Photodiagnosis and Photodynamic Therapy 25 (2019) 142–147

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

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

Comparison of fluorescence parameters between three generations of QLF devices for detecting enamel caries *in vitro* and on smooth surfaces

Seok-Woo Park[a], Sang-Kyeom Kim[a], Hyung-Suk Lee[a], Eun-Song Lee[a], Elbert de Josselin de Jong[a,b,c], Baek-Il Kim[a,*]

[a] *Department of Preventive Dentistry & Public Oral Health, BK21 PLUS project, Yonsei University College of Dentistry, Seoul, Republic of Korea*
[b] *Department of Health Services Research, University of Liverpool, Liverpool, United Kingdom*
[c] *Inspektor Research Systems BV, Amsterdam, the Netherlands*

ARTICLE INFO

Keywords:
Enamel caries
Inspektor Pro
QLF-D
Qraycam
Quantitative light-induced fluorescence (QLF)
Validity

ABSTRACT

Background: This study compared two fluorescence parameters (fluorescence loss [ΔF] and red fluorescence gain [ΔR]) among three generations of quantitative light-induced fluorescence (QLF) systems with the aim of determining the validities of these parameters in the three devices for differentiating the severity of enamel caries.
Methods: Forty-one extracted human premolars and molars with suspected enamel caries were selected. Fluorescence images of all teeth were obtained using first-, second-, and third-generation QLF systems (Inspektor Pro, QLF-D, and Qraycam, respectively). Fluorescence parameters were then calculated using proprietary software. All of the specimens were also categorized histologically using polarized-light microscopy (PLM) based on histological levels related to the lesion depth into sound enamel (S), caries limited to the outer half of the enamel (E_1), and caries involving the inner half of the enamel (E_2). The Mann-Whitney test with Bonferroni correction was used to compare fluorescence parameters among the three generations of systems. The sensitivity, specificity, and area under the receiver operating characteristics curve (AUC) at two thresholds (S/E_1 for detecting enamel caries lesions and E_1/E_2 for differentiating the caries severity) were calculated for evaluating the validities of the fluorescence parameters obtained using all three generations of QLF devices.
Results: ΔF did not differ significantly between the devices at any histological level. In addition, ΔF showed large AUCs at the thresholds of S/E_1 and E_1/E_2 (0.97–0.98 and 0.89–0.90, respectively). On the other hand, ΔR was significantly higher for the third-generation device than for the first- and second-generation devices for E_2 lesions ($P < 0.001$). At the S/E_1 threshold, ΔR values of the first- and third-generation devices showed larger AUCs (0.96–0.97) compared with that of the second-generation device (0.91), whereas at the E_1/E_2 threshold the AUC was the largest for the third-generation device (0.87).
Conclusions: The ΔF fluorescence parameter did not differ between the three generations of QLF devices, and showed high validity values. In terms of ΔR, the devices of all generations also showed good diagnostic performance for quantifying and detecting enamel caries lesions, but the third-generation QLF system produced superior results.

1. Introduction

Dental caries has been defined as a cavitated lesion that can be clearly detected by the naked eye in conventional dental paradigm mainly focused on restorative treatment [1]. These cavitated lesions must be treated surgically because they cannot recover reversibly due to the irreversible physiochemical loss of dental hard tissue [2]. There has recently been a paradigm shift in the early detection of noncavitated caries lesions and treating them nonsurgically before they become cavitated [3–5]. Radiography has been used as a traditional diagnostic method until now, but it is not suitable for detecting noncavitated caries lesion due to its low reported sensitivity (0.17–0.83) [6].

Various optical diagnostic devices such as fiber-optic transillumination, optical coherence tomography, the DIAGNOdent device, and quantitative light-induced fluorescence (QLF) have been developed in recent years to overcome the limitations of conventional diagnostic methods [6–8]. QLF technology has in particular been widely used

* Corresponding author at: Department of Preventive Dentistry & Public Oral Health, BK 21 PLUS Project, Yonsei University College of Dentistry, 50-1 Yonsei-ro, Seodaemun-gu, Seoul, 03722, Republic of Korea.
E-mail address: drkbi@yuhs.ac (B.-I. Kim).

https://doi.org/10.1016/j.pdpdt.2018.11.019
Received 27 April 2018; Received in revised form 17 November 2018; Accepted 29 November 2018
Available online 30 November 2018
1572-1000/

Fig. 1. First-, second-, and third- generation QLF devices: (A) Inspektor Pro, (B) QLF-D, and (C) Qraycam, respectively.

since it was introduced in the 1980s, and it can be used to observe two kinds of fluorescence (changes in autofluorescence from the tooth and in red fluorescence) using visible light in the blue spectral range [9]. Changes in the mineral contents involved in the caries process cause light scattering, and the intensity of autofluorescence emitted from fluorophores located in the dentinoenamel junction (DEJ) is reduced. One of the fluorescence parameters employed in QLF technology to indicate the reduced autofluorescence intensity of tooth is the fluorescence loss (ΔF). ΔF allows minute changes in dental hard tissues such as those associated with dental caries, dental fluorosis, and dental erosion to be quantified nondestructively [10–12]. The other fluorescence parameter is the red fluorescence gain (ΔR), which relates to the red fluorescence generated by porphyrin produced by bacterial metabolism. Red fluorescence can be found in mature plaques, the tongue coating, dental cracks, and active caries lesions [13–17].

Various types of devices based on the QLF technology have been developed (Fig. 1). The first-generation QLF system, Inspektor Pro (Inspektor Research Systems BV, Amsterdam, the Netherlands), was introduced in 2004 and comprises a 404 nm peak wavelength of light (with a full width at half maximum of 22 nm), a high-pass filter ($>$ 520 nm), and a micro − CCD camera. The Quantitative Light-Induced Fluorescence—Digital Biluminator 2 + device (QLF-D, Inspektor Research Systems BV), which was introduced as the second-generation device in 2012, mainly uses a single-lens reflex (SLR) camera in which the Biluminator system containing 405 nm peak wavelength of violet-blue light LEDs with full width at half maximum of 15 nm and white-light LEDs and an Inspektor filter (high-pass filter $>$ 480 nm in combination with a pink filter to emphasize on the 630–640 nm band) are embedded in the main body. The Qraycam device (AIOBIO, Seoul, Republic of Korea) introduced in 2014 is a third-generation QLF device, comprising a set of LEDs (in the same configuration as QLF-D), a CCD camera, and an Inspektor glass filter (with the same filter as in the QLF-D device).

These various generations of QLF devices use visible light of the same wavelength but different cameras, capturing methods, and filters. Nevertheless, few studies have directly compared the fluorescence patterns from the different generations of QLF systems in evaluations of the same caries lesion. The aims of this *in vitro* study were therefore to compare the fluorescence parameters for quantifying enamel caries on smooth surfaces among the generations of QLF system and to confirm the validities of fluorescence parameters for the detection of caries lesions.

2. Materials and methods

2.1. Selection and preparation of teeth samples

The Ethics Committee of the Dental Hospital of Yonsei University approved the study (IRB 2-2017-0044), and all of the voluntary participants were provided informed consent before teeth were extracted. Forty-one permanent human premolars and molars with suspected enamel caries on smooth surfaces (including proximal surfaces, partially) were selected after excluding teeth with enamel dysplasia or dental fluorosis. Dental plaque and residues including periodontal tissue remaining on the tooth surfaces were removed using toothbrushes and curettes. The teeth were stored at − 20 °C in a container blocked from external light. Tooth specimens were kept stable when capturing surface images by fixing them vertically into a 15-mm × 12-mm × 8-mm acrylic mold with a 9-mm-diameter hole using a self-cured resin (Ortho-Jet, Lang Dental Manufacturing, Wheeling, IL, USA).

2.2. Acquisition of fluorescence images and quantitative analysis of fluorescence parameters

Before fluorescence images were captured, the specimens were dried for 15 s using compressed air [18]. According to the results of the preliminary experiment, the order of capturing the fluorescence images with the different devices did not affect the fluorescence loss. Therefore, without randomization, the first-, second-, and third-generation devices (Inspektor Pro, QLF-D, and Qraycam, respectively) were used in the same sequence to obtain fluorescence images of each specimen. Images of the specimens were captured using the proprietary software supplied with each QLF device. The QLF-D fluorescence images were captured using an ISO of 1600, a shutter speed of 1/30, and an aperture value of 6.7, while the Qraycam settings were an exposure of 0 and a contrast of 0. According to the manufacturer's instructions, the distances between the specimens and the first-generation device and the LEDs of the second- and third-generation devices were 0.5, 13, and 8 cm, respectively. Since the size of the fluorescence images differ among the devices, patches were drawn around the same region to be analyzed in each image and set as the area of interest (AOI). The fluorescence intensity in the AOI was quantified using the two kinds of fluorescence parameters, ΔF and ΔR, which were both measured as percentages using analysis software (QA2 version 1.24, Inspektor Research Systems BV). ΔF represents the percentage decrease in the autofluorescence intensity in a caries lesion compared with that in sound enamel, and it reflects changes in the mineral contents of enamel. ΔR is the percentage increase in red fluorescence intensity due to porphyrins from bacterial metabolism compared with that for sound enamel, and it reflects the activity of oral microorganisms.

2.3. Histological analysis

All of the teeth used in this study were histologically analyzed to calculate the goldstandard, lesion depth. Before cutting the specimens, wax was used to cover the crown to prevent destruction of enamel. The specimens were cut at the center of the AOI using a microtome (TechCut 4™, Allied High Tech Products, Compton, CA, USA). The slide containing the specimen was ground to a thickness of 100 μm using 600-grit silicon-carbide sandpaper (R&B, Daejeon, Republic of Korea). The ground specimens were photographed at 40× and 100× using polarized light microscope (PLM, CX31-P, Olympus, Tokyo, Japan). The results based for the depths of the caries lesion observed in the PLM images were classified into three categories: code S refers to sound enamel, code E_1 refers to enamel demineralization limited to the outer half of the enamel layer, and code E_2 refers to enamel demineralization involving the inner half of the enamel, up to the DEJ.

2.4. Statistical analysis

Box-and-whisker plots were drawn to display the distribution of fluorescence parameters. The Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction were applied to identify significant differences in fluorescence parameters among the three generations of QLF devices. Receiver operating characteristics curves were calculated to evaluate the validities of the fluorescence parameters for detecting enamel caries lesions among the QLF devices. The sensitivity, specificity, and area under the receiver operating characteristics curve (AUC) were calculated at two thresholds: one for detecting enamel caries lesions (S/E_1) and the other for differentiating the caries severity (E_1/E_2). All statistical analyses were conducted using PASW version 23.0 (IBM, Somers, NY, USA) and MedCalc® (MedCalc Software, Ostend, Belgium).

3. Results

The histological results showed that 15 teeth were code S (36.6%), 17 teeth had E_1 lesions (41.5%), and 9 teeth had E_2 lesions (22.0%). The fluorescence properties observed for sound enamel differ among the three generations of devices (Fig. 2). The first-generation device visualizes the sound enamel as green, whereas tooth-colored fluorescence appeared in the second- and third-generation devices. When observing the caries lesion area, the decreased intensity of autofluorescence simply appeared dark. On the other hand, red fluorescence in the fluorescence images of the second- and third-generation devices appeared together with decreased autofluorescence of the caries lesion.

Comparison of ΔF between all three devices showed no statistically significant difference for each of the histological codes (Fig. 3A). In the red fluorescence analysis, there was no statistically significant difference in ΔR between codes S and E_1. However, for code E_2, ΔR was significantly larger for the second- and third-generation devices than for the first-generation device (Fig. 3B, $P < 0.001$).

For distinguishing enamel caries from sound enamel (at the S/E_1 threshold), cutoff ΔF values of the QLF devices ranged from –11.0 to –9.7 (Fig. 4A). The sensitivity, specificity, and AUC for ΔF showed excellent values of 0.92, 1.00, and 0.97–0.98, respectively. The cutoff values for ΔR ranged from 20.0 to 29.9 (Fig. 4B). The sensitivity, specificity, and AUC for ΔR also showed excellent values, of 0.85–0.89, 0.93–1.00, and 0.91–0.97, respectively.

At threshold E_1/E_2 for distinguishing the severity of enamel caries, the cutoff ΔF values of the devices ranged from –16.1 to –13.1. Validity analyses showed that the devices of all generations had good accuracies, with high sensitivities (0.89–1.00), specificities (0.69–0.78), and AUCs (0.89–0.90) (Fig. 5A). The cutoff ΔR values were similar in the first- and second-generation devices, while it was 1.4-fold higher in the third-generation device (Fig. 5B). The sensitivity, specificity, and AUC were higher for the third-generation device than for the first-generation. In addition, the AUC was smallest (0.81) for the first-generation device.

4. Discussion

The comparisons among three generations of QLF devices performed in this study revealed that ΔF values (which reflect the mineral contents of teeth) did not differ among the devices and had excellent diagnostic accuracies for detecting enamel caries on smooth surfaces and differentiating lesion severity. Meanwhile, ΔR (representing the microbial activity) was significantly higher for the second- and third-generation devices than for the first-generation device for code E_2 ($P < 0.001$). Two kinds of fluorescence parameters from QLF devices have been demonstrated to be useful for distinguishing enamel caries lesions. Nonetheless, for the red fluorescence, the third-generation device with an integrated glass filter could provide clearer fluorescence images and showed better performance for differentiating the enamel caries severity than the first-generation device (with one filter) and the second-generation device (with two filters).

The fluorescence images produced by the three generations of QLF devices looked different to the naked eye even though there were no significant differences in the calculated ΔF values. The main reason for this apparent discrepancy is the different filters in the three QLF devices. As QLF technology has developed, new filters have been developed for the second- and third-generation devices to emphasize the minute differences in fluorescence intensities by broadening the red and blue spectral ranges. This has resulted in the colors of sound enamel and caries lesions differing among the fluorescence images produced by the devices. However, ΔF is not affected by the filters because it is calculated only from the green spectral components of the autofluorescence of teeth. This resulted in the ratios of the decrease in green fluorescence intensity in caries lesions relative to sound enamel being similar among all of the devices.

Both red fluorescence and green fluorescence can be observed in

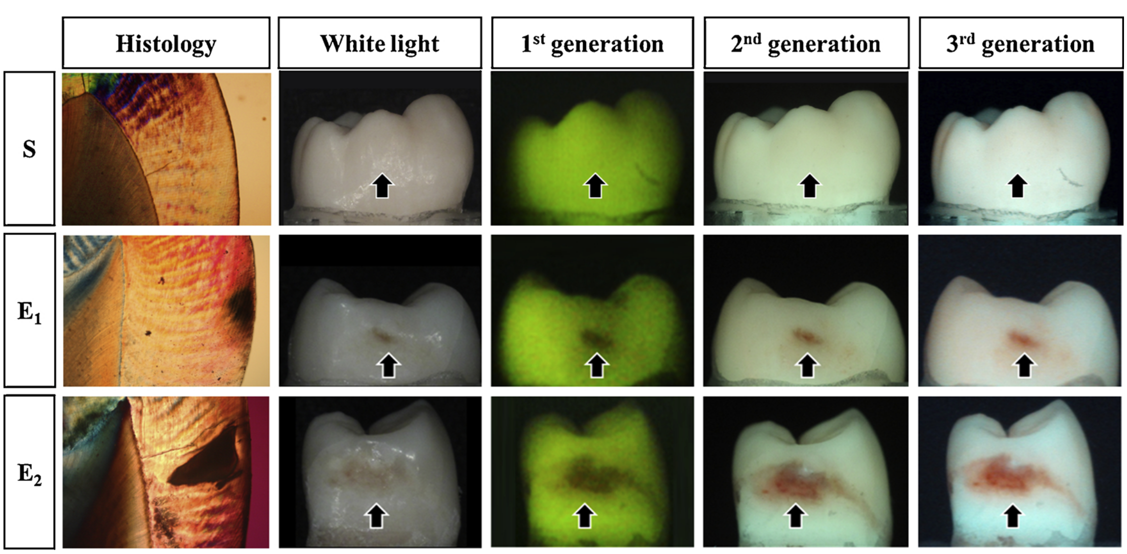

Fig. 2. Representative polarized light microscope (PLM, magnified 100x), white light, and fluorescence images obtained using each QLF device.
Black arrows indicate the AOI for QLF image analysis. S, sound enaeml; E_1, demineralization limited to the outer half of the enamel; E_2, demineralization involving the inner half of the enamel.

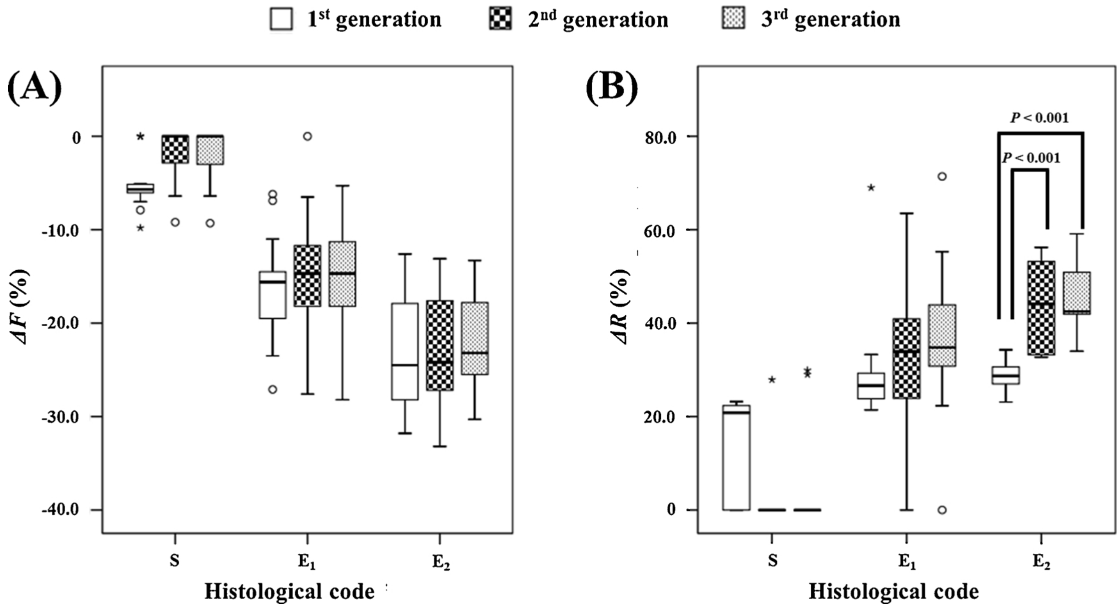

Fig. 3. Comparison of ΔF (A) and ΔR (B) values between the three generations of QLF devices for different histological codes.
S, sound enamel; E_1, demineralization limited to the outer half of the enamel; E_2, demineralization involving the inner half of the enamel.

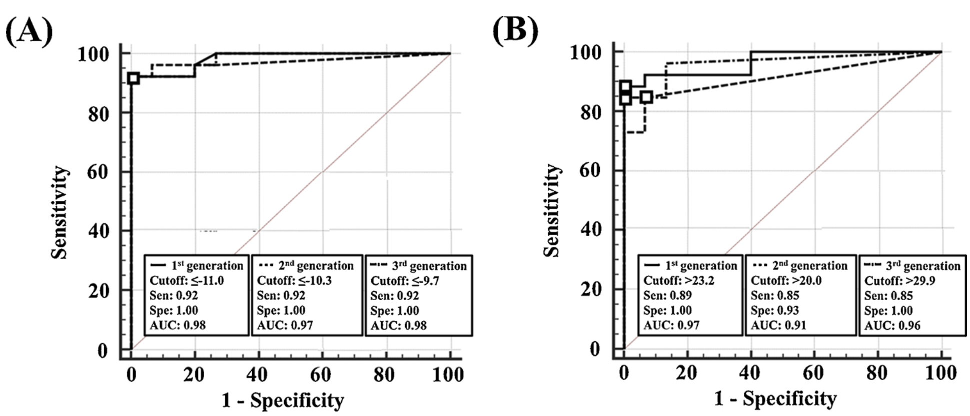

Fig. 4. Receiver operating characteristics (ROC) curves of ΔF (A) and ΔR (B) at the threshold for detecting enamel caries lesions (S/E_1).
Sen, sensitivity; Spe, specificity; AUC, area under the receiver operating characteristics curve.

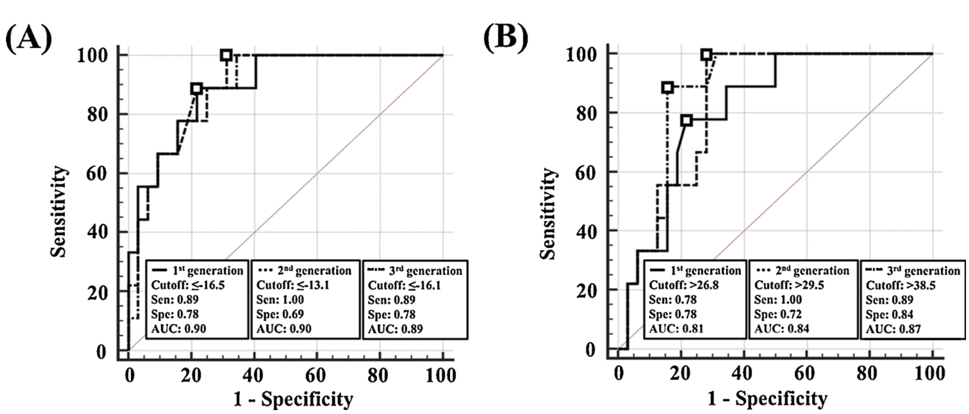

Fig. 5. Receiver operating characteristics (ROC) curves of ΔF (A) and ΔR (B) at the threshold for differentiating the severity of enamel caries lesions (E_1/E_2).
Sen, sensitivity; Spe, specificity; AUC, area under the receiver operating characteristics curve.

caries lesions. Demineralized enamel caries lesions contain a lower volume of minerals and a higher volume of organic matter and water compared to sound enamel [19,20]. This can result in the formation of porous pathways connecting between the inside and outside of the lesion. Previous studies using QLF systems have mainly investigated the red fluorescence in mature and pathogenic biofilms [21,22]. Lee et al. were the first to report that the red fluorescence that had been observed in mature plaques could also be observed in cross-sectional images of occlusal caries lesions in *in vitro* experiments [23]. The red fluorescence inside caries lesions has also been reported for some *in vivo* studies that evaluated how ΔR varied with increased depths of occlusal and proximal caries lesions [24,25]. These results indicate that QLF technology demonstrated that mature and pathogenic biofilms can potentially penetrate enlarged porous structures, and hence this technology can be regarded as a useful tool for detecting red fluorescence inside enamel caries lesions.

The ΔR values differed among the devices of different generations. Kim and Kim evaluated the progression of caries lesions using the total intensity of red fluorescence, and reported that the second-generation QLF device had an improved capability for detecting red fluorescence and could improve the ability to predict the progression of caries compared to the first-generation device [17,26]. As stated above, the validity for distinguishing between shallow enamel caries lesions (E_1) and deep caries lesions (E_2) was highest for the third-generation device and lowest for the first-generation device (Fig. 5B). In particular, for deep lesions there were no differences in ΔF among the three devices, but differences in ΔR were present due to the use of different filters (Fig. 3B). Therefore, there are no differences in the low levels of red fluorescence for shallow caries lesions, while differences can occur for the high intensity of red fluorescence for deep caries lesions due to ΔR being higher in the second- and third-generation devices than in the first-generation device.

The results of this study suggest that the ΔR value—which reflects not only the microbial activity but also the mineral content—could be a useful parameter for evaluating enamel caries lesions. This is supported by the present *in vitro* study finding excellent validity values for the use of red fluorescence in distinguishing enamel caries lesions. A previous *in vivo* study found a validity of 0.69 for ΔR in distinguishing proximal enamel caries using the second-generation device [24]. That validity value is lower than those in the present study due to inherent limitations of an *in vivo* study. Proximal caries lesions generally occur mainly below the contact points of adjacent teeth, which makes them difficult to observe directly [27]. The previous assessments of proximal caries only captured fluorescence images of the occlusal surface, and the indirect evaluations had limitations in demonstrating the relationship between lesion depth and fluorescence parameters despite the good validity. For this reason, we obtained direct images of lesions, which make accurate validation tests of the fluorescence parameters possible. Another potential limitation is the type of gold-standard method used. The previous study used conventional bitewing radiography, which showed a low sensitivity of 0.41 for detecting proximal caries lesions [28]. In contrast, the present *in vitro* study used a histological analysis with the aid of PLM to calculate the precise lesion depth, which reconfirmed the possibility of using red fluorescence to detect proximal caries and thereby augmented the previous *in vivo* study by yielding clear evidence.

To the best of our knowledge, this is the first *in vitro* study to confirm differences in representative three QLF devices for detecting enamel caries lesions, and also the first to use the latest-generation device and confirm the excellent validities of the devices of all generations in detecting enamel caries. From our results we suggest that the most-appropriate choice of QLF device depends on the capturing environment and desired usage, as follows:

1 The first-generation device (Inspektor Pro) comprises of an intraoral camera that makes it easy to obtain fluorescence images of a single tooth and to monitor lesions due to its video repositioning capability to capture sequential images at the same position.
2 The second-generation device (QLF-D) is suitable for use in both clinics and laboratories because it can continuously capture high-resolution white-light and fluorescence images every 2 s. It is possible to image a complete quadrant (or sextant) with high resolution so that individual teeth can be envisioned with the same image quality as the first generation device. Posterior teeth can be imaged indirectly by using a mirror. Due to the use of the double filter of this device, it shows a much higher contrast in red fluorescence enabling the detection of oral anomalies which could not detected with the previous device.
3 The third-generation QLF device (Qraycam), which is similar to the second-generation QLF device, is capable of full-range imaging. With the second and third generation devices it is easy to detect lesions in the clinic. The quality of the images obtained with the third-generation device is similar to that of the second generation device. It is also both light weight and portable as well, which makes it easier for dental professionals to capturing image in the clinical environment. Additionally, it is a practical device to be used large-scale epidemiological surveys and for providing oral-care education in schools and public health centers.

Considering the above characteristics, the various generations of QLF devices can be effective detection and education tools for enamel caries in a variety of situations. Future studies should attempt to confirm their utility in other diverse cases and situations.

5. Conclusion

This *in vitro* study was aimed to compare the difference of the fluorescent parameters of enamel caries on smooth surfaces with three different QLF generation devices. It was found that ΔF (fluorescence loss) did not differ among the first-, second-, and third-generation QLF devices of smooth enamel caries lesions. In contrast, ΔR (red fluorescence gain) had higher validity for the second- and third-generation devices than for the first-generation device.

Conflicts of interest

Inspektor Research Systems BV provided the salary for author EdJdJ, but did not have any additional role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. EdJdJ's involvement in this research was under the auspices of his status as adjunct professor at Yonsei University College of Dentistry supported by BK21 PLUS Project. The specific role of EdJdJ was to provide his expertise regarding the fluorescence technology. This does not alter the author's adherence to the policies of Photodiagnosis and Photodynamic Therapy on sharing data and materials. EdJdJ holds several patents with respect to QLF technology. The remaining authors declared no conflicts of interest.

Acknowledgments

This study was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (316071031HDD020).

References

[1] N.B. Pitts, D.T. Zero, White Paper on Dental Caries Prevention and Management, FDI World Dental Press Ltd., 2016.
[2] N.B. Pitts, Modern concepts of caries measurement, J. Dent. Res. 83 (2004) C43-C7.
[3] N.B. Pitts, J.W. Stamm, International consensus workshop on caries clinical trials (ICW-CCT)—final consensus statements: agreeing where the evidence leads, J. Dent. Res. 83 (2004) 125–128.
[4] J. Fisher, M. Glick, A new model for caries classification and management: the FDI world dental federation caries matrix, J. Am. Dent. Assoc. 143 (2012) 546–551.
[5] N.B. Pitts, Are we ready to move from operative to non-operative/preventive treatment of dental caries in clinical practice? Caries Res. 38 (2004) 294–304.
[6] J. Gomez, C. Zakian, S. Salsone, S.C. Pinto, A. Taylor, I.A. Pretty, et al., In vitro performance of different methods in detecting occlusal caries lesions, J. Dent. 41 (2013) 180–186.
[7] I.A. Pretty, Caries detection and diagnosis: novel technologies, J. Dent. 34 (2006) 727–739.

[8] M.H. van der Veen, Detecting short-term changes in the activity of caries lesions with the aid of new technologies, Curr. Oral Health Rep. 2 (2015) 102–109.
[9] H. Bjelkhagen, F. Sundstrom, B. Angmar-Mansson, H. Ryden, Early detection of enamel caries by the luminescence excited by visible laser light, Swed. Dent. J. 6 (1982) 1–7.
[10] H.Y. Ko, S.M. Kang, H.E. Kim, H.K. Kwon, B.I. Kim, Validation of quantitative light-induced fluorescence-digital (QLF-D) for the detection of approximal caries in vitro, J. Dent. 43 (2015) 568–575.
[11] S.K. Kim, H.S. Lee, S.W. Park, E.S. Lee, E. de Josselin de Jong, H.I. Jung, et al., Quantitative light-induced fluorescence technology for quantitative evaluation of tooth wear, J. Biomed. Opt. 22 (2017) 1–6.
[12] D.M. Cuevas-Espinosa, E.A. Martinez-Mier, M. Ando, G.A. Castiblanco, F. Cortes, C.M. Rincon-Bermudez, et al., *In vitro* validation of quantitative light-induced fluorescence for the diagnosis of enamel fluorosis in permanent teeth, Caries Res. 51 (2017) 515–526.
[13] S.Y. Han, B.R. Kim, H.Y. Ko, H.K. Kwon, B.I. Kim, Validity and reliability of auto-fluorescence-based quantification method of dental plaque, Photodiagn. Photodyn. Ther. 12 (2015) 587–591.
[14] S.Y. Han, B.R. Kim, H.Y. Ko, H.K. Kwon, B.I. Kim, Assessing the use of Quantitative Light-induced Fluorescence-Digital as a clinical plaque assessment, Photodiagn. Photodyn. Ther. 13 (2016) 34–39.
[15] M.K. Jun, H.M. Ku, E.S. Kim, H.E. Kim, H.K. Kwon, B.I. Kim, Detection and analysis of enamel cracks by quantitative light-induced fluorescence technology, J. Endod. 42 (2016) 500–504.
[16] E.S. Lee, H.K. Yim, H.S. Lee, J.H. Choi, J.H. Lee, B.I. Kim, Clinical assessment of oral malodor using autofluorescence of tongue coating, Photodiagn.Photodyn. Ther. 13 (2016) 323–329.
[17] G. Felix Gomez, G.J. Eckert, A. Ferreira Zandona, Orange/Red fluorescence of active caries by retrospective quantitative light-induced fluorescence image analysis, Caries Res. 50 (2016) 295–302.
[18] I.A. Pretty, W.M. Edgar, S.M. Higham, The effect of dehydration on quantitative light-induced fluorescence analysis of early enamel demineralization, J. Oral Rehab. 31 (2004) 179–184.
[19] Barbosa de Sousa F, Soares J. Dias, Vianna S. Sampaio, Natural enamel caries: a comparative histological study on biochemical volumes, Caries Res. 47 (2013) 183–192.
[20] M.H. van der Veen, R.Z. Thomas, Huysmans MCDNJM, J.J. de Soet, Red auto-fluorescence of dental plaque Bacteria, Caries Res. 40 (2006) 542–545.
[21] 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.
[22] E.S. Lee, E. de Josselin de Jong, H.I. Jung, B.I. Kim, Red fluorescence of dental biofilm as an indicator for assessing the efficacy of antimicrobials, J. Biomed. Opt. 23 (2018) 1–6.
[23] H.S. Lee, S.K. Kim, S.W. Park, Jong EdJd, H.K. Kwon, S.H. Jeong, et al., Caries detection and quantification around stained pits and fissures in occlusal tooth surfaces with fluorescence, J. Biomed. Opt. 23 (2018) 7.
[24] E.S. Kim, E.S. Lee, S.M. Kang, E.H. Jung, E. de Josselin de Jong, H.I. Jung, et al., A new screening method to detect proximal dental caries using fluorescence imaging, Photodiagn. Photodyn. Ther. 20 (2017) 257–262.
[25] E.H. Jung, E.S. Lee, H.I. Jung, S.M. Kang, E. de Josselin de Jong, B.I. Kim, Development of a fluorescence-image scoring system for assessing noncavitated occlusal caries, Photodiagn. Photodyn. Ther. 21 (2018) 36–42.
[26] H.E. Kim, B.I. Kim, Analysis of Orange/Red fluorescence for bacterial activity in initial carious lesions may provide accurate lesion activity assessment for caries progression, J. Evid. Dent. Pract. 17 (2017) 125–128.
[27] W.H. Arnold, P. Gaengler, L. Kalkutschke, Three-dimensional reconstruction of approximal subsurface caries lesions in deciduous molars, Clin. Oral Invest. 2 (1998) 174–179.
[28] J. Bader, D. Shugars, A. Bonito, Systematic reviews of selected dental caries diagnostic and management methods, J. Dent. Educ. 65 (2001) 960–968.