An In Vitro Evaluation of the Effect of Sealant Characteristics on Laser Fluorescence for Caries Detection

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Abstract

Purpose: The purposes of this study were to: (1) evaluate the ability of a laser fluorescence (LF) unit to detect simulated caries under pit and fissure sealants; (2) determine the effect of an opacifying agent in sealants on LF values; and (3) determine interexaminer reproducibility values of the unit in a highly controlled, laboratory setting. Sealant characteristics specifically considered were: (1) filler content; (2) opacity; and (3) intrinsic fluorescence.

Methods: Three sealants were used in this study: 2 unfilled and 1 filled. To evaluate the effect of an opacifying agent, titanium dioxide powder was added to both filled and unfilled sealants. 0.5-mm thick sealant discs were prepared for all samples. The sealant discs were individually placed on top of 3 wells filled with varying amounts of protoporphyrin IX, a fluorescent material that mimicked dental caries. A total of 270 readings were made through the different sealant discs to evaluate signal attenuation of the laser fluorescence unit.

Results: Clear sealants, without an added opacifying agent, attenuated LF readings. At baseline protoporphyrin IX levels yielding DIAGNOdent readouts of 20 and 60, there was a significant difference in the LF readings between the baseline protoporphyrin (uncovered) and with sealant disc covered in all 3 sealant types ($P<.001$). Furthermore, the filled sealant attenuated LF signals significantly more than the unfilled sealant ($P<.001$). Sealants with titanium dioxide added had variable levels of intrinsic fluorescence. Titanium dioxide added to the sealants also had a profound effect on fluorescence transmission of the underlying simulated caries. As the concentration of titanium dioxide approached 0.5%, the fluorescence signal was almost fully attenuated.

Conclusion: Clinical detection of caries under dental sealants with the use of laser fluorescence units is unreliable and not recommended due to a high likelihood of inaccurate readings caused by: (1) intrinsic fluorescence of sealant material; and (2) attenuation of fluorescence signals by the sealant. (Pediatr Dent 2006;28:445-450)

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For children who receive regular dental care, sealants are considered and applied to newly erupted teeth. Dentists evaluate and diagnose the extent of caries on a given tooth surface and decide the best course of treatment for the tooth. Treatment options include: (1) observation; (2) sealant placement; (3) enameloplasty followed by sealant placement; (4) preventive resin restoration; or (5) traditional restoration.

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Studies have shown that properly sealed incipient carious lesions have ceased in progression and arrested over time.[1,2] It has also been reported that sealant treatment of carious lesions yielded bacterial cultures that were predominantly negative, with an 89% reversal from a caries active to caries inactive state.[3] Others have specifically recommended targeting teeth with incipient caries for sealants due to the fact that initially sound tooth surfaces were unlikely to become decayed in 5 years and perhaps did not benefit greatly from the application of sealants.[4]

Despite evidence-based studies, general practitioners and pediatric dentists are reluctant to place sealants on teeth with incipient lesions. Chapko found that 82% of dentists who offer sealants in their practices did not use sealants on "incipient" or "superficial" lesions.[5] The major reasons given for not sealing apparent lesions were: (1) concern about

failure or leakage; (2) lack of confidence in the technique's success; and (3) the determination that a restoration would be better.[5] In 2001, however, Primosch and Barr reported less reluctance among pediatric dentists to seal questionable carious surfaces and incipient caries.[6] Practitioners may be more receptive to the use of a sealant on borderline incipient occlusal caries if they had the ability to quantitatively monitor the lesion's progression under the sealant.

In clinical dentistry, dental practitioners have sought to find accurate techniques to diagnose caries in their patients. Traditional methods of diagnosing caries have included: (1) tactile examination; (2) visual examination; (3) visual examination with magnification; (4) transillumination; and (5) radiographic assessment. In 1998, a laser fluorescence (LF) unit (DIAGNOdent, KaVo America, Lake Zurich, Ill) for the detection of dental caries was introduced and used with some success as an adjunct to conventional methods of caries detection.[7,8] This low-power diode laser unit operates at 100 mW, producing energy in the visible spectrum (400-700 nm wavelength).[9] The main advantage of this laser system has been a high sensitivity for caries detection.[9]

There are limited studies in the literature to show the LF's ability to detect caries under fissure sealants. Concerns have been expressed that active caries under sealants may potentially progress, leading to undesirable outcomes such as: (1) unnecessary loss of tooth structure; (2) larger restorations; and (3) pulpal proximity issues.[5] Furthermore, there is no published research specifically addressing the role of sealant opacifying agent and filler content in attenuation of LF signals.

One of the most commonly used opacifying agents added to dental sealants is titanium dioxide (TiO_2), ranging from 0% up to 3% by volume. This pigment is added to some dental sealants to aid in the placement and subsequent visualization of sealants by dental practitioners. When incorporated in dental sealants, the effect of the TiO_2 pigment on LF signals is unknown.

Filler materials, usually consisting of quartz and silica particles, are also added to some resin sealants. The amounts of added filler vary considerably depending on the manufacturer, but generally range from 0% up to 60% by weight. The role of the filler within the sealant resin matrix is to improve properties such as: (1) compressive strength; (2) modulus of elasticity; (3) hardness; (4) bond strength; and (5) resistance to abrasion and wear.[10] The effect of filler on LF signals, when incorporated in dental sealants, is also unknown.

Protoporphyrin IX (Sigma-Aldrich, Saint Louis, Mo) has been proposed as a chromophore present in carious lesions—which, at specific excitation wavelengths, will fluoresce when stimulated by a LF system—thus allowing practitioners to detect carious tooth structure.[11-13] Synthetic protoporphyrin IX has also been previously used in experimental models to simulate dental caries.[11-13]

The purposes of this in vitro study were to:

1. evaluate the ability of a LF unit to detect simulated caries under pit and fissure sealants;
2. determine the effect of opacifying agent in sealants on LF reading values; and
3. determine interexaminer reproducibility of LF reading values of the DIAGNOdent unit in a highly controlled, laboratory setting.

Methods

Clear pit and fissure sealants were obtained from 3 different manufacturers for this study's purposes. Sealants were selected based on: (1) the amount of filler material contained within the filled or unfilled sealant; and (2) lack of TiO_2 as an opacifying agent. Clear sealant 1 (unfilled), clear sealant 2 (unfilled), and clear sealant 3 (filled 58%) were obtained from Delton DDS Clear (Dentsply International, York, Pa), Helioseal Clear (Ivoclar Vivadent, Liechtenstein, Germany), and Ultraseal XTplus Clear (Ultradent Products, Inc, South Jordan, Utah), respectively.

TiO_2 is commonly used as opacifying agent in dental commercial sealants. To investigate the effect of TiO_2 in attenuating LF, TiO_2 was added by weight to each of the 3 uncured sealants at 10%, 1%, 0.5% 0.25%, 0.1%, and 0%. Care was taken to protect the uncured sealant solutions by wrapping the containers with aluminum foil so that the sealant was not exposed to any ambient light during processing. Sealant discs were subsequently prepared by placing a standard volume of the sealant solutions into uniform stainless steel washers measuring 0.5 mm thick with a hole diameter of 3.5 mm. Washers were placed on top of glass microscope slides. Sealant solutions were vortexed to ensure a homogeneous mixture and then flowed into the washers. Glass microscope slide covers were then placed on top of the washers to preserve the desired sealant disc thickness. Five discs were prepared for each of the 6 dilution levels.

A 0.5-mm sealant disc thickness was used to mimic the average sealant thickness when applied in a clinical setting. This was based on a study of 240 teeth sealed by 12 dental hygienists.[14] Sealant disc thickness was rounded to 0.5 mm+0.05 mm due to experimental limitations.

Care was taken to avoid entrapping air voids within the sealant. The sealant was cured with a conventional halogen light source from both the top and bottom for 20 seconds. The cured discs were then: (1) removed with finger pressure; (2) examined for uniformity; and (3) measured with a Boley gauge to ensure proper thickness. Once all discs were prepared, rough edges were polished with an 8-inch emery polishing wheel mounted on a variable speed grinder/polisher with water irrigation (Ecomet 4 polisher, Buehler, Lake Bluff, Ill). Discs were stored in vials at room temperature and protected from ambient and artificial lighting until used.

A clear thermoplastic acrylic polymer rod was cut with a thin-bladed handsaw to yield 3 round blocks measuring 12 mm thick and 25 mm in diameter. This block of acrylic polymer was selected to house the protoporphyrin IX because it is nonporous and has negligible fluorescent properties. The cylinder surfaces were polished with the emery polishing wheel, mounted on the polisher, using

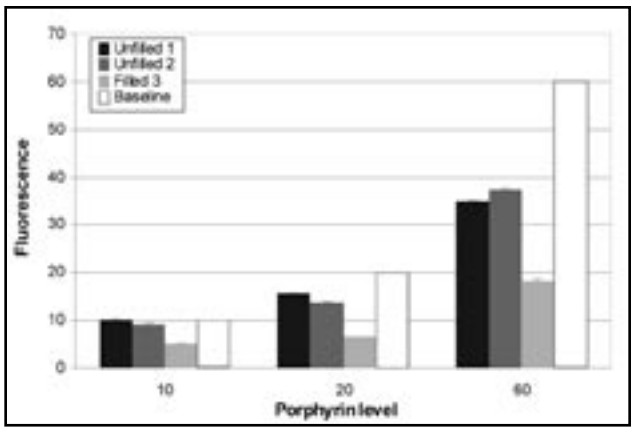

Figure 1. Protoporphyrin IX fluorescence values covered by 0% opacified sealant discs. The figure shows the laser fluorescence readings across the 3 levels of protoporphyrin IX (10, 20, 60), covered by an unopacified sealant disc. Note the significant attenuation of laser fluorescence readings of the "Filled 3" sealant discs at all the protoporphyrin IX levels relative to the baseline readings. Also note the significant attenuation of laser fluorescence for "unfilled 1" and "unfilled 2" at the 20 and 60 protoporphyrin IX levels relative to the baseline readings.

water irrigation (Ecomet 4 polisher, Buehler, Lake Bluff, Ill). A drill press-mounted drill bit was used to prepare standardized wells measuring 2 mm in depth and 2.5 mm in diameter in each of the acrylic blocks to accommodate the protoporphyrin IX powder.

Protoporphyrin IX disodium salt was used in this study, as it has been demonstrated to be the fluorescing chromophore naturally found in carious lesions. The material was stored per the manufacturer's recommendations, and the determined amounts were dispensed as needed into the acrylic blocks' wells. The powder was weighed by a gram scale (Mettler AE 240, Mettler Instruments Corp. Hightstown, NJ) to the nearest 10,000th of a gram, eliciting the specific readings from the LF unit within the treatment categories described in the manufacturer operating instructions.

The LF unit was used according to manufacturer specifications. The unit was calibrated with the calibration disc provided by the manufacturer, and a 0 baseline was established prior to each set of readings. For each measurement, the probe tip was placed at the sealant disc's center and swept around in a circular fashion for 2 revolutions to ensure that all readings were standardized and uniform. Only the Peak readings were recorded for data points, with the operator being blinded to the reading panel during the measurement period to avoid experimental bias.

The wells in the acrylic blocks were incrementally filled with 0.3 mg, 0.5 mg, and 1.2 mg of protoporphyrin IX powder to yield 3 baseline DIAGNOdent readout values of 10, 20, and 60, respectively.

Next, each of the 5 sealant discs—with the varying levels of opacifying agent—were measured for intrinsic fluorescence. This was accomplished by holding the discs individually with cotton forceps and placing the LF unit tip against the discs. Peak intrinsic fluorescence values were then recorded. Each sealant disc was then placed on top of the protoporphyrin IX-filled well and a second reading was made.

Attenuation of the laser signal was determined by subtracting the fluorescence of the sealant disc covered protoporphyrin IX wells from the baseline value. This was carried out for each of the 3 sealant types. A total of 270 measurements were made of the sealant discs.

Statistical analysis

The data were collected and analyzed using SPSS (SPSS Inc, Chicago, Ill). A 3-way split-plot analysis of variance (ANOVA) of the data, with 1 grouping factor (material, 3 levels) and 2 repeated factors (protoporphyrin IX level and TiO_2 level), was conducted. The results of the analysis were evaluated for presence of main effects and/or interactions, with a value of $P<.05$.

Interexaminer reproducibility for the LF unit was also analyzed. Eighteen protoporphyrin IX and 18 intrinsic fluorescence disc readings were made by the principal investigator and repeated 1 week later, within the same laboratory setting, by a second examiner using the same set of instructions. The second examiner had no previous experience with the unit before being given the instructions by the principal investigator. The data were recorded and analyzed with a paired sample *t* test and Pearson correlation coefficient using SPSS.

Results

Preliminary analyses demonstrated reproducibility of the LF readings. Pearson correlation coefficient relating interexaminer reliability exceeded 0.99, and the absolute difference between paired observations averaged less than 0.2 (±1.1 SD), which was very close to the theoretical minimum of 0, especially considering that the LF unit does not provide readings less than whole units.

Furthermore, results demonstrated that all 3 sealants interfered with LF signals (Figure 1). Signal attenuation for unfilled sealants was 0%, 25%, and 40% for protoporphyrin levels of 10, 20, and 60, respectively. Signal attenuation for the filled sealant was 50%, 70%, and 70% for protoporphyrin levels of 10, 20, and 60 respectively. ANOVA revealed an interaction of group and protoporphyrin IX level ($F[4,24]=16.6$; $P<.001$). Post-hoc *t* tests showed that, for the protoporphyrin IX level of 10, there was only a significant difference due to the filled sealant, where the average reading was 5.0 (±0.7). For the protoporphyrin IX level of 20, both unfilled sealants provided average readings significantly less than the baseline 20, and the filled sealant provided reading significantly less than either of the unfilled sealants. For the protoporphyrin IX reading of 60, both unfilled sealant provided readings significantly less than the baseline 60, while the filled sealant provided mean readings significantly less than either unfilled sealant.

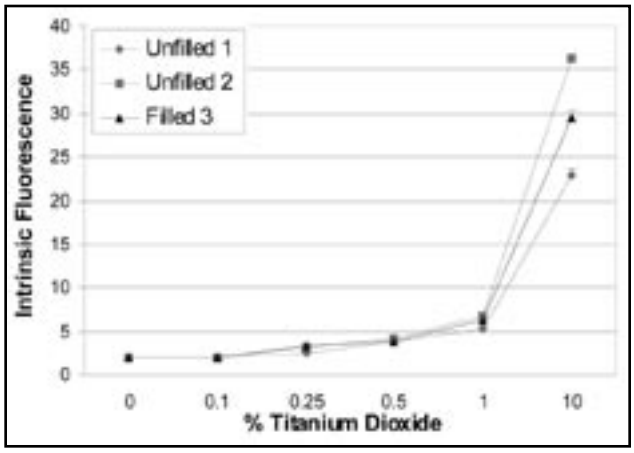

Figure 2. The effect of opacifying agent on intrinsic fluorescence of discs. This figure shows a direct correlation between percentage of titanium dioxide contained within the sealant discs and intrinsic fluorescence of the discs. As titanium dioxide increased, so did the intrinsic fluorescence.

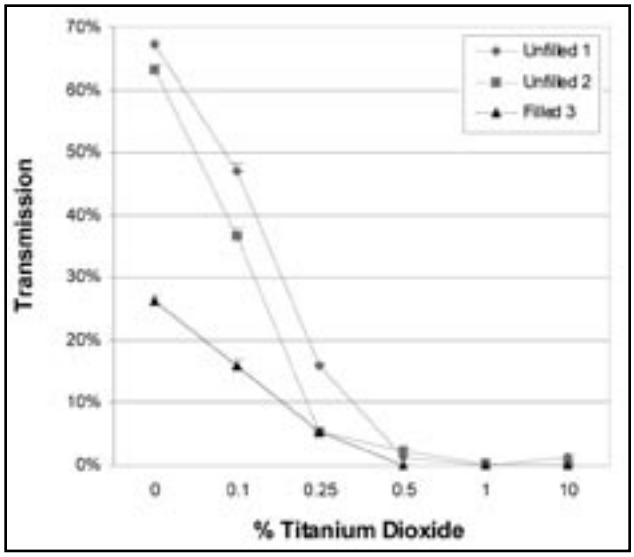

Figure 3. True net percentage transmission through sealant discs with intrinsic fluorescence removed. This figure shows that there is a notable reduction of readable fluorescence through the sealant discs for all three sealant types. Additionally, as indicated by the steepness of the slopes, the unit's ability to read through sealants as titanium dioxide levels increase becomes greatly reduced. Above 0.5% titanium dioxide, there is complete attenuation of fluorescence readings through all 3 sealant disc varieties.

The intrinsic fluorescence values from the sealant discs alone, without protoporphyrin IX, are shown in Figure 2. Relevant levels of intrinsic fluorescence (<5), even for completely unopacified discs, and a gradual increase in fluorescence were observed up to TiO_2 concentrations of 0.5%. A marked increase in intrinsic fluorescence due to TiO_2 was observed above 0.5%. The relationship of TiO_2 concentration and intrinsic fluorescence showed a direct correlation. As the percentage of TiO_2 increased, so did the intrinsic fluorescence of the sealant discs. ANOVA revealed an interaction effect of group by TiO_2 concentration on fluorescence ($F_{[8,20]}=7.4$; $P<.001$). Post-hoc *t* tests showed a significant difference between all pairs of sealants at TiO_2 concentrations of 1% and 10%. Thus, sealant discs containing TiO_2 and unopacified sealant discs have variable levels of intrinsic fluorescence.

To determine a true or net fluorescence, which removes the intrinsic fluorescence factor from the fluorescence readings, the following formula was used:

$$\text{Net fluorescence}=\frac{(\text{raw fluorescence reading-intrinsic fluorescence})}{\text{baseline fluorescence}}\times 100$$

where:
raw fluorescence=fluorescence reading of protoporphyrin IX filled well covered by a given sealant disc;
intrinsic fluorescence=fluorescence of a given disc by itself
baseline fluorescence=fluorescence of a protoporphyrin IX filled well

Figure 3 shows the net transmission data, expressed as a percent of the baseline. The effect of increasing concentrations of TiO_2 on transmission of protoporphyrin IX fluorescent signal transmission through the 3 sealant disc groups can be seen. The relationship of TiO_2 concentration and percent transmission was inversely correlated; as the concentration of TiO_2 increased, the percentage of transmission decreased. As the percentage of TiO_2 approached 0.5%, the fluorescence signal was almost completely attenuated. ANOVA revealed an interaction between material and TiO_2 concentration on net transmission ($F_{[10,18]}=53.6$; $P<.001$). Post-hoc *t* tests showed significant differences between materials at each TiO_2 concentration from 0% to 0.5%. There were no differences at 0.5 % or greater concentrations, as the signal was negligible or nonexistent in all materials. Thus, TiO_2 may be seen to further reduce transmission of a fluorescence signal through sealant discs, over and above that attributable to the unopacified sealant material.

Discussion

Pearson correlations for interexaminer reliability exceeded 0.99 in this study. This is compared to 0.84 obtained by Lussi et al.[15] Results of a 1999 study by Lussi et al were based on caries detection measurements made on extracted molars by 11 different raters.[15] The results of the present study were based on measurements of simulated caries within acrylic blocks in a highly controlled laboratory setting. The different conditions in which the data was collected in the 2 studies might account for the differing results.

There has been limited previous information available in the literature on the ability of LF units to reliably detect caries under dental sealants. In this study, the efficacy of the LF unit to accurately read through 3 sealant varieties was evaluated; 2 of the sealants were unfilled, and 1 was 58% filled by weight. In all 3 cases, at a 0.5 mm sealant thickness the sealant discs attenuated LF signals to some degree. As the level of protoporphyrin IX was increased from 10 to 20 to 60, the amount of transmission through

all 3 sealant discs was reduced. At the 10 level of protoporphyrin IX, both unfilled sealants maintained high levels of fluorescence transmission similar to the baseline level. At the other 2 protoporphyrin IX levels (20 and 60), however, there was a marked amount of signal attenuation regardless of sealant type.

These findings were consistent with a previous study by Takamori et al,[16] who evaluated the ability of the DIAGNOdent unit to detect florescence under clear, red, and white sealants. They found that diagnosis of caries under sealants was possible up to a level of 10 on the LF unit for all 3 sealant types. In teeth registering higher fluorescence values (greater than 10), however, they found that this was no longer the case. Under "white" sealants, they found that caries was properly diagnosed only 54% of the time. In the present study, the percentage of proper diagnosis was not directly measured. Rather, the amount of transmission through the sealants was evaluated. Furthermore, sealants were not categorized based on color, as in the Takamori study, but on levels of filler and TiO_2.

Regarding filler, the 58% filled sealant consistently "filtered out" approximately 50% more of the fluorescence signals at all protoporphyrin IX levels compared to the unfilled sealants. This finding suggests that filler particles within sealant discs serve as scattering centers within the resin matrix. The scattering effect is dependent on the size, shape, and number of scattering centers.[17] The scattering effect would influence: (1) exciting radiation carried by the excitation fiber; and (2) returning fluorescence carried via detection fibers. Since other components are incorporated in filled sealant and were not controlled for in this study, it could not be definitively concluded that the filtering of fluorescent signals seen in filled sealants was completely due to filler particles.

Another factor observed in this study was that of the intrinsic fluorescence of dental sealants. The authors are not aware of any other studies that have specifically addressed the impact of intrinsic fluorescence on LF readings. In this study, each sealant disc was measured for intrinsic fluorescence prior to placement over the protoporphyrin IX-filled wells. The objective was to determine the amount of fluorescence value attributable to the sealant and to the underlying protoporphyrin IX (ie, simulated caries). The sealant discs with varying amounts of opacifying agent further demonstrated that TiO_2 had an effect on intrinsic fluorescence. As TiO_2 levels increased, the levels of intrinsic fluorescence increased. It was also noted that the sealant with 58% filler demonstrated approximately the same intrinsic fluorescence values as the unfilled sealants. This suggests that filled sealant material does not impart an additional intrinsic fluorescence effect compared to unfilled materials.

In previous studies, the effects of intrinsic fluorescence were not taken into consideration. The present study, however, demonstrates that it has a profound effect on LF readings. This is clinically significant; if an LF unit is used to detect caries under a sealant, the sealant's intrinsic fluorescence will register on the unit, as well as the true caries fluorescence under the sealant, but the 2 cannot be differentiated.

Opacity is the property of a material that prevents the passage of light. It should be noted that TiO_2 is a commercially used opacifying agent which, when serially added to sealants, makes them whiter in color. Additionally, increasing TiO_2 levels results in a distinct reduction of signal transmission with all 3 sealants tested. For all 3 sealants, a TiO_2 concentration of 0.5% and greater resulted in no net transmission of the LF signal at all. The readings registered by the unit were purely intrinsic fluorescence of the sealant discs and not of the underlying protoporphyrin IX. These findings indicate that TiO_2 particles act as scattering centers equivalent to the effects seen in the filled sealant materials. This scattering phenomenon again affects both the laser energy directed toward the fluorescent substance as well as the emitted fluorescence returning to the unit. The effect of TiO_2, however, was controlled in this study and demonstrated a dose-response relationship.

In assessing the ability of LF units to allow signal transmission through sealants, the outcome is not straightforward. The present study was conducted under optimal conditions with one examiner and consistently measured levels of simulated caries provided with the use of protoporphyrin IX powder. Based on this study's results, the "best-case" scenario is with unfilled 1 sealant with no added opacifying agent. Even in this case, however, the outcome is poor—with the amount of true signal transmission equal to 68% after removing intrinsic fluorescence. With other factors included, such as higher baseline protoporphyrin IX levels, increasing amounts of filler, increasing amounts of TiO_2, and increasing thickness, the situation only worsens.

Previous studies on this specific topic have concluded that, "fissure sealants on permanent molars do not affect DIAGNOdent values"[16] and "laser diagnosis system (DIAGNOdent) makes it easy to detect the existence of caries under pit and fissure sealant during routine check-ups."[7] Both previous studies were conducted on human teeth and not with simulated caries, as in the case of the present study. The benefit of the laboratory condition utilized in the present study was that it enabled a determination of intrinsic fluorescence values. This is not possible once a sealant is bonded to a tooth, as a bonded sealant cannot be removed and assessed for intrinsic fluorescence. Both the Anttonen et al[7] and Takamori et al[16] studies have supported the use of LF in caries detection under dental sealants. In contrast, the present study's results identified inherent problems with this practice. The possibility of false positives—due to intrinsic fluorescence of sealant material as well as false negatives due to scattering centers caused by filler and TiO_2—could account for the differences in outcomes. Additionally, there may be other components in sealant materials that may further affect LF readings.

Based on this study's outcomes, the use of LF units to detect fluorescence under dental sealants is unpredictable and requires caution due to a high likelihood of inaccurate readings. This study provides only preliminary data for clinicians and does not relate directly to clinical practice. Similar studies should be conducted with other methodologies such as using extracted human teeth with pre-existing caries lesions covered using commercially available sealants.

Conclusions

Based on this in vitro study's results, the following conclusions can be made:

1. Clear and opaque dental sealants demonstrate levels of intrinsic fluorescence and attenuate laser fluorescence signals.
2. Clinical detection of caries under dental sealants with the use of laser fluorescence units is not advised due to a high likelihood of inaccurate readings.

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