DOI: 10.1111/j.1365-263X.2010.01076.x

INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

Effects of different fissure sealant applications on laser fluorescence measurements

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International Journal of Paediatric Dentistry 2011; 21: 29–34

Objective. The aim of this *in vitro* study was to evaluate the effects of using only phosphoric acid or a self-etch bonding agent under clear and opaque fissure sealants on laser fluorescence (LF) readings and the reproducibility of the laser device.

Methods. Eighty extracted permanent molars, ranged from sound to carious, were randomly divided into four groups: phosphoric acid + opaque sealant (group I), Clearfil S3 Bond (Kuraray, Kurashiki, Japan) + opaque sealant (group II), phosphoric acid + clear sealant (group III), and Clearfil S3 Bond + clear sealant (group IV). The teeth were measured using an LFpen device, before and after sealing. Data were analysed using the Spearman's correlation, Wilcoxon signed rank, and Mann–Whitney *U*-test.

Results. Except group IV, there was a statistically significant decrease in fluorescence after the application of sealants ($P < 0.05$). The decrease of LFpen readings in the opaque sealant groups was more significant than the clear sealant groups ($P < 0.05$). But for both sealants, the difference between phosphoric acid and Clearfil S3 Bond groups was nonsignificant ($P > 0.05$).

Conclusions. There was a statistically significant decrease in fluorescence for both clear and opaque sealant groups. However, clear sealant with Clearfil S3 Bond does not influence the LFpen readings.

Introduction

Tooth surfaces with pits and fissures have always been the earliest and most prevalent of carious areas therefore new methods of caries prevention focus on pit and fissure caries[1]. Pit and fissure sealants are recognized as effective caries-preventive agents[2] and for sealing questionable occlusal carious lesions as part of the preventive strategies used in the management of incipient caries[3]. If a sealant is placed over a lesion, for a long-term success of the sealant, the practitioner needs to monitor and assess any changes in the status of the sealed surface[4]. Also depending on the incorrect application of the sealant, leakage and microorganisms underneath sealants could lead to caries development[5]. Therefore, the long-term success of the sealant treatment is dependent upon regular follow-up[1]. Even on unsealed occlusal surfaces, lesions are difficult to detect[5], because recent changes in lesion morphology mean that occlusal dentinal caries can be present under a fissure that seems intact to the naked eye[6]. As visual inspection is difficult on sealed surfaces, practitioners need adjunct diagnostic methods that could quantitatively monitor the lesion's progression under the sealant[7]. Several methods have been developed and recommended as diagnostic aids to identify and quantify early caries lesions on occlusal surfaces[8]. Among the new methods, laser fluorescence (LF) has led more acceptable results, in many studies the DIAGNOdent device (Kavo, Biberach, Germany), which uses the LF method, has reported as a valuable tool for caries detection[6,7,9–12]. The more recent laser fluorescence pen (LFpen) device (DIAGNOdent pen) was introduced in 2005, which has been studied *in vivo* and *in vitro* for caries detection[10,13–15]. Only one previous study[5] has evaluated the performance of the LFpen in detecting caries under sealants. They have investigated the influence of different sealants on fluorescence readings using lasers (DIAGNOdent and DIAGNOdent pen devices).

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Since the introduction of the sealant technique, the standard clinical procedure has involved etching with phosphoric acid of cleaned enamel, rinsing with water spray, establishing of a dry field followed by application and curing of the sealant[16]. In the development of the acid-etch technique, isolation is a key to the success of the clinical sealant procedure[17]. In the last decade, self-etching adhesives have been introduced[18]. Some laboratory studies conclude that the use of self-etching adhesive systems may be a promising alternative to acid etching with phosphoric acid[17,19], as it eliminates a separate etching–rinsing–drying procedure[18–21]. Especially when treating paediatric patients, having less operative steps, reducing chair time and the need for compliance is particularly interesting[18,19]. Therefore, the present study included groups with a self-etch bonding agent under a sealant besides phosphoric acid groups.

Besides, no study has evaluated whether the influence of using only phosphoric acid or self-etch bonding agent under clear and opaque sealants affects the LFpen readings. Thus, the aims of this *in vitro* study were to evaluate the effects of using only phosphoric acid or self-etch bonding agent (Clearfil S3 Bond, Kuraray, Kurashiki, Japan) under clear and opaque fissure sealant applications on LF readings and to assess the reproducibility of the laser device. The null hypotheses of this study were: (i) clear and opaque sealants affect the LF measurements; and (ii) application of only phosphoric acid or self-etch bonding agent under sealants have no influence on the materials' fluorescence.

Material and methods

Eighty extracted permanent human molars (exposed to no water fluoridation) were selected for the study. One examiner, who also took part in the LF measurements, visually examined the teeth for caries. Their occlusal surfaces ranged from apparently sound ($n = 21$) to carious (37 had microcavities and 22 were noncavitated). Teeth with restorations, sealants, large cavitations, or hypoplasia were excluded. After cleaning the occlusal surfaces by a brush mounted in a slow-speed handpiece without pumice or polishing paste, the teeth were numbered (1–80). LF measurements were performed using DIAGNOdent pen 2190 with fissure probe being used according to the manufacturer's instructions. The tip of the DIAGNOdent pen probe was moved along the fissure, the whole fissure was scanned, and the highest (peak) value was recorded. Measurements were made before and after sealing. All assessments were independently carried out twice by two dentists, with a 1-week interval between the measurements. To avoid dehydration of the teeth, between all measurements the teeth were stored in distilled water. The teeth were randomly divided into the following sealing treatment groups (each group containing 20 teeth):

Group I: 37% phosphoric acid (Superetch; SDI, Victoria, Australia) + opaque, filled sealant (Fissurit FX; Voco, Cuxhaven, Germany).

Group II: self-etch bonding agent (Clearfil S3 Bond; Kuraray, Kurashiki, Japan) + opaque, filled sealant.

Group III: 37% phosphoric acid + clear sealant (Helioseal Clear; Vivadent, Schaan, Liechtenstein).

Group IV: self-etch bonding agent + clear sealant.

In groups I and III, occlusal surfaces were etched for 30 s, rinsed with water for 15 s, and dried for 5 s with an air syringe. Teeth were visually inspected to ensure a frosty appearance. In groups II and IV, the enamel was treated with Clearfil S3 Bond according to the manufacturer's instructions. Clearfil S3 Bond was applied on the enamel, then left in the place for 20 s, air dried for 5 s, and light-cured for 10 s with dental curing light (Radii Plus; SDI, Victoria, Australia). After enamel-conditioning procedures, sealants were applied using an explorer. Time for penetration of the sealants was 15 s, then sealants were light-cured for 20 s. After sealing, the same examiners remeasured the teeth using the DIAGNOdent pen device.

The data from the two measurements performed by each examiner in each phase were combined and the average for the LFpen measurement was obtained for each tooth.

The quality of the intra- and inter-examiner reproducibility was calculated using the pair-wise Spearman's correlation coefficient. Differences before and after sealing for each material were analysed by Wilcoxon signed rank test, and Mann–Whitney *U*-test was used for comparison between the phosphoric acid and self-etch bonding agent groups and the two kinds of sealant materials, clear, and opaque.

Results

Table 1 presents intra- and inter-examiner reproducibility values before and after sealing.

DIAGNOdent pen device showed good intra- and inter-examiner reproducibility according to the Spearman's correlation coefficient.

In all the groups except IV (self-etch bonding agent + clear sealant), mean LFpen readings showed significant decrease after sealing ($P < 0.05$, Table 2). In group IV, there is no statistically significant difference between before and after sealing ($P > 0.05$).

Comparison of the difference in the LFpen readings before and after sealing between the materials by the Mann–Whitney *U*-test showed that LFpen readings were affected more by opaque sealant than clear sealant. But there is no statistically significant difference between phosphoric acid (I + III) and self-etch bonding agent (II + IV) groups ($P > 0.05$).

Table 1. Intra- and inter-examiner reproducibility values before and after sealing (Spearman's correlation).

	Intra-examiner		Inter-examiner
	Examiner 1	Examiner 2	Examiner 1–2
Before sealing	0.791*	0.824*	0.847**
After sealing	0.725*	0.715*	0.846*

Correlation significance level: *0.01, **0.05.

Table 2. Mean laser fluorescence and *z*-values from the comparisons before and after sealing.

Groups	LFpen values before sealing	LFpen values after sealing	Wilcoxon signed rank *z*-values
I	34.2 ± 36.3	12.6 ± 19.7	−3.290*
II	41.5 ± 31.2	15.7 ± 15.8	−3.622*
III	47.4 ± 37.4	38 ± 34.8	−2.301*
IV	46.4 ± 35.9	41.5 ± 35.1	−0.568
I + II (opaque)	37.8 ± 33.6	14.2 ± 17.7	−4.923*
III + IV (clear)	46.9 ± 36.2	39.7 ± 34.5	−2.133*

Values represent mean ± SD.
*$P < 0.05$.

Discussion

General practitioners and paediatric dentists are reluctant to place sealants on teeth with incipient lesions, in spite of evidence-based studies, that show that it is safe to do so[22]. Chapko[23] have stated that the major reasons given by dentists for not sealing apparent lesions were: concern about failure or leakage; lack of confidence in the technique's success; and the determination that a restoration would be better[23]. However, there is evidence that an intact sealant can prevent the progression of caries[24,25].

As the presence of a sealant may interfere with occlusal caries diagnosis[26], using methods for diagnosis that could quantitatively monitor lesion's progression gives the clinician opportunity to ensure the long-term success of the sealant treatment. A laser fluorescence device (DIAGNOdent) has been developed to aid with occlusal caries diagnosis. This device contains a laser diode as the excitation light source and a photo-diode combined with a long-pass filter as the detector. The photo-detector measures the amount of fluorescent light and displays a value between 0 and 99. As the carious lesion progresses, the fluorescence increases. In the present study, we used the more recent laser fluorescence device, DIAGNOdent pen.

In the literature, there are limited studies about the influence of sealants on the LF measurements. In addition to the effects of sealants, taking into consideration the concern of practitioners about sealant application success, in the present study we included a self-etch bonding agent as an alternative to acid etching that does not require sensitivity as acid etching requires. Saliva and moisture contamination of etched enamel before sealant placement is the most common reason for sealant failure and loss[19]. As self-etching adhesive systems reduce the adhesive technique sensitivity by eliminating etching, rinsing, and drying steps, they are user-friendly

to dental community[19,21]. Although the benefits of adding a bonding agent layer between the etched enamel and the sealant have been demonstrated[17–19], in our study the main reason that we have chosen one-step self-etch adhesive is difficulties in isolation depending on poor cooperation when treating paediatric patients. This is the first study to investigate whether using only phosphoric acid or self-etch bonding agent (Clearfil S3 Bond) under clear and opaque fissure sealant applications affect the LFpen readings.

According to the results of this study, the LFpen readings were significantly lower after sealing with clear and opaque sealants (Table 2). After the placement of opaque sealants, low LF values have also been reported in other studies[5,7,25,27]. The opaque sealant used in this study, Fissurit FX, had a filler content of 55% w/w and contains titanium dioxide as an opacifying agent. In a previous study, authors have stated that the titanium dioxide may be the confounding factor[25], as opacity is the property of a material that prevents the passage of light[22].

Our results agree with Deery *et al.*[4] who showed lower measurement after sealing, using a clear sealant (Delton Clear Pit and Fissure Sealant; Dentsply, Konstanz, Germany). It has been suggested that this interference may be due to fluorescence, absorption, and scatter of the laser beam. Reduction of the DIAGNOdent signal through clear sealant discs has been reported in an *in vitro* study[22] where caries were simulated with protoporphyrin. Although Hosoya *et al.*[27] stated that residual polishing paste left on the tooth surface may influence the LF readings, in our study no polishing paste was used. Also in the present study, LFpen readings of all groups except IV (self-etch bonding agent + clear sealant) showed a significant decrease after sealing ($P < 0.05$). In group IV, there is also a reduction in the LFpen readings but it is not statistically significant ($P > 0.05$). According to these results, it could be an effect of acid etching[4], as the decrease level was higher in group III (phosphoric acid + clear sealant) than group IV.

Several studies have[5,25,28] reported that clear sealants did not affect LF measurements. Even, an increase in fluorescence readings after sealing with a clear sealant was reported in a previous study[7]. Differences between *in vitro* studies may be attributed to the differences in the design, such as teeth, storage medium, disease level at the examination sites, the materials used, etc.[7] In the present study, the teeth were stored in distilled water, whereas in previous studies samples were stored in different solutions such as 1% aqueous thymol solution[4], physiological saline solution[25], or frozen at −20°C[5]. Francescut *et al.*[29] examined whether different storage methods influence LF values and stated that storing solutions (chloramine, formalin, thymol solutions) have a significant influence on the fluorescence yield, whereas frozen teeth at −20°C showed nonsignificant difference in fluorescence[29].

These differences between the depths of caries in the selected teeth for the studies may be another factor affecting the results. According to the LFpen measurements, the teeth with dentin caries were 51% in our study. Deery *et al.*[4] have stated that 57% of the teeth had dentin caries in their study, which displayed a decrease in LF readings with the clear sealant. However in a recent study[7] in which an increase after sealing with clear sealant was reported, the rate of teeth with dentin caries was 10%.

In the present study, we used Helioseal Clear (Helioseal Clear; Vivadent, Schaan, Liechtenstein) as the clear sealant; other clear fissure sealants have also been tested: Delton Clear (Dentsply)[4,5,7,25], experimental nano-filled clear (Fissurit nano; Voco)[5,25], and Fissurit Clear (Voco)[25]. Considering the results of different studies, different compositions of the materials may have an influence on the LF readings[25].

Comparison of the difference in the LFpen readings before and after sealing between the materials showed that LFpen readings were affected more by opaque sealant than clear sealant (Table 2). Also the effect of group II (self-etch bonding agent + opaque sealant) was more pronounced than group IV (self-etch bonding agent + clear sealant), and the reason for the difference between these groups could be based on the opaque sealant. But there is no statistically significant differ-

ence between phosphoric acid (group I + III) and self-etch bonding agent (group II + IV) groups ($P > 0.05$). Besides when groups are individually reviewed, no statistically significant difference was found between groups I and II, and groups III and IV. According to these findings, using only phosphoric acid or self-etch bonding agent under clear and opaque fissure sealants did not affect LFpen readings. The reason for no significant difference between groups III and IV, could be the level of decrease was low in the clear sealant group, so LF readings could be more reliable for caries detection under clear sealants as suggested in other studies[4,5,25,28].

As visual inspection for caries detection is a subjective method, new methods of caries diagnosis are required to provide some objectivity, therefore a high reproducibility would be necessary[4,5]. In the present study, DIAGNOdent pen device showed good intra- and inter-examiner reproducibility according to the Spearman's correlation coefficient (Table 1).

This *in vitro* study showed that DIAGNOdent pen readings, through clear and opaque sealant materials, are influenced by both materials. The effect of the opaque material was more pronounced. It can be concluded that both hypotheses could be approved and LFpen could be a useful adjunct to detect occlusal caries under a clear sealant with a self-etch bonding agent. However, further *in vivo* and *in vitro* studies are necessary to evaluate the effect of phosphoric acid, self-etch bonding agent, or phosphoric acid with self-etch bonding agent, which was not tested in our study, under sealants on LF measurements.

What this paper adds

- This paper provides a perspective on the influence of different fissure sealant applications on the laser fluorescence measurements. Using only phosphoric acid or self-etch bonding agent under clear and opaque fissure sealants have no affect on LFpen readings.
- The paper also presents the efficiency of DIAGNOdent pen device in detecting caries under clear sealant with the application of self-etch bonding agent.

Why this paper is important to paediatric dentists

- The paper informs significant decrease in fluorescence for both clear and opaque sealant groups.

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