluorescence of the anterior dental biofilm was significantly correlated with oral health indicators, including PI, GI, and BOP. Particularly, the distribution of RF was found to influence the severity of gingivitis. These results suggest that the biofluorescence of anterior dental biofilms could be effectively utilized as a tool for screening individuals at a high risk of gingivitis.

The correlation coefficient between the RF area and PI in this study was 0.499, indicating a significant correlation, although it was somewhat lower than the results reported in previous studies regarding fluorescence variables and PI. In a previous study comparing the plaque area observed in QLF images with that observed using two-tone blue staining, the correlation coefficient was reported to be 0.62 [21]. Another study demonstrated a strong correlation (0.730) between the simple plaque score analyzed using QLF images and the clinically assessed plaque index [22]. The relatively lower correlation observed in this study compared to previous studies may be attributed to the fact that the PI was evaluated for the entire oral cavity, whereas the RF area was analyzed specifically for the anterior teeth. According to previous research, the PI in posterior teeth is reported to be approximately 33 % higher than that in anterior teeth, likely because of greater plaque accumulation in the posterior region [23]. Despite calculating the RF only for the anterior region in this study, while assessing the PI for the entire oral cavity, the observed correlation is noteworthy, as it remains significantly comparable to the findings of previous studies.

In this study, a significant moderate correlation was observed between the anterior RF and GI ($r = 0.422, p = 0.001$), confirming that the level of the RF is an important factor influencing the severity of gingivitis. Specifically, in Model 2, which was adjusted for sociodemographic characteristics, the OR increased to 4.35, indicating a significant impact. In Model 3, which additionally accounted for risk factors, such as alcohol consumption and smoking, the OR further increased to 6.07 ($p = 0.036$). This demonstrates that RF is an independent and strong factor affecting the severity of gingivitis. Notably, the significant effect of RF persisted even after adjusting for well-known risk factors for periodontal disease, such as alcohol consumption and smoking. These findings suggest that RF may serve as a key indicator in the assessment and management of gingival health.

The emission of red biofluorescence from dental biofilms is likely associated with the presence of thick plaque layers, plaque maturation, or inflamed gingival tissues [24]. According to previous study, the red biofluorescence expressed in cultured biofilms increased with biofilm maturation and was observed in mature biofilms cultured for more than three days [25]. Aged or mature biofilm are thought to induce gingivitis and promote the onset of periodontitis [26]. High levels of red biofluorescence in dental biofilms indicate biofilm maturation, which increases the risk of gingivitis [18]. Additionally, the red biofluorescence of dental biofilms is associated with pathogenicity and is driven by changes in the microbial composition of the dental biofilm. Previous research has shown that dental biofilms exhibiting red biofluorescence are more abundant in gingivitis-associated bacteria, such as *Leptotrichia* and *Selenomonas*, compared than in biofilms without red fluorescence [15]. Dental biofilms exhibiting red biofluorescence are associated with 50 % prevalence of clinical signs of gingivitis at adjacent gingival margins [15]. Although microbiological analyses of the dental biofilms

Table 2
Comparison of oral health status variables according to red biofluorescence area of anterior teeth.

	Low-RF ($n = 27$)	High-RF ($n = 28$)	p-value
PI	0.49 ± 0.28	0.63 ± 0.34	0.117
GI	1.02 ± 0.32	1.24 ± 0.48	0.049
BOP	0.25 ± 0.14	0.37 ± 0.25	0.029

PI: plaque index, GI: gingival index, BOP: bleeding on probing.
Mean ± standard deviation. p-values were calculated based on the independent t-test.

Table 3
Correlation analysis between oral health status variables and red biofluorescence area of anterior teeth.

	RF area	PI	GI	BOP
RF area	1			
PI	0.499**	1.000		
GI	0.422*	0.467**	1.000	
BOP	0.376*	0.355*	0.863**	1.000

RF area: red biofluorescence area, PI: plaque index, GI: gingival index, BOP: bleeding on probing.
* $p < 0.01$
** $p < 0.001$
by Pearson's correlation analysis.

Table 4
Logistic regression analysis of red biofluorescence area of anterior teeth and gingivitis severity.

Variables	Model 1		Model 2		Model 3	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
RF						
Low	ref.		ref.		ref.	
High	2.40	0.77–7.53	4.35*	1.01–18.85	6.07*	1.13–32.65

RF: red biofluorescence.
Model 1: Unadjusted model. Model 2: Age. sex, educational level, household income, number of systemic diseases adjusted model. Model 3: Age. sex, educational level, household income, number of systemic diseases, smoking, drinking adjusted model.
* $p < 0.05$.

with red biofluorescence detected in this study were not performed, based on previous research, it can be inferred that the high pathogenicity of dental biofilms with red biofluorescence likely influenced the severity of periodontitis. Furthermore, the primary biofluorescence variable (RF area) used in this study encompassed not only the presence of red biofluorescence in dental biofilms, but also the amount of dental biofilm. Considering that individuals with periodontitis tend to have a greater accumulation of dental biofilms on average than healthy individuals [27], the increase in gingivitis severity with a higher RF area can be attributed to the combined effects of the pathogenicity associated with red biofluorescence and the amount of accumulated biofilm. Therefore, the RF area can serve as a crucial indicator that provides both pathogenic and quantitative information for assessing gingival health. This study aimed to predict the overall oral health status using biofluorescence analysis of anterior dental biofilms alone. According to a previous study reporting the association between dental plaque and BOP using logistic regression analysis, the OR for the front teeth was higher than that for the posterior teeth in both the maxilla and mandible [28]. Another study demonstrated that the correlation coefficient between the red biofluorescence variable (ΔR70) measured via QLF and the GI was approximately 23 % higher in the anterior teeth than in the posterior teeth [22]. These findings suggest that the biofilm-related status of anterior teeth provides more effective information for predicting gingivitis than that of posterior teeth. One concern raised by dental professionals regarding teledentistry is the significant amount of time required for image acquisition and data transmission [29]. Generally, capturing images of the posterior teeth is time consuming and challenging because it requires retraction of the buccal mucosa and access to the rearmost molars. By contrast, acquiring front-view images is relatively simple and straightforward. Therefore, the approach used in this study to evaluate overall oral health status by capturing images exclusively of the anterior teeth is both clinically and practically valid. Moreover, this method demonstrates the potential for efficient application in teledentistry.

However, based on the findings of this study, it would be an overstatement to conclude that individuals with high levels of anterior red biofluorescence are affected by moderate gingivitis. Caution is needed when interpreting these results as the evaluation was based solely on the biofluorescence of the anterior dental biofilm rather than a direct comparison between biofluorescence and gingivitis indicators at specific sites. Therefore, red biofluorescence information of dental biofilms is recommended as a supplementary tool for identifying high-risk individuals during initial screening, followed by further examinations (e.g., palpation and periodontal pocket assessment) to ensure an accurate diagnosis and appropriate intervention. Additionally, red biofluorescence in dental biofilms, given its association with biofilm accumulation and the pathological state, can serve as an intuitive tool for assessing oral hygiene status. Providing red biofluorescence information about dental biofilms has been reported to be effective in education as it visually demonstrates the degree of biofilm adherence to patients [30]. In particular, the information provided through teledentistry has been shown to be effective in promoting patients' self-oral care [31]. Therefore, intuitively visualizing the state of dental biofilm accumulation could effectively motivate patients to improve their oral hygiene. Utilizing biofluorescence examinations for dental biofilms has positive implications, as it allows the remote assessment of gingival health and oral hygiene management through images, while simultaneously motivating patients for self-care.

Most participants of this study were patients who voluntarily visited a university dental hospital. This indicates that the majority of the participants were likely individuals with a relatively high interest in oral health, as they chose to visit a university dental hospital on their own. Consequently, despite the inclusion of individuals with gingivitis, this study has limitations in adequately representing patients with severe dental biofilm accumulation. Another limitation is the inability to perform analyses related to the diagnostic potential of biofluorescence information, such as sensitivity and specificity, making direct comparisons with studies using other imaging modalities challenging. Despite these limitations, this study is significant because it represents the first attempt to predict the overall gingival health status using only the biofluorescence of the anterior dental biofilm. This study focuses on exploring the applicability of this variable. Future research should involve larger-scale studies, including patients with diverse levels of dental biofilm accumulation, and assess the diagnostic accuracy of biofluorescence information to validate its clinical utility.

5. Conclusion

The red biofluorescence observed in the dental biofilms of the anterior teeth was associated with overall dental plaque accumulation and gingivitis. Notably, the level of red biofluorescence influenced the severity of gingivitis. Therefore, the red biofluorescence of dental biofilms observed in the anterior teeth holds potential as an effective screening tool for evaluating gingival health, and is expected to be useful in the early detection and intervention of gingivitis.

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CRediT authorship contribution statement

Hyo-Jung Kim: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Eun-Song Lee:** Writing – review & editing, Visualization, Formal analysis. **Baek-Il Kim:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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