©Operative Dentistry, 2019, **44-4,** 405-413

In Vitro Red Fluorescence as an Indicator of Caries Lesion Activity

BR Kim • SM Kang • E de Josselin de Jong • HK Kwon • BI Kim

Clinical Relevance

Red fluorescence measurement may be useful for objectively evaluating activity in smooth surface caries lesions.

SUMMARY

This *in vitro* study examined the utility of comparing red fluorescence between active and inactive caries lesions and investigated whether changes in red fluorescence and fluorescence loss are influenced by lesion activity following remineralization. Sixty-two noncavitated smooth surface caries lesions on extracted human teeth were classified into active or inactive lesions using the Nyvad system prior to a 12-day pH-cycling procedure. Quantitative light-induced fluorescence-digital images were used to measure fluorescence parameters before and after pH cycling. At baseline, the intensity (ΔR) and area ($A_{\Delta R}$) of red fluorescence were 1.5- and 2.2-fold higher in active lesions than in inactive lesions ($p<0.05$). The ratio of $A_{\Delta R}$ to lesion area was associated with classification of active lesions (odds ratio = 1.031; 95% confidence interval = 1.005–1.058). After pH cycling, the active lesions showed about 2- and 8-fold greater reductions in the median values of $A_{\Delta R}$ and fluorescence loss related to lesion volume (ΔQ) compared with inactive lesions ($p<0.05$). In conclusion, red fluorescence differs depending on lesion activity, and the red fluorescence area and lesion volume change following remineralization. The results suggest that measuring red fluorescence may be a useful way of objectively evaluating lesion activity of smooth surface lesions.

Bo-Ra Kim, PhD, Yonsei University College of Dentistry, Brain Korea 21 PLUS Project, Department of Preventive Dentistry & Public Oral Health, Seoul, Republic of Korea

Si-Mook Kang, PhD, Yonsei University College of Dentistry, Department of Preventive Dentistry & Public Oral Health, Seoul, Republic of Korea

Elbert de Josselin de Jong, PhD, Yonsei University College of Dentistry, Brain Korea 21 PLUS Project, Department of Preventive Dentistry & Public Oral Health, Seoul, Republic of Korea

Ho-Keun Kwon, PhD, Yonsei University College of Dentistry, Department of Preventive Dentistry & Public Oral Health, Seoul, Republic of Korea

*Baek-Il Kim, PhD, Yonsei University College of Dentistry, Brain Korea 21 PLUS Project, Department of Preventive Dentistry & Public Oral Health, Seoul, Republic of Korea

*Corresponding author: 50-1 Yonsei-ro, Seodaemun-Gu, Seoul 03722, Republic of Korea; e-mail: drkbi@yuhs.ac

DOI: http://doi.org/10.2341/17-369-L

INTRODUCTION

The dynamic balance of dental caries can proceed toward net demineralization underneath cariogenic plaque,[1,2] but the process can be arrested or shifted toward net remineralization if plaque removal and/or fluoride treatment are performed.[3,4] These lesions could be called potentially "active" at a certain point in time, whereas those with arrested progression could be called "inactive."[3,5] The status of caries lesions is visible on the surface by different relative refractive indices or light scattering, depending on

the porosity.[3,6,7] Therefore, lesion activity is currently assessed with visual-tactile inspection including evaluation of color, roughness, gloss, or the presence of plaque.[8-11] Considering that examining lesion activity status can help in determining and performing appropriate noninvasive management of noncavitated caries lesions,[4,5] it is worth focusing on the activity characteristics of caries lesions, especially in the early stage of caries. However, the assessment of caries lesion activity requires careful attention because it depends on specific clinical features,[12] and monitoring changes over time is needed to confirm progression.

Auto-fluorescence emission under a certain violet-blue wavelength is an intrinsic optical property of tooth tissue.[13] Tooth mineral loss leads to increased light scattering and changed fluorescence,[14,15] so early caries lesions can be identified by their reduced fluorescence intensity compared to that of sound teeth.[13] In addition, the fluorescence emission spectra of caries lesions contain additional emission peaks at 590-700 nm (red wavelength), which is not observed in healthy tissue.[16-19] Although it was not clear exactly which material emits the fluorescence, red fluorescence is detected from mature dental plaque and/or some oral bacteria that synthesize porphyrins as metabolites.[16,18,20,21] It has also been reported that the spectral characteristics of red fluorescent porphyrins such as protoporphyrin IX, coproporphyrin, and Zn-protoporphyrin correspond with that of caries lesions emitting red fluorescence by absorbing light at wavelength of 407 nm.[16,18]

Red fluorescence can be found in caries lesions; however, little is known about how its emission from caries relates to lesion activity status. A preliminary study reported differences in the ratios of the area under the 480- to 520-nm and 620- to 640-nm bands (green/red) of the fluorescence emission profile of natural caries lesions, depending on the activity assessed at a point in time.[22] A recent *in vitro* investigation demonstrated that the red fluorescence of caries-like lesions developed with a bacterial model could be measured by fluorescence-based devices over a progression of bacterial cariogenic challenges.[23] These findings indicate that the red fluorescence may be an objective predictor of activity status. Additionally, because lesion activity would respond differently to environmental conditions such as remineralization,[11] it could be expected that further changes in their fluorescent properties are dependent on lesion activity. Therefore, the present *in vitro* study aimed to compare the red fluorescence properties between active and inactive caries lesions and to investigate whether changes in red fluorescence and the degree of intrinsic fluorescence loss are influenced by lesion activity following remineralization.

METHODS AND MATERIALS

Study Design

A total of 62 noncavitated lesions on smooth surfaces of extracted permanent teeth were used. Caries lesions were classified into either active or inactive lesions by a visual-tactile inspection method based on the Nyvad system,[10] and the lesions were then subjected to a 12-day pH-cycling procedure. The light-induced fluorescence images of the lesions were taken before and after the pH cycling with a fluorescence camera (Quantitative Light-induced Fluorescence-Digital [QLF-D] Biluminator 2+, Inspektor Research Systems BV, Amsterdam, The Netherlands). The fluorescence images were used to measure the values of fluorescence parameters that were related to fluorescence loss and the increase in red fluorescence compared with that of sound teeth. Statistical analyses were used to verify the differences in the red fluorescence or fluorescence loss values at baseline and differences in the changes of the parameter values after pH cycling between the active and inactive lesion groups.

Tooth Selection and Caries Lesion Activity Assessment

Extracted human premolars and molars with at least one caries lesion on one smooth surface were selected (Institutional Review Board Approval 2-2014-0024). Any debris and calculus attached on the surfaces were removed with a hand scaler, and the teeth were brushed with nonfluoride toothpaste and a toothbrush. Among 62 lesions from 62 teeth, most were present on the proximal (mesial or distal) surface. If there were two or more lesions on one surface or tooth, one lesion was selected randomly.

The activity status (active or inactive) was determined by color (white/yellow or brown/black), texture (rough or hard/smooth), and luster (opaque with loss of luster or shiny) according to the criteria described by Nyvad and others.[10] Lesions were classified as active if the surface was rough/opaque with loss of luster or as inactive if the surface was smooth, hard, or shiny. Plaque presence was not considered due to tooth extraction. A trained examiner assessed lesion activity after air drying using a WHO probe and an explorer (Osung, Gimpo, Republic of Korea). The teeth were stored in a dark

Table 1: *Descriptions of the Fluorescence Parameters Obtained by the White Spot Patch of the QA2 Analysis Program of the QLF-D*[27]

Parameter (Unit)	Description	Expression for Changes in the Values of Parameters[a]
ΔR (%)	Percent increase in the ratio of red over green units observed in the lesion region compared to that of the sound tooth region around the lesion in fluorescence images	DΔR
ΔRmax (%)	Maximum value of ΔR in the lesion region	DΔRmax
$A_{\Delta R}$ (pixel)	The area within the lesion where $\Delta R > 30\%$	$DA_{\Delta R}$
ΔF (%)	Percent decrease in fluorescence intensity observed in the lesion region compared to that of the sound tooth region in fluorescence images. This parameter was presented as negative values due to fluorescence loss in the lesions.	DΔF
ΔFmax (%)	Maximum value of ΔF in the lesion region	DΔFmax
$A_{\Delta F}$ (pixel)	The area within the lesion where $\Delta F < -5\%$	$DA_{\Delta F}$
ΔQ (% × pixel)	The value produced by multiplying ΔF and $A_{\Delta F}$	DΔQ
$A_{\Delta R}/A_{\Delta F}$ (%)	Ratio of red fluorescence area with respect to inside the lesion region. Values were calculated using the following equation: $A_{\Delta R}/A_{\Delta F} = A_{\Delta R \text{ before pH-cycling}}/A_{\Delta F \text{ before pH-cycling}} \times 100$	—

[a] *Change in each parameter was calculated as follows:* $D\Delta X = \Delta X_{\text{after pH-cycling}} - \Delta X_{\text{before pH-cycling}}$.

container and only exposed to light when the assessment was performed. Reexamination was performed 10 days after the first examination using 23 teeth (37%) that were randomly selected from among the samples.

pH-Cycling Procedure

Aciduric nail varnish was applied to the sound region around a caries lesion. The lesions were exposed to a modified version of a pH-cycling model[24] by using an acidic solution (0.1 M lactic acid, 50% saturation with hydroxyapatite, 1.0% v/v of Carbopol 2050, pH 4.8), an artificial saliva (0.22% gastric mucin, 6.5 mM NaCl, 1.45 mM $CaCl_2 \cdot 2H_2O$, 5.42 mM KH_2PO_4, 14.94 mM KCl, 0.02% NaN_3, pH 6.8), and a sodium fluoride (NaF) solution of 367 ppm. The fluoride concentration of the NaF solution was equivalent to that of a 33% v/v diluted slurry (ratio 1:2) of 1100 ppm NaF toothpaste with water.[25] The lesions were treated with NaF solution for one minute and artificial saliva for two hours. These steps were performed twice in the same order. After a two-hour acid challenge followed by a 30-minute artificial saliva treatment, the lesions were exposed to NaF solution for one minute and artificial saliva treatments for two hours. Then, a one-minute NaF solution treatment was performed, and the lesions were immersed in artificial saliva until they were subjected to the first treatment with NaF solution on the next day. These daily solution phases were repeated for 12 days. All treatments were performed under agitation at room temperature while blocking ambient light.

Analysis of Fluorescence Properties Observed in Caries Lesions

The fluorescence images were taken before and after pH-cycling treatment using a QLF-D, which can detect red fluorescence,[26] with photography conditions as follows: ISO sensitivity of 1600, aperture value of 13.0, and shutter speed of 1/8. The quantitative data were calculated using the white spot patch function of the QLF-D analysis software (QA2, v1.26, Inspektor Research System BV, Amsterdam, The Netherlands). With respect to red fluorescence, the average (ΔR [%]), maximum (ΔRmax [%]), and area ($A_{\Delta R}$ [pixel]) of red fluorescence were measured. In addition, the average and maximum values of fluorescence loss (ΔF [%] and ΔFmax [%]), lesion area ($A_{\Delta F}$ [pixel]), and lesion volume (ΔQ [% × pixel]) were measured. The detailed descriptions of the fluorescence parameters are listed in Table 1.[27] The values of the parameters related to red fluorescence were expressed as positive figures because the red fluorescence was increased by external materials derived from bacterial metabolism. On the other hand, the values of the parameters related to fluorescence loss were expressed as negative figures except for area parameters because caries lesions revealed darker fluorescence than the adjacent sound teeth. The degree of change in each parameter after pH cycling was calculated as follows: $D\Delta X = \Delta X_{\text{after pH-cycling}} - \Delta X_{\text{before pH-cycling}}$. As an additional parameter, this study calculated the percentage of area ratio of red fluorescence area and the lesion area ($A_{\Delta R}/A_{\Delta F}$ [%]) using the area parameters measured at baseline.

Table 2: *Values of the Fluorescence Parameters Detected in the Active or Inactive Lesions Before pH Cycling*

	Group			*p* value
	Active	**Inactive**	**Total**	
N	32	30	62	
Red Fluorescence Parameters at Baseline				
ΔR (%)	39.85 (28.73, 53.35)	27.35 (21.88, 44.80)	33.05 (24.08, 50.63)	0.049
ΔRmax (%)	85.00 (46.00, 187.75)	42.00 (0.00, 118.25)	69.50 (32.75, 149.50)	0.053
$A_{\Delta R}$ (pixel)	3289.00 (1584.75, 6849.25)	1488.50 (51.75, 3810.00)	2365.00 (504.75, 5256.50)	0.018
Fluorescence Loss Parameters at Baseline				
ΔF (%)	−17.47 (−25.92, −12.63)	−13.58 (−18.16, −10.71)	−16.01 (−23.01, −11.69)	0.045
ΔFmax (%)	−47.03 (−77.32, −30.63)	−34.16 (−57.38, −24.51)	−41.27 (−68.73, −25.51)	0.057
$A_{\Delta F}$ (pixel)	7681.50 (4584.50, 15,497.75)	6201.00 (3672.50, 9083.75)	7198.00 (4105.00, 12,661.75)	0.128
ΔQ (%×pixel)	−125,427.13 (−387,155.45, −65,496.71)	−102,424.88 (−172,647.16, −40,846.63)	−112,059.36 (−251,958.88, −47,193.44)	0.069
$\%A_{\Delta R}/A_{\Delta F}$ (%)	43.61 (30.18, 54.52)	24.60 (1.18, 43.82)	37.32 (11.04, 51.73)	0.021

All values are presented as median (25th, 75th percentiles).
For each parameter, statistical significance between the groups was determined using the Mann-Whitney U test at $\alpha=0.05$.

Statistical Analysis

All data were analyzed statistically after a normal distribution test using the SPSS software (IBM SPSS Statistics version 20, IBM, Armonk, NY, USA) with statistical significance set at $\alpha=0.05$. Intraexaminer reproducibility regarding lesion activity assessment was evaluated by calculating unweighted Cohen's κ values and percentage of agreement.

Because the data revealed a non-normal distribution, the Wilcoxon signed rank test was used to compare the fluorescence data obtained before and after pH cycling within the same activity lesion group. The Mann-Whitney *U* test was used to verify the differences in each fluorescence parameter obtained before pH cycling ($\Delta X_{\text{before pH-cycling}}$) and the differences in the changes of those data after pH cycling (DΔX) between active and inactive lesions. Logistic regression analyses were performed to identify the red fluorescence parameter obtained at baseline, which was associated with the classification of active lesions. In multiple logistic regression analysis, the variable selection criteria were 0.05 for inclusion and 0.20 for exclusion using a backward elimination method. Finally, the odds ratios (ORs) of independent variables were obtained.

RESULTS

The intraexaminer consistency was substantial (unweighted $\kappa=0.65$; %agreement=82.6%) for classifying the caries lesions into active or inactive lesions. Among the 62 lesions, 32 were classified as active lesions and 30 as inactive lesions.

The median values and interquartile ranges (IQRs) of the baseline fluorescence parameters are shown in Table 2. With respect to red fluorescence, the ΔR and $A_{\Delta R}$ of active lesions were 1.5-fold and 2.2-fold higher, respectively, than those of inactive lesions ($p=0.049$ and $p=0.018$). With respect to fluorescence loss, there was a significant difference in ΔF values between activity groups: 1.3-fold larger in active lesions than in inactive lesions ($p=0.045$). However, the $A_{\Delta F}$ and ΔQ (lesion volume) values did not show significant intergroup differences. Accordingly, the ratio of the red fluorescence area to the lesion area, $A_{\Delta R}/A_{\Delta F}$, was significantly larger in active lesions than in inactive lesions ($p=0.021$). This value was also significantly associated with classification of the active lesions, according to the multiple logistic regression analysis (OR=1.031, 95% confidence interval [CI]=1.005-1.058). Classification accuracy of 75% and 53% was found for the active and inactive lesions, respectively.

The distributions of DΔR, DΔRmax, and $DA_{\Delta R}$ are plotted in Figure 1. The $A_{\Delta R}$ displayed a significant downward trend within each activity group ($p<0.05$). The $DA_{\Delta R}$ in active lesions (median=−644.00; IQR=−1781.50, −74.75) was significantly higher than that in inactive lesions (median=−84.00; IQR=−798.75, 0.00; $p=0.018$). However, the ΔR and ΔRmax displayed minimal changes within each group, and the DΔR and DΔRmax showed no differences between activity groups.

Overall, lesions in both activity groups tended to recover all the fluorescence loss after pH cycling (Figure 2). The values of each parameter obtained

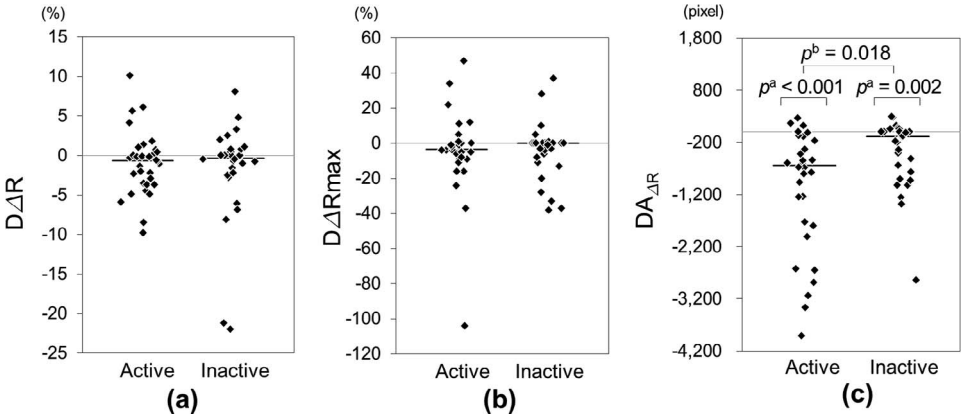

Figure 1. *Jitter plots showing the distribution of DΔR (a), DΔRmax (b), and DA$_{ΔR}$ (c). A black line in each activity group indicates the median value. A negative number denotes a reduction in each parameter value after pH cycling.* p *values are presented on the top of plotted dots if the values of ΔR, ΔRmax, or A$_{ΔR}$ obtained before pH cycling were significantly different from those obtained after pH cycling within the same group (*p^a*), or if the values of DΔR, DΔRmax, or DA$_{ΔR}$ were significantly different between the active and inactive lesion groups (*p^b*).*

after pH cycling were significantly different with those obtained before pH cycling within each group ($p<0.05$). Active lesions revealed the larger numerical values of changes in each fluorescence loss parameter (DΔF, DΔFmax, DA$_{ΔF}$, DΔQ), but a significant difference ($p=0.038$) was observed only in DΔQ between active lesions (median=28,420.48; IQR=4871.46, 83,120.68) and inactive lesions (median=14,743.28; IQR=795.26, 32,553.41). Representative white light and fluorescence images for active and inactive lesions obtained before and after pH cycling are presented in Figure 3.

DISCUSSION

The primary goal of this *in vitro* study was to observe red fluorescence under 405-nm light and compare active and inactive lesions on smooth surfaces. The results showed that there were significant differences in the red fluorescence intensity and area depending on lesion activity, with greater values in active lesions. The ratio of red fluorescence area to lesion area (A$_{ΔR}$/A$_{ΔF}$) was associated with classification of active lesions; hence, examining red fluorescence may help identify active caries lesions on smooth surfaces.

In this study, lesions with higher red fluorescence intensity (ΔR) were more likely to be considered "active" and showed greater fluorescence loss (ΔF). Regarding the fluorescence emitted from dental caries lesions, more severe caries lesions emit spectra with longer dominant wavelengths (ie, red-shifted; from green to yellow) and exhibit stronger red fluorescence because of the existence of organic materials in the lesion volume.[28,29] This indicates that active lesions have greater mineral loss and organic material accumulation relative to inactive lesions. However, it is known that noncavitated lesion activity is affected by surface layer porosity.[1,30] Regarding the clinical assessment of lesion activity, a rough surface may be due to enhanced porosity and intercrystalline spaces caused by large mineral loss in the surface layer compared with a nonrough or sound surface.[1] Moreover, water in the larger porous structure of a lesion evaporates more rapidly than in healthy tissue, and air in the pores can affect light scattering.[14,31] This might be

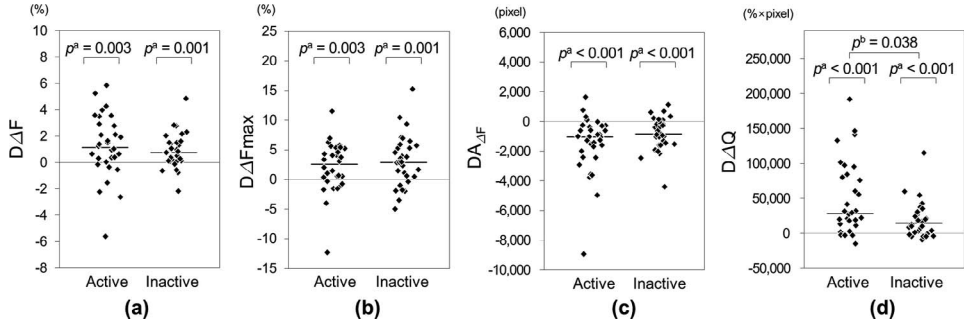

Figure 2. *Jitter plots showing the distribution of DΔF (a), DΔFmax (b), DA$_{ΔF}$ (c), and DΔQ (d). A black line in each activity group indicates the median value. A negative number denotes a reduction in A$_{ΔF}$ (c), whereas a positive number denotes a recovery in ΔF (a), ΔFmax (b), and ΔQ (d) after pH cycling.* p *values were presented on the top of plotted dots if the values of ΔF, ΔFmax, A$_{ΔF}$, or ΔQ obtained before pH cycling were significantly different from those obtained after pH cycling within the same group (*p^a*) or if the values of DΔF, DΔFmax, DA$_{ΔF}$, or DΔQ were significantly different between the active and inactive lesion groups (*p^b*).*

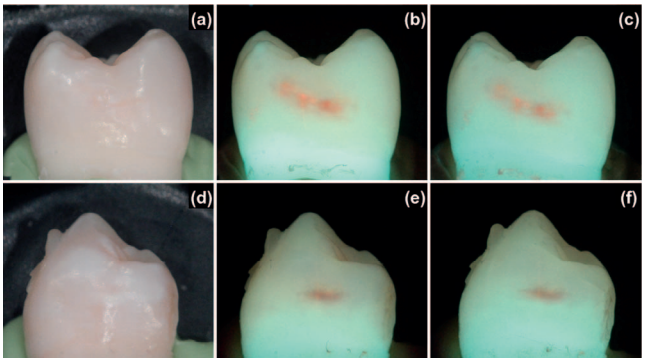

Figure 3. *Representative white light and corresponding fluorescence images of active (a-c) and inactive (d-f) lesions captured before pH cycling (a, b, d, e) and after pH cycling (c, f).*

reflected by the findings of Ando and others[32,33] that showed greater fluorescent changes in active caries lesions with dehydration compared to inactive lesions. Thus, the fluorescence related to lesion activity in the present study could be explained with regard to the mineral content in a lesion's surface layer, as well as lesion severity.

The observation that active and inactive lesions exhibit differences in red fluorescence intensity and area could be explained by the degree of infiltration of bacteria and their metabolites, which is dependent on the porosity. This is possible because bacteria and/or porphyrin derivatives may more easily penetrate into the inner areas of lesions with more porous microstructures.[34,35] In terms of porosity, it is inferred that bacteria and their metabolites may be continuously infiltrating the tooth surface to access the inner part of active lesions, as the lesions are usually covered with undisturbed plaque.[10] Conversely, disease arrested in inactive lesions may result in a less porous surface layer following remineralization.

Differences in surface layer microstructure between active and inactive lesions can also influence remineralization capability, because surfaces with higher mineral content may block ion migration during remineralization.[36] This is one possible explanation of the finding of different $D\Delta Q$ and $DA_{\Delta R}$ values for the active and inactive lesion groups after pH cycling. Lynch and others[37] reported that a weaker response to fluoride treatment was observed in carious lesions with higher surface mineral contents than surface-softened lesions; the two types of lesions have similar total mineral loss but different mineral distribution with regard to lesion depth. Additionally, Al-Khateeb and others[38] reported improved remineralization in the inner lesion body following surface etching. Taking lesion activity into consideration, inactive lesions usually have a smaller pore size and higher mineral content in the surface,[30] which are unfavorable for the migration of external ions and materials into inner lesion regions.[32] Therefore, inactive lesions appeared to have diminished potential to change in response to fluoride treatment following pH cycling in the current study.

This study showed the potential of red fluorescence as an indicator for classifying smooth caries lesion activity. The results support the hypothesis of Felix Gomez and others[39] that red fluorescence measured on noncavitated caries lesions using QLF can be used to differentiate between lesions that will progress to cavitation from those that will not. One interesting finding of the present study was that higher $A_{\Delta R}/A_{\Delta F}$ values were found in active lesions compared with the values in inactive lesions; $A_{\Delta R}/A_{\Delta F}$ was a new variable used in this study. This means that fluorescence loss, in addition to red fluorescence, should be considered in the evaluation of lesion activity. In addition, this variable showed classification accuracies of 75% and 53% for active and inactive lesions, respectively. This result shows this variable would be more useful in detection of active lesions than of inactive lesions. Although the parameter showed somewhat lower discrimination accuracy in the detection of inactive lesions, it is more important not to miss active lesions because the presence of an active lesion in the noncavitation stage of caries, which usually progresses, can affect the decision to apply nonoperative treatment.[4,5]

The visual-tactile inspection for lesion activity in this study was performed on noncavitated caries lesions. In the process, seven lesions were judged to be "mixed" with active and inactive characteristics, and the lesions were eventually classified as active according to a previous study (7/32, 22%).[10] It is conceivable that such lesion severity and the final classification of mixed lesions affected the reproducibility of assessment of lesion activity in this study.[8] Several studies have reported that the intra- or interexaminer reliability of assessment to rate the activity of early stage caries lesions was lower than for cavitated lesions.[10,40,41] Moreover, earlier studies attempting to distinguish lesion activity statuses were based on results for lesions with sharply different features that were observed on the surfaces of root or dentin lesions with different activities.[4] Accordingly, the existing lesion activity assessment criteria would be valuable to dichotomize lesions with the classic characteristics. However, Nyvad and Fejerskov[4] noted that this is difficult if lesions have

both active and inactive characteristics or if they are in a transitory state; lesion activity may ultimately be determined by examiner opinion, which could be subjective and ambiguous. This suggests that it is necessary to find an alternative method to overcome the disadvantage associated with this clinical method.

Although the validity and reliability of clinical lesion activity assessment criteria have been reported,[9-11] they are subjective and require substantial clinical training and experience.[10,41,42] Nevertheless the most commonly used method to assess caries lesion activity is visual-tactile inspection.[8,10] Optical detection/diagnostic techniques provide enhanced visibility and quantitative analysis,[43] so they can be used as adjuncts for minimizing subjectivity and improving clinical assessment reliability. The present results suggest that measuring red fluorescence observed on smooth surface caries could aid the clinical decision making for assessing lesion activity and predicting remineralization potential. However, this *in vitro* study investigated extracted teeth; therefore, clinical studies are required to validate our findings. *In vivo* caries lesions were classified as active when the lesions were covered with plaque or showed evidence of plaque stagnation,[8,10] but this parameter could not be evaluated in extracted teeth. Additionally, further confirmation is needed for occlusal surface caries lesions as they progress in a different way compared to smooth surface caries lesions.

Another limitation was that there was no histologic examination in this study because the study design was such that the same total lesion areas were subjected to the remineralization treatment and the detection of the changes in the lesion fluorescence before and after the treatment. Instead of histologic analysis, the fluorescence variables that are related to the ΔF of the QLF system, which has high correlation and good validity in quantifying and monitoring changes in the mineral loss of non-cavitated lesions,[29,43] were used to evaluate the changes in the lesions after the treatment. As a result, the different changes in the fluorescence loss variable related to lesion volume (ΔQ) after the remineralization were observed between active and inactive lesions. However, further confirmation of whether these fluorescence changes are related to the histologic changes, which can be detected from the caries lesions surface after a certain treatment, would be needed to generalize the findings of the present study. The histologic analysis can help to provide evidence of the changes in the inner part of the lesions that are reflected as the changes in the fluorescence observed on the lesion surface.

CONCLUSIONS

In conclusion, our *in vitro* results demonstrate that the parameters of light-induced red fluorescence observed on smooth surfaces differed depending on caries lesion activity, and red fluorescence area and fluorescence loss related to lesion volume significantly changed during remineralization. Collectively, these findings suggest that in conjunction with the fluorescence loss, red fluorescence may be a valuable parameter for classifying lesion activity and predicting the remineralization potential of smooth surface caries lesions.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education under grant (2016R1D1A1A09916934).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institutional Review Board of Yonsei University Dental Hospital. The approval code for this study is: 2-2014-0024.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 3 July 2018)

REFERENCES

1. Holmen L, Thylstrup A, Øgaard B, & Kragh F (1985) A scanning electron microscopic study of progressive stages of enamel caries *in vivo Caries Research* **19(4)** 355-367.
2. Holmen L, Thylstrup A, Øgaard B, & Kragh F (1985) A polarized light microscopic study of progressive stages of enamel caries *in vivo Caries Research* **19(4)** 348-354.
3. Thylstrup A, Bruun C, & Holmen L (1994) *In vivo* caries models: mechanisms for caries initiation and arrestment *Advances in Dental Research* **8(2)** 144-157.
4. Nyvad B & Fejerskov O (1997) Assessing the stage of caries lesion activity on the basis of clinical and microbiological examination *Community Dentistry and Oral Epidemiology* **25(1)** 69-75.
5. Nyvad B (2004) Diagnosis versus detection of caries *Caries Research* **38(3)** 192-198.
6. Kidd EAM & Fejerskov O (2004) What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms *Journal of Dental Research* **83(Special Issue C)** C35-C38.

7. Ekstrand KR, Zero DT, Martignon S, & Pitts NB (2009) Lesion activity assessment *Monographs in Oral Science* **21** 63-90.
8. Ekstrand KR, Martignon S, Ricketts DJN, & Qvist V (2007) Detection and activity assessment of primary coronal caries lesions: A methodologic study *Operative Dentistry* **32(3)** 225-235.
9. Guedes RS, Piovesan C, Ardenghi TM, Emmanuelli B, Braga MM, Ekstrand KR, & Mendes FM (2014) Validation of visual caries activity assessment: a 2-yr cohort study *Journal of Dental Research* **93(Supplement 7)** 101S-107S.
10. Nyvad B, Machiulskiene V, & Baelum V (1999) Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions *Caries Research* **33(4)** 252-260.
11. Nyvad B, Machiulskiene V, & Baelum V (2003) Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity *Journal of Dental Research* **82(2)** 117-122.
12. Gimenez T, Bittar DG, Piovesan C, Guglielmi CAB, Fujimoto KY, Matos R, Novaes TF, Braga MM, & Mendes FM (2013) Influence of examiner experience on clinical performance of visual inspection in detecting and assessing the activity status of caries lesions *Operative Dentistry* **38(6)** 583-590.
13. Benedict HC (1928) A note on the fluorescence of teeth in ultra-violet rays *Science* **67(1739)** 442.
14. Angmar-Månsson B & ten Bosch JJ (1987) Optical methods for the detection and quantification of caries *Advances in Dental Research* **1(1)** 14-20.
15. ten Bosch JJ, Borsboom PCF, & ten Cate JM (1980) A nondestructive method for monitoring de- and remineralization of enamel *Caries Research* **14(2)** 90-95.
16. Koenig K, Hibst R, Meyer H, Flemming G, & Schneckenburger H (1993) Laser-induced autofluorescence of carious regions of human teeth and caries-involved bacteria *Proceedings of the SPIE 2080, Dental Applications of Lasers* **(December 31)** The International Society for Optics and Photonics, Bellingham, WA http://dx.doi.org/10.117/12.116180.
17. Buchalla W (2005) Comparative fluorescence spectroscopy shows differences in noncavitated enamel lesions *Caries Research* **39(2)** 150-156.
18. Koenig K, Flemming G, & Hibst R (1998) Laser-induced autofluorescence spectroscopy of dental caries *Cellular Molecular Biology (Noisy-le-grand)* **44(8)** 1293-1300.
19. Alfano RR & Yao SS (1981) Human teeth with and without dental caries studied by visible luminescent spectroscopy *Journal of Dental Research* **60(2)** 120-122.
20. Kim YS, Lee ES, Kwon HK, & Kim BI (2014) Monitoring the maturation process of a dental microcosm biofilm using the quantitative light-induced fluorescence-digital (QLF-D) *Journal of Dentistry* **42(6)** 691-696.
21. Kim YS, Kang SM, Lee ES, Lee JH, Kim BR, & Kim BI (2016) Ecological changes in oral microcosm biofilm during maturation *Journal of Biomedical Optics* **21(10)** 101409.
22. Ribeiro A, Rousseau C, Girkin J, Hall A, Strang R, John Whitters C, Creanor S, & Gomes AS (2005) A preliminary investigation of a spectroscopic technique for the diagnosis of natural caries lesions *Journal of Dentistry* **33(1)** 73-78.
23. Diniz MB, Campos PH, Sanabe ME, Duarte DA, Santos MTBR, Guaré RO, Duque C, Lussi A, & Rodrigues JA (2015) Effectiveness of fluorescence-based methods in monitoring progression of noncavitated caries-like lesions on smooth surfaces *Operative Dentistry* **40(6)** E230-E241.
24. White DJ (1987) Reactivity of fluoride dentifrices with artificial caries. I. Effects on early lesions: F uptake, surface hardening and remineralization *Caries Research* **21(2)** 126-140.
25. Lippert F & Juthani K (2015) Fluoride dose-response of human and bovine enamel artificial caries lesions under pH-cycling conditions *Clinical Oral Investigations* **19(8)** 1947-1954.
26. Lee ES, Kang SM, Ko HY, Kwon HK, & Kim BI (2013) Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by quantitative light-induced fluorescence-digital (QLF-D) *Journal of Dentistry* **41(12)** 1264-1270.
27. Waller E, van Daelen CJ, & van der Veen MH (2012) Application of QLF™ for diagnosis and quality assessment in clinical practice. White paper on QLF, 11. Retrieved online April 20, 2016 from: http://www.inspektor.nl/download/WhitepaperQLF-11.pdf
28. Chen Q, Zhu H, Xu Y, Lin B, & Chen H (2015) Discrimination of dental caries using colorimetric characteristics of fluorescence spectrum *Caries Research* **49(4)** 401-407.
29. Shi XQ, Tranaeus S, & Angmar-Månsson B (2001) Comparison of QLF and DIAGNOdent for quantification of smooth surface caries *Caries Research* **35(1)** 21-26.
30. Cochrane NJ, Anderson P, Davis GR, Adams GG, Stacey MA, & Reynolds EC (2012) An X-ray microtomographic study of natural white-spot enamel lesions *Journal of Dental Research* **91(2)** 185-191.
31. Al-Khateeb S, Exterkate RAM, de Josselin de Jong E, Angmar-Månsson B, & ten Cate JM (2002) Light-induced fluorescence studies on dehydration of incipient enamel lesions *Caries Research* **36(1)** 25-30.
32. Ando M, Stookey GK, & Zero DT (2006) Ability of quantitative light-induced fluorescence (QLF) to assess the activity of white spot lesions during dehydration *American Journal of Dentistry* **19(1)** 15-18.
33. Ando M, Ferreira-Zandona AG, Eckert GJ, Zero DT, & Stookey GK (2017) Pilot clinical study to assess caries lesion activity using quantitative light-induced fluorescence during dehydration *Journal of Biomedical Optics* **22(3)** 35005.
34. de Josselin de Jong E, Higham SM, Smith PW, van Daelen CJ, & van der Veen MH (2009) Quantified light-induced fluorescence, review of a diagnostic tool in prevention of oral disease *Journal of Applied Physics* **105(10)** 102031.

35. Parolo CCF & Maltz M (2006) Microbial contamination of noncavitated caries lesions: A scanning electron microscopic study *Caries Research* **40(6)** 536-541.

36. Larsen MJ & Fejerskov O (1989) Chemical and structural challenges in remineralization of dental enamel lesions *Scandinavian Journal of Dental Research* **97(4)** 285-296.

37. Lynch RJ, Mony U, & ten Cate JM (2007) Effect of lesion characteristics and mineralizing solution type on enamel remineralization *in vitro Caries Research* **41(4)** 257-262.

38. Al-Khateeb S, Exterkate R, Angmar-Månsson B, & ten Cate JM (2000) Effect of acid-etching on remineralization of enamel white spot lesions *Acta Odontologica Scandinavica* **58(1)** 31-36.

39. Felix Gomez G, Eckert GJ, & Ferreira Zandona A (2016) Orange/red fluorescence of active caries by retrospective quantitative light-induced fluorescence image analysis *Caries Research* **50(3)** 295-302.

40. Braga MM, Ekstrand KR, Martignon S, Imparato JCP, Ricketts DNJ, & Mendes FM (2010) Clinical performance of two visual scoring systems in detecting and assessing activity status of occlusal caries in primary teeth *Caries Research* **44(3)** 300-308.

41. Tikhonova SM, Feine JS, Pustavoitava NN, & Allison PJ (2014) Reproducibility and diagnostic outcomes of two visual-tactile criteria used by dentists to assess caries lesion activity: A cross-over study *Caries Research* **48(2)** 126-136.

42. Ekstrand KR, Ricketts DN, Longbottom C, & Pitts NB (2005) Visual and tactile assessment of arrested initial enamel carious lesions: An *in vivo* pilot study *Caries Research* **39(3)** 173-177.

43. Pretty IA (2006) Caries detection and diagnosis: novel technologies *Journal of Dentistry* **34(10)** 727-739.