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In vitro performance of a pen-type laser fluorescence device and bitewing radiographs for approximal caries detection in permanent and primary teeth

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Abstract

Aim: To evaluate the performance of a pen-type laser fluorescence device (DIAGNOdent 2190; LFpen, KaVo, Germany) and bitewing radiographs (BW) for approximal caries detection in permanent and primary teeth. **Materials and Methods:** A total of 246 anterior approximal surfaces (102 permanent and 144 primary) were selected. Contact points were simulated using sound teeth. Two examiners assessed all approximal surfaces using LFpen and BW. The teeth were histologically assessed for the reference standard. Optimal cut-off limits were calculated for LFpen for primary and permanent teeth. Sensitivity, specificity, accuracy and area under the receiver operating characteristic curve (Az) were calculated for D1 (enamel and dentin lesions) and D3 (dentin lesions) thresholds. The reproducibility was assessed by intraclass correlation coefficient (ICC) and Cohen«SQ»s weighted kappa values. **Results:** For permanent teeth, the LFpen cut-off were 0– 27 (sound), 28– 33 (enamel caries) and >33 (dentin caries). For primary teeth, the LFpen cut-off were 0– 7 (sound), 8– 32 (enamelcaries) and >32 (dentin caries). The LFpen presented higher sensitivity values than BW for primary teeth (0.58 vs. 0.32 at D1 and 0.80 vs. 0.47 at D3) and permanent teeth (0.80 vs. 0.57 at D1 and 0.94 vs. 0.51 at D3). Specificity did not show a significant difference between the methods. Rank correlations with histology were 0.59 and 0.83 (LFpen) and 0.36 and 0.70 (BW) for primary and permanent teeth, respectively, considering all lesions. ICC values for LFpen were 0.71 (inter) and 0.86 (intra) for permanent teeth and 0.94 (inter) and 0.90/0.99 for primary teeth. Kappa values for BW were 0.69 (inter) and 0.68/0.90 (intra) for permanent teeth and 0.64 (inter) and 0.89/0.89 for primary teeth. **Conclusion:** LFpen presented better reproducibility for primary and permanent teeth and higher accuracy in detecting caries lesions at D1 threshold than BW for permanent teeth. LFpen should be used as an adjunct method for approximal caries detection.

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Full Text

Despite the decline in caries prevalence over the last few decades, [1],[2] dental caries is still a problem. Moreover, changes in the progression and pattern of caries lesions have led to a greater difficulty in their early detection, especially on approximal surfaces. [3]

In clinical practice, conventional methods such as visual and radiographic examination are the most common methods applied for detecting approximal caries lesions. The visual examination is an easy method and could estimate the roughness of the caries lesion. [4],[5] However, it is not quantitative and is subjective as well as examiner-dependent, which leads to a low reproducibility. [5],[6],[7] Moreover, there is agreement in the literature that visual and radiographic examinations demonstrate high specificity but lower sensitivity. [5],[8],[9]

Radiography may underestimate the lesion depth and is unable to detect the initial enamel demineralization process, in addition to the disadvantage of exposing the patient to the hazard of ionizing radiation. [5],[8],[9],[10] Furthermore, conventional methods perform better at detecting advanced caries lesions than noncavitated lesions. [5],[6],[11]

Approximal noncavitated carious lesions are difficult to detect directly by visual exam, due to the contact area. Three-dimensional reconstruction of caries lesions in the primary dentition found that 75% of the lesions were in the contact area, and 25% were beneath, obscuring visual detection. [6],[12] Therefore, tooth separation using orthodontic rubber rings may be used in cases of clinical signs of lesion to indicate an operative treatment on approximal surfaces. This procedure may cause children discomfort, as well as bitewing radiographs (BW) and laser fluorescence methods. [13],[14] The early detection of noncavitated caries lesions provides proper management of caries lesions and allows their monitoring over time. [15],[16]

Adjunct methods have been investigated, offering accurate detection, quantification and monitoring of caries lesions. [5],[7],[16],[17] One quantitative method for occlusal and approximal caries detection is a pen-type laser fluorescence device (LFpen, DIAGNOdent 2190, KaVo, Biberach, Germany). It is based on the same principle of the previous device, the DIAGNOdent (KaVo) in which carious tissue fluoresces differently than sound tissue when excited by light at a specific wavelength. [18] The LFpen uses a laser diode that emits red light at 655 nm and a photodetector that quantifies the reflected fluorescence from bacterial metabolites (fluorophores) in carious lesions, showing values varying from 0 to 99. [7],[17],[19] The LFpen has a probe tip of solid single sapphire fiber with a prismatic shape that is rotatable around its long axis and has a small diameter, which permits its application on approximal surfaces where the light has been deflected laterally. [17]

Lussi et al. [7] demonstrated in an in vitro study that the LFpen device showed higher values of validity and reproducibility than radiography in detecting enamel and dentin approximal caries lesions in permanent teeth. However, Braga et al. [5] and Novaes et al. [8] observed in in vivo and in vitro studies that LFpen performed similarly to the radiographic examination in primary teeth. Moreover, Celiberti et al. [20] found in an in vitro study that LFpen readings presented low correlation with approximal caries depth and low reproducibility in primary teeth.

Considering the controversial results in the literature about the performance of LFpen in detecting approximal caries lesions, as well as the differences presented between primary and permanent teeth such as anatomical and compositional characteristics, [12],[21] the aim of this in vitro study was to compare the performance of the LFpen device and BW in detecting approximal caries lesions in primary and permanent teeth.

MATERIALS AND METHODS

This study was conducted in accordance with the declaration of Helsinki, and it was approved by the Local Ethics Committee in Research (#58/08). Prior to extraction, the patients were informed about the use of their teeth for research purposes, and their consent was obtained. The teeth were extracted by dental practitioners in Araraquara, Brazil (0.7 mg/L fluoride in

the water supply).

Sample preparation

A total of 51 permanent (102 surfaces) and 72 primary (144 surfaces) upper incisors with approximal surfaces varying from sound to having different stages of carious lesions were selected and kept frozen at −20°C in small containers. Exclusion criteria for approximal surfaces were the presence of sealants, restorations, hypoplasia, hypomineralization and frank approximal cavitation extending to facial or buccal surfaces, to avoid some interference on the approximal readings. A wet cotton roll was placed at the bottom of each container to guarantee 100% humidity, with no contact between the tooth and the water. This storage method has not been associated with any significant change in the fluorescence signal. [22]

Sample size determination was based on the study of Chawla et al., [6] who evaluated approximal surfaces in primary teeth using LFpen and digital radiography. The authors found an average of the area under the receiver operating characteristic (ROC) curve of 0.61 ± 0.02 (LFpen) and 0.68 ± 0.02 (digital radiography). A conservative assumption of specificity and sensitivity of a detection test of 75% was chosen.

After defrosting for 3 h, the teeth were thoroughly cleaned for 10 s using tap water and a toothbrush. The teeth were additionally cleaned with sodium bicarbonate powder and the water-powder blasting device Profi III Bios® (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) for 10 s. [23] This procedure does not affect the fluorescence. [22] The teeth were rinsed with a 3-in-1 syringe for 10 s to remove any remnants. [23],[24]

In order to simulate proximal contact areas of the maxillary anterior teeth and to standardize the tooth position during the examination, each test tooth was placed between two sound upper incisors with a fixed position, making an anterior three-tooth group within an arch model. These arch models were constituted by primary or by permanent teeth. The teeth were fixed with a condensation silicone (Zetaplus, Zhermack SpA, Badia Polesine, Italy). Care was taken to simulate tooth contacts as best as possible, using dental floss to confirm. The evaluations were performed only on the approximal surfaces of the test teeth. The surfaces of the fixed teeth were not evaluated. [5]

Examinations

A total of 246 selected approximal surfaces were independently assessed twice with a 1-week interval by two experienced examiners (J.F.S. and T.B.) by radiographic and LFpen examinations in a random order. The examiners were dentists with <5 years of clinical practice, who have experience in using the different methods and participated of other published study. [15]

Radiographic examination

Standardized BW were taken for each test tooth in contact with fixed teeth using an X-ray machine (Spectro 60X, Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) and Kodak insight films (22 mm × 35 mm, Eastman Kodak, Rochester, NY, USA) at 60 kV, 10 mA and an exposure time of 0.2 s and 0.4 s for primary and permanent teeth, respectively. All radiographs were processed by an automatic X-ray film developer (Dent-X 9000, Dent-X, Elmsford, NY, USA), placed in transparent cards and identified. [25]

The radiographs were then examined using an X-ray viewer and an X-ray film magnifier (magnification × 2; VRX-Fabinject, Taubaté, Sãão Paulo, Brazil) in a darkroom. The approximal caries lesions were classified according to Lussi et al.: [7] (0) No radiolucency visible; (1) radiolucency visible in the outer half of the enamel; (2) radiolucency visible in the inner half of the enamel; (3) radiolucency in the outer half of the dentin; and (4) radiolucency in the inner half of the dentin.

Assessments with a pen-type laser fluorescence device

The device was calibrated for each tooth using a ceramic standard in accordance with the manufacturer's instructions. For the LFpen measurements, the wedge-shaped probe for approximal surfaces (width 0.7 mm and thickness 0.4 mm) was used. On every tooth, the device was also calibrated on a sound smooth surface, and the zero value was recorded. [17] The probe was guided in the interdental spaces without pressure. During the exam, the probe was positioned underneath the contact area, first from the facial side and then from the oral side. The highest value from the two measurements was recorded and then subtracted from the zero value. [7]

Histological validation

The teeth were longitudinally sectioned near the periphery of the center of the approximal surface with a water-cooled diamond disk in a sectioning machine (ISOMET 1000, Buehler, Lake Bluff, IL, USA). Progression of the grinding process (papers of grain size 600 and 1200) was constantly checked under a stereomicroscope (SZ2-ILST, Olympus, Tokyo, Japan) until the periphery of the site was reached. The teeth surfaces were then colored with saturated rhodamine B (Fluka, Buchs, Switzerland). The site was histologically assessed at × 10 magnification (SZ2-ILST, Olympus) for caries extent. The histological analysis was performed by an experienced senior researcher, who did not take part in the examination and scored according to Lussi et al.: [19] (0) Caries free, (1) caries extending up to halfway through the enamel, (2) caries extending into the inner half of the enamel, (3) caries in the outer half of dentin and (4) caries in the inner half of dentin.

Statistical analysis

Statistical analyses were performed using MedCalc version 9.3.0.0 (MedCalc Software Mariakerke, Belgium). Optimal cut-off limits for LFpen for primary and permanent teeth were determined by the point at which the sum of sensitivity and specificity was maximal, considering the histology scores 1-2 as enamel caries and scores 3-4 as dentin caries. Based on that, sensitivity, specificity, accuracy and area under the ROC curve (A z) were calculated at D 1 (enamel and dentin lesions- scores 1-4) and D 3 (dentin lesions- scores 3-4) thresholds for the methods. The McNemar test was used to compare the sensitivity, specificity and accuracy among the methods. A nonparametric statistical test was used to compare the differences among the A z values. [26] The significance level was set at $P < 0.05$. The correlation between the methods and histological scores was determined using Spearman's correlation coefficient, considering all lesions. A correlation coefficient of 0.70 or above indicates a strong relationship between two variables. [27]

The intraclass correlation coefficient (ICC) and Cohen's weighted kappa values were calculated for LFpen and BW, respectively, to assess inter- and intra-examiner reproducibility. The ICC was calculated using the original values of the LFpen device. The correlation was interpreted according to Fleiss [28] as follows: Values above 0.75 denoted excellent agreement while values between 0.40 and 0.75 indicated good agreement. The kappa values for BW were calculated and interpreted according to Landis and Koch [29] as follows: ≤0 = Poor agreement, 0.01-0.20 = slight agreement, 0.21-0.40 = fair agreement, 0.41-0.60 = moderate agreement, 0.61-0.80 = substantial agreement, and 0.81-1.00 = almost perfect agreement.

The Bland and Altman method were applied to the methods to identify systematic differences and the 95% limits of agreement. [28],[30] The graphical method showing the differences between the two measurements was plotted against the averages of the two measurements. Horizontal lines were drawn at the mean difference and at the limits of agreement, which were defined as the mean difference plus and minus 1.96 times the standard deviation. [30]

RESULTS

From the 246 approximal surfaces, histological examination revealed that 147 were caries free (score 0 - 53 permanent and 94 primary), 24 surfaces had caries extending up to halfway through the enamel (score 1 - 6 permanent and 18 primary), 25 surfaces had caries extending into the inner half of enamel (score 2 - 8 permanent and 17 primary), 30 surfaces had caries extending up to halfway through the dentin (score 3 - 19 permanent and 11 primary) and 20 surfaces had caries extending into the inner half of dentin (score 4 - 16 permanent and 4 primary).

The optimal cut-off limits for the LFpen device are shown in [Table 1]. For permanent teeth, the LFpen cut-off were 0-27 (sound), 28-33 (enamel caries) and > 33 (dentin caries). For primary teeth, the LFpen cut-off were 0-7 (sound), 8-32 (enamel caries) and >32 (dentin caries).{Table 1}

[Table 2] and [Table 3] present the specificity, sensitivity, accuracy and A z at D 1 and D 3 thresholds for the methods in permanent and primary teeth, respectively. LFpen had statistically significantly higher accuracy values than BW at the D 1 threshold while at the D 3 threshold the methods did not exhibit a statistically significant difference. In addition, LFpen exhibited statistically significantly higher sensitivity values than BW at the D 1 and D 3 thresholds (0.80 and 0.94, respectively). Both methods presented high specificity values (from 0.90 to 0.96) at the D 1 and D 3 thresholds, with no statistically significant differences among them. For both primary and permanent teeth, LFpen presented higher sensitivity values at D 1 and D 3 thresholds than BW. The highest accuracy values were observed at the D 3 threshold for both methods, with no statistically significant difference. Besides the fact that BW presented the lowest sensitivity, its accuracy was not statistically significantly different from the LFpen accuracy values at the D 3 threshold.{Table 2}{Table 3}

Although the Spearman's rank correlation coefficients (Rs) showed a significant positive correlation ($P < 0.01$) with histology scores, the BW method exhibited lower correlation coefficients than LFpen. Moreover, the correlation of both methods was found be lower for primary teeth (for LFpen Rs = 0.59, 95% confidence interval [CI]: 0.481-0.693; for BW Rs = 0.36, 95% CI: 0.210-0.496) than permanent teeth (for LFpen Rs = 0.83 95% CI: 0.757-0.882; for BW Rs = 0.70 95% CI: 0.586-0.788).

[Table 4] and [Table 5] present the ICC and weighted kappa values for LFpen and BW, respectively, for inter- and intra-examiner reproducibility. The ICC values revealed excellent intra-examiner reproducibility for the LFpen device for permanent (0.86 for examiners A and B) and primary teeth (0.99 and 0.90 for examiners A and B, respectively). The inter-examiner reproducibility for LFpen varied from 0.71 to 0.94 for permanent and primary teeth, indicating good and excellent agreement, respectively. The weighted kappa values for BW method for intra-examiner reproducibility showed for examiner. A substantial agreement (0.68) for permanent teeth and almost perfect agreement (0.89) for primary teeth and for examiner B almost perfect agreement for permanent (0.90) and primary teeth (0.89). For inter-examiner reproducibility, kappa values showed substantial agreement for BW for permanent (0.69) and primary teeth (0.64).{Table 4}{Table 5}

The limits of agreement (mean ± 1.96 standard deviation) for both inter- and intra-examiner reproducibility for the methods can be observed in the Bland and Altman plots for permanent and primary teeth [Figure 1] and [Figure 2].{Figure 1}{Figure 2}

DISCUSSION

Noncavitated approximal caries lesions are difficult to detect visually due to the wide contact areas. Commonly, the first sign visually detected may be cavitation, especially when the overlying weakened marginal ridge breaks down. [6] Despite the limitation of radiography in detecting caries lesions, many clinical guidelines used throughout the world recommend radiographs in children as an useful auxiliary tool to detect approximal caries. [31] In the present study, the visual examination was not performed once the contact points were simulated to assess the BW and LFpen examinations. Therefore, the performance of LFpen was compared with that of BW in primary and permanent teeth, as earlier studies have shown controversial results when approximal surfaces were evaluated. [4],[5],[6],[7],[8],[20],[32]

LFpen readings may be influenced by several factors such as calculus, plaque, prophylactic pastes and conditions of the adjacent approximal surfaces. [23],[24],[33] Within the limitation of an in vitro study, the experimental set-up was designed to avoid these biases through a consistent cleaning procedure of the surfaces and the storage method of the teeth. The storage method was to keep the teeth frozen at −20°C until the examinations, which allowed for the evaluation of the LFpen values without confounding factors. [22] Moreover, during the examination each test tooth was mounted in an arch model in a three-tooth group to simulate the oral relationship and maintain the approximal contact.

Regarding the cut-off presented for LFpen, the limits were calculated considering all examinations of the sample and the lesion depths for permanent and primary teeth as described by Rodrigues et al. [34] Primary teeth presented lower fluorescence values due to the different levels of mineralization, morphology and thickness of dental tissue, justifying the different cut-off between permanent and primary teeth. The optimal cut-off calculated in this study were similar to the values suggested by Braga et al. [5] for primary teeth, which found values of 0-4 for sound enamel, 5-38 for enamel lesions and above of 38 for cavitation. For both dentitions, it could be observed that the LFpen device could differentiate enamel from dentin carious lesions, but it was not able to differentiate the lesion depth (such as outer from inner carious lesions) for enamel or dentin. Although the actual depth of the carious lesion could not be evaluated using LFpen, the presence of cavitation is indicative of operative treatment, since this lesion is more prone to progress to more advanced stages. In addition, the presence of noncavitated lesions would be indicative of preventive treatment. [8],[35] Although the cavitation threshold was not assessed in the present study, only the lesion depth, it could be agreed with the statement by Novaes et al. [8] that the surface integrity is more important than the lesion depth for treatment decisions. This fact could be considered a limitation of the present investigation.

For primary and permanent teeth, LFpen presented high values of specificity at the D 1 threshold, showing that LFpen tends to be more specific than sensitive. The high amount of sound surfaces could have influenced this result; [36],[37] however, it is in agreement with findings reported in the literature. [4],[7],[8] Similar results were found by Lussi et al. [7] in an earlier in vitro study when approximal surfaces were evaluated in permanent teeth, and by Novaes et al. [8] who assessed the performance of the method in approximal surfaces using primary teeth. Another in vivo study has shown that LFpen presented higher specificity for approximal caries detection in primary teeth. [4]

At the D 1 threshold, LFpen and BW showed a reduced ability to detect approximal caries lesions in primary teeth than in permanent teeth. This was confirmed by the correlation between the methods with the histology scores. Although no studies were found in the literature comparing these methods in permanent and primary teeth, these results were expected due to the thinner enamel, lower mineral content, greater porosity and faster caries lesion progress in primary teeth, supporting the difficulty in detecting caries lesions in these teeth. [5],[12],[21],[34]

Comparing the performance of the methods for caries detection in primary teeth, LFpen and BW presented similar accuracy at both thresholds. This corroborated previous studies evaluating approximal caries lesions in primary teeth: Braga et al. [5] and Chawla et al. [6] who evaluated the in vitro performance of LFpen, visual examination and BW, and the in vivo studies of Novaes et al. [8] and Mendes et al. [32]

In addition, BW and LFpen presented lower A z values at the D 1 threshold than at the D 3 threshold. This result was expected and corroborated previous in vitro studies from Chawla et al. [6] who observed similar A z values for LFpen and BW at the D 1 threshold, and at the D 3 threshold. In addition, Braga et al. [5] found similar A z values for LFpen and BW at the D 1 and D 3 thresholds. The A z values for the laser fluorescence device confirm the difficulty of using this method for detecting enamel caries lesions, such as noncavitated lesions on approximal surfaces [5],[8],[16],[32] and occlusal surfaces. [11],[15],[36] This could be explained by the manner in which the device functions, [5] whereas the fluorescence of the carious lesions induced by the diode laser reflects changes in the organic content due to the bacterial metabolites present there, [38] because cavitated lesions are infected to a greater degree by those metabolites than noncavitated lesions. [39]

In permanent teeth, LFpen showed higher A z values at the D 1 threshold and higher sensitivity at D 1 and D 3 thresholds, while the specificity values were not significantly different between the methods. This is in agreement with Lussi et al. [7] who found better performance with LFpen compared to radiography. On the other hand, Novaes et al. [8] did not find differences between LFpen and BW performance in detecting approximal caries in primary teeth in a clinical study. Differences in the methodology with respect to the validation method could explain some disagreements, but more in vivo investigations are necessary.

LFpen presented statistically significantly higher sensitivities and A z values than BW at the D 1 threshold for primary and permanent teeth. On the other hand, Chawla et al. [6] found no statistically significant differences between the methods in primary teeth at the D 1 and D 3 thresholds. These differences can be explained due to the differences in the cut-off limits. In the present study, the optimal cut-off limits for LFpen were determined by the point at which the sum of sensitivity and specificity was maximal, classifying the caries lesion with three reference points (sound enamel, enamel lesion and dentinal lesion), while Chawla et al. [6] used four cut-off limits (sound enamel, inner and outer of enamel and dentin).

With regard to BW in this study, the A z values were lower than those obtained in earlier studies. [5],[6],[8] In fact, the radiographic method is more efficient at detecting more advanced caries lesions. [5],[40] Moreover, no significant differences have been found under clinical and laboratory conditions. Different results can reflect the examiner's experience with the method. [5],[35] In the present study, the examiners did not receive extensive training sessions for both methods. According to a previous study by Diniz et al. [41] the clinical experience of the examiners might influence the reproducibility and accuracy of radiographic examination.

In the present study, the LFpen showed excellent reproducibility for primary teeth and moderate to excellent reproducibility for permanent teeth, corroborating previous studies. [4],[7] However, some studies using primary teeth showed low reproducibility values for LFpen, especially when cavitated lesions were excluded from the analysis. [20] The disagreement could be attributed to the lesion thresholds. On the other hand, the BW reproducibility values showed substantial to almost perfect agreement for permanent and primary teeth. This difference could be explained by the subjectivity of the BW examination, which depends on the examiner's experience, skills and other factors. For these reasons, it could be agreed with Diniz et al. [41] that caution must be taken when treatment decisions are made based only on BW.

In order to evaluate the variability of the LFpen readings and BW scores, and to detect deviations from measurements, the Bland and Altman [30] method was adopted. Kóhnisch et al. [42] suggested that the range of the limits should not exceed ± 20 LF units (range of 40 units). The plots presented the diamond shape as described by Huysmans et al., [43] which was defined by the concentration of points close to 99 and 0 representing perfect agreement of the extreme measurements, and clusters of points at the top and bottom corners of the diamond representing disagreement of the extreme measurements. For the LFpen plots, the values were clustered in the left and in the right borders of the line, suggesting that the methods were more reliable in the lower values. In the present study, the lowest range between the upper and the lower limits was approximately 6.6 of the agreement for the LFpen intra-examiner reproducibility in primary teeth. These findings are in agreement with Celiberti et al. [20]

On the other hand, the BW plots did not show a specific shape, whereas the values presented deviations from measurements. Although it presented lower limits of agreement, BW

presented the lowest range of agreement (range of 1.3) for the intra-examiner reproducibility. According to Celiberti et al., [20] this comparison is not acceptable, due to the range dependence on the measurement value available for the method.

CONCLUSION

LFpen presented higher accuracy in detecting both enamel and dentin caries lesions (D 1 threshold) than BW for permanent teeth. Besides, the LFpen device presented better reproducibility in detecting approximal caries lesions than BW for both primary and permanent teeth. It could be suggested that LFpen may be a suitable alternative method to BW.

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