

Ability of laser fluorescence device associated with fluorescent dyes in detecting and quantifying early smooth surface caries lesions

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Abstract. A laser fluorescence (LF) device is a portable tool, but it does not measure minor mineral changes. Our *in vitro* study aim is to propose the association of an LF with two fluorescent dyes and to evaluate the performance in detecting and quantifying early demineralization. Artificial caries lesions are created in 40 primary canine teeth using a demineralizing solution (pH=4.8) for 12, 24, 48, and 96 h. LF measurements are performed with DIAGNOdent after demineralization in these samples and in 20 sound primary teeth. Measurements with LF with 0.2-mM tetrakis(*N*-methylpyridyl)porphyrin (LF TMPyP) and with 4-mM protoporphyrin IX (LF PPIX) are made. The amount of calcium loss is determined by atomic emission spectrometry. A correlation between LF and LF with dyes and mineral loss and receiver operating characteristics analysis are performed, as well as comparisons of sensitivity, specificity, and accuracy values. Significant correlation is obtained with LF TMPyP and mineral loss of lesions demineralized for 24, 48, and 96 h. Better performance is achieved with LF TMPyP for all parameters than with LF alone. LF PPIX does not present good results. In conclusion, LF TMPyP provides good performance in detecting and quantifying very early enamel caries lesions.
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1 Introduction

Early detection of caries lesions provides for more efficient arrestment of the caries process, avoiding operative treatment.¹ Current clinical procedures for caries detection, such as visual and radiographic methods, are able to detect caries lesions only at an advanced stage.¹⁻⁵ When dentists use visual inspection with extensive drying of the suspected site, an earlier and more accurate detection of initial demineralization can be achieved.⁶ Nevertheless, visual inspection is not a quantitative method and minor changes due to the caries process cannot be detected within short periods.²

New technologies are being developed to detect and quantify early caries lesions on smooth and occlusal surfaces. A quantitative laser/light-induced fluorescence (QLF) device measures the intrinsic fluorescence of mineralized tooth tissues. This method provides a good correlation between mineral loss^{3,7-9} and early detection of enamel smooth surface caries lesions.^{3,10} Optical coherence tomography (OCT) is another advanced method that has achieved good results in detecting and quantifying caries lesions on occlusal and smooth surfaces.^{11,12} However, QLF is not suitable in occlusal sur-

faces or on dentin caries lesions¹³ and few clinical studies have been performed with OCT.

Another device, based on emission of a light ($\lambda=655$ nm) from a diode laser, is called DIAGNOdent (KaVo, Biberach, Germany). This laser fluorescence (LF) device captures the fluorescence emitted by dental tissues and translates it on a numerical scale from 0 to 99. The higher the number, the deeper the caries lesion.¹⁴ Previous studies with LF have presented good results in detecting occlusal caries lesions.¹⁵ However, the device does not provide a good correlation with mineral loss on smooth surface caries lesions in permanent^{7,16} and primary teeth.¹⁷ Furthermore, despite the fact that some authors have claimed that the device is able to detect early enamel caries lesions,^{14,18} studies do not confirm this assertion.^{15,17} In fact, LF reflects changes in the organic material, rather than in the inorganic content of the teeth,^{7,14,16} probably because of the presence of porphyrins in caries lesions, mainly protoporphyrin IX (PPIX).^{14,19}

Because of these facts, LF is not indicated to detect very early demineralization. Nevertheless, the LF device is portable, easy to use, and available for clinical practice. Thus, the aim of the present *in vitro* study is to propose the association of the LF device with two fluorescent dyes and to evaluate the performance of the methods in detecting and quantifying very

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early demineralization from smooth surface caries lesions in primary teeth.

2 Material and Methods

2.1 Dye Selection

The Ethical Committee of the School of Dentistry of University of São Paulo approved this study. Since porphyrins have been pointed out as the molecules responsible^{7,14,16,19} for the fluorescence increase in caries lesions when excited at 655 nm, we chose two porphyrins for the experiments, PPIX (Aldrich, Milwaukee, Wisconsin) and tetrakis(*N*-methylpyridyl)porphyrin (TMPyP) (Aldrich, Milwaukee, Wisconsin). PPIX is an anionic and lipophilic porphyrin produced by some bacteria related with caries lesions.¹⁹ TMPyP is a cationic and hydrophilic porphyrin with a great affinity for surfaces. TMPyP has been used in photodynamic therapy against malignant cells and virus.²⁰ There was no concern regarding toxicity regarding the use of low-concentration PPIX or TMPyP solutions in sound human tissues.

For this study, different solutions of each dye were measured with an LF device (DIAGNOdent). The solution (0.4 ml) was put in a 1-ml quartz cuvette with a 1-cm optical path length, and LF measurements were performed with a tip appropriate for smooth surfaces (tip B). The tip was put in contact with the external cuvette wall and the maximum LF value was recorded. The measurements were performed in two cuvettes, three readings were done on each cuvette, and the mean value was calculated. Linear regression analysis was performed between dye concentration and LF values.

The solutions were tested on primary teeth with natural smooth surface caries lesions to observe the difference in the LF values associated with the dyes in evaluating sound and carious surfaces. However, we did not obtain good results using PPIX in water to differentiate carious and sound sites. Thus, we evaluated the PPIX dissolved in water and dimethyl sulfoxide (1:1) (DMSO). In this way, we obtained better differentiation. TMPyP was tested with the same solvents, but better results were found with the water solution of the dye. Linear regression was obtained with the two dyes: $y = 719.54x - 1.585$ and $R^2 = 0.997$ for TMPyP, and $y = 12.67x + 24.66$ and $R^2 = 0.946$ for PPIX in water and DMSO, where y is related to LF values and x is dye concentration. New readings in natural caries were performed. Based on these experiments, we chose solutions of 0.2-mM TMPyP in water and 4.0-mM PPIX in water:DMSO (1:1) as the best concentrations of the porphyrins to differentiate between caries lesions and sound sites.

2.2 Sample Selection and Demineralization Induction

Sixty exfoliated primary upper human canine teeth were donated by the Bank of Teeth of the School of Dentistry of the University of São Paulo. The teeth were polished with pumice/water slurry and rinsed with tap water. Then, the teeth were sectioned in two halves. The left portion was destined for experiments with TMPyP and the right one for experiments with PPIX.

In 40 teeth, two windows of about 2- × 3-mm dimensions (one in each separated portion) were delimited in the buccal

surface of each primary tooth using nail varnish to create the artificial caries lesions. The demineralizing solution contained 2.2-mM CaCl₂, 2.2-mM NaH₂PO₄, and 50-mM acetic acid, and the pH was adjusted to 4.8 using KOH. All the reagents were analytical grade (Merck, Darmstadt, Germany) and the solutions were prepared with deionized water. The samples were divided into four groups according to the period of demineralization (12, 24, 48, and 96 h). Each specimen was individually immersed in 5 ml of demineralizing solution. This procedure was carried out at room temperature and without shaking.

Twenty teeth were used for LF evaluation of sound surfaces, and consequently, were not submitted to demineralization. LF readings were performed on buccal surfaces without and with dyes.

2.3 LF Measurements

LF readings were performed with DIAGNOdent following the manufacturer's instructions. Probe tip B (for smooth surface) was selected. The laser device was calibrated against a porcelain reference object prior to the examination and recalibrated after taking the readings of 10 teeth. The calibration on the sound surface of each tooth was not performed. The nail varnish was removed to avoid interference with the LF readings. The teeth were taken out of the solution, wiped with a filter paper for 5 s, and submitted to LF readings by one operator. In sound teeth, readings were performed on all buccal surfaces and for the teeth submitted to demineralization, readings were carried out on and 2 mm around the lesions. Three readings were performed at each site and the mean value was calculated.

The left portions of the teeth were destined for the experiment to evaluate the association of LF with TMPyP dye (LF TMPyP). After initial measurements with LF, the samples were immersed in 5 ml of 0.2-mM TMPyP for 60 s, removed and dipped in distilled water (two dippings), dried with filter paper for 5 s and evaluated with the LF device as already described. With the right portions of the teeth, the same procedures were performed, but the dye was 4-mM PPIX in water and DMSO to evaluate the association of the LF with PPIX (LF PPIX).

2.4 Mineral Loss Evaluation

The mineral loss of the teeth was evaluated by determining the amount of calcium loss in the solution during the demineralization induction. After demineralization, the samples were removed from the solutions and 5 ml of 10% nitric acid was added to the remaining solution. The calcium concentration in this solution was analyzed by inductively coupled argon plasma with atomic emission spectrometry (Spectroflame Modula, Spectro Analytical Instruments, Kleve, Germany). The sensitivity of the technique is 0.008 ppm. The measurements were performed twice for each sample, and the relative standard deviation was up to 1.5%. The amount of calcium loss was calculated by subtracting the amount of calcium present in the solution after and before the demineralizing treatment.

After demineralization and prior to the removal of nail varnish, the teeth were evaluated in a stereomicroscope coupled to a CCD camera and the actual area of the windows

Table 1 Pearson's correlation coefficient between amount of calcium loss and LF values without dye (LF), and LF associated with tetrakis(N-methylpyridyl)porphyrin (LF TMPyP), and LF and LF with protoporphyrin IX (LF PPIX), and areas under ROC curve (Az) in the measurements performed in teeth subjected to different demineralization times and sound teeth.

Demineralization Time (h)	Correlation Coefficients		Az		Significance between Az
	LF	LF TMPyP	LF	LF TMPyP	
12	-0.167 ns	0.237 ns	0.685	0.943	*
24	0.275 ns	0.735*	0.728	0.990	*
48	0.173 ns	0.821***	0.883	0.985	ns
96	0.407 ns	0.896***	0.878	0.993	ns
Demineralization Time (h)	Correlation Coefficients		Az		Significance between Az
	LF	LF PPIX	LF	LF PPIX	
12	0.258 ns	0.287 ns	0.673	0.570	ns
24	-0.240 ns	0.316 ns	0.743	0.570	ns
48	0.223 ns	0.488 ns	0.953	0.503	***
96	-0.493 ns	0.507 ns	0.790	0.750	ns

ns=statistically nonsignificant difference ($p \geq 0.05$); *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$.

were determined by an image analysis software (Leica Qwin, Leica microsystems, Heidelberg, Germany). Thus, the calcium loss of each tooth was determined in parts per million per square millimeter.

2.5 Statistical Analyses

Initially, the samples were evaluated separately according to time of demineralization. Pearson's correlation coefficients between LF readings performed on teeth with different periods of demineralization, with no dye and with TMPyP and PPIX, and calcium loss was calculated. A receiver operating characteristic (ROC) analysis was conducted to assess the LF performance in detecting smooth surface caries lesions in samples with different caries lesion induction periods. ROC analysis is a good statistical approach for new methods with numerical values. The sensitivity is plotted as a function of 1—specificity for various possible cutoff points. The area under the curve can be calculated, and the closer is the curve to the upper left corner, the greater is the overall accuracy of the test.²¹ In our study, for these analyses, teeth submitted to demineralization were considered as carious ones (presence of lesion), while teeth not submitted to demineralization were considered as sound ones (absence of lesion). Accordingly, areas under ROC curves for each method were calculated to evaluate how early the caries process was detected.

After these initial analyses, the samples were jointly analyzed and Pearson's correlation coefficients between LF measurements (with and without dyes) and calcium loss were determined for all the teeth submitted to artificial caries induction. We also performed new ROC analyses considering all the teeth submitted to demineralization as carious, and teeth not demineralized as sound ones. With ROC analysis, the best cutoff points to discriminate sound and carious sites

can be also calculated.²¹ Thus, area under ROC curves and best cutoff points between carious and sound teeth were calculated for each method. With these cutoff limits, sensitivity, specificity, and accuracy were calculated. Sensitivity is the ability of the test to detect a carious tooth when it is truly affected by dental caries, and specificity is the probability that the diagnostic test will be negative among the sound teeth. Accuracy is the number of true results (true positive diagnoses+true negative ones) divided by the number of examined sites.²² The comparison between these values for each method was performed by the McNemar change test. The significance level for all tests was chosen as $p < 0.05$.

3 Results

With the sample destined for TMPyP experiment, there was a significant correlation between LF TMPyP values and calcium loss of the caries lesions demineralized for 24, 48, and 96 h. No significant correlation was found between LF TMPyP readings and calcium loss of lesions produced for 12 h, as well as the LF values and calcium loss with all teeth (demineralized for 12, 24, 48, and 96 h) (Table 1). The calcium losses [mean±standard deviation (SD)] in these samples were 12 h; 0.49 ± 0.31 ppm/mm²; 24 h; 2.15 ± 1.03 ppm/mm²; 48 h; 3.66 ± 1.59 ppm/ppm/mm²; and 96 h; 8.02 ± 2.33 ppm/mm².

Concerning the performance, we observed high values of the area under ROC curves with the LF TMPyP method to detect early caries lesions (higher than 0.94, for all periods of demineralization in contrast with measurements in sound surfaces). The areas under ROC curves obtained with the LF method were lower than with LF TMPyP, but there were sig-

Table 2 Best cutoff points to distinguish between teeth with early caries lesions and sound teeth, and performance of laser fluorescence (LF) and LF associated with tetrakis(N-methylpyridyl)porphyrin (LF TMPyP), and LF and LF associated with protoporphyrin IX (LF PPIX) methods in detecting early artificial demineralization expressed in sensitivity, specificity, accuracy and area under ROC curve (Az)

Methods	Best Cutoff Points	Sensitivity	Specificity	Accuracy	Az
LF	>3.0	0.875 a	0.600 a	0.783 a	0.793 a
LF TMPyP	>9.3	0.950 a	0.850 b	0.917 b	0.978 b
Methods	Best Cutoff Points	Sensitivity	Specificity	Accuracy	Az
LF	>3.0	0.769 a	0.750 a	0.763 a	0.785 a
LF PPIX	>8.7	0.179 b	0.950 a	0.441 b	0.528 b

Different letters express statistically significant difference between values within the same column ($p < 0.05$)

nificant differences only in lesions demineralized for 12 and 24 h (Table 1).

When the lesions were jointly assessed, considering all demineralized teeth (for 12, 24, 48, and 96 h) as carious teeth and the measurements performed in teeth not submitted to demineralization as sound teeth, higher specificity, accuracy, and area under ROC curve was achieved by LF TMPyP association. There was no significant difference in the sensitivity values between the methods, although the value was higher with LF TMPyP method (Table 2). Moreover, when considering all samples, there was significant correlation between LF TMPyP and calcium loss ($R=0.707$, $p < 0.001$), while with LF without TMPyP, there was no significant correlation ($R=0.310$, $p \geq 0.05$).

In the LF PPIX experiments, one tooth demineralized for 48 h was excluded, because it presented a much higher LF value (20.3) than other samples. There was no significant correlation of LF and LF PPIX values with the amount of calcium loss in any period of demineralization (Table 1). Furthermore, there was no significant difference between areas under ROC curves with LF and LF PPIX methods, except in teeth demineralized for 48 h, where LF without PPIX presented better performance than with the dye (Table 1). The calcium losses in the samples destined for PPIX experiment were (mean \pm SD) 12 h; 0.74 ± 0.40 ppm/mm²; 24 h, 2.44 ± 1.37 ppm/mm²; 48 h; 4.03 ± 2.01 ppm/mm²; and 96 h; 7.95 ± 4.01 ppm/mm².

When the samples were assessed in combined groups, there was no significant correlation between LF readings and amount of calcium loss ($R=0.091$, $p \geq 0.05$), but this correlation was significant between LF PPIX values and calcium loss ($R=0.597$, $p < 0.001$). With regard the performance, the LF method showed significantly higher values of sensitivity, accuracy, and area under ROC curve, and similar specificity compared with the LF PPIX method (Table 2).

4 Discussion

The LF device is portable and available for clinical practice, but it is not able to detect very early mineral loss. Thus, we tested an association of the LF device with two porphyrins to improve the detection of the LF device in very early caries

lesions. This is a new approach for the utilization of the LF device.

The dyes were selected based on literature and properties of the products. In the absorption spectrum of TMPyP and PPIX, there are^{20,23} two strong features at around 400 nm (Soret band) and a set of bands around 520 nm (*Q* bands). Excitation at 655 nm is resonant with the *Q* bands for both porphyrins,^{20,23} thus, originating emission at longer wavelengths used in the dye-assisted caries lesion detection presented in our study.

For this purpose, we used an artificial caries-induction method for shorter periods than other studies. While some studies used periods of demineralization of about 14 to 21 days,²⁴⁻²⁷ other authors have used shorter periods to evaluate methods to detect early demineralization,^{3,8-12} similar to our study.

Our results showed good performance in detecting early demineralization with the LF TMPyP method, even with lesions produced in 12 h. With different periods of demineralization, we accomplished good performance of LF TMPyP in detecting the caries lesions, since the areas under ROC curves were higher than 0.94. This association (LF TMPyP) presented significant correlation with calcium loss in caries lesions produced for 24, 48, and 96 h. Without dye the LF device did not achieve significant correlation with any lesion.

We observed that the earlier the caries lesions, the lower the areas under ROC curves with the LF TMPyP method. The performance of the LF device on natural smooth surface caries lesions in primary teeth was also better in more advanced caries lesions.¹⁷ These lesions could retain a higher quantity of TMPyP. Thus, the performance in more advanced lesions could improve, as observed in our study.

When the analyses were performed with all samples (independent of demineralization periods), the LF TMPyP also provided good results in detecting (area under ROC curve=0.978) and quantifying early caries lesions (Pearson's correlation coefficient=0.707). The other methods (LF and LF PPIX) did not present good results. In a previous study, the performance of LF in detecting artificial caries lesions was better than that obtained in this study.²⁶ Those differences could be related to different methods of caries lesions induction, since the artificial caries lesions in the earlier study were

created in 14 days using a pH cycling method. The disparity in methodology could explain these differences.²⁶ Inequalities related to the sample drying before the measurements could also explain the differences.²⁸ Furthermore, in the previous study,²⁶ measurements were taken on the same sample before and after the demineralization, while in this study, we carried out the measurements on different samples. Other authors have claimed that LF is not adequate in detecting artificial caries lesions.¹⁴

The better performance obtained by the LF TMPyP method than that achieved with the LF PPIX method could be explained by the chemical properties of the dyes. While the PPIX is a lipophilic substance,¹⁹ the TMPyP is a hydrophilic one, and it has a high affinity for surfaces.²⁰ As the initial demineralization occurs in surface,²⁵ TMPyP should have an advantage over PPIX in the early caries lesions. For longer periods of demineralization, a characteristic subsurface enamel caries lesion is formed, with an apparently intact surface.²⁵ In this way, PPIX could interact better with more advanced caries lesions since it presents anionic properties.¹⁹ In fact, better performance was obtained in quantifying mineral loss with LF PPIX, and a similarity in detecting artificial caries lesions created from 8- to 16-day lesions with two dyes (unpublished data).

Previous research has reported methods of early caries lesions detection. A QLF device (using an argon-ion laser) has shown a significant correlation with artificial caries lesions depth created from 2 to 24 h. Another study proposed the association of a fluorescent dye with the QLF method.³ The dye-enhanced laser fluorescence method (DELF) showed a very early detection of demineralization in artificial caries lesions formed in 2 h. However, the DELF method has not achieved significant correlation with mineral loss. A QLF device (without dye) was able to detect caries lesions with 8 h of demineralization and showed better correlation with mineral loss.³ A comparison between these studies and our results is complicated, because the earlier studies have used bovine teeth and a different method of caries induction, while our study uses human primary teeth.

Another study, using primary teeth, observed that a QLF device was able to detect 60% of the teeth demineralized for 24 h, and 100% of the samples demineralized for 48 h. This study did not present performance values, such as ROC curves, sensitivity, specificity, or accuracy values.¹⁰ This study used primary teeth and an artificial caries method using a demineralizing solution (pH=4.5), with shaking, and at 37°C. In our study, we achieved a good performance in detecting tooth demineralized for 12 h. We also used primary teeth, the pH of demineralizing solution was higher (pH=4.8), and the experiment was carried out at room temperature and with no shaking. Thus, it is possible that the method of artificial caries induction used in the earlier study¹⁰ was more aggressive than the method used in our research.²⁴ If this is the case, LF TMPyP could detect earlier caries lesions than the QLF device

Regarding OCT, it seems that the method is adequate to detect minor mineral changes due to demineralization and remineralization.^{11,12} Nevertheless, the comparison with a LF device associated with fluorescent dyes is complicated on account of the differences in the methodology used. In fact,

further studies aiming to compare these methods, using the same sample and procedures, are necessary to corroborate these findings. Moreover, different concentrations of the dyes, different periods of immersion into dyes, and other kinds of dyes should be tested to improve the performance of association between LF and fluorescent dyes.

Some authors have suggested that methods of early detection of caries lesions could also provide more false positive diagnosis.^{4,5} Furthermore, a great number of non-cavitated early caries lesions could be arrested without professional intervention. Therefore, these methods should not be cost-effective.⁴ Nevertheless, in primary teeth, caries lesions progress faster than in permanent teeth.²⁷ Besides, we obtained high specificity and sensitivity values with LF TMPyP method, and therefore, a low number of false positive results. A quantitative method that detects minor changes in mineral content due to the caries process could be suitable for evaluation of a patient's caries activity and for use in short-term clinical studies.²⁶ Moreover, better remineralization has been achieved in small enamel caries lesions than in larger ones.¹

Despite the good results provided by our study, we used an artificial caries method. There are significant differences between artificial and natural caries lesions. While natural caries lesions take a long time to form, the artificial ones are formed in a few days. Thus, the surface zone of artificial caries is more softened than that of natural lesions.²⁵ The method used in our study, however, simulates early demineralization, which occurs in the enamel surface. Nevertheless, other *in vitro* studies with natural enamel caries must be carried out. After that, *in situ* and *in vivo* studies must be designed to confirm the good performance of the LF TMPyP method in early detection of the enamel demineralization and to prove the efficacy in monitoring caries development.

5 Conclusion

The LF TMPyP association provides excellent performance in detecting early enamel caries lesions (even with 12 h of demineralization) and a good correlation with mineral loss of these lesions in primary teeth.

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