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Effectiveness of Fluorescence-based Methods in Monitoring Progression of Noncavitated Caries-like Lesions on Smooth Surfaces

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Clinical Relevance

In this investigation, the authors observed that fluorescence-based methods were able to identify progressive enamel demineralization on smooth surfaces in the presence of biofilm.

SUMMARY

Although there has been a significant decrease in caries prevalence in developed countries, the slower progression of dental caries requires methods capable of detecting and quantifying lesions at an early stage. The aim of this study was to evaluate the effectiveness of fluorescence-based methods (DIAGNOdent 2095 laser fluorescence device [LF], DIAGNOdent 2190 pen [LFpen], and

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VistaProof fluorescence camera [FC]) in monitoring the progression of noncavitated caries-like lesions on smooth surfaces. Caries-like lesions were developed in 60 blocks of bovine enamel using a bacterial model of *Streptococcus mutans* and *Lactobacillus acidophilus*. Enamel blocks were evaluated by two independent examiners at baseline (phase I), after the first cariogenic challenge (eight days) (phase II), and after the second cariogenic challenge (a further eight days) (phase III) by two independent examiners using the LF, LFpen, and FC. Blocks were submitted to surface microhardness (SMH) and cross-sectional microhardness analyses. The intraclass correlation coefficient for intra- and inter-examiner reproducibility ranged from 0.49 (FC) to 0.94 (LF/LFpen). SMH values decreased and fluorescence values increased significantly among the three phases. Higher values for sensitivity, specificity, and area under the receiver operating characteristic curve were observed for FC (phase II) and LFpen (phase III). A significant correlation was found between fluorescence values and SMH in all phases and integrated loss of surface hardness (ΔKHN) in phase III. In conclusion, fluorescence-based methods were effective in monitoring noncavitated caries-like lesions on smooth surfaces, with moderate correlation with SMH, allowing differentiation between sound and demineralized enamel.

INTRODUCTION

Although there has been a significant decrease in caries prevalence in children from most developed countries, dental caries make up one of the most prevalent oral diseases.[1] In recent decades, major changes have occurred in the pattern of dental caries due to the widespread use and availability of fluoride. Thus, the slower progression of caries lesions[2] requires methods capable of detecting and quantifying lesions at an early stage.[3]

The early detection of smooth-surface caries lesions is important for determining the appropriate management and monitoring of dental caries at a time when preventive measures could still be introduced.[4] It is known that conventional methods for caries detection are subjective[5] and not capable of quantifying the mineral loss caused by the disequilibrium in the process of demineralization and remineralization of hard dental tissues.[6]

Thus, noninvasive quantitative methods have been evaluated to detect lesions at an initial stage and subsequently monitor lesion changes over time.[3] Fluorescence methods have received considerable attention as technology-based approaches to caries detection because bacterial porphyrins and other chromophores present on the demineralized dental tissues emit fluorescence when excited by a light source with a specific excitation wavelength.[3]

The laser fluorescence devices DIAGNOdent 2095 (LF, KaVo, Biberach, Germany) and DIAGNOdent 2190 pen (LFpen, KaVo) are based primarily on fluorescence absorption by bacterial by-products in porous carious lesions when the surface is illuminated by the device's diode laser with a wavelength of 655 nm.[3] Some studies have evaluated the performance of the LF and LFpen devices in detecting or monitoring caries development on smooth surfaces, with contradictory results.[7-16]

The intraoral fluorescence camera (FC, VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) was developed for the detection of caries and emits blue light at 405 nm to capture and digitalize images from the teeth while they are emitting fluorescence.[17] In incipient carious lesions, red porphyrin fluorescence is emitted, whereas such fluorescence is not emitted by sound enamel.[18] However, there is little evidence on the FC device's efficacy in detecting caries lesions on smooth surfaces. An *in vitro* study has shown good reliability of the FC device in detecting caries on smooth surfaces, similar to the reliability shown by the LF and LFpen devices.[19] In more recent studies, however, the FC device showed poor effectiveness in detecting demineralization and remineralization on smooth surfaces.[15,16]

Fluorescence-based methods have been proposed to aid caries detection, as they can offer objective assessments of the carious process.[20] However, there are still many questions regarding their performance when evaluating smooth surfaces. To date, no study has evaluated the efficacy of the LF, LFpen, and FC in monitoring the progression of caries lesions on smooth surfaces.

Therefore, the aim of this *in vitro* study was to evaluate the effectiveness of fluorescence-based methods (LF, LFpen, and FC) in monitoring progression of noncavitated caries-like lesions developed using a bacterial model. The null hypothesis is that there is no difference between the results obtained using the three different fluorescence-based devices on smooth surfaces.

METHODS AND MATERIALS

Sample and Specimen Preparation

Two hundred enamel blocks (4 × 4 × 2 mm) were obtained from bovine incisors and were stored frozen at −20°C. Each block was embedded in epoxy resin in order to expose only the buccal surface. This procedure was performed to allow polishing of the enamel surface as needed for an appropriate SMH analysis since such evaluation requires a stable specimen during the indentation process.[4]

All blocks were then stored individually at 100% humidity. The enamel surface was then sequentially polished with carbide papers (600, 1200, and 1500 grit) and diamond abrasive on a polishing paper, resulting in the removal of about 100 μm of the outer enamel, which was checked with a micrometer. Surface microhardness (SMH) analysis was performed using a microhardness tester (HMV-2, Shimadzu Corp, Tokyo, Japan) with a Knoop diamond under a 25-g load for five seconds.[6] Five indentations spaced 100 μm apart were made, and their average was recorded. Of the original 200 enamel blocks, only 120 with hardness of 310.9 ± 25.5 Knoop hardness (KHN) were selected. Each of the 120 enamel blocks was randomly allocated into one of two groups: control (n=60) or experimental (n=60).

The 60 enamel blocks in the experimental group were used to evaluate the effectiveness of fluorescence-based methods in monitoring the development of noncavitated caries lesions on smooth surfaces.

Experimental Design

This *in vitro* study involved three phases of treatment of enamel blocks in the experimental group: baseline (phase I), after the first cariogenic challenge (phase II), and after the second cariogenic challenge (phase III).

Measurement With Fluorescence-Based Methods

Each enamel block was assessed by two examiners using LF, LFpen, and FC. The examiners had experience handling the devices and had participated in previous published studies. The enamel blocks were removed from the 100% humidity storage environment, fixed in clear acrylic resin disks, and dried with a paper tissue.[6]

The LF and LFpen measurements were performed using a fiber-optic conical tip (tip B), specifically designed for smooth surfaces, and a cylindrical sapphire fiber tip, respectively, according to the manufacturer's instructions. Before each measurement, the devices were calibrated against a ceramic standard and were recalibrated after testing 10 blocks.[6,21] After calibration, the laser point was placed in the center of each enamel block and swept across the surface. The maximum fluorescence value detected by the devices was recorded. Each block was dried with a paper tissue and air-dried for five seconds and analyzed three times consecutively by each examiner, after which the mean values were calculated.[4,16]

The FC measurements were performed in a dark environment to block external light when examining the enamel blocks. The tip of the device was placed perpendicular to the enamel surface using a distance holder. After capturing the images of the enamel blocks, they were analyzed by the FC-specific software (DBSWIN, Dürr Dental), translating the red and green rate of fluorescence to numbers that correspond to the lesion severity.[17] The maximum value displayed by each sample was recorded for further analysis (Figure 1). The FC measurements were also done three times by each examiner, and the mean values were calculated.[16] The FC images were taken twice with a one-week interval.

Cariogenic Challenge

The enamel blocks in the experimental group (n=60) were used for the development of caries-like lesions using a bacterial model adapted from previous studies[22,23] and assessed by a previous study.[24] The experimental period for initial assessment of caries lesions was determined at eight days (phase II) and the second assessment after a further eight days (phase III).

Each enamel block was coated with a layer of epoxy adhesive and a layer of acid-resistant varnish, except for the buccal surface, leaving exposed a 16-mm^2 enamel window. Then each block was individually attached to orthodontic wire to allow the free enamel window to be immersed in 25 mL of distilled water in a Falcon tube without touching the tube walls and autoclaved at 121°C for 20 minutes. The 60 enamel blocks in the control group were used to evaluate the influence of the autoclave sterilization process on the enamel SMH.

The microorganisms used in this study were *Streptococcus mutans* (ATCC 25175) and *Lactobacillus acidophilus* (ATCC 4356). The organisms were grown overnight in brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) under anaerobic

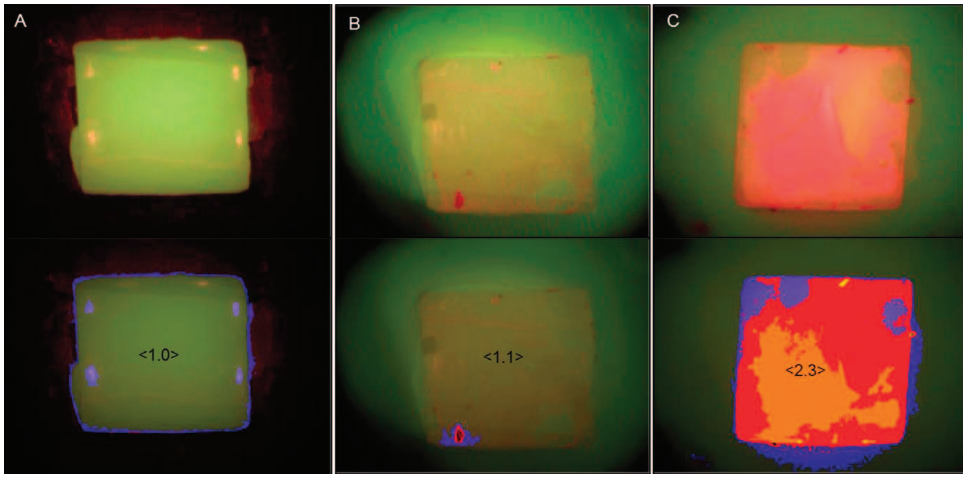

Figure 1. *Fluorescence images taken with an FC device from the buccal surface of an enamel block in all phases of the study. Upper pictures represent the digitalized images from the surface while it is emitting fluorescence. Lower pictures represent the fluorescence images analyzed by the FC-specific software DBSWIN. (A): At baseline (FC value = 1.0). (B): After the first cariogenic challenge (FC value = 1.1). (C): After the second cariogenic challenge (FC value = 2.3).*

conditions at 37°C. To standardize the inoculum density, a visible turbidity of McFarland 0.5 standard (equivalent to a bacterial quantity of 1.5×10^8 colony-forming units/mL) was applied. In addition, Gram staining was performed in order to differentiate between the two species of microorganism.

After sterilization, each enamel block was removed from the distilled water and transferred aseptically to another Falcon tube filled with 25 mL of a modified artificial caries solution (brain heart infusion culture medium supplemented with yeast extract, 0.5% glucose, 1% sucrose, and 0.5% young primary culture broth of *S. mutans* and *L. acidophilus*). The enamel blocks were incubated at 37°C in a candle jar. Every 48 hours, the specimens were transferred to another Falcon tube containing a new sterile artificial caries solution for a continuous cariogenic fresh supply.[22,23] The pH of the medium decreased from 7.0 to 4.5 within 48 hours of bacterial inoculation.

At the end of eight days (phase II), the biofilm was removed from the enamel blocks with sterile gauze, and the blocks were then washed with deionized water for 60 seconds. The samples exhibited a dull, whitish change resembling opacity under the microscope. SMH and fluorescence-based measurements were obtained. Then the enamel blocks exhibiting incipient caries lesions were autoclaved again and submitted to cariogenic challenge for a further eight days (phase III) in order to simulate the progression of caries lesions in the enamel. At the end of the experimental period, SMH and fluorescence-based measurements were obtained again. Steam autoclaving is effective for sterilizing the enamel to be used in cariogenicity tests and does not interfere with the demineralization pattern.[25]

Cross-Sectional Microhardness

After SMH analysis, all enamel blocks were longitudinally sectioned through the center of the exposed enamel for cross-sectional microhardness (CSMH) determination. Half of each block was embedded in acrylic resin, and the cut surfaces were exposed and polished. CSMH was determined according to the method described by Spiguel and others[6] using a Knoop indenter with a 25-g load for five seconds (Shimadzu HMV-2). Three rows of eight indentations at 10, 30, 50, 70, 90, 110, 220, and 330 μm from the outer enamel surface were made: one row in the central region of the exposed enamel and the other two spaced 100 μm from the first (one of these rows was 100 μm above the first row, and the second was 100 μm below it). The mean value of each distance indentation was calculated.[16]

Integrated hardness (KHN × μm) of sound and demineralized enamel was calculated to a depth of 220 μm using the trapezoidal rule[26] (GraphPad Prism, version 3.02, GraphPad Software Inc, La Jolla, CA, USA). The integrated loss of hardness (ΔKHN) was calculated by subtracting the demineralized integrated hardness from the integrated hardness of sound enamel.[6]

Polarized Light Microscopy

The other half of each enamel block was cut into sections of approximately 500-μm thickness using a diamond saw. The sections were then manually ground and polished to a thickness of 100 μm, mounted on slides with distilled/deionized water, and covered with a cover glass. The sections were examined by polarized light microscopy (Leica DM750, Leica Microsystems, Wetzlar, Germany) at 400× magnification. Three areas in the central

regions of the sections were analyzed by recording the thickness of the superficial enamel layer and the depth of the lesion using ImageJ 1.38x software (National Institutes of Health, Bethesda, MD, USA).[6,16]

Control Group

In order to evaluate the influence of the autoclave sterilization process on the enamel surface microhardness, the enamel blocks (n=60) were autoclaved using the same intervals used for the experimental group. There was no development of caries-like lesions in this group. There were three experimental phases in the control group: baseline (phase I), after the first autoclave process (phase II), and after the second autoclave process (phase III).

First, the enamel blocks were autoclaved at 121°C for 20 minutes and stored in distilled/deionized water for eight days (phase II). SMH and fluorescence-based measurements were obtained after this period. The enamel blocks were then autoclaved once more and stored in distilled/deionized water for a further eight days (phase III). SMH and fluorescence-based measurements were obtained again at the end of this period.

Statistical Analysis

The data were analyzed using the statistical software MedCalc for Windows, version 12.3.0 (MedCalc Software, Mariakerke, Belgium), and the level of significance was α=0.05. Outcome variables were the mean values of LF, LFpen, FC, SMH, and ΔKHN and the phases (I, II, and III) as variation factors.

The intraclass correlation coefficient (ICC) was used to assess intra- and interexaminer reproducibility for fluorescence-based methods. The ICC was considered poor when the values were below 0.40, fair for values between 0.40 and 0.59, good for values between 0.60 and 0.75, and excellent for values above 0.75.[27]

The Kolmogorov-Smirnov test was used to verify the normal distribution of the data. Nonparametric tests for paired samples were used because of the lack of normality of the data. In order to compare the three phases of the experiment for measurements made using SMH and fluorescence-based methods, the nonparametric Friedman test and multiple comparison tests were performed. The Wilcoxon test was used to compare the integrated hardness of sound and demineralized enamel.

The percentage change of SMH (%SMHC), determined in relation to the baseline measurement, was calculated for each enamel block according to the method of Cury and others: %SMHC = (SMH after demineralization − baseline × 100)/baseline.[26] The Mann-Whitney test was used to compare the %SMHC between phases II and III.

A receiver operating characteristic (ROC) analysis was conducted to evaluate the performance of each fluorescence-based method in monitoring the development of caries lesions in enamel. ROC analysis is a good statistical approach for methods with numerical values. The sensitivity is plotted as a function of 1 − specificity for various possible cutoff points. The area under the ROC curve (Az) can be calculated for each method, and the closer the curve is to the upper left corner, the greater is the overall accuracy of the test. With ROC analysis, the best cutoff points for discriminating between sound and carious teeth can be also calculated.[28] Thus, Az values and cutoff limits for differentiating between carious and sound teeth were calculated for each method in phases II and III. With these optimal cutoff points, sensitivity and specificity were also calculated for each method. The comparison between these values was performed by the McNemar test. In the present study, enamel blocks submitted to the cariogenic challenge were considered as carious ones (presence of noncavitated lesion), while enamel blocks that had not yet been submitted to the cariogenic challenge were considered as sound ones (absence of lesion).

Spearman's rank correlation coefficient (rho) was used to test the strength of a relationship between the different fluorescence-based methods and SMH, considering all phases or ΔKHN and lesion depth (μm), in phase III. The Spearman coefficient varies between −1 and 1; the closer these extremes, the greater the association between variables.

RESULTS

Table 1 represents the intra- and interexaminer reproducibility assessed by calculating ICC for LF, LFpen, and FC for the experimental group in all three phases. ICC values for intra- and interexaminer reproducibility indicated fair to excellent agreement for the fluorescence-based methods in phases I and II and good to excellent agreement in phase III.

With respect to the experimental group, the fluorescence-based methods showed significant differences between the three phases, with the highest values being recorded for phase III ($p<0.05$; Table 2). With regard to the SMH analysis of the enamel blocks, statistically significant differences ($p<0.05$)

Table 1: *Intraclass Correlation Coefficient (ICC) for Intra- and Interexaminer Reproducibility for LF, LFpen, and FC in the Experimental Group for All Phases of Treatment (n=60)*

Phase	Method	ICC (95% confidence interval)		
		Intraexaminer reproducibility		Interexaminer reproducibility
		Examiner A	Examiner B	
I	LF	0.93 (0.88-0.96)	0.87 (0.79-0.92)	0.61 (0.44-0.73)
	LFpen	0.85 (0.75-0.91)	0.84 (0.74-0.91)	0.70 (0.57-0.79)
	FC	0.76 (0.60-0.86)	0.74 (0.56-0.84)	0.49 (0.27-0.64)
II	LF	0.65 (0.41-0.79)	0.86 (0.77-0.92)	0.53 (0.32-0.67)
	LFpen	0.76 (0.60-0.86)	0.89 (0.81-0.93)	0.87 (0.81-0.91)
	FC	0.51 (0.18-0.71)	0.66 (0.43-0.80)	0.74 (0.63-0.82)
III	LF	0.87 (0.79-0.92)	0.94 (0.90-0.97)	0.90 (0.85-0.93)
	LFpen	0.92 (0.86-0.95)	0.94 (0.90-0.97)	0.93 (0.90-0.95)
	FC	0.90 (0.83-0.94)	0.82 (0.69-0.89)	0.82 (0.75-0.88)

were observed between the three phases, with the lowest values being recorded for phase III. The %SMHC was statistically significant between phases II and III ($p<0.05$). Integrated hardness (KHN×μm) was significantly different between sound enamel (88.101±10.489) and demineralized enamel (42.335±14.598) (Wilcoxon test, $p<0.05$). Integrated loss of hardness (ΔKHN) was 45.766.6 ± 16.067.6. With respect to the control group, there was no difference in the fluorescence values for LF, LFpen, FC and SMH values between all the three phases ($p>0.05$) (Table 2).

Table 3 shows sensitivity, specificity, area under the ROC curve (Az), and cutoff points for LF, LFpen, and FC in phases II and III of the treatment in the experimental group. Applying the best cutoff points to differentiate between sound and carious teeth, it was observed that FC and LFpen demonstrated statistically higher sensitivity, specificity, and Az values in phases II and III, respectively. In addition, LF showed the lowest values in phase II, and LF and FC presented similar values in phase III.

Spearman's rank correlation coefficients (rho) are shown in Table 4. There was a significant negative correlation between SMH and fluorescence values in phase I and a significant positive correlation between SMH and fluorescence values in phases II and III ($p<0.05$). There was also a significant positive correlation between fluorescence values and ΔKHN in phase III ($p<0.05$). No correlation was observed between fluorescence values and lesion depth ($p>0.05$). The highest correlation was found for LFpen in phase II (rho = 0.380), meaning that the higher the SMH values after the first cariogenic challenge, the higher the LFpen measurements.

Figure 1 shows the digital and the fluorescence images taken with the FC device from the surface of an enamel block in all phases of the study. An increase in fluorescence values was observed in each consecutive phase.

Figure 2 shows a polarized light photomicrograph after the second cariogenic challenge (phase III). The demineralized enamel seems dark under polarized

Table 2: *Fluorescence Values, Surface Microhardness (SMH) Values, and Percentage of SMH Change (%SMHC) in the Experimental (n=60) and Control (n=60) Groups for All Phases of Treatment*[a]

Group	Phase	Mean ± standard deviation				
		LF*	LFpen*	FC*	SMH* (KHN)	%SMHC**
Experimental	I	7.8 ± 2.5 A	15.9 ± 4.8 A	1.0 ± 0.0 A	310.9 ± 25.5 A	—
	II	12.3 ± 7.0 B	30.0 ± 11.3 B	1.2 ± 0.1 B	104.1 ± 43.6 B	−66.4 ± 5.0 A
	III	24.8 ± 10.7 C	60.2 ± 20.2 C	1.6 ± 0.3 C	43.6 ± 10.1 C	−85.9 ± 3.5 B
Control	I	6.8 ± 2.3 A	14.8 ± 4.5 A	1.0 ± 0.0 A	305.7 ± 45.4 A	—
	II	6.4 ± 2.0 A	14.9 ± 4.3 A	1.0 ± 0.0 A	305.4 ± 42.5 A	—
	III	6.9 ± 2.3 A	14.1 ± 4.9 A	1.0 ± 0.0 A	306.1 ± 43.2 A	—

[a] *Significant differences are represented by different letters within the same column (*Friedman and multiple comparison tests/**Mann-Whitney test;* $p<0.05$).

Table 3: *Sensitivity, Specificity, Area Under the Receiver Operating Characteristic Curve (Az), and Cutoff Points for LF, LFpen, and FC in the Experimental Group for Phases II and II of the Treatment (n=60)*[a]

Phase	Method	Sensitivity	Specificity	Az	Cutoff points
II	LF	0.68 A	0.69 A	0.757 A	>8
	LFpen	0.75 B	0.90 B	0.889 B	>21
	FC	0.99 C	0.88 B	0.983 C	>1
III	LF	0.89 A	0.98 A	0.983 A	>13
	LFpen	0.98 B	0.96 B	0.997 B	>25
	FC	0.87 A	0.99 A	0.989 A	>1.2

[a] *Significant differences are represented by different letters within the same column (McNemar test; p<0.05).*

light microscopy. The mean lesion depth was 103.9 ± 29.3 μm.

DISCUSSION

The present study evaluated the effectiveness of fluorescence-based methods in monitoring the development of noncavitated caries-like lesions on smooth surfaces. To our knowledge, this is the first study to use a bacterial model for caries generation to evaluate the ability of the LF, LFpen, and FC devices to detect initial caries-like lesions in enamel and monitor their progression. It should be emphasized that conventional methods for the detection of caries lesions do not comply with the criteria for an ideal caries detection method because they rely on subjective interpretation and are insensitive to early caries detection. Therefore, methods capable of detecting and quantifying early caries lesions are required as adjunct tools in clinical practice, allowing preventive intervention before irreversible destruction of tooth substance occurs.[3]

It was verified that the *in vitro* methodology used was capable of forming caries-like lesions in enamel using a bacterial model composed of *S. mutans* and *L. acidophilus*, simulating the process of dental caries.[24,29] The bacterial model offers the opportunity to evaluate the variability and exchangeability of the species involved in the carious process and factors such as lesion site or availability of fermentable carbohydrates.[30-32]

To evaluate the effectiveness of fluorescence-based methods for monitoring enamel caries lesions, an *in vitro* model using bacterial films is likely to be more realistic than chemical systems since the devices have the potential to identify bacterial metabolites such as porphyrins (fluorophores and other chromophores) produced by cariogenic bacteria.[3,17-19,33,34] Even though some studies have shown that cultures of selected oral bacteria, such as *S. mutans* and *Lactobacillus* species, seem to show no typical porphyrin fluorescence,[5,36] other studies demonstrated that *S. mutans* induced enamel lesions exhibited increased fluorescence in the red region.[37,38] Fluorescent properties of dental caries can be attributed to tissue demineralization and an increase in bacterial flora and its metabolism.[39,40] In addition, pH seems to have an important influence

Table 4: *Spearman's Rank Correlation Coefficient (rho) Between Fluorescence-Based Methods and Surface Microhardness (SMH), integrated loss of hardness (ΔKHN), and Lesion Depth (μm) in the Experimental Group for All Phases of Treatment (n=60)*[a]

Phase	Method	Spearman's rank correlation coefficient		
		SMH	ΔKHN	Lesion depth (μm)
I	LF	−0.235*	—	—
	LFpen	−0.328*	—	—
	FC	−0.198*	—	—
II	LF	0.328*	—	—
	LFpen	0.380*	—	—
	FC	0.191*	—	—
III	LF	0.297*	0.224*	0.091 ns
	LFpen	0.343*	0.246*	0.110 ns
	FC	0.356*	0.285*	0.114 ns

[a] *Variables statistically correlated.*
* *p<0.01.*
Abbreviation: ns, not significant.

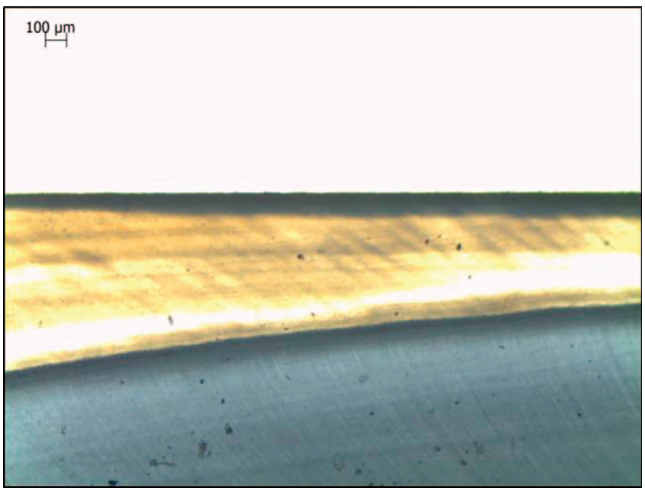

Figure 2. *Polarized light micrograph of an enamel block after the second cariogenic challenge. (Magnification = 400×). The demineralized enamel seems dark on the top surface.*

on the light absorption and emission of porphyrins.[41] In the present investigation, it could be suggested that the red fluorescence recorded by the devices was probably due to the synergistic effects between the carious process and the bacterial species and their environments.

In the present study, CSMH analysis was used in combination with polarized light microscopy to determine lesion mineral distribution and area of demineralization. Other studies on enamel demineralization have also used CSMH profiling for validation and evaluated the artificial caries lesion depth in a polarized light microscope.[6,16,24,42] It is important to point out that researchers have used a considerable number of analytical techniques to quantify changes in the mineral content of enamel during caries lesion formation.[43] Transverse microradiography (TMR) provides a quantitative measure of the mineral content, lesion depth, and attributes of the surface layer in enamel lesions.[44] CSMH measures the mechanical resilience (physical strength) of enamel, which cannot be determined by TMR.[45] According to ten Bosch and Angmar-Mansson,[46] SMH is a noninvasive technique and does not damage the enamel's macrostructure. SMH can be used in caries research, as it measures an important parameter with structural integrity and mechanical properties that cannot necessarily be derived from TMR variables.[43] The results of superficial microhardness analysis after the first and second cariogenic challenges showed that KHN values were significantly lower than the baseline values, demonstrating that the present methodology was able to create and cause progression of caries-like lesions, a finding confirmed by ΔKHN and polarized light microscopy. These results corroborate those of Spiguel and others[6] and Moriyama and others,[16] who used *in situ* methodologies to create caries-like lesions. It is known that polarized light microscopy is a good method for assessing lesion depth, and CSMH is an indirect method for the assessment of mineral loss in smooth-surface caries lesions.[47]

It should be emphasized that a caries detection method should offer good reproducibility, allowing consistent and reliable results to be produced by different evaluations and examiners. According to Lussi and Hellwig,[34] a high level of agreement for the LF device means that it could be useful for monitoring the carious process. In general, good to excellent agreement was observed for LF and LFpen in all phases for both intra- and interexaminer reproducibility, except for LF in phase II (interexaminer reproducibility). These results confirm Aljehani and others,[13] Bahrololoomi and others,[14], Moriyama and others,[16] and De Benedetto and others,[19] who also observed good to excellent reproducibility values for LF and LFpen devices for caries detection on smooth surfaces. In contrast, fair to good agreement was found for the LF device for intraexaminer reproducibility.[4] These differences might be attributed to the experimental design, which was an *in vitro* study using a buffer system to produce caries-like lesions, and to subjective errors in the measurements.

Generally, the FC device also showed good to excellent agreement for intra- and interexaminer reproducibility, supporting the findings of De Benedetto and others.[19] Fair interexaminer agreement at baseline might be explained by the specimen size and procedure for polishing of the enamel surfaces, which reflected the six light-emitting diode (LED) light sources, interfering with image capturing and fluorescence analysis by the different examiners.[16]

To date, no information about the effectiveness of different fluorescence-based methods in monitoring the demineralization process on smooth surfaces is available. In this study, the LF, LFpen, and FC fluorescence values showed significant differences between the three phases. This suggests that the devices were able to identify progressive enamel demineralization on smooth surfaces in the presence of biofilm.[3,6] In other words, an increase in fluorescence values might be attributed to bacterial endogenous porphyrins and related compounds present in enamel caries lesions. Moriyama and others[16] and Spiguel and others[6] also observed differences in fluorescence values after *in situ* enamel demineralization on smooth surfaces. The LF device showed a significant increase in fluorescence values after artificial demineralization, different from the *in vitro* studies of Mendes and others[21] and Diniz and others,[4] in which the caries-like enamel lesions were induced without oral bacteria and no differences found between sound and demineralized enamel for the LF values.

It was observed that the fluorescence values obtained with the LF and LFpen devices were different in all phases of this study. The LFpen fluorescence values were higher than the values recorded with the LF device, corroborating the findings of previous studies.[16,17,20,48] This finding could be attributed to the smaller diameter and architecture of the LFpen tip. Also, inside the tip of the LFpen device, the excitation and fluorescence follows the same optical path of propagation in

opposite directions, which is different in the LF device.[34]

When assessing the performance of fluorescence-based methods for caries detection, the cutoff points recommended by the manufacturers should be discussed because they could affect treatment decision making in clinical practice. It was observed that changes in LF fluorescence values at baseline and after the first and second cariogenic challenges were within the cutoff points proposed by the manufacturer. The manufacturer proposed that values of 0-10 indicate sound teeth and that values of 11-30 indicate enamel caries. In contrast, the values obtained with LFpen fluorescence did not fit within the cutoff points proposed by KaVo, which state that values of 0-14 indicate a healthy surface and that values of 15-20 indicate enamel caries. According to the manufacturer, the fluorescence values observed in phases II and III with the LFpen would indicate dentin caries lesions, and operative and preventive care is advised. Moriyama and others[16] observed that changes in LF values at baseline and in LFpen values at baseline and after *in situ* demineralization were within the cutoff points proposed by the manufacturer. These differences might be attributed to the different degree of demineralization obtained by the different methodologies. The present study was conducted *in vitro* using a bacterial model with no mimicry of the diverse conditions present in the oral cavity that might affect development of dental caries.

In regard to the FC device, according to the manufacturer, the numbers between 0.0 and 1.0 represent a healthy tooth, values from >1.0 to 1.5 indicate incipient enamel caries, and values from >1.5 to 2.0 indicate deep enamel caries. For Diniz and others,[20] values between 0.0 and 1.0 indicated a sound surface, and those between 1.1 and 1.2 indicated enamel lesion. In the present study, the FC values were statistically different between the phases and lend support to the cutoff points proposed by the manufacturer, allowing monitoring of the development of enamel caries on smooth surfaces. On the other hand, Moriyama and others[16] obtained FC values very close to each other at baseline and after *in situ* demineralization, making it difficult to monitor incipient caries lesions. It should be stressed that care must be taken in choosing to adopt the cutoff points proposed by the manufacturer and by other studies for interpreting the FC fluorescence values.

The VistaProof FC is a system with blue LEDs emitting at 405 nm (blue-violet light), and it is similar in design to the quantitative light-induced fluorescence (QLF) system, presenting the same excitation wavelength. The QLF device is considered a valuable instrument for early caries detection, capable of monitoring demineralization and remineralization and quantifying changes in the mineral content of noncavitated lesions. The fluorescence image of incipient caries lesions is digitized, and the fluorescence loss is quantified in comparison to the fluorescence radiance level of sound enamel. Three parameters are analyzed: fluorescence loss (ΔF; %), area of the lesion (A; mm^2), and fluorescence loss integrated over the lesion area (ΔQ; $\Delta F \times A$; $\% \times mm^2$).[49-51] The only significant difference is that QLF measures mainly the loss of intrinsic fluorescence of the dental enamel caused by demineralization, and VistaProof fluorescence camera is based on the increase in fluorescence of carious tissues due to the presence of bacterial metabolites, such as porphyrins.[17-19]

The results obtained from the control group verified that fluorescence and SMH values were statistically similar among the three phases of the present investigation. Thus, it was demonstrated that the autoclave sterilization process did not influence the fluorescence values and enamel surface microhardness. Parsell and others[52] reported that steam sterilization did not interfere with the enamel hardness of extracted teeth. By contrast, Chandler[53] showed by microhardness testing before and after autoclaving that some modification of enamel does occur under the influence of moist heat, pressure, and air drying. The differences found in Chandler's study[53] might be related to the autoclaving process, which was performed for five minutes at 132°C followed by air drying at subatmospheric pressure for 10 minutes.

According to the results of the ROC analysis, the optimal cutoff points to indicate initial enamel caries were >8 (LF), >21 (LFpen), and >1.0 (FC), and cutoff points to indicate deep enamel caries were >13 (LF), >25 (LFpen), and >1.2 (FC). With these cutoff points, the sensitivity and specificity values were high. Fluorescence-based methods were observed to perform well in monitoring the development of enamel caries lesions. The highest area under the ROC curve was found for FC in phase II and for LFpen in phase III. Mendes and Nicolau[10] and Mendes and others[11] reported good performance of the LF device in detecting incipient caries lesions since the area under the ROC curve was >0.8. Diniz and others[20] also described areas >0.9 for LF and LFpen devices in detecting occlusal caries lesions in

permanent molars. After the first cariogenic challenge, LF and LFpen devices showed lower values for sensitivity and specificity, whereas after the second cariogenic challenge, the values were greater. These findings support those of previous research by Mendes and others,[11] who observed that the less developed the caries lesions on a smooth surface, the worse the performance of the LF device. Thus, at inner enamel caries, the performance was better than at outer enamel caries in primary teeth.

There was a significant moderate correlation between SMH and fluorescence values for all methods in all phases of the study and between ΔKHN and fluorescence values after the second cariogenic challenge. At baseline, the correlation between SMH and fluorescence values was negative, indicating that higher SMH measurements lower the fluorescence values. These results show that the devices were able to monitor the development of noncavitated enamel lesions, in agreement with the results for the LF device reported by Mendes and Nicolau.[10] Spiguel and others[6] described a positive significant correlation between ΔKHN and LF values after *in situ* demineralization, as verified for LF, LFpen, and FC devices in the present investigation. Conversely, Moriyama and others[16] found no significant correlation between fluorescence values and SMH at baseline and after demineralization and between ΔKHN and fluorescence values after demineralization. These differences might be attributable to the *in situ* methodology used by Moriyama and others[16] to create caries-like lesions of the enamel. Also in line with Moriyama and others,[16] the present study found no significant correlation between fluorescence values and lesion depth. On the other hand, Mendes and others[11] found a good positive correlation between LF values and lesion depth on smooth-surface natural caries in primary teeth.

It is important to emphasize that fluorescence-based methods should be considered as adjunct tools to the visual examination for caries detection and monitoring of smooth surfaces.[16] A systematic review and meta-analysis have shown that fluorescence-based devices have similar overall performance; however, better accuracy in detecting more advanced caries lesions has been observed.[54] Further *in vivo* studies are needed to elucidate the efficacy of fluorescence-based methods in monitoring the development of enamel caries lesions on smooth surfaces.

CONCLUSION

It can be concluded that fluorescence-based devices were effective in monitoring the development of noncavitated caries lesions on smooth surfaces *in vitro*, using a bacterial model for caries induction. The FC device showed good performance with regard to indicating incipient noncavitated caries lesions, while the LFpen device performed better at indicating deep noncavitated caries lesions. The fluorescence values showed significant moderate correlation with SMH, allowing differentiation between sound and demineralized enamel.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Cruzeiro do Sul University–UNICSUL.

Conflict of Interest

The authors of this article 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.

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