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The Influence of Pit and Fissure Sealants on Infrared Fluorescence Measurements

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Key Words
Dental caries, diagnosis · Laser fluorescence · Pit and fissure sealants

Abstract
The aim of this in vitro study was to evaluate the influence of pit and fissure sealants on fluorescence readings using lasers. We selected 166 permanent molars and randomly divided them into 4 groups which were each treated with a different sealant (a commercially available clear sealant, 2 opaque sealants and an experimental nanofilled clear sealant). The teeth were independently measured twice by 2 experienced dentists using conventional laser fluorescence (LF) and a laser fluorescence pen device (LFpen), before and after sealing, and again after thermocycling to simulate the thermal stressing between the tooth and the dental materials. Friedman test showed no statistically significant changes using LF and LFpen for the commercial clear sealant group, although values tended to increase after sealing. However, the values increased significantly after thermocycling. There was a statistically significant decrease in fluorescence after application of opaque sealants. After application of the experimental nanofilled clear sealant, LF values increased only after thermocycling, whereas the LFpen values increased after sealing and after thermocycling as well. The intraclass correlation coefficient ranged from 0.87 to 0.96 for interexaminer and 0.82 to 0.94 for intraexaminer reproducibility. It was shown that pit and fissure sealants influence LF and LFpen readings, with the values increasing or decreasing according to the material used. In conclusion, both laser fluorescence devices could be useful as an adjunct to detect occlusal caries under unfilled clear sealants. Nevertheless, surfaces sealed with clear nanofilled material could be assessed using only the LF device.

While pit and fissure sealants have been demonstrated to be effective in occlusal caries prevention [Wendt et al., 2001], their efficacy may be related to the background caries prevalence in the population [Ahovuo-Saloranta et al., 2004]. Incorrect application of the sealant could result in leakage, and microorganisms trapped underneath sealants could lead to caries lesion development. Consequently, undesirable outcomes, such as loss of tooth structure, larger restorations and endodontic treatment can occur [Chapko, 1987]. Besides, incorrect application of sealants can lead to failure and sealant loss. Therefore, regular follow-up is required to ensure the long-term success of the sealant treatment [Feigal, 2002]. Even on unsealed occlusal surfaces lesions are difficult to detect because of the surface morphology, fissures, and the presence of plaque and stain, which can mask caries lesions. As visual inspection is difficult to perform on sealed surfaces, adjunct methods must be used to improve the fol-

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low-up assessments and to increase the diagnostic accuracy. Some of the newer methods are based on fluorescence. Porphyrins present in caries lesions fluoresce when stimulated at specific excitation wavelengths [Hibst et al., 2001]. This formed the basis for the first laser fluorescence device (LF) as an adjunct to detecting caries, and for the more recent laser fluorescence pen device (LFpen), which has been studied for both occlusal and approximal caries detection [Lussi and Hellwig, 2006; Lussi et al., 2006].

Several studies have evaluated the LF devices for occlusal caries detection with good results for validity and reproducibility, both in vivo and in vitro [Lussi et al., 1999; Shi et al., 2000; Sheehy et al., 2001; Lussi and Hellwig, 2006]. Nevertheless, the presence of composite filling materials might influence the LF readings, leading to false-positive results [Lussi and Reich, 2005].

There are conflicting data on the influence of sealants on LF measurements. Some studies suggest that fissure sealants do not affect LF readings, implying that the device could be used during routine checkups to detect caries under fissure sealants [Takamori et al., 2001; Anttonen et al., 2003; Deery et al., 2006; Krause et al., 2008]. However, other studies have shown contradictory results and problems with this practice [Hosoya et al., 2004; Gostanian et al., 2006]. Besides, no study has evaluated the performance of the LFpen in detecting caries under fissure sealants. Therefore, the aim of this in vitro study was to assess the influence of different fissure sealants, clear and opaque, on laser fluorescence readings for caries diagnosis.

Materials and Methods

From a pool of teeth, 166 extracted third permanent human molars were selected. Their conditions ranged from sound to carious (108 had microcavities and 52 were noncavitated, of which 22 were apparently sound). The teeth were stored frozen at –20°C until use and during the experiments; this method of storage does not change the red fluorescence significantly [Francescut et al., 2006]. All teeth were extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm F in table salt). Prior to extraction, the patients were informed about the use of their teeth for research purposes and their written consent was obtained. The teeth were defrosted for 3 h, and calculus and debris were removed using a scaler (Cavitron). They were cleaned for 15 s with water and toothbrush (Trisa ultra super-sensitive, Triengen, Switzerland) and for 10 s with a water-powder jet cleaner (Prophyflex II, Kavo, Biberach, Germany) and sodium hydrogen carbonate powder. To remove powder remnants from the fissures, the teeth were finally rinsed with a 3-in-1 syringe for 10 s [Lussi and Reich, 2005].

Photographs of the occlusal surfaces were taken at a magnification of ×6.25 using a light microscope (Leica DC300, Leica, Heerbrugg, Switzerland) equipped with a video camera linked to a computer (Leica M420, Leica, Heerbrugg, Switzerland) and the test site was marked. All assessments were independently carried out twice by 2 experienced dentists, with a 1-week interval between the measurements. The following devices were used: Diagnodent 2095 (LF) and Diagnodent 2190 (LFpen; both from Kavo, Biberach, Germany). During the measurements, the teeth were stored in 100% humidity. Measurements were made before sealing (baseline), after sealing and after thermocycling.

Baseline Measurements

Measurements were performed with LF using the probe tip 'A' and with the LFpen using the cylindrical sapphire fiber tip, according to the manufacturer's instructions. Before each measurement, the devices were calibrated with a ceramic standard and the zero value of fluorescence of a sound part of the cuspal area on the buccal surface was recorded. The tip was placed on the selected site and rotated around the vertical axis until the highest fluorescence reading was obtained. The peak values were recorded and the zero value of fluorescence was subtracted.

After measuring, we used the average LF values and the cutoff limits suggested by Lussi and Hellwig [2006] (D_0 = 0–7; D_1 = 7.1–14; D_2 = 14.1–24; D_3, $D_4 \geq 24$) to form groups with the same numbers of teeth with enamel and dentine caries and sound teeth. The teeth were then randomly divided into the following sealing treatment groups:

- Group I: Delton Unfilled Clear (Dentsply, Konstanz, Germany), 41 teeth;
- Group II: Delton Opaque (Dentsply, Konstanz, Germany), 42 teeth;
- Group III: Helioseal Opaque (Ivoclar Vivadent, Schaan, Liechtenstein), 41 teeth;
- Group IV: experimental nanofilled clear (Voco, Cuxhaven, Germany), 42 teeth.

Sealant Placement

Occlusal surfaces were etched with 35% phosphoric acid gel (Vococid etching liquid, Voco) for 60 s. The etchant was gently stirred on the occlusal surfaces using a soft microbrush. Teeth were then rinsed with water/air spray for 15 s and dried with an air syringe for 5 s. The sealant was applied directly onto the etched and dried surface with a round-ended applicator (BR 06/08, A. Deppeler SA, Rolle, Switzerland), taking care not to overfill the fissures and to avoid contact between the applicator and the enamel surfaces. The sealant was left undisturbed for 20 s in order to allow it to flow into the fissure system and over the etched surface, and it was then light-cured for 40 s (Optilux 400, 300 mW/cm^2, Demetron Research Corp.) [Celiberti and Lussi, 2007]. Photographs at a magnification of 6.25× were taken.

After sealing, the same examiners remeasured the teeth using the laser fluorescence devices as described above.

Thermocycling

The sealed teeth were thermocycled in deionized water for 1,000 cycles between 5 ± 2 and 55 ± 2°C, with a dwell time of 30 s. Photographs at a magnification of ×6.25 were taken again and the fluorescence measurements repeated.

Material Samples

In order to assess the intrinsic fluorescence of the materials, 2 disk-shaped samples of each material were light-cured for 60 s and polished to 0.5 or 1 mm of thickness. These specimens were placed on a piece of round glass 25-cm high and fluorescence measurements made with LF and the LFpen, after calibration on a ceramic standard. The samples were thermocycled as described above and measured again.

Etch-Only Experiment

Twelve permanent molars were selected from a pool of teeth to test whether the fluorescence values would change after sealing using the clear sealants without acid etching. The teeth were initially measured with both devices. Six teeth were etched for 60 s (as for the whole sample), measured, sealed using both clear sealants (3 teeth for each material) and measured again. The other 6 teeth were sealed using both materials, without previous acid etching, and measured by the same devices.

Statistical Analysis

The data from the 2 measurements performed by each examiner in each phase were combined and the average for the laser fluorescence methods (LF and LFpen) was obtained for each tooth. For each method, the median values obtained at baseline, after sealing and after thermocycling were compared by performing the Friedman test, because the data were not normally distributed. The first and third quartiles, as well as the minimum and maximum values, were calculated. Intraclass correlation was used to assess inter- and intraexaminer reproducibility [Lin, 1989]. Reproducibility indicates the closeness of the agreement between the results of measurements carried out under changed conditions of measurement. The significance level was set at $p < 0.05$.

Results

The fluorescence results are shown as box plots in figure 1. Generally, fluorescence increased after placing clear sealants and decreased after placing opaque sealants. For the Delton Clear group, there was no statistically significant change in either the LF or LFpen values after sealing, although values tended to increase. However, in this group the LF and LFpen values increased significantly after thermocycling. Fluorescence decreased significantly after sealing with the opaque sealants. In teeth sealed with the experimental nanofilled clear sealant, the LF values increased only after thermocycling, whereas the LFpen values increased both after sealing and thermocycling.

The intraclass correlation ranged from 0.87 to 0.96 for interexaminer reproducibility and from 0.82 to 0.94 for intraexaminer reproducibility (table 1).

The fluorescence values, at baseline and after thermocycling, of sealant samples 0.5 and 1.0 mm thick are shown in table 2.

Table 1. Intraclass correlations for intraexaminer and interexaminer reproducibility in the 3 phases

	Before sealing		After sealing		After thermocycling	
	inter	intra	inter	intra	inter	intra
LF	0.87	0.82	0.96	0.93	0.94	0.94
LFpen	0.92	0.87	0.94	0.90	0.94	0.92

Table 2. Fluorescence values of sealant samples with thicknesses of 0.5 and 1.0 mm

	Baseline				After thermocycling			
	0.5 mm		1.0 mm		0.5 mm		1.0 mm	
	LF	LFpen	LF	LFpen	LF	LFpen	LF	LFpen
Delton Clear	2	1	7	6	2	1	4	5
Delton Opaque	5	4	5	5	5	4	5	5
Helioseal Opaque	2	2	2	1	1	2	1	1
Experimental clear	2	1	1	1	1	1	1	1

In the etch-only experiment, both clear sealants showed practically the same results. After 60 s of etching, there was an average increase of 36 units for both devices. These teeth, after sealing, presented no changes on the fluorescence values. However, when the teeth were not etched, the values after sealing showed an average decrease of 3 units compared to the initial measurements.

Discussion

The LF and LFpen readings were significantly lower after sealing with both opaque sealants. Low LF values have also been reported after placement of opaque [Takamori et al., 2001; Hosoya et al., 2004; Krause et al., 2008] and glass ionomer sealants [Hosoya et al., 2004]. Some authors have suggested that the titanium dioxide used as a pigment in opaque resin composites, or even the residual polishing paste left on the surface [Hosoya et al., 2004; Deery et al., 2006; Krause et al., 2008], could absorb either the light emitted by the devices or the fluorescence emitted by the carious tissue. In this study, however, no polishing paste was used. All teeth were cleaned by air-polishing to remove calculus and plaque from the occlusal surfaces. In addition, Delton Opaque contains silicon di-

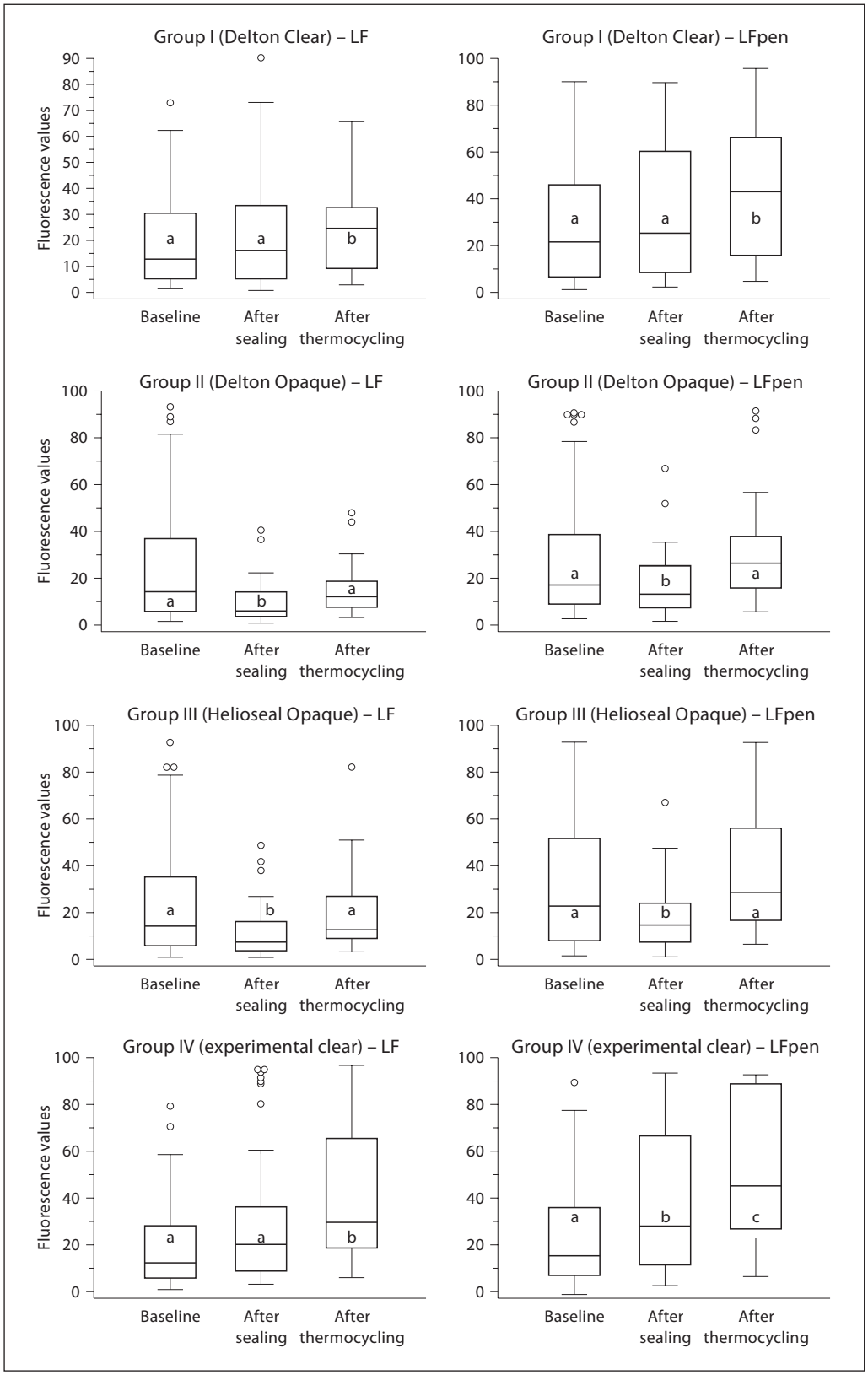

Fig. 1. Box plots for fluorescence values measured at baseline, after sealing and after thermocycling using the LF and LFpen devices for the different groups. The median, first and third quartiles, minimum and maximum values and outliers (open circles) are shown. The different lowercase letters show statistically significant differences (Friedman test $p < 0.05$).

oxide and titanium dioxide as opacifiers, and these are probably related to the higher intrinsic fluorescence of this material [Gostanian et al., 2006]. Takamori et al. [2001] attributed the lower LF values in opaque sealants to the fluorescence, absorption and scattering of irradiation and reflected beams, as these would be different from those in the clear sealants.

Although in the present investigation the LF and LFpen values tended to increase, no statistically significant difference was found after sealing using Delton Clear. These results disagree with those from Deery et al. [2006], who showed lower LF measurements after sealing teeth stored in 1% aqueous thymol solution using the same material. This difference could be due to the method of storage. However, Anttonen et al. [2003], in an in vivo study, and Krause et al. [2008] reported that clear sealants did not affect LF measurements. Our results suggest that both devices could be used as an adjunct for caries diagnosis, as already suggested by Deery et al. [2006] in relation to occlusal surfaces sealed with a clear sealant. The tendency of values to increase would not be clinically influential. Nevertheless, it should be kept in mind that placement of the Delton Clear sealant could increase fluorescence values.

Thermocycling was performed to simulate the daily ingestion of the hot and cold liquids and solids that are part of a normal diet, and the consequent thermal stressing between 2 materials with different coefficients of thermal expansion [Simmons et al., 1976]. After this procedure, the LF and LFpen values increased significantly for the Delton Clear and experimental nanofilled clear sealants. However, for the opaque sealants there was no difference from baseline for either device, although values tended to increase, possibly because of color changes in the sealant materials or loss of marginal integrity between sealant and enamel. Also, after thermocycling, some cracks on the materials were observed which could have allowed more infrared light to reach the occlusal surface. This could be the reason for the LF and LFpen values increasing after thermocycling of the teeth sealed with both clear materials.

Although some studies have shown that an unfilled sealant penetrates more deeply into fissures and is better retained [Rock et al., 1990; Simonsen, 2002], nanotechnology has allowed considerable improvement in the physical and mechanical characteristics of dental materials. The LFpen values increased after sealing with the experimental nanofilled clear sealant but there was no statistically significant difference in LF values, even though they tended to increase. These results agree with those of Krause et al. [2008], who found that this material did not influence the LF readings. Some differences between the studies should be pointed out, such as the etching time used (60 s) which modifies the scattering properties of the treated enamel and the translucency of the material which allowed the light to reach the etched surface. The results of the etch-only experiment confirm this statement. We suggest that this might be why values tended to increase after sealing. Besides, Krause et al. based their conclusions on a sample of 15 teeth (more than 1 site per tooth) stored in physiological saline solution, while our sample comprised teeth stored frozen by a method that does not change the red fluorescence significantly.

New methods of caries detection are required to present high validity and reproducibility for reliable results. The intraclass correlations for both intra- and interexaminer reproducibility for both LF and the LFpen were high, as previously shown by Kühnisch et al. [2007].

Gostanian et al. [2006] found that the intrinsic fluorescence of the sealants (clear and opaque) had a considerable effect on the LF readings, since the sealant's intrinsic fluorescence was not differentiated from the true caries fluorescence. This phenomenon could increase the possibility of false-positive results. In the present study, tests on sealant samples 0.5 or 1.0 mm thick showed that only the fluorescence readings of the Delton Clear samples increased in the thicker sample. There was no change in fluorescence values of either the opaque materials or the experimental nanofilled clear sealant. Consequently, the thickness of an opaque sealant does not play an important role in the fluorescence readings.

For clinical use, clear sealants seem to be the best option to allow caries detection, since they allow visual examination of the lesion. Nevertheless, clinical studies are necessary to evaluate the influence of pit and fissure sealants on occlusal caries detection.

This in vitro study showed that pit and fissure sealants influence fluorescence readings, values increasing or decreasing according to the material used. It can be concluded that both LF and LFpen could be useful as adjuncts to detect occlusal caries under clear unfilled sealant, although surfaces sealed with clear nanofilled material could only be assessed using the LF device.

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