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Effectiveness of Fluorescence-Based Methods to Detect in situ Demineralization and Remineralization on Smooth Surfaces

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Key Words
Caries detection · Demineralization · Dental caries · Laser fluorescence · Remineralization

Abstract
This study aimed to evaluate the effectiveness of fluorescence-based methods (DIAGNOdent, LF; DIAGNOdent pen, LFpen, and VistaProof fluorescence camera, FC) in detecting demineralization and remineralization on smooth surfaces in situ. Ten volunteers wore acrylic palatal appliances, each containing 6 enamel blocks that were demineralized for 14 days by exposure to a 20% sucrose solution and 3 of them were remineralized for 7 days with fluoride dentifrice. Sixty enamel blocks were evaluated at baseline, after demineralization and 30 blocks after remineralization by two examiners using LF, LFpen and FC. They were submitted to surface microhardness (SMH) and cross-sectional microhardness analysis. The integrated loss of surface hardness (ΔKHN) was calculated. The intraclass correlation coefficient for interexaminer reproducibility ranged from 0.21 (FC) to 0.86 (LFpen). SMH, LF and LFpen values presented significant differences among the three phases. However, FC fluorescence values showed no significant differences between the demineralization and remineralization phases. Fluorescence values for baseline, demineralized and remineralized enamel were, respectively, 5.4 ± 1.0, 9.2 ± 2.2 and 7.0 ± 1.5 for LF; 10.5 ± 2.0, 15.0 ± 3.2 and 12.5 ± 2.9 for LFpen, and 1.0 ± 0.0, 1.0 ± 0.1 and 1.0 ± 0.1 for FC. SMH and ΔKHN showed significant differences between demineralization and remineralization phases. There was a negative and significant correlation between SMH and LF and LFpen in the remineralization phase. In conclusion, LF and LFpen devices were effective in detecting demineralization and remineralization on smooth surfaces provoked in situ.

The early detection of smooth surface caries lesions is important to provide proper and noninvasive management; lesions at this stage have the potential to be remineralized and can be monitored over time [Diniz et al., 2009]. Conventional methods for caries detection are not capable of quantifying the mineral loss or gain occurring as a result of demineralization and remineralization processes, respectively [Spiguel et al., 2009].

In this context, quantitative methods have been developed for caries detection and for monitoring changes in

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the mineral content [Pretty and Maupomé, 2004]. Some of these methods are based on the fluorescence phenomenon emitted by bacterial porphyrins (fluorophores), molecules that are excited by a light source with a specific excitation wavelength [Hibst et al., 2001; Bader and Shugars, 2004].

The laser fluorescence devices DIAGNOdent (LF; DIAGNOdent 2095, KaVo, Biberach, Germany) and DIAGNOdent pen (LFpen; DIAGNOdent 2190, KaVo) are able to capture, analyze and quantify the fluorescence emitted from bacterial porphyrins and other chromophores [Hibst et al., 2001]. Some studies have evaluated the performance of the LF and LFpen devices in detecting or monitoring caries development on smooth surfaces, with contradictory results [Hibst et al., 2001; Shi et al., 2001a, b; Pinelli et al., 2002; Mendes and Nicolau, 2004; Mendes et al., 2005; Aljehani et al., 2006, 2007], and in monitoring the remineralization process [Mendes et al., 2003; Andersson et al., 2004; Ferreira et al., 2008; Diniz et al., 2009; Kiertsman et al., 2009; Spiguel et al., 2009].

The intraoral fluorescence camera (FC; VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) was developed for caries detection and emits blue light at 405 nm and captures fluorescent images from dental surfaces [Rodrigues et al., 2008]. In initial carious lesions, red porphyrin fluorescence is emitted whereas it is absent in sound enamel [Thoms, 2006]. However, there is no scientific evidence available about the cutoff limits used to determine caries lesions on smooth and occlusal surfaces. An in vitro study has shown good reliability in detecting caries on occlusal and smooth surfaces, similar to the LF and LFpen devices [De Benedetto et al., 2011].

To our knowledge, only two studies have evaluated the LF device to detect caries-like lesions created in in situ conditions [Kiertsman et al., 2009; Spiguel et al., 2009]. Furthermore, to date no study has evaluated the effectiveness of LFpen and FC to monitor the de-/remineralization process on smooth surfaces. For that reason, it is relevant to verify the ability of the fluorescence-based methods to provide accurate and reliable measurements to monitor the development and regression of incipient caries lesions.

Therefore, the aim of this study was to evaluate the effectiveness of fluorescence-based methods (LF, LFpen and FC) in detecting demineralization and remineralization provoked on smooth surfaces in situ. The null hypothesis is that there is no difference among the fluorescence-based methods in differentiating demineralization and remineralization on smooth surfaces.

Materials and Methods

Ethical Aspects

This study was conducted in accordance with the Declaration of Helsinki and it was approved by the Ethics in Research Committee of Cruzeiro do Sul University (204/2011), São Paulo, Brazil. The study aim, procedures, possible discomforts and risks, safety, and benefits were fully explained to the subjects. Informed consent was obtained from all volunteers prior to the investigation.

Experimental Design

This in situ study involved three phases performed over 21 days: baseline (I), demineralization (II) and remineralization (III).

I. Baseline

Specimen Preparation

One hundred enamel blocks (4 × 4 × 2 mm) were obtained from bovine incisors and were stored in 0.1% thymol solution (pH 7.0) at room temperature. Each block was embedded in epoxy resin in order to expose only the buccal surface. This procedure is needed for an appropriate surface microhardness (SMH) analysis [Diniz et al., 2009].

The blocks were then stored individually at 100% humidity. Afterwards, the enamel surface was serially polished with carbide paper (600, 1,200 and 1,500 grid, in sequence; Buehler, Lake Bluff, Ill., USA) and diamond abrasive on a polishing paper, resulting in removal of about 100 μm of the outer enamel, which was controlled with a micrometer. SMH analysis was performed using a microhardness tester (HMV-2; Shimadzu Corp., Tokyo, Japan) with a Knoop diamond under a 25-gram load for 5 s [Spiguel et al., 2009]. Five indentations spaced 100 μm from each other were made and the average was recorded. From 100 enamel blocks, only 60 with a hardness of 351.1 ± 18.0 KHN were selected. Enamel blocks were sterilized with gamma radiation (25 kGy).

Measurements with Fluorescence-Based Methods

Each enamel block was assessed by two examiners using LF and LFpen devices and FC. The examiners have experience in using and handling the devices, since they had participated in previous studies [Rodrigues et al., 2008; Diniz et al., 2009, 2012]. The enamel blocks were removed from the 100% humidity storage environment, fixed in clear acrylic resin disks and dried with a paper tissue [Spiguel et al., 2009].

The LF and LFpen measurements were performed using a fiber-optic conical tip (tip B), specifically designed for smooth surfaces, and the cylindrical sapphire fiber tip, respectively, according to the manufacturer's instructions. Before each measurement, the devices were calibrated against a ceramic standard, and then they were recalibrated after testing 10 blocks [Mendes et al., 2003; Spiguel et al., 2009]. 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 5 s and analyzed 3 times in sequence by each examiner and the mean values were calculated [Diniz et al., 2009].

The FC measurements were performed in a dark environment. After capturing the images of the enamel blocks, they were analyzed by FC-specific software (DBSWIN, Dürr Dental), which translates the red and green rate of fluorescence into numbers that correspond to the lesion severity [Rodrigues et al., 2008]. The val-

ues were recorded for further analysis. The FC measurements were also done 3 times by each examiner and the mean values were calculated.

Participant Selection

Ten volunteers (4 males and 6 females, aged 20–30 years) who lived in an area whose water supply contained fluoride (0.7 mg F/l) were selected for the investigation. They were in good general and oral health and presented a normal salivary flow rate. The volunteers were clinically evaluated after professional dental prophylaxis for the detection of active caries lesions and periodontal disease. The participants were willing to cooperate with the clinical research protocol and to abstain from their own oral hygiene products, except those provided for this study. The exclusion criteria were current or recent use of any form of medication that affects salivary flow, use of fixed or removable orthodontic appliances, dental treatment, presence of active caries lesions or periodontal disease, pregnant or breast-feeding, smoker, or systemic illness [Spiguel et al., 2009].

A meeting was organized with the volunteers in order to present the research project, its objectives and the experimental design. The volunteers received oral and written information regarding the procedures to be performed during the experiment and to refrain from using any antibacterial or fluoridated product.

II. Demineralization Phase

During this phase, the volunteers wore intraoral acrylic palatal appliances. Six spaces were created and one sterilized enamel block was placed in each, leaving a 1.0-mm space for plaque accumulation. Dental plaque was formed on the enamel blocks, which were protected from mechanical disturbance by a plastic mesh fixed in the acrylic surface [Cury et al., 2000].

The volunteers were instructed to remove the intraoral appliance 8 times per day for 14 days and to place 2 drops of 20% sucrose solution onto each enamel block (at 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00 and 22.00) [Spiguel et al., 2009]. The appliance was placed back into the mouth 5 min after each cariogenic challenge. Volunteers were instructed to wear the appliances continuously, including at night, except during meals, when drinking water, when consuming any acidic beverage or during oral care [Aires et al., 2006]. They brushed their natural teeth with nonfluoride dentifrice (Cocoricó, Bitufo, Itupeva, São Paulo, Brazil) [Spiguel et al., 2009].

After the demineralization phase, the enamel blocks (n = 60) were removed from the appliances and gently brushed. SMH and fluorescence-based measurements were obtained. For SMH analysis, 5 indentations spaced 100 μm from each other and from the baseline indentations were made [Vieira et al., 2005]. Afterwards, 30 enamel blocks (3 enamel blocks from each volunteer) were selected randomly for cross-sectional microhardness (CSMH) analysis and polarized light microscopy. A 7-day period was allowed between demineralization and remineralization phases to allow the examinations. Francescut et al. [2006] showed that the fluorescence values significantly decreased only after 7–14 days of storage.

III. Remineralization Phase

The other 30 enamel blocks were rinsed in deionized water and replaced in each appliance without the plastic mesh protection. The volunteers wore the intraoral appliances again for 7 days and were instructed to wear them all the time, including at night, but to remove them during meals (1 h per meal) [Spiguel et al., 2009].

They were instructed to perform oral hygiene 3 times a day and the appliances were to be brushed in the mouth, including the enamel blocks, using fluoridated dentifrice (Colgate Total 12 Professional Gengiva Saudável, Colgate-Palmolive Ind. E Com. Ltda., São Paulo, Brazil, 1,450 μg F/g) for 2 min. After the remineralization phase, SMH and fluorescence-based measurements were obtained again. For SMH analysis, 5 indentations spaced 100 μm from each other and 200 μm from the baseline indentations were made [Vieira et al., 2005]. CSMH analysis and polarized light microscopy were performed.

Cross-Sectional Microhardness

After SMH analysis, all enamel blocks were longitudinally sectioned through the center of the exposed enamel for CSMH determination. Half of each block was embedded in acrylic resin and the cut surfaces were exposed and polished. The CSMH was performed according to Spiguel et al. [2009] using a Knoop indenter with a 25-gram load for 5 s (Shimadzu HMV-2). Three rows of 8 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. The mean value of each distance was calculated.

Integrated hardness (KHN × μm) of sound, demineralized and remineralized enamel was calculated to a depth of 220 μm using the trapezoidal rule [Cury et al., 2000] (GraphPad Prism, version 3.02; GraphPad Software Inc., La Jolla, Calif., USA). The integrated loss of hardness (ΔKHN) was calculated by subtracting the integrated hardness (demineralized or remineralized) from the integrated hardness of sound enamel [Spiguel et al., 2009].

Polarized Light Microscopy

The other half of the enamel block was sectioned to 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 glass coverslip. The sections were examined by polarized light microscopy (Leica DM750, Leica Microsystems, Wetzlar, Germany) at ×400 magnification. Three areas in the central region 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, USA) [Spiguel et al., 2009].

Statistical Analysis

The data were analyzed using the statistical software MedCalc for Windows (version 12.3.0, Mariakerke, Belgium), and the level of significance was $p < 0.05$. Outcome variables were the mean values of LF, LFpen, FC, SMH, integrated hardness and ΔKHN, and the phases (baseline, demineralization and remineralization) as variation factors.

The intraclass correlation coefficient (ICC) was used to assess 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 [Lin, 1989].

The percentage change of surface microhardness (%SMHC), determined in relation to the baseline measurement, was calculated for each enamel block according to the method of Cury et al. [2000]: %SMHC = (SMH after demineralization – baseline × 100)/

Table 1. ICC for interexaminer reproducibility for LF, LFpen and FC in all phases (n = 10 volunteers)

Phases	ICC (95% confidence interval)		
	LF	LFpen	FC
Baseline	0.60 (0.33–0.76)	0.45 (0.09–0.67)	0.21 (–0.33 to 0.53)
Demineralization	0.70 (0.49–0.82)	0.86 (0.76–0.92)	0.47 (0.12–0.68)
Remineralization	0.77 (0.52–0.89)	0.51 (–0.03 to 0.77)	0.68 (0.33–0.85)

Table 2. SMH values, %SMHC, %SMR, integrated hardness (KHN × μm) and ΔKHN (mean ± standard deviation) in all phases (n = 10 volunteers)

Phases	SMH, KHN	%SMHC	%SMR	Integrated hardness, KHN × μm	ΔKHN
Baseline	351.1±18.0[a]	–	–	36,940±861[a]	–
Demineralization	113.7±69.4[b]	–67.8±18.9[a]	–	19,160±1,553[b]	17,780±3,544[a]
Remineralization	196.4±85.9[c]	–43.9±25.2[b]	36.6±35.5	29,993±1,883[c]	6,947±3,738[b]

Significant differences ($p < 0.05$) are indicated within the same column by different superscript letters.

Table 3. Fluorescence values (mean ± standard deviation) for LF, LFpen and FC in all phases (n = 10 volunteers)

Phases	LF	LFpen	FC
Baseline	5.4±1.0[a]	10.5±2.0[a]	1.0±0.0[a]
Demineralization	9.2±2.2[b]	15.0±3.2[b]	1.0±0.1[b]
Remineralization	7.0±1.5[c]	12.5±2.9[c]	1.0±0.1[b]

Significant differences ($p < 0.05$) are indicated within the same column by different superscript letters.

baseline or (SMH after remineralization – baseline × 100)/baseline. The percentage of enamel surface microhardness recovery (%SMR) was calculated for each enamel block remineralized according to the method of Cury et al. [2005]: %SMR = [100 (SMH post-treatment – SMH caries)/baseline SMH – SMH caries].

In order to compare the three phases of the experiment for SMH, fluorescence-based measurements and integrated hardness, the nonparametric Friedman's and multiple comparison tests were performed. The Mann-Whitney test was used to compare the %SMHC and ΔKHN for demineralization and remineralization phases.

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

Results

Table 1 presents the interexaminer reproducibility assessed by calculating the ICC values for LF, LFpen and FC in all phases, ranging from poor to excellent agreement between the examiners.

With regard to the SMH analysis, statistically significant differences were observed among the three phases ($p < 0.05$). The %SMHC was also significantly different between demineralization and remineralization phases ($p < 0.05$). The %SMR was 36.6 ± 35.0. Integrated hardness was significantly different among all phases ($p < 0.05$). ΔKHN was also significantly different between demineralization and remineralization phases ($p < 0.05$, table 2).

Table 3 presents the mean fluorescence values for LF, LFpen and FC in all phases. The LF and LFpen measurements showed significant differences among the three phases, with the highest values obtained for the demineralization phase ($p < 0.05$). With respect to the FC measurements, there was no difference between demineralization and remineralization phases ($p > 0.05$); however, those phases presented significant differences compared to baseline.

Spearman's rank correlation coefficients are shown in table 4. There was a negative correlation between SMH/ΔKHN and fluorescence values in all phases, with a statistically significant difference in the remineralization

Table 4. Spearman's rank correlation coefficients between fluorescence-based values and SMH and ΔKHN in all phases (n = 10 volunteers)

Phases	Spearman correlation coefficient (p value)					
	SMH			ΔKHN		
	LF	LFpen	FC	LF	LFpen	FC
Baseline	–0.155 (p = 0.2370)	–0.062 (p = 0.6395)	–0.187 (p = 0.1526)	–	–	–
Demineralization	–0.075 (p = 0.5675)	–0.030 (p = 0.8203)	–0.151 (p = 0.2499)	–0.107 (p = 0.4166)	–0.087 (p = 0.5088)	–0.160 (p = 0.2228)
Remineralization	–0.339* (p = 0.0081)	–0.445* (p = 0.0004)	–0.166 (p = 0.1877)	–0.167 (p = 0.2023)	–0.180 (p = 0.1694)	–0.057 (p = 0.6658)

Variables statistically correlated: * $p < 0.05$.

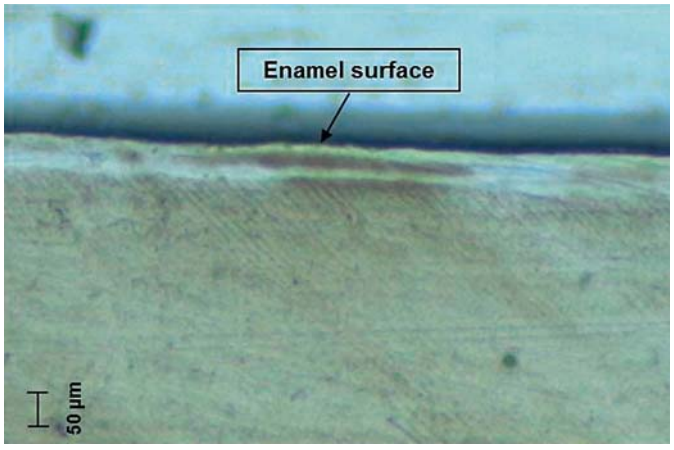

Fig. 1. Polarized light micrograph of an enamel block after demineralization. ×400.

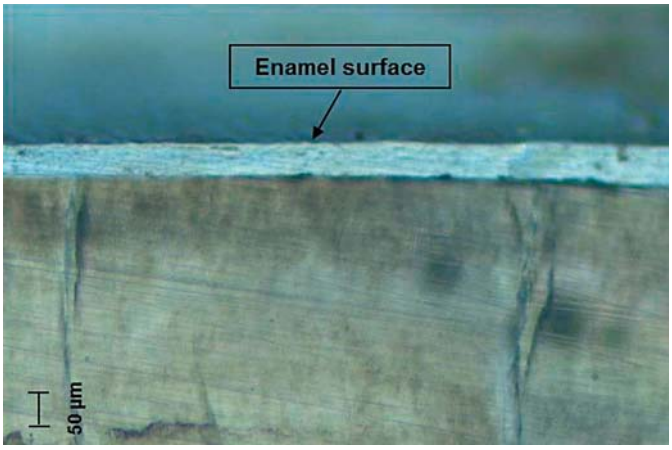

Fig. 2. Polarized light micrograph of an enamel block after remineralization. ×400.

phase ($p < 0.05$) for LF and LFpen measurements and SMH. The highest correlation was found for LFpen-remineralization (r = –0.445), which means the higher the SMH values after remineralization, the lower the LF and LFpen measurements.

Figure 1 shows a polarized light photomicrograph after demineralization. The mean lesion depth was 70.6 ± 21.1 µm and the thickness of the surface layer was 8.7 ± 2.4 µm. Spearman's correlation coefficients between fluorescence values and lesion depth after demineralization were low and nonsignificant: r = 0.013 for LF, r = 0.032 for LFpen and r = 0.152 for FC.

Figure 2 shows a polarized light photomicrograph after remineralization. The mean lesion depth was 35.8 ± 14.3 µm and the thickness of the surface layer was 9.9 ± 2.0 µm. Spearman's correlation coefficients between fluorescence values and lesion depth after remineralization were also nonsignificant: r = 0.234 for LF, r = 0.278 for LFpen and r = 0.122 for FC.

Discussion

In this study, the effectiveness of fluorescence-based methods was evaluated in the detection of demineralization and remineralization on smooth surfaces. It is important to emphasize that a caries detection method should present good reproducibility, allowing for the acquisition of consistent and reliable results between different evaluations and examiners. According to Lussi and Hellwig [2006], a high level of agreement for the LF device means that it could be useful for monitoring the carious process. In this study, ICC values for interexaminer reproducibility showed great variability among the fluorescence-based methods as well as in all phases of the study. Higher ICC values were observed after demineralization for LFpen and after remineralization for LF, indicating excellent agreement between the examiners. These results corroborate the studies by Aljehani et al. [2007] and De Benedetto et al. [2011], who also observed high reproduc-

ibility values for LF and LFpen devices for caries detection on smooth surfaces. On the other hand, moderate agreement was found for the LF device at baseline and after demineralization phases, a fact also reported by Diniz et al. [2009]. In general, LFpen was less reproducible than LF. This could be attributed to the difficulty of handling the LFpen device by the different examiners and its more fragile tip made of sapphire fiber.

It was observed that the FC device showed poor agreement between examiners in the baseline phase. This might be due to specimen size and the polishing procedure of the enamel surfaces, which reflected the six-LED light sources and may have interfered with image capture and fluorescence analysis by the different examiners and led to subjective errors during measurements. On the other hand, fair and good agreement was observed in the demineralization and remineralization phases, respectively. This might be explained by the lack of reflection of the six-LED light sources on the opaque surface due to the demineralization process. Controversially, De Benedetto et al. [2011] observed that the FC device showed high reproducibility on smooth surfaces of primary teeth, resembling the LF and LFpen devices. These differences may be explained by the fact that in that study natural caries lesions were assessed on smooth surfaces.

To date, no information about the effectiveness of LFpen and FC devices to monitor the de-/remineralization process on smooth surfaces is available. The FC fluorescence values were statistically significantly different between baseline and demineralization or remineralization phase. However, no difference was found between demineralization and remineralization phases. It could be suggested that FC was not able to identify small changes in enamel mineral content. These results were also found when occlusal dentin lesions were analyzed in previous studies [Rodrigues et al., 2008; Diniz et al., 2012].

In the present study, the LF and LFpen fluorescence values showed significant differences among the three phases, while FC did not. Therefore, the null hypothesis can be rejected. In previous studies, the LF device was suitable for detecting caries on smooth surfaces and it showed a significant increase in fluorescence values after the cariogenic challenge [Mendes and Nicolau, 2004; Ferreira et al., 2008; Spiguel et al., 2009] and between demineralization and remineralization [Spiguel et al., 2009], as was found in the present investigation. Divergent results were found in other studies. Kiertsman et al. [2009] found no statistically significant difference in LF fluorescence values among baseline, demineralization and remineralization of occlusal surfaces of human premolars, which might be due to the complex invaginated anatomy of pits and fissures. Mendes et al. [2003] and Diniz et al. [2009] reported that the LF device was not appropriate for monitoring the demineralization and remineralization processes. This fact could be explained by differences in methodologies, such as the type of dental substrate (human or bovine enamel), natural versus artificial caries lesions and remineralizing agent (pH-cycling models, fluoride dentifrice or fluoride gel). Since LF detects changes in organic tooth content, such as fluorophores and other chromophores produced by cariogenic bacteria rather than inorganic content [Lussi et al., 1999; Shi et al., 2000; Hibst et al., 2001], its effectiveness is questionable in artificial caries-like enamel lesions, which are induced without metabolites from oral bacteria. The LF and LFpen fluorescence values were significantly greater after demineralization when compared to the baseline values, which might be explained by increase in the surface porosity of the enamel and light scattering [Mendes and Nicolau, 2004] and/or the increase in organic content in the presence of biofilm [Spiguel et al., 2009]. The presence of microorganisms has been shown within active and inactive incipient caries lesions by scanning electron microscopy [Parolo and Maltz, 2006]. Thus, it is possible that the penetration of metabolic products into enamel tissue in early caries lesions, producing significant amounts of endogenous porphyrins and related compounds, results in an increase in fluorescence values.

The fluorescence values obtained using LFpen were higher than those obtained using the LF device in agreement with previous studies [Diniz et al., 2008; Rodrigues et al., 2008; Diniz et al., 2012]. The reasons for this finding could be attributed to the different diameters and materials of the tips in both devices, which might influence the amount of the excitation light transmitted through the tip and the degree of capture of the fluorescence emitted by the dental tissues.

Another important aspect to be discussed is related to the cutoff limits proposed for each device. The performance of fluorescence-based methods is dependent on the cutoff limits used and the difference in cutoff values for sound teeth, enamel or dentine caries will affect the treatment decision-making in clinical practice [Heinrich-Weltzien et al., 2003]. It was observed that changes in LF (baseline) and LFpen (baseline, after demineralization and remineralization) fluorescence values were within the cutoff limits proposed by the manufacturer. Although the manufacturer indicates the cutoff values for all surfaces, they were based on studies assessing occlusal lesions. This should be considered when LF devices are

used in clinical practice on smooth surfaces. In the present study, the FC values obtained in all phases were very close to each other, ranging from sound to demineralized when classified according to the cutoff limits proposed by the manufacturer, making it difficult to monitor incipient caries lesions. These results suggest that care should be taken in the use of the cutoffs, since there is no consensus in the literature for active and inactive incipient caries lesions on smooth surfaces. Thus, fluorescence-based methods should be used as complementary methods in monitoring incipient caries lesions on smooth surfaces.

There was a negative and moderate correlation between SMH and fluorescence values (LF and LFpen) in the remineralization phase ($p < 0.05$). This means that when there was an increase in SMH values, indicating remineralization, fluorescence values decreased. These results show that LF and LFpen devices might be able to detect the remineralization process of early enamel lesions, which differs from the results described for the LF device by Spiguel et al. [2009]. However, there was no significant correlation between fluorescence values and lesion depth, which means that fluorescence might be related to the softening of the surface rather than to subsurface demineralization.

The lack of correlation between hardness and LF and LFpen for demineralization can be explained by the fact that the devices were not capable of detecting small changes in mineral content due to the scattering phenomenon [Shi et al., 2001a; Mendes et al., 2003], which might be due to the porosity of caries lesions [Mendes and Nicolau, 2004; Diniz et al., 2009]. However, there was a significant correlation for LF and LFpen after the remineralization process when slight surface hardness recovery was obtained and LF values increased, which might be related to the decrease in porosity and the scattering phenomenon. The absence of correlation between FC and hardness for any phase could be attributed to the small range in the cutoff limits of the device for sound and enamel caries [Rodrigues et al., 2008; Diniz et al., 2012].

It is important to discuss the cost-benefit ratio of the fluorescence-based methods, which should be considered as adjunct tools to visual examination for caries detection and monitoring on smooth surfaces, when bitewing radiographs are not indicated. Fluorescence values should be carefully interpreted in the treatment decision-making in clinical practice. To date, studies regarding the efficacy of fluorescence-based methods in monitoring the caries process are limited, especially with respect to LFpen and FC devices on smooth surfaces. Moreover, clinical studies should be performed in order to evaluate the effectiveness of the fluorescence-based methods in monitor demineralization and remineralization processes.

It is reasonable to conclude that LF and LFpen devices were effective in differentiating demineralization and remineralization on smooth surfaces in situ, with moderate correlation with SMH for the remineralization of enamel. However, the results should not be considered as indicating exact threshold measurements. Besides, FC was not able to differentiate between demineralization and remineralization phases.

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Disclosure Statement

The authors declare that they have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that might introduce bias or affect their judgment or that could be construed as influencing the position presented herein or the review of the manuscript entitled 'Effectiveness of Fluorescence-Based Methods to Detect in situ Demineralization and Remineralization on Smooth Surfaces'.

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