Journal of Dentistry 136 (2023) 104600

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Real-time optical detection of endodontic infection using bacterial autofluorescence

Eun-Song Lee [a], Elbert de Josselin de Jong [a,b,c], Euiseong Kim [d,e], Baek-Il Kim [a,*]

[a] Department of Preventive Dentistry & Public Oral Health, BK21 FOUR Project, Yonsei University College of Dentistry, Seoul, Korea
[b] Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, Amsterdam, The Netherlands
[c] Inspektor Research Systems BV, Amsterdam, The Netherlands
[d] Microscope Center, Department of Conservative Dentistry and Oral Science Research Center, Yonsei University College of Dentistry, Seoul, Korea
[e] Department of Electrical & Electronic Engineering, Yonsei University College of Engineering, Seoul, Korea

ARTICLE INFO

Keywords:
Diagnostic systems
Endodontic infection
Optical detection
Quantitative light-induced fluorescence
Red fluorescence

ABSTRACT

Objectives: For successful root canal treatment (RCT), it is essential to objectively assess the presence and activity of bacteria in the root canal system. However, current methods rely on subjective observations of root canal exudates. This study aimed to confirm whether real-time optical detection using bacterial autofluorescence can evaluate endodontic infection status by assessing the red fluorescence (RF) detected from root canal exudates.
Methods: During RCT, endodontic paper points were used to collect root canal exudates scored using conventional organoleptic tests to assess the severity of root canal infections. RF on the paper points was assessed using quantitative light-induced fluorescence (QLF) technology. RF intensity and area from the paper points were quantified, and their correlations with infection severity were assessed using their organoleptic scores. The oral microbiome composition of RF samples was compared with non-red fluorescent (non-RF) samples.
Results: The RF detection rate was nil and >98% in the non-infectious and severe groups. The RF intensity and area significantly increased with infection severity ($p<0.001$) and showed strong correlations with organoleptic scores (r=0.72, 0.82, respectively). The diagnostic accuracy for detecting root canal infection using RF intensity was good to excellent (AUC = 0.81−0.95) and increased with infection severity. The microbial diversity of the RF samples was significantly lower than that of the non-RF samples. Gram-negative anaerobic bacteria such as *Prevotella* and *Porphyromonas* were more predominant in RF samples.
Conclusions: Optical detection using bacterial autofluorescence can objectively evaluate endodontic infection status in real-time by assessing the RF of endodontic root canal exudates.
Clinical significance: This real-time optical technology can be utilised to detect endodontic bacterial infection without conventional incubation, allowing clinicians to determine the endpoint of chemomechanical debridement and increase the positive outcomes of RCTs.

1. Introduction

Endodontic root canal infection is caused by obligate and facultative anaerobic bacteria that survive in nutrient-deficient environments [1,2]. These bacteria present in the root canal must be eliminated before endodontic treatment is completed [3,4], as persistent infection due to residual pathogenic bacteria is one of the main causes of root canal treatment (RCT) failure [5,6]. Thus, dentists need to detect and eradicate pre-existing root canal infections and determine appropriate endpoints for chemomechanical preparation, as the success rate of RCT increases if these procedures are performed correctly [3].

Current clinical practices for detecting root canal bacteria use subjective assessment methods, such as the colour or odour of exudates on paper points during RCT [5]. However, these conventional methods have low reliability; it is difficult to determine the endpoint of biomechanical treatment as they depend solely on the examiner's subjective judgment. Several approaches have been developed to objectively evaluate and quantify the bacterial status of infected root canals to overcome these limitations. They require a staining process before evaluation or a time-consuming process of sample collection, pre-treatment, or incubation, making it impractical in a clinical setting. Furthermore, they focus on the presence of pathogenic bacteria, do not

* Corresponding author at: Department of Preventive Dentistry & Public Oral Health, BK21 FOUR Project, Yonsei University College of Dentistry, Seoul, Korea.
E-mail address: baekil.kim@gmail.com (B.-I. Kim).

https://doi.org/10.1016/j.jdent.2023.104600
Received 12 May 2023; Received in revised form 21 June 2023; Accepted 23 June 2023
Available online 29 June 2023

detect bacterial pathogenicity, and cannot detect very low bacterial quantities [7,8]. A recent fluorescence-based chair-side method utilising fluorescent dyes to detect vital bacteria still requires a 5-min incubation time [7]. Some laser fluorescence approaches for measuring bacterial metabolites have shown potential for real-time assessment of endodontic infection. However, they require specialised flexible tips for easier access to the root canal [5]. Real-time imaging assessment has the advantage of obtaining imaging data in real-time for detecting and diagnosing disease status and immediately quantifying them as a numeric value. This imaging assessment has been widely used in the medical field, but not in the dental field. Therefore, it is essential to develop a rapid and reliable method for detecting endodontic infection by assessing the pathogenicity of overall endodontic bacteria and minimizing additional clinical steps, enabling clinicians to rapidly and objectively execute RCTs and increase positive outcomes.

Active oral bacteria related to the dysbiotic change in oral biofilm composition produces red autofluorescence. Previous studies reported that this red fluorescence (RF) is associated with dental plaque maturity, pathogenicity, and risk of oral diseases; thus, quantifying it can help assess and predict bacteria-related oral diseases [9–14]. RF originates from the metabolic by-products of bacteria, and high levels of endogenous metal-free fluorescent porphyrins are mainly synthesised by late colonisers of plaque, such as anaerobes, resulting in a strong RF in a mature plaque [9,15].

It is important to evaluate pathogenicity induced by microbial community interactions rather than focusing on specific pathogens for infectious disease assessment. Previous studies have found that the obligate anaerobic bacterial community could be associated with infected root canals and periapical periodontitis [16,17]. Particularly, the bacterial community in necrotic pulp is frequently dominated by *Fusobacterium, Porphyromonas, Bacteroides,* and *Prevotella* [17,18]. Therefore, evaluating the bacterial products of microbial communities associated with endodontic infections would be a useful technique for optical assessment of the increased pathogenicity of root canal infections.

Based on these principles, optical devices for detecting the pathogenicity of biofilms have been developed and are currently being utilised for diagnosing oral diseases. Among them, quantitative light-induced fluorescence (QLF) is an optical technology that uses narrowband blue-light irradiation (centred at 405 nm) to induce bacterial autofluorescence [9,19,20]. It provides an enhanced image using a special filter and enables the examiner to immediately observe the fluorescent reaction and obtain the results as a numeric value. Particularly, it is possible to evaluate the root canal exudate collected on the paper point used during RCT without any special additional equipment or devices, allowing for efficient and timely assessment of treatment progress. It is also possible to objectively and quantitatively evaluate root canal infection by visualizing and evaluating the dysbiotic status of the bacterial community in the root canal using the RF observed.

This study aimed to confirm whether bacterial red autofluorescence can objectively evaluate endodontic infection status by assessing the RF detected from root canal exudates. The specific objectives are as follows: First, to compare the RF characteristics of exudates evaluated using QLF technology with the degree of contamination evaluated using conventional organoleptic test methods. Second, to identify the microbial characteristics of RF samples using 16S rRNA sequencing.

2. Materials and methods

2.1. Study design and patient selection

This cross-sectional study was approved by XXX (2-2014-0003), conformed to the tenets of the Declaration of Helsinki, and followed the STROBE guidelines. All patients provided written informed consent.

Initially, 65 patients were screened, of which seven were excluded due to systemic diseases. Of the 172 samples from 58 patients, 12 were excluded due to missing data. Finally, 58 patients (33 men and 25 women, mean age of 40.6 years) participated in this study, and 160 paper points were used for the final analysis. Samples were taken from adult patients (aged 19 to 80 years) referred to our department for RCT between March 2015 and December 2017. Only patients without systemic diseases, such as diabetes and hypertension, who had not received antibiotic treatment within the previous three months were included in this study. Selected teeth were those scheduled for RCT due to a pulp or periradicular disease. Sample size calculation was conducted using G*power software, considering the data collected in the preliminary study. Based on the data, at least 150 samples were required to identify a significant correlation, with an alpha-type error of 0.05, a power of 0.8, and an effect size of 0.2.

2.2. Sample collection and organoleptic test scoring

After anaesthesia, a rubber dam was applied, and the tooth surface was sterilized using cotton pellets soaked in 5.25% sodium hypochlorite. Rubber dam leaks were prevented during each access cavity preparation. The temporary filler was removed with a #4 round bur without water spray, and a diamond chamfer bur was used to remove the temporary filler and cotton attached to the access cavity wall. For exudate sampling, a size 20, 0.02 tapered sterilised paper point was inserted into the root canal as deeply as possible for 1 minute. Samples were excluded from analysis when the paper point became contaminated with blood or other debris. Infection severity was evaluated and classified into four categories from 0 to 3 based on visual and olfactory testing (the clinical standard for assessing endodontic infection status). Two trained dentists performed this to prevent bias. Where there was disagreement, the score was decided until an agreement was reached. The criteria are as follows: score 0 (no contamination) - no exudate (no change in colour of the paper points) and no foul odour; score 1 (slight) – exudate present in one-third of the paper points and no foul odour; score 2 (moderate) - exudate present in more than one-third of the paper points and no foul odour; and score 3 (severe) - exudate present with a foul odour.

2.3. Assessment of bacterial fluorescence from root canal exudate

We obtained fluorescence images using the QLF device to evaluate bacterial autofluorescence of paper points with exudates from the root canal immediately after the organoleptic assessment. A trained examiner captured white-light and fluorescence images of the paper points using a QLF device (QLF-D Biluminator, Inspektor Research Systems BV, Amsterdam, The Netherlands) equipped with a high-specification digital single-lens reflex camera (550D, Canon, Tokyo, Japan) with blue and white LED lights with a peak wavelength of 405 $\pm$ 7 nm and a modified filter set (high-pass filter $>$ 480 nm and a pink filter). A proprietary software (C3 version 1.23, Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to maintain the same conditions (shutter speed of 1/45 s, aperture value of 3.2, and ISO 1600) for all samples.

Immediately after imaging, samples were stored in 1 mL phosphate-buffered saline for DNA extraction at -20 ℃. The fluorescence properties of images were quantified using image analysis software (Image-Pro Plus 6.0, Media Cybernetics, Rockville, Maryland). We calculated two fluorescence variables (area and intensity) as numerical values from the fluorescence images of paper points. The fluorescent area of each paper point was calculated as the ratio (%) of the area where RF was detected out of the total paper point area. RF intensity was obtained by calculating the maximum value of the ratio of red and green values [Red/Green ratio] of each pixel. The RF properties in each paper point fluorescent image were quantified based on these two variables.

2.4. Bacterial community analysis based on 16S rRNA gene sequencing

To confirm the difference in the microbial composition according to the presence or absence of fluorescence, 16S rRNA gene sequencing

analysis was carried out for 20 randomly selected paper point samples: ten each from the RF and non-red fluorescent (non-RF) groups. DNA was extracted from the samples using the PowerBiofilm DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA).

PCR amplification was performed using primers targeting the V3 to V4 regions of the 16S rRNA gene with extracted DNA. An amplicon library was constructed from individual samples by PCR amplification targeting the 16S rRNA V3-V4 region. Bacterial amplification was performed using the primers 341F (5′-TCGTCG GCAAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′; underlined sequence indicates the target region primer) and 805R (5′-GTCTCGTGGGCTCGG-AGATG TGTATAAGAGACAG -GACTACHVGGGTATCTAATCC-3′).

A secondary amplification for attaching the Illumina NexTera barcode was performed with the i5 forward primer (5′-AATGA TACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAGCGTC-3′; X indicates the barcode region) and i7 reverse primer (5′-CAA GCAGAAGACGGCATACGAGAT-XXXXXXXX-AGTCTCGTGGGC TCGG-3′).

The amplified products were purified using the QIA quick PCR purification kit (Qiagen, Valencia, CA, USA). Equal concentrations of purified products were pooled, and short fragments (nontargeted products) were removed using the AMPure beads kit (Agencourt Bioscience, Beverly, MA, USA). PCR product quality and size were evaluated on the Bioanalyzer 2100 device (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Mixed amplicons were pooled, and sequencing was carried out using the Illumina MiSeq Sequencing system (Illumina, San Diego, CA, USA).

2.5. Data processing

Raw sequence reads were processed with a quality-control check and filter in go flow quality (<Q25) reads using the Trimmomatic 0.321 [21]. Output paired-end reads were merged using the PANDASeq assembler. The primers were trimmed using EzBioCloud (https://www.ezbiocloud.net/) at a similarity cut-off of 0.8. Sequences were denoised using Mothur's 3 preclustering program [22]. The EzTaxon database was used for taxonomic assignment with BLAST 2.2.224, and the similarity was quantified using pairwise alignment [23]. UCHIME and the non-chimeric 16S rRNA database from EzTaxon were used to detect chimera on reads for which the best-hit similarity rate was less than 97%. Sequence data were then clustered using CD-HIT7 and UCLUST [24,25]

2.6. Statistical analysis

Alpha diversity indices were calculated at 97% identity, and the differences between the two groups were confirmed by independent samples t-test. Taxonomy composition and abundances at phylum, genus, and species levels were analysed, and differences in relative abundances of taxa in the two groups were compared using the Wilcoxon signed-rank test. Differential abundances of operational taxonomical units (OTUs) between the two groups were determined using linear discriminant analysis (LDA) effect size (LEfSe).

The root canal microbiota statuses were compared between the RF and non-RF groups. Kruskal-Wallis analysis and Bonferroni corrections were used to confirm differences in the fluorescence variables among the infection severity score groups determined by the organoleptic test. Correlations between fluorescence variables and organoleptic scores were assessed using Spearman correlation coefficients. A receiver operating characteristic (ROC) analysis was used to investigate the overall performance of fluorescence in detecting endodontic infection. With the organoleptic score (OLS) as a clinical standard for the severity of endodontic infection, the sensitivity and specificity values of the RF intensity were calculated along with 95% confidence intervals. The area under the ROC curve (AUC) for the RF intensity was calculated to evaluate the accuracy in detecting each diagnostic threshold of endodontic infection. The optimal cutoff values of RF intensity were determined based on the highest sum of sensitivity and specificity for each threshold. Cases with missing data were excluded from the final analysis. A significance level of 5% was adopted for all analyses, and the data were analysed using PASW Statistics software (version 23.0, SPSS, IBM Corporation, Somers, New York).

3. Results

Organoleptic test results, a conventional method for evaluating the severity of root canal infection, revealed different levels of infection: no contamination (code 0) occurred in 38%, slight-moderate (code 1−2) in 29%, and severe (code 3) in 33% of all root canals. Based on the results from QLF technology, approximately 61% were non-RF samples, and 39% were RF samples. The representative QLF images observed a difference in the fluorescence intensity and distribution area according to the OLS (Fig. 1).

The fluorescence variables for the four severity groups classified according to OLS are listed in Table 1. Non-RF was observed in all samples, with a score of 0 determined to be free of bacterial infection. As the OLS increased, the proportion of samples with RF also increased. The fluorescence variables, such as intensity and area, which quantified the degree of RF, tended to increase significantly with increasing infection severity ($p < 0.001$). According to the results of the correlation analysis between fluorescence variables and organoleptic test results, the correlation coefficient of the fluorescence intensity was the highest ($r = 0.82$, $p < 0.0001$), and that of the fluorescent area was also significantly strong ($r = 0.72$, $p < 0.0001$). The diagnostic accuracy for detecting root

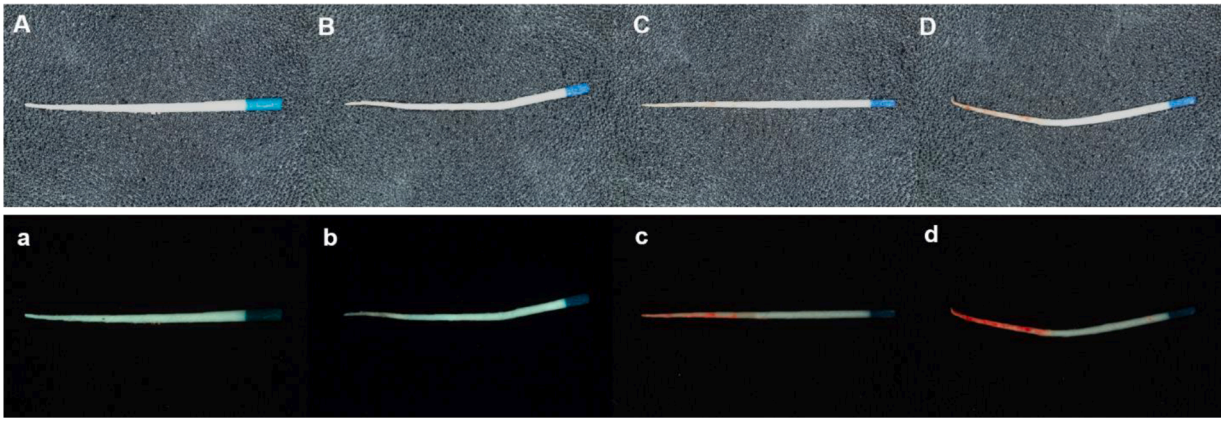

Fig. 1. Representative white-light (A-D) and fluorescent (a-d) images obtained from quantitative light-induced fluorescence (QLF) for the collected paper points according to the organoleptic scores (score 0: A, a; score 1: B, b; score 2: C, c; score 3: D, d).

Table 1
Red fluorescence (RF) variables according to organoleptic scores (OLS) and correlations between the OLS and RF variables.

OLS	N (%)	RF detection rate (%)	RF variables	
			Intensity	Area
0	60 (38)	0	0	0
1	35 (22)	15.3	0.20 ± 0.52 [a]	0.07 ± 2.20 [a]
2	12 (7)	58.3	0.68 ± 0.60 [a]	3.73 ± 4.86 [a]
3	53 (33)	98.2	2.09 ± 1.17 [b]	19.52 ± 16.92 [b]
p-value*			<0.001	<0.001
r (*p*-value)			0.82 (<0.001)	0.72 (<0.001)

Values of red fluorescence variables are represented as the mean ± SD
Different letters in the same row indicate significant differences between groups as determined using Bonferroni's correction for multiple analysis at $\alpha = 0.05$.
p-value* was determined by analysis of variance (ANOVA)
r indicates Spearman correlation coefficients between organoleptic scores and each fluorescence variable.

Table 2
The area under the ROC curve, sensitivity, and specificity for red fluorescence intensity in detecting endodontic infection at each diagnostic threshold categorised by organoleptic score.

Infection severity	AUC	95% CI	*p*-value	SE (%)	SP (%)	Cutoff value
OLS 0/1-3 slight	0.810	0.74-0.87	<0.0001	62.0	100.0	0
OLS 0-1/2-3 moderate	0.917	0.86-0.95	<0.0001	88.7	94.7	0
OLS 0-2/3 severe	0.953	0.90-0.98	<0.0001	94.3	89.7	0.25

AUC, area under the ROC curve; CI, confidence interval; OLS, organoleptic score; ROC, receiver operating characteristic; SE, sensitivity; SP, specificity.

Table 3
Microbial diversity of the two groups based on the presence of red fluorescence.

Groups	N	Number of valid reads	OTUs	Shannon index
non-RF	10	104536	877.7 ± 240.2	3.07 ± 1.03
RF	10	105369	380.4 ± 233.5	2.62 ± 0.55
p-value			0.04	0.30

OTU, operational taxonomical units; RF, red fluorescence

canal infection using the RF intensity, mostly correlated with the infection severity among the fluorescence variables, was good to excellent (AUC=0.81−0.95) and increased with the threshold level (Table 2). The sensitivity was the highest (94.3%) in detecting a severe level of infection.

In comparison, the specificity was the highest (100%) in detecting a slight level of infection using RF presence (RF intensity cutoff > 0). The microbial properties of each group classified according to fluorescence expression are shown in Table 3. Microbial diversity was significantly higher in the non-RF samples (mean OTUs at 97% identity = 878) than that in the RF samples (mean OTUs at 97% identity = 380). The Shannon diversity index showed that the non-RF samples had more diverse bacterial communities than the RF samples, although the difference was not statistically significant (Table 3).

Taxonomic composition analysis indicated that the two groups had distinctively different dominant phyla (Fig. 2-A). In the RF samples, *Bacteroidetes* was the most abundant at about 40%, followed by *Firmicutes* (30%), *Actinobacteria* (13%), *Synergistetes* (11%), and *Fusobacteria* (3%). Meanwhile, in the non-RF samples, *Proteobacteria* was the most abundant at 44%, which was about 2% in the RF samples, followed by *Firmicutes* (38%), *Actinobacteria* (11%), and *Bacteroidetes* (4%). The ratio of RF samples to non-RF samples (RF/non-RF ratio) was 33 for *Synergistetes*, nine for *Bacteroidetes*, and seven for *Fusobacteria*. The relative abundance of *Spirochaetes* was less than 0.5% in both groups but about three times higher in the RF samples than in the non-RF samples.

The dominant genera in the RF samples were *Prevotella, Porphyromonas, Pyramidobacter, Olsenella*, and *Streptococcus*, whereas those in the non-RF samples were *Streptococcus, Acinetobacter, Enterococcus*, and *Actinomyces* (Fig. 2-B). Based on the LEfSe, which indicates the degree of consistent difference in relative abundance at the genus level, *Prevotella* (3.43) and *Fusobacterium* (2.79) showed significant differences in abundance in the RF samples, whereas *Enterococcus* (4.61), *Neisseria* (4.16), *Rothia* (3.88), and *Actinomyces* (3.02) were found to be over-represented in the non-RF plaque.

Beta diversity analysis indicated that the bacterial community structures among the RF group samples were more similar than those in the non-RF samples, based on weighted UniFrac distances. Principal coordinate analysis of weighted UniFrac distances revealed that samples in the RF group were relatively more clustered, suggesting more similar community structures (Fig. 3).

4. Discussion

The present study suggests that the QLF technology using bacterial autofluorescence could be used to objectively detect and evaluate the status of bacterial infection in the root canal in real-time during RCT. Moreover, it can minimise additional equipment and clinical steps using endodontic paper points for sampling and immediately evaluating the bacterial pathogenicity. Compared to conventional microbiology techniques such as PCR and colony-forming unit counting, it has the potential advantage of being a chair-side detection tool that saves time in the evaluation process. Since the activity and resilient nature of residual bacteria in the root canal lead to persistent infection and RCT failure [26–28], this method is expected to increase the success rate of RCT and prevent unnecessary secondary treatment by immediately detecting residual bacterial communities.

According to the results for the fluorescence variables (RF intensity and area), non-RF was detected in all score 0 samples, which were judged to have no infection according to the organoleptic test. In score 3 samples with severe bacterial infection status and foul odour, RF was distinctively detected at the highest level in almost all samples (98%). Based on these results, the absence of RF indicates no bacterial metabolic activity in the root canal, whereas strong RF indicates severely infected root canals.

Organoleptic test scores 1 and 2, assessed by visually evaluating the exudate in the absence of odour, are likely to be classified as score 0 because the exudate is difficult to detect visually, leading to poor validity and reliability. This study detected RF in about 15% of score 1 and 58% of score 2 samples. Thus, RF detection from root canal exudates

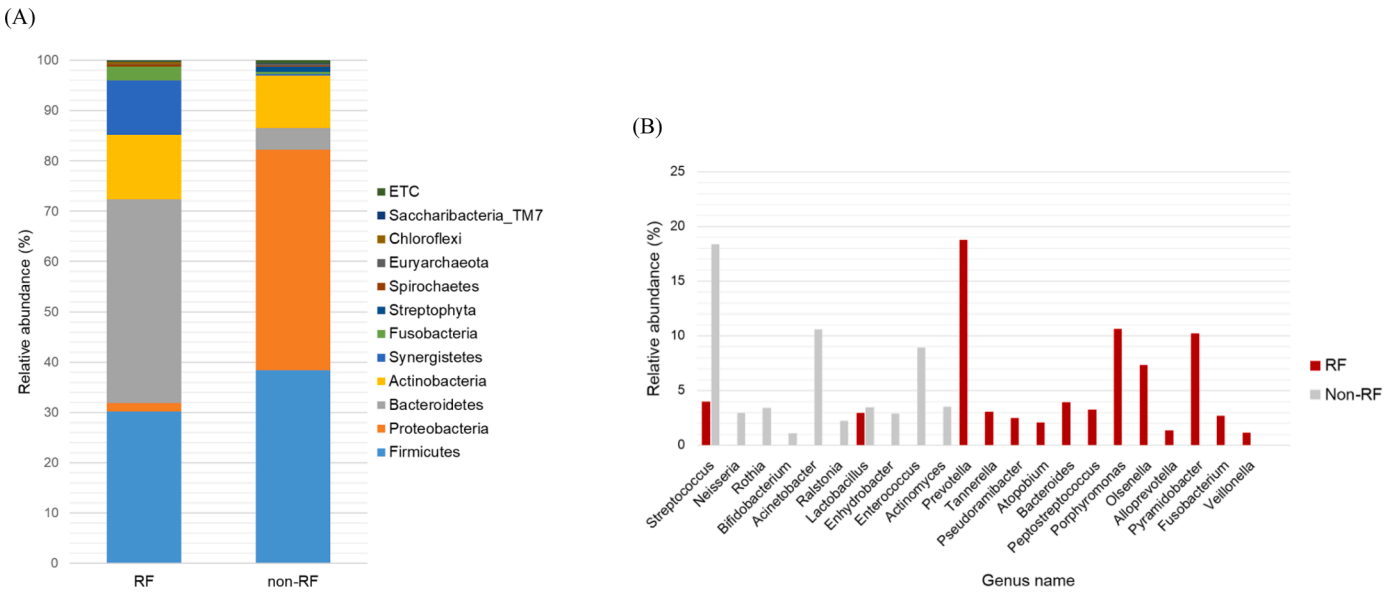

Fig. 2. Relative abundance of phylum-level (A) and genus-level communities (B) identified in the non-RF and RF group samples.

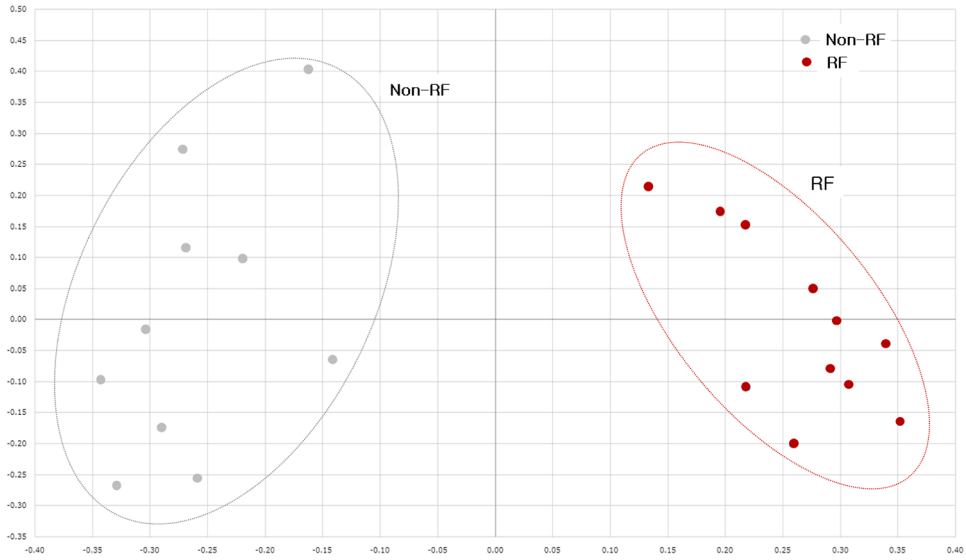

Fig. 3. Principal coordinates analysis (PCoA) for microbial community variation within each group (RF and non-RF groups).

may identify bacterial infection in ambiguous cases where it is difficult to determine the presence of infection.

Although the absence of fluorescence does not confirm the complete absence of bacteria, it can be assumed that these samples have bacteria that are not pathogenically active in the root canal. In this respect, QLF technology is beneficial for assessing bacterial metabolic activity following recent theories that the development of endodontic infections and apical inflammatory lesions is related to the bacterial community as a unit of pathogenicity rather than the presence of a specific species [29, 30]. Furthermore, in cases where even a faint RF is detected in the sample prior to permanent filling treatment after all the instrumentation and irrigation procedures, it can indicate residual bacterial activity within the root canal system. Therefore, detecting RF at this endpoint determination stage holds clinical significance in guiding additional disinfection and treatment interventions.

Our results revealed that the fluorescence variables significantly increased as the level of root canal infection assessed by the organoleptic test increased, showing strong correlations between the two. It is inferred that the RF exhibited by endodontic bacteria is related to the pathogenicity of the bacterial community in the infected root canal. When irradiated with a 405 nm light source, RF from the bacterial community mainly originates from porphyrin compounds, metabolites of gram-negative anaerobic bacteria that exhibit strong fluorescence in the red spectral region when irradiated with 400–420 nm violet light. These porphyrin compounds, produced by microorganisms that become dominant late-colonizers in dysbiotic biofilms through haeme biosynthesis, can be detected as RF [31]. Previous studies utilising a biofilm model revealed that early biofilms with low pathogenicity did not exhibit RF, while mature biofilms with increased pathogenicity showed an increase in RF [11].

Furthermore, evaluating RF in biofilm surrounding dental calculus revealed a higher proportion of periodontal pathogenic bacteria in biofilms with RF than in biofilms without it [12]. This study also confirmed bacterial dysbiosis within the root canal in RF samples through the 16S rRNA sequencing analysis results.

More than 70% of bacteria in infected root canals are obligate

anaerobes; the predominant bacteria in necrotic pulp and primary infection are *Fusobacterium, Prevotella*, and *Porphyromonas* spp. [32,33]. Based on our results, the relative abundance of these bacteria was higher in the RF than in the non-RF samples, and *Prevotella* (19%) and *Porphyromonas* (11%) were especially predominant in the RF group. These bacteria require porphyrin as an absolute requirement in the metabolic pathways to induce pathogenicity. As metabolic activity increases in an anaerobic environment, they need protoporphyrin IX to substitute hemin to promote their growth [34,35]. Therefore, it can be inferred that the metabolic activity of the dominant anaerobic bacteria within the root canal can be detected through RF of porphyrin. This allows the detection of an increased pathogenic state resulting from the root canal ecosystem's dysbiosis.

The strong RF from score 3 samples with foul odour can be explained by the results of microbial analysis showing that the relative abundance of obligate anaerobic bacteria was higher in the RF samples than in the non-RF samples. When anaerobic bacteria, such as *Prevotella, Porphyromonas,* and *Fusobacterium,* are present in the root canal, a foul odour emanates, and the odour intensity is higher than without the bacteria [36]. However, odour assessment by organoleptic measurement has low reliability due to examiner subjectivity and can differ depending on the environment. Thus, it is meaningful to apply the QLF technology proposed in this study to identify pulp necrosis and apical periodontitis, which can be diagnosed by the presence of metabolic activity of these bacteria [17,18,36]. However, for clinical application, there is a need to validate the association between the RF from the root canal and treatment outcomes by conducting longitudinal follow-up studies.

This study confirmed a distinct difference in the bacterial communities according to the presence of RF. According to the phylum level analysis results, Bacteroidetes, which had the highest abundance (about 40%) in the RF samples, accounted for only about 4% of bacteria in the non-RF samples. This agrees with a previous study that used next-generation sequencing technology and reported that Bacteroidetes are the most abundant and predominant in primary and persistent cases of infected root canals [37].

Concerning the genus level results, *Porphyromonas* and *Prevotella*, found to be less than 0.5% in the non-RF group, were the most predominant in the RF group at 19% and 11%, respectively. These bacteria play an important role in inducing virulence in the pathogenesis of endodontic infection and are known to be mainly related to primary endodontic infection [38]. In particular, more than 70% of the bacteria in the primarily infected root canal were found to be obligate anaerobes, such as *Fusobacterium, Prevotella,* and *Porphyromonas*, which exhibit rapid, violent host responses in periapical tissue. Based on the clinical diagnosis of the samples in this study, 70% of RF samples predominantly with these bacteria were diagnosed with primary apical periodontitis and apical abscess (data not shown).

It is known that the bacterial community shows distinct patterns corresponding to clinical conditions, such as chronic apical periodontitis and acute apical abscess [39]. According to one review, *Treponema, Tannerella, Porphyromonas, Fusobacterium, and Prevotella* spp. were found to be most frequently detected in primary apical periodontitis and apical abscess [40]. According to recent studies that evaluated the fluorescence spectra of some pathogenic oral bacteria, *Prevotella intermedia* exhibited the strongest RF intensity when cultured on blood-containing media [41]. It is known that various types of porphyrins are actively produced during haeme biosynthesis [31], which supports the high proportion of *Prevotella intermedia* detected in the RF group in this study. Based on these findings, it may be useful to identify the activity of pathogenic bacteria in the root canal by assessing the RF of the exudate as an indicator for predicting the onset of apical lesions.

From the microbial analysis at the phylum level, Synergistetes, which accounted for 0.3% relative abundance in the RF group, showed about 32 times higher predominance in the RF group, with the most significant difference in relative abundance. A previous study demonstrated that Synergistetes increased in teeth with chronic endodontic infection, such as apical periodontitis [42]. Apical periodontitis is mainly caused by dental caries, and if infection persists in the root canal, the pulp tissue degrades, leading to endodontic infection, eventually causing apical periodontitis. Although the clinical presentations of each sample in the RF group need to be considered, it can be predicted that the teeth exhibiting root canal exudates with RF are at high risk for apical periodontitis.

In conclusion, the QLF technology can effectively detect the presence of anaerobic bacteria and assess their activity in the root canal in the clinical setting. This would help determine the end point of endodontic debridement and conduct a successful RCT. Studies have shown that dental hygiene students with relatively limited clinical experience who use QLF technology improve their competency in detecting dental restorations in the oral cavity [43]. Real-time fluorescence detection methods could be useful in developing the competency of dental students in objectively evaluating root canal infection during their root canal therapy.

This is the first clinical trial confirming the utility of the QLF technology on RCT as a chair-side tool to ascertain the level of bacterial infection and help guide proper treatment accordingly. However, further analysis of RF in the root canal and its association with clinical symptoms and apical lesions is needed to verify its association with endodontic infection. In addition to establishing clinical guidelines, further studies evaluating changes in RF after chemomechanical debridement or during treatment procedures would be needed to extend the scope of application in the clinical setting.

CRediT authorship contribution statement

Eun-Song Lee: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization. **Elbert de Josselin de Jong:** Formal analysis, Data curation, Software. **Euiseong Kim:** Investigation, Resources, Data curation. **Baek-Il Kim:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (No. 2022M3A9F3016364).

References

[1] F. Foschi, F. Cavrini, L. Montebugnoli, P. Stashenko, V. Sambri, C. Prati, Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients, Oral Microbiol. Immunol. 20 (2005) 289–295, https://doi.org/10.1111/j.1399-302X.2005.00227.x.

[2] M.E. Vianna, H.P. Horz, B.P. Gomes, G. Conrads, Microarrays complement culture methods for identification of bacteria in endodontic infections, Oral Microbiol. Immunol. 20 (2005) 253–258, https://doi.org/10.1111/j.1399-302X.2005.00221.x.

[3] J.F. Siqueira, I.N. Rocas, Clinical implications and microbiology of bacterial persistence after treatment procedures, J. Endod. 34 (2008) 1291–1301, https://doi.org/10.1016/j.joen.2008.07.028, e3.

[4] A.S. Garcez, S.C. Nunez, J.L. Lage-Marques, M.R. Hamblin, M.S. Ribeiro, Photonic real-time monitoring of bacterial reduction in root canals by genetically engineered bacteria after chemomechanical endodontic therapy, Braz. Dent. J. 18 (2007) 202–207, https://doi.org/10.1590/s0103-64402007000300005.

[5] A.L. Sainsbury, P.S. Bird, L.J. Walsh, DIAGNOdent laser fluorescence assessment of endodontic infection, J. Endod. 35 (2009) 1404–1407, https://doi.org/10.1016/j.joen.2009.07.006.

[6] L.E. Chavez de Paz, Redefining the persistent infection in root canals: possible role of biofilm communities, J. Endod. 33 (2007) 652–662, https://doi.org/10.1016/j.joen.2006.11.004.

[7] D.B. Herzog, N.A. Hosny, S.A. Niazi, G. Koller, R.J. Cook, F. Foschi, T.F. Watson, F. Mannocci, F. Festy, Rapid bacterial detection during endodontic treatment, J. Dent. Res. 96 (2017) 626–632, https://doi.org/10.1177/0022034517691723.

[8] T. Sato, K. Yamaki, N. Ishida, M. Shoji, E. Sato, Y. Abiko, K. Hashimoto, Y. Takeuchi, J. Matsuyama, H. Shimauchi, N. Takahashi, Rapid quantification of bacteria in infected root canals using fluorescence reagents and a membrane filter: a pilot study on its clinical application to the evaluation of the outcomes of endodontic treatment, Int. J. Dent. 2012 (2012), 172935, https://doi.org/10.1155/2012/172935.

[9] Y.S. Kim, E.S. Lee, H.K. Kwon, B.I. Kim, Monitoring the maturation process of a dental microcosm biofilm using the quantitative light-induced fluorescence-digital (QLF-D), J. Dent. 42 (2014) 691–696, https://doi.org/10.1016/j.jdent.2014.03.006.

[10] M.H. van der Veen, C.M. Volgenant, B. Keijser, J.B. Ten Cate, W. Crielaard, Dynamics of red fluorescent dental plaque during experimental gingivitis—A cohort study, J. Dent. 48 (2016) 71–76, https://doi.org/10.1016/j.jdent.2016.02.010.

[11] E.S. Lee, S.M. Kang, H.Y. Ko, H.K. Kwon, B.I. Kim, Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by quantitative light-induced fluorescence-digital (QLF-D), J. Dent. 41 (2013) 1264–1270, https://doi.org/10.1016/j.jdent.2013.08.021.

[12] E.S. Lee, E. de Josselin de Jong, B.I. Kim, Detection of dental plaque and its potential pathogenicity using quantitative light-induced fluorescence, J. Biophotonics. 12 (2019), https://doi.org/10.1002/jbio.201800414 e201800414.

[13] E.S. Lee, H.K. Yim, H.S. Lee, J.H. Choi, H.K. Kwon, B.I. Kim, Plaque autofluorescence as potential diagnostic targets for oral malodor, J. Biomed. Opt. 21 (2016) 85005, https://doi.org/10.1117/1.JBO.21.8.085005.

[14] H.M. Ku, Y.R. Oh, E.S. Lee, E. Kim, B.I. Kim, Using autofluorescence to detect bacterial contamination in root fractures, J. Dent. 86 (2019) 27–32, https://doi.org/10.1016/j.jdent.2019.05.024.

[15] L. Coulthwaite, I.A. Pretty, P.W. Smith, S.M. Higham, J. Verran, The microbiological origin of fluorescence observed in plaque on dentures during QLF analysis, Caries. Res. 40 (2006) 112–116, https://doi.org/10.1159/000091056.

[16] J.C. Baumgartner, B.J. Watkins, K.S. Bae, T. Xia, Association of black-pigmented bacteria with endodontic infections, J. Endod. 25 (1999) 413–415, https://doi.org/10.1016/S0099-2399(99)80268-4.

[17] J.F. Siqueira, I.N. Rôças, J.C. Oliveira, K.R. Santos, Molecular detection of black-pigmented bacteria in infections of endodontic origin, J. Endod. 27 (2001) 563–566, https://doi.org/10.1097/00004770-200109000-00002.

[18] M. Haapasalo, Black-pigmented gram-negative anaerobes in endodontic infections, FEMS Immunol. Med. Microbiol. 6 (1993) 213–217, https://doi.org/10.1111/j.1574-695X.1993.tb00329.x.

[19] R. Heinrich-Weltzien, J. Kühnisch, M. van der Veen, E. de Josselin de Jong, L. Stösser, Quantitative light-induced fluorescence (QLF)–a potential method for the dental practitioner, Quintessence. Int. 34 (2003) 181–188.

[20] I.A. Pretty, W.M. Edgar, P.W. Smith, S.M. Higham, Quantification of dental plaque in the research environment, J. Dent. 33 (2005) 193–207, https://doi.org/10.1016/j.jdent.2004.10.017.

[21] A.M. Bolger, M. Lohse, B. usadel, Trimmomatic: A flexible trimmer for illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.

[22] P.D. Schloss, S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R. A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G. G. Thallinger, D.J. Van Horn, Weber, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities, Appl. Environ. Microbiol. 75 (2009) 7537–7541, https://doi.org/10.1128/AEM.01541-09.

[23] D.W. Mount, Using the basic local alignment search tool (BLAST). CSH protocols. (2007) pdb.top17. 10.1101/pdb.top17.

[24] R.C. Edgar, Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26 (2010) 2460–2461, https://doi.org/10.1093/bioinformatics/btq461.

[25] L. Fu, B. Niu, Z. Zhu, S. Wu, W. Li, Cd-hit: Accelerated for clustering the next-generation sequencing data, Bioinformatics 28 (2012) 3150–3152, https://doi.org/10.1093/bioinformatics/bts565.

[26] U. Sjögren, D. Figdor, S. Persson, G. Sundqvist, Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis, Int. Endod. J. 30 (1997) 297–306, https://doi.org/10.1046/j.1365-2591.1997.00092.x.

[27] P.J. Lumley, P.S. Lucarotti, F.J. Burke, Ten-year outcome of root fillings in the General Dental Services in England and Wales, Int. Endod. J. 41 (2008) 577–585, https://doi.org/10.1111/j.1365-2591.2008.01402.x.

[28] A.C. Anderson, E. Hellwig, R. Vespermann, A. Wittmer, M. Schmid, L. Karygianni, A. Al-Ahmad, Comprehensive analysis of secondary dental root canal infections: a combination of culture and culture-independent approaches reveals new insights, PLoS One 7 (2012) e49576, https://doi.org/10.1371/journal.pone.0049576.

[29] J.F. Siqueira, I.N. Rôças, Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 107 (2009) 870–878, https://doi.org/10.1016/j.tripleo.2009.01.044.

[30] M. Zehnder, G.N. Belibasakis, On the dynamics of root canal infections-what we understand and what we don't, Virulence 6 (2015) 216–222, https://doi.org/10.4161/21505594.2014.984567.

[31] J.E. Choby, E.P. Skaar, Heme synthesis and acquisition in bacterial pathogens, J. Mol. Biol. 428 (2016) 3408–3428, https://doi.org/10.1016/j.jmb.2016.03.018.

[32] G. Sanchez-Sanhueza, H. Bello-Toledo, G. Gonzalez-Rocha, A.T. Goncalves, V. Valenzuela, C. Gallardo-Escárate, Metagenomic study of bacterial microbiota in persistent endodontic infections using Next-generation sequencing, Int. Endod. J. 51 (2018) 1336–1348, https://doi.org/10.1111/iej.12953.

[33] B.P. Gomes, R.C. Jacinto, E.T. Pinheiro, E.L. Sousa, A.A. Zaia, C.C. Ferraz, F. J. Souza-Filho, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia and Prevotella nigrescens in endodontic lesions detected by culture and by PCR, Oral Microbiol. Immunol. 20 (2005) 211–215, https://doi.org/10.1111/j.1399-302X.2005.00214.x.

[34] J. Mysak, S. Podzimek, P. Sommerova, Y. Lyuya-Mi, J. Bartova, T. Janatova, J. Prochazkova, J. Duskova, Porphyromonas gingivalis: major periodontopathic pathogen overview, J. Immunol. Res. (2014), 476068, https://doi.org/10.1155/2014/476068.

[35] K.P. Leung, S.P. Folk, Effects of porphyrins and inorganic iron on the growth of Prevotella intermedia, FEMS Microbiol. Lett. 209 (2002) 15–21, https://doi.org/10.1111/j.1574-6968.2002.tb11103.x.

[36] Y. Yamada, Y. Takahashi, K. Konishi, I. Katsuumi, Association of odor from infected root canal analyzed by an electronic nose with isolated bacteria, J. Endod. 33 (2007) 1106–1109, https://doi.org/10.1016/j.joen.2007.05.020.

[37] J.M. Shin, T. Luo, K.H. Lee, D. Guerreiro, T.M. Botero, N.J. McDonald, A. H. Rickard, Deciphering endodontic microbial communities by next-generation sequencing, J. Endod. 44 (2018) 1080–1087, https://doi.org/10.1016/j.joen.2018.04.003.

[38] J.F. Siqueira, I.N. Rôças, Diversity of endodontic microbiota revisited, J. Dent. Res. 88 (2009) 969–981, https://doi.org/10.1177/0022034509346549.

[39] J.F. Siqueira, I.N. Rôças, A.S. Rosado, Investigation of bacterial communities associated with asymptomatic and symptomatic endodontic infections by denaturing gradient gel electrophoresis fingerprinting approach, Oral Microbiol. Immunol. 19 (2004) 363–370, https://doi.org/10.1111/j.1399-302x.2004.00170.x.

[40] J.F. Siqueira, I.N. Rocas, Distinctive features of the microbiota associated with different forms of apical periodontitis, J. Oral. Microbiol. 1 (2009), https://doi.org/10.3402/jom.v1i0.2009.

[41] A.M. Lennon, L. Brune, S. Techert, W. Buchalla, Fluorescence spectroscopy shows porphyrins produced by cultured oral bacteria differ depending on composition of growth media, Caries. Res. 57 (2023) 74–86, https://doi.org/10.1159/000528731.

[42] C.D.C. Fernandes, D.K. Rechenberg, M. Zehnder, G.N. Belibasakis, Identification of Synergistetes in endodontic infections, Microb. Pathog. 73 (2014) 1–6, https://doi.org/10.1016/j.micpath.2014.05.001.

[43] H.Y. Oh, H.I. Jung, J.W. Lee, E.J. de Jong, B.I. Kim, Improving the competency of dental hygiene students in detecting dental restorations using quantitative light-induced fluorescence technology, Photodiagnosis. Photodyn. Ther. 17 (2017) 245–249, https://doi.org/10.1016/j.pdpdt.2016.12.010.