

***In Vitro* Detection of Secondary Caries Associated with Composite Restorations on Approximal Surfaces Using Laser Fluorescence**

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Clinical Relevance

Detecting secondary caries is difficult when using the visual-tactile examination. This study showed that the laser fluorescence device can be an auxiliary method for the detection of lesions associated with composite restorations on approximal surfaces.

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SUMMARY

This study evaluated the performance of the DIAGNOdent pen laser fluorescence device (LFpen) in comparison with visual examination (VE), bitewing radiographs (BW) and visual examination combined with bitewing radiographs (VEBW) in detecting secondary approximal caries associated with composite restorations. In total, 60 approximal surfaces from 43 permanent molars with composite restorations were assessed twice by two examiners using the LFpen, VE, BW and VEBW. After histological preparation and hardness measurements, the sample was assigned to either a crown or root caries group, depending on the location of the lesions as the gold standard. For crown caries at D1, the highest values of specificity and sensitivity were observed for the LFpen at a cutoff value of 18 (1.00) and for the VEBW (0.89). At D3 (cutoff of 30), the LFpen showed the highest values of sensitivity and specificity. For root caries, the

LFpen and VEBW showed the highest values of specificity (0.54), sensitivity (0.81) and accuracy (0.69). The Spearman rank correlation coefficients for crown/root caries with histology were 0.54/0.37 (LFpen), 0.29/0.10 (BW), 0.29/0.18 (VE) and 0.23/0.37 (VEBW). For the LFpen, the ICC varied from 0.80 (interexaminer) to 0.97 (intraexaminer B); the kappa value was 0.19 for BW and 0.35 for VE (interexaminer). Intraexaminer kappa values for BW were 0.25 (A) and 0.29 (B), and those for VE were 0.31 (A) and 0.32 (B). The LFpen device exhibited a performance comparable to that of conventional methods but with higher interexaminer reproducibility. Therefore, the LFpen should be considered an auxiliary method for the detection of secondary approximal caries associated with composite restorations.

INTRODUCTION

Secondary caries has been described as the result of a new attack (recurrent) on the tooth surface or as residual caries (old) adjacent to a restoration area. Both are common reasons for restorative material replacement.^{1,2} Secondary caries has also been characterized as primary caries next to a restoration site.³ These lesions usually occur as an “outer lesion” that is histologically similar to the primary lesion, whereas a “wall lesion” is a narrower defect in the enamel or dentin at the cavity wall.⁴

Carious lesions adjacent to restorations on approximal surfaces are difficult to detect using visual-tactile examination, unless the lesion is relatively advanced with considerable loss of tooth structure.^{5,6} Moreover, any color change at the buccal or lingual surfaces around the restoration site are also difficult to interpret.⁷ Bitewing radiographs have been recommended to aid and improve the diagnostic process of approximal carious lesions. However, this method has limited value during diagnosis and might lead to an underestimation of lesion size due to the shading effect of radio-opaque restorative materials.⁸

In cases of a restoration extending to or close to the enamel-cementum junction, a secondary lesion in the root-dentin can occur. This region of the tooth is highly irregular and represents a particularly vulnerable retention site for bacteria.⁹ The classification of root lesions (active or inactive) has been accepted as only being made after successive patient examinations where the clinician monitors visual changes. However, such changes need to be detected first, but they can be difficult to quantify. A system that permits the detection and monitoring of these lesions would be of great use to clinicians and researchers.¹⁰

A new laser fluorescence device (LFpen) was recently developed and has been tested for the detection of occlusal and approximal caries. It emits red light with a wavelength of 655 nm, while a filter blocks light below 665 nm that eliminates reflected and ambient light. A photodetector quantifies the fluorescent light passing through the filter, which is placed in the optical path in front of the photodetector and separates the fluorescent light from the excitation light. The photodetector shows the real time (moment) and maximum (peak) values via a digital display. In contrast to previous LF devices, the excitation and fluorescence light follow the same optic path of propagation inside the solid fiber tip, but in opposite directions.¹¹⁻¹²

The first generation LFs have been previously evaluated, and it was concluded that they have the potential to detect secondary caries in occlusal surfaces restored with composite materials.⁵ Thus, these devices may be valuable adjuncts to conventional methods for the detection of secondary caries.⁶ Nevertheless, LFs have limitations regarding penetration of the proximal space. The tip of the new LFpen forms a prismatic shape that allows access to the approximal space and permits the direction of the excitation light to be targeted laterally along the long axis of the tip.¹³⁻¹⁴ Lussi and others have shown that the LFpen is capable of detecting decay on approximal surfaces with good accuracy.¹⁴

Therefore, this *in vitro* study evaluated the performance of the LFpen in comparison with bitewing radiographs, visual examination and a combination of both in detecting secondary approximal carious lesions associated with composite restorations. In addition, secondary lesions were characterized in terms of gap size between the restoration and the teeth and its correlation with histology.

METHODS AND MATERIALS

Setup

In total, 43 permanent human molars with 60 approximal surfaces having Class II composite restorations were selected by visual inspection from a pool of extracted teeth. All the teeth had been extracted for periodontal or orthodontic reasons by dental practitioners in Switzerland (no water fluoridation, 250 ppm F⁻ in table salt). The authors were not involved in placement of the restorations. Prior to extraction, consent was obtained and the patients were informed regarding the use of their teeth for research purposes. No approval by the Ethical Committee was obtained, since, in Switzerland, this is not necessary for *in vitro* studies. The teeth had been stored frozen at -20°C until use. Previous study has shown that fluorescence and light cutoff values did not change when the teeth were stored

under these conditions.¹⁵ Calculus was removed using a scaler (Cavitron, Dentsply, York, PA, USA), and the teeth were cleaned for 15 seconds with water, then for 10 seconds with Prophyflex (Kavo, Biberach, Germany) and sodium bicarbonate powder. To remove powder remnants from fissures, the teeth were rinsed for 10 seconds using a 3-in-1 syringe.¹⁶

Approximal sites (sound and carious) were chosen visually, and photographs (6.5x magnification) were taken of both the occlusal and approximal surfaces in order to identify the teeth and the exact approximal sites for examination and subsequent histological preparation. The LFpen was used to assess the lesions before the approximal contacts were simulated and the value recorded. The tooth under study was placed between two sound teeth that had their roots embedded in a composite simulating the contact points of adult teeth. Soft tissue was not simulated in the blocks. The blocks were stored frozen at 20°C under 100% humidity throughout the study. In order to calculate reproducibility, two examiners assessed the teeth twice. No calibration training was performed and the examiners were informed about using the device. The following methods were used.

Radiographic and Visual Examination

Bitewings radiographs (BW) were taken of all blocks using an x-ray machine (HDX, DentaleEZ, Lancaster, PA, USA) and twin Kodak Insight films (22 x 35 mm, Eastman Kodak Company, Rochester, NY, USA) at 65 kV, 7 mA and an exposure time of 0.09 seconds. The source-to-film distance was 6100 mm. A 5-mm wide plastic mold was placed close to the object on the focus side to simulate soft tissue. The radiographs were examined independently by two experienced examiners (A and B) on a backlit screen using an x-ray viewer (Imatec Röntgentechnik, Basel, Switzerland) and an x-ray film magnifier (magnification 2x; Svenska Dental Instrument, Upplands Vasby, Sweden). Examinations were performed in a dark room, and the degree of radiotranslucency at the approximal surfaces was determined. For crown and root caries, the following scoring system was used: no radiolucency (0), radiolucency on the enamel (1) and radiolucency in the dentin (2).

The teeth were also examined visually (VE) by the same examiners under clinical lighting from both the buccal and lingual sides three weeks after radiographic examination. The approximal surfaces observed were classified according to the presence of visible marginal color changes surrounding the restoration site, ditches or even cavities. The following visual scoring system was used: sound surface (0), enamel caries (1) and dentin caries (2).

After three weeks, both examiners analyzed the sites together (following normal procedures for clinical practice) by combining the visual and radiographic exami-

nations (VEBW). For this assessment, the examiners considered the visual (discolorations, ditches or cavities) and radiographic (radiolucencies) characteristics and coded the sites using the same criteria used for VE. The lowest score given by the examiners was registered.

Assessments with the LFpen

The LFpen (DIAGNOdent 2190, Kavo) measurements were carried out using a wedge-shaped sapphire fiber tip (1.1 mm) for the approximal surface. The device was calibrated for every tooth against a ceramic reference that had a known fluorescence value in accordance with the manufacturer's instructions. The fluorescence value of a sound part of the buccal-cuspal surface (zero value) was recorded. The approximal surfaces were carefully assessed with the LFpen by both examiners and moved from the buccal towards the lingual side underneath the contact area. The procedure was repeated, moving from the lingual towards the buccal side. Both peak values were recorded.

Histological Validation (gold standard)

Upon completion of these assessments, the teeth were ground longitudinally until 1 mm before the site of measurement using a rotating polishing machine (Knuth-Rotor, Struers, Copenhagen, Denmark) with 60-µm grain size silicon carbide paper under cooled tap water. When the periphery of the site was reached by the grinding process, grain size papers 30, 18, 8 and 5 µm were sequentially used. The cut surfaces were then cleaned and dehydrated in solutions of alcohol at increasing concentrations with the addition of basic 0.5% fuchsin (Inselspital-Apotheke, Bern, Switzerland) so as to stain the block. The alcohol was removed with acetone and the teeth embedded in methylmethacrylate. The embedded samples containing tooth sections were sectioned perpendicular to the approximal surface in order to produce slides containing the clinically examined spot. Each section was then contrast-stained with light green in acetic acid solution (0.25% light green dissolved in 0.20% acetic acid) for two minutes, cut and ground until 300 µm-thick slices were obtained. For cutting, a diamond abrasion wheel (Isomet, 11-1, 180 Low Speed Saw, Buehler Ltd, Lake Bluff, IL, USA) and the polishing machine described above were used. Hardness measurements of the histological specimens were performed to aid the histological classification in cases of doubt. This measure used a Knoop diamond (KHN) under a force of 100g, which is equivalent to a force of 0.981 N, with a load time of 15 seconds (Leitz Wetzlar, Germany). Sound enamel and dentin surfaces were measured, and hardness values below 200 KHN in enamel and below 70 KHN in dentin were considered indicative of caries. Photographs of the cut colored surfaces were taken (Leica DC300 camera, Leica, Heerbrugg, Switzerland). Two examiners assessed the

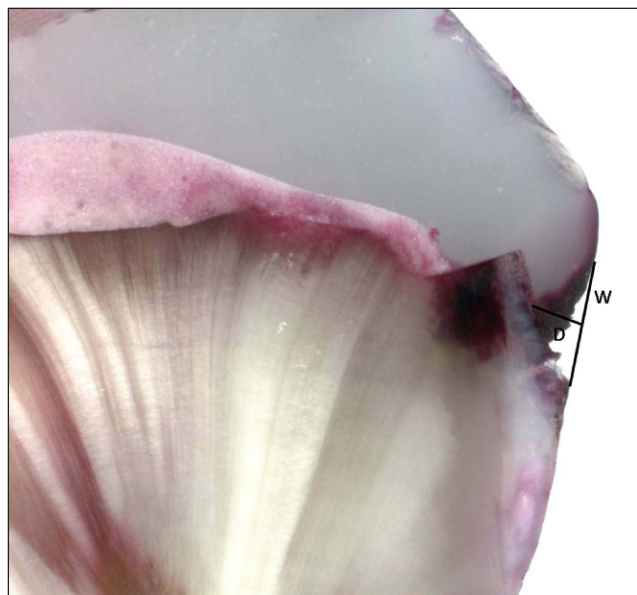


Figure 1. Illustration of how measurements for the depth (D) and width (W) of the gap border between the tooth and the composite restoration were performed.

sites (magnification 10x) and the hardness measurements, and both reached a consensus before classifying the lesion. The sample was divided according to histological assessments into “crown caries” and “root caries” groups. The samples were considered to have “root caries” if no enamel was observed and if the restoration reached at least the enamel-cementum junction. The crown lesions under the restoration were then classified according to the following criteria: caries-free (0), caries extending up to halfway through the enamel (1), caries extending into the inner half of the enamel (2), caries in dentin (3) and deep dentin caries (4). For root caries, the sites were classified according to the following criteria: caries-free (0) and root caries (1). Using digital photographs, the maximal depth and width of the gap between the restoration and tooth were measured (IM500, Leica) (Figure 1).

Statistical Analyses

LF measurements starting from both the buccal and lingual sides underneath the contact area were collected and the highest mean value (between the two examiners) was used for the calculation. The Wilcoxon signed-rank test was performed to compare the mean fluorescence values obtained before the contact point had been simulated with those obtained after, as described above.

Sensitivity, specificity and accuracy were calculated for both crown and root caries at D1 (considering scores 1, 2, 3 and 4 as decayed) and D3 (considering scores 3 and 4 as decayed), and thresholds were calculated using the ROC (Receiver Operating Characteristics) curve. Performance results were obtained using the

cutoff values of the LFpen suggested for primary approximal caries.¹⁴ Additionally, optimal cutoff values for secondary approximal caries lesions on the crown and root surfaces were determined by the ROC curve at the point for which the sum of the sensitivity and specificity was maximal. The McNemar test was used to compare the sensitivity, specificity and accuracy among the methods. The Spearman rank correlation coefficient (r) was determined so as to compare the depth and width of the gap size with histology. The statistical significance level was set at $\alpha=0.05$.

The intraclass correlation coefficient (ICC) and weighted kappa measure were used to assess the reproducibility by means of intra- and interexaminer agreement. The program MedCalc for Windows (version 10.4.0.0, MedCalc Software bvba, Mariakerke, Belgium) was used for these analyses.

RESULTS

Of the 60 approximal lesions studied, histological assessments revealed that 29 lesions were located at the root and extended beyond the enamel-cementum junction. Of these, 13 were caries-free and 16 showed root caries. From a total of 31 lesions, eight had caries extending halfway through the enamel, six had caries scored as two and 13 had caries scored as three.

The Wilcoxon signed-rank test showed a statistically significant difference between the fluorescence values measured before and after the contact point simulation. The mean fluorescence values before and after the contact point simulation were 55 and 41 for root caries ($p=0.0147$) and 32 and 25 for crown caries ($p=0.0002$), respectively.

Table 1 shows the values of specificity, sensitivity and accuracy for the BW, VE, VEBW and LFpen examinations at the D1 and D3 thresholds. The results from the LFpen are shown using the cutoff value previously published by Lussi and others for primary approximal caries lesions and the optimal cutoff values for secondary approximal lesions.¹⁴ The highest values of specificity and sensitivity for crown caries at D1 were observed with the LFpen at a cutoff of 18 (1.00) and with VEBW (0.89). At D3 (cutoff 30), the LFpen showed the highest values for sensitivity and specificity. For root caries (Table 2), the LFpen and VE showed the highest values of specificity (0.54), sensitivity (0.81) and accuracy (0.69) at a cutoff of 18. The Spearman rank correlation coefficients for the approximal lesions/root caries with histology were 0.54/0.37 (LFpen), 0.29/0.10 (BW), 0.29/0.18 (VE) and 0.23/0.37 (VEBW).

Figure 2 shows a scatter diagram representing the correlation between depth and width of the gap border with the histology for crown and root caries. For crown caries, a positive and statistically significant Spearman

Table 1: Specificity, Sensitivity and Accuracy of the BW, VE, VEBW and LFpen at D1 and D3 Thresholds for Secondary Caries on Crown Surfaces						
	Specificity		Sensitivity		Accuracy	
	D1	D3	D1	D3	D1	D3
BW	0.50 ^a	0.71 ^b	0.33 ^a	0.23 ^b	0.35 ^a	0.68 ^a
VE	0.75 ^b	0.65 ^{a,b}	0.41 ^a	0.65 ^a	0.45 ^a	0.67 ^a
VEBW	0.25 ^c	0.77 ^c	0.89 ^b	0.79 ^a	0.81 ^b	0.51 ^b
LFpen ¹	0.00 ^d	0.61 ^{a,b}	0.85 ^b	0.74 ^a	0.74 ^b	0.68 ^a
LFpen ²	1.00 ^a	1.00 ^d	0.52 ^{a,c}	0.92 ^c	0.58 ^c	0.81 ^c
<i>Within columns, significant differences are represented by different superscript letters (McNemar test, p=0.05).</i>						
¹ D1= 6; D3=15 (Lussi & others, 2006): primary caries lesions.						
² D1=18; D3=30 (optimal): current study.						

Table 2: Specificity, Sensitivity and Accuracy of the Methods at D1 Thresholds for Secondary Caries on Root Surfaces			
	Specificity D1	Sensitivity D1	Accuracy D1
BW	0.31 ^a	0.65 ^a	0.50 ^a
VE	0.42 ^a	0.75 ^b	0.60 ^b
BWVE	0.54 ^b	0.81 ^b	0.69 ^b
LFpen	0.54 ^b	0.81 ^b	0.69 ^b
<i>Results for the LFpen are shown using optimal cut-off values for the presence of caries (cutoff: 18). Within columns, significant differences are represented by different superscript letters (McNemar test, p=0.05).</i>			

rank correlation coefficient (r) was observed between the depth of the gap and histological findings (mean = 344.62 µm; SD = ± 70.68; r=0.41; p=0.02). A positive but non-significant correlation was found between the width of the gap border and histology (mean = 291.88 µm; SD = ± 81.84; r=0.33; p=0.08). For root caries, neither the depth (mean = 510.31 µm; SD = ± 90.20; r=-0.23; p=0.22) nor width (mean = 234.23 µm; SD = ± 110.39; r= -0.21; p=0.26) of the gap was significantly correlated with histology.

Reproducibility is represented in Table 3. For the LFpen, the ICC varied from 0.80 (interexaminer) to 0.97 (intraexaminer B). The weighted kappa value was 0.19 for BW and 0.35 for VE (interexaminer). Intraexaminer kappa values for BW were 0.25 (A) and 0.29 (B), and those for VE were 0.31 (A) and 0.32 (B).

DISCUSSION

In the current study, the LFpen showed good performance in terms of sensitivity, specificity and accuracy, which was comparable to that of bitewing radiographs

Table 3: ICC, Weighted Kappa (k) Values and 95% Confidence Intervals (CIs) for the Inter- and Intraexaminer Reproducibility of the Methods				
	LFpen (ICC and 95% CI)	LFpen (k and 95% CI)	BW (k and 95% CI)	VE (k and 95% CI)
Intra A	0.93 (0.88 to 0.95)	0.93 (0.84 to 1.00)	0.25 (0.05 to 0.42)	0.31 (0.14 to 0.48)
Intra B	0.97 (0.94 to 0.98)	0.80 (0.65 to 0.95)	0.29 (0.09 to 0.33)	0.32 (0.13 to 0.48)
Inter	0.80 (0.73 to 0.85)	0.62 (0.43 to 0.80)	0.19 (0.02 to 0.35)	0.35 (0.17 to 0.52)

combined with visual examination.

No statistically significant correlation was observed between gap width and histology for crown caries; however, the p-value obtained was close to

the established significance level. This failure to find a significant correlation may be due to the sample size, because it is obvious that an area of restricted access during cleaning (for example, approximal filling excess) was subject to a higher risk of developing caries. Notably, this correlation has already been suggested by Hodges and others in the assessment of occlusal caries. The depth of the gap size (mean = 344.62 µm; SD = ± 70.68) showed a statistically significant correlation with the histology in crown caries. Microgaps between 50 to 280 µm seemed to be sufficient for correlation, even without statistical differences for mineral loss or lesion development around composite fillings. In addition, secondary caries seemed to be mainly influenced by the bacterial composition of marginal plaque and oral hygiene habits of the patient. As there was no significant correlation between leakage and histology in root caries, the authors of the current study assumed that cleaning access in the root region of 16 teeth was sufficient to impede the progression of secondary caries under such conditions.

The LFpen cutoff values for the detection of approximal caries that had already been published for primary lesions¹⁴ could not be used to assess approximal surfaces restored with composite material. The current study showed the highest values of sensitivity and specificity at different points (18 and 30, respectively) when evaluating the presence of enamel and dentin carious lesions. These cutoff values were slightly higher than those previously published. The authors of the current study speculate that the different sensitivity and specificity values found using both cutoff values (for primary and secondary caries lesions) arose due to

the difference between bacterial ecology on the surface of primary lesions and that adjacent to composite restorations.¹⁷ This assumption was supported by the observation that laser fluorescence values were directly related to the microflora in

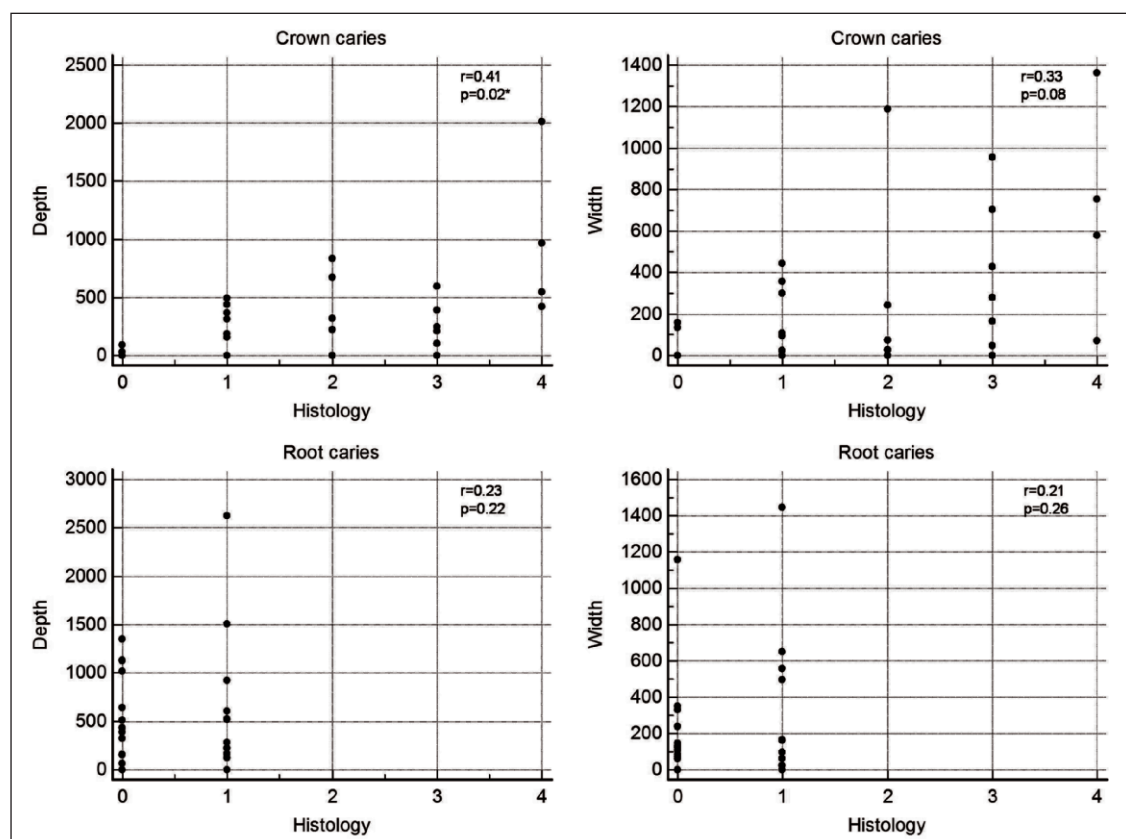


Figure 2. Scatter diagrams showing the correlation between depth and width of the gap border with the histology for crown and root caries (r : Spearman rank correlation coefficient; p : statistical difference; * $p < 0.05$: statistically significant difference).

carious tissue.¹⁸ Bamzahim and others⁶ assessed *in vivo* occlusal surfaces restored with composite materials and also suggested a cutoff of 20 for the optimal assessment of carious lesions. This value is closer to the value found in the current study. Additionally, differences in the prevalence of caries, the criteria for the selection of teeth, the distribution of lesion types, the gold standards applied and the approaches for determining sensitivity and specificity (for example, choosing cutoff values) between these studies may also explain these inconsistent results.

The current study also confirmed the effectiveness of the LFpen for the detection of secondary root caries. No previous study has evaluated the performance of the LFpen in assessing such surfaces. For active root caries, a cutoff between five and 10 was suggested based on an *in vivo* study with 266 subjects comparing laser fluorescence and visual-tactile lesion detection.¹⁹ However, the best cutoff value found in the current study for the presence of root caries (D1) was 18. Therefore, during assessment of the approximal surfaces restored with composite materials, LFpen values ranging from 18 to 20 seemed to suggest the presence of carious lesions. These higher cutoff values might be due to a higher sensitivity resulting from histological

analysis, which was not performed in the aforementioned study.

Radiography is a more sensitive method than clinical inspection for detecting primary approximal lesions in dentin.²⁰ However, the sensitivity of BW radiography for assessing approximal enamel lesions is low,²⁰⁻²¹ and sensitivity is even lower for the detection of approximal secondary caries.²² Moreover, the results also depend on the adhesive system used. For example, non-filled bonding agents are radiolucent and may cause false-positive errors. Furthermore, concavities

at the approximal surface cause radiolucencies and also lead to false-positive findings. False-negative results reflected by the higher specificity at both the D2 and D3 thresholds may have been caused by the superimposition of radio-opaque filling material and radiolucent secondary caries, depending on the projection.

VE showed accuracy similar to that of BW. Color changes, such as white, brown or gray spots, or even just a line of staining between the restoration and tooth, are more easily identified in composite restorations.²³ In order to decrease the number of bitewing radiographs taken, the combination of this technique and the LFpen could be recommended for monitoring the regression or progression of caries. However, it is important to bear in mind that LF values may be influenced in cases where staining is present.

For the detection of root caries, the values for sensitivity, specificity and accuracy of the LFpen and VEBW were the same. This finding supports the use of the LFpen instead of BW and illustrates another advantage of the LFpen method.¹⁴

The ICC and kappa values obtained for the LFpen (intra- and interexaminer) were high. However, BW and VE interexaminer reproducibility assessed by the

kappa statistic showed low values. Lussi and others,¹⁴ who assessed primary approximal lesions, also observed high reproducibility with the LFpen. To date, no study has evaluated reproducibility of the LFpen on approximal surfaces restored with composite materials in the presence of a contact point. Interestingly, very poor kappa values were observed for BW and VE. These assessments involved subjective aspects (for example, knowledge and clinical experience of the examiners) that could have affected the intra- and interexaminer agreement.²⁴ Additionally, lesions associated with composite restorations are more difficult to observe in radiographs, because an unfilled bonding layer could mimic the presence of a lesion. These results should be interpreted with caution when the methods are compared.

Secondary caries associated with composite restorations may be related to a failure of the adhesive bond and marginal degradation over time. Therefore, one potential complication is the fact that these failures, or even the restorative material itself, may emit fluorescence at different wavelengths and subsequently influence the correct measurement of the lesion. In addition, the accumulation of plaque and calculus was reported to influence LF measurements.^{14,25} Therefore, the teeth should be cleaned before assessment. For approximal surfaces, the same concept applies. However, tight approximal contact and the difficulty in directly assessing this area may make this procedure difficult, which was observed in the comparison of the Wilcoxon signed-rank test values. Separating the teeth is required for restoration placement and increases accessibility. Consequently, the detection of secondary caries on those surfaces should be easier, since the restoration margins are often in an area with enough space between the teeth.

Franscescut and others¹⁵ have suggested that the storage method does not change fluorescence values; therefore, the results obtained in this *in vitro* study may be extrapolated to *in vivo* conditions. However, the contact point simulation for approximal surfaces *in vitro* is obviously not the same as that *in vivo*. Moreover, except for BW, it is important to consider the fact that soft tissue cannot be faithfully simulated *in vitro*. Additionally, access with the LFpen probe is far more easily obtained *in vitro* than *in vivo*, because the contact point allows better penetration of the probe into the approximal space. Clinicians should keep this fact in mind when assessing approximal (crown and root) surfaces on patients. As the values provided by the LFpen comprise a range of fluorescence values rather than a fixed border, the LFpen should not be used as the only tool for detecting caries. For this reason, its combination with conventional methods is recommended for the assessment of approximal surfaces. Furthermore, the decision to replace a restoration

should also be based on other factors (for example, dietary habits, increased exposure of fluoride, reduction in frequency of fermentable carbohydrate intake and carious activity), because secondary lesions with incipient caries can be controlled if proper cleaning is feasible. Further *in vivo* studies should evaluate the performance of this device in the detection of secondary caries on restored approximal surfaces.⁷

CONCLUSIONS

The authors of the current study conclude that leakage at the composite restoration margin may lead to reduced clinical longevity of a filling due to the development of secondary caries. In addition, the LFpen device shows a performance comparable to that of bitewing radiographs combined with visual examination, but with a higher interexaminer reproducibility. Therefore, the LFpen should be considered as an auxiliary method for the detection of secondary approximal carious lesions associated with composite restorations.

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