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ORIGINAL ARTICLE

In situ and in vitro comparison of laser fluorescence with visual inspection in detecting occlusal caries lesions

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Abstract The aim of this study was to compare the in situ and in vitro performances of a laser fluorescence (LF) device (DIAGNOdent 2095) with visual inspection for the detection of occlusal caries in permanent teeth. Sixty-four sites were selected, and visual inspection and LF assessments were carried out, in vitro, three times by two independent examiners, with a 1-week interval between evaluations. Afterwards, the occlusal surfaces were mounted on the palatal portion of removable acrylic orthodontic appliances and placed in six volunteers. Assessments were repeated and validated by histological analysis of the tooth sections under a stereomicroscope. For both examiners, the highest intra-examiner values were observed for the visual inspection when in vitro and in situ evaluations were compared. The inter-examiner reproducibility varied from 0.61 to 0.64, except for the in vitro assessment using LF, which presented a lower value (0.43). The methods showed high specificity at the D_1 threshold (considering enamel and dentin caries as disease). In vitro evaluations showed the highest values of sensitivity for both methods when compared to the in situ evaluations at D_1 and D_2 (considering only dentinal caries as the disease) thresholds. For both methods, the results of sensitivity (at D_1 and D_2) and accuracy (at D_1) showed significant differences between in vitro and in situ conditions. However, the sensitivity (at D_1 and D_2), specificity and accuracy (both at D_1) of the methods were not significantly different when the same condition was considered. It can be concluded that visual inspection and LF showed better performance in vitro than in situ.

Keywords Laser fluorescence · DIAGNOdent · Caries detection · Visual inspection · Occlusal caries

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Introduction

Dental caries is an important worldwide public health problem [1, 2]. Early diagnosis is fundamental for the establishment of a proper treatment plan as conservative as possible, reducing the involvement of tooth structures in restorative procedures, when indicated. However, the detection of small lesions, especially on occlusal surfaces, is still difficult for dental professionals [3]. The difficulty of precise detection is related to factors such as the complex anatomy of pits and fissures [4, 5], superposition of structures in the radiographic evaluation, and increase in the number of hidden carious lesions due to the wide utilization of fluorides [6–8]. Thus, the combination of methods for caries diagnosis has been suggested for a more accurate diagnosis [5].

Visual inspection and bite-wing radiographic examination are the methods most commonly employed for the detection of occlusal caries in clinical practice [9–14], and a combination of both methods has shown good results in the detection of dentinal occlusal caries [5]. However, these have been described as limited, due to their subjectivity [15, 16] and the difficulty in detecting incipient caries lesions, from which the loss of tooth structure is nearly imperceptible.

With the technological evolution, new auxiliary methods for detection of carious lesions, such as the air abrasion system [17], digital radiography and utilization of laser fluorescence, have been investigated [5, 7, 8, 17–21].

The laser fluorescence (LF) device (DIAGNOdent, Kavo, Biberach, Germany) provides a quantitative method for caries detection and has the ability to emit red light at 655 nm wavelength and to measure the fluorescence emitted by metabolites of oral bacteria [22]. This new method allows quantifiable, non-invasive examination of the tooth surface, thereby favoring early detection of the lesion, so that preventive measures may be adopted to prevent caries progression [23, 24].

Most studies on the effectiveness of this method for the detection of occlusal caries lesions have been performed in vitro [5, 17, 25, 26] and thus are limited with regard to the integral extrapolation of results to the clinical situation. The difficulty in obtaining a reliable gold standard in an in vivo situation could justify the necessity of studies which show conditions closer to that in clinical practice. Therefore, the aim of this study was to compare the in situ and in vitro performances of laser fluorescence with visual inspection for the detection of occlusal caries in permanent teeth. The null hypothesis established for this study was that there is no difference between laser fluorescence and visual examination under in situ and in vitro conditions.

Materials and methods

This study was approved by the Ethics Committee of the School of Dentistry, São Paulo State University (UNESP), Araraquara, Brazil. Sixty-four sites on the occlusal surfaces of 26 permanent (third maxillary and mandibular) molars suggested to have initial caries lesions on occlusal surfaces were selected. The teeth were donated by the Tooth Bank of the Dentistry Faculty of the University of São Paulo, Brazil.

The occlusal surfaces were separated from the roots by transverse sectioning, perpendicular to the tooth long axis, with a low-speed diamond saw mounted in a sectioning machine (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under constant water cooling. The teeth were cleaned with pumice slurry and water with a polishing brush, rinsed with a 3-in-1 syringe for 10 s [27], and stored in distilled water at room temperature for no longer than 1 week.

Digital photographs were taken of all surfaces on which the sites to be evaluated were marked, to guide the examiners during their visual inspections and LF assessments. The assessments, both in vitro and in situ, were carried out by two experienced dentists, in triplicate, with a 1-week interval between measurement.

For in vitro visual inspection, the teeth were air-dried and the sites were examined by naked eye under artificial light and awarded the following scores: 0—no caries; 1—carious lesion in the enamel; 2—carious lesion in the dentin.

For LF assessment, the device was initially calibrated on a ceramic standard for which the fluorescence was known, following the manufacturer's instructions using the tip A, indicated for occlusal surfaces. The tip was moved around the test site and rotated on its central axis until the highest value was obtained. The values displayed on the screen were recorded. The performance was assessed according to the cut-off limits suggested by the manufacturer, as follow: score 0 (0–10, sound); score 1 (11–20, caries in the enamel); score 2 (21–99, caries in the dentin).

After in vitro evaluation, the occlusal surfaces were autoclaved for 15 min at 120°C, mounted on the palatal portion of removable acrylic orthodontic appliances, and placed in six volunteers, who had signed and agreed to the informed consent terms for their participation in the study. They were asked to wear the appliance for one hour before each evaluation, so that the occlusal surfaces would be moistened with saliva. Visual inspections and LF assessments were conducted following the same criteria described for the in vitro analysis, except for the utilization of a dental mirror.

After in situ evaluation, the teeth were carefully removed from the orthodontic appliances and stored under 100% humidity. They were stored for 1–2 days after they had been removed from the appliances and before the validation had been started.

For validation of the results, the teeth were bisected buccolingually through the center of the carious lesion with a water-cooled diamond disc at low-speed. Histological analysis of the sites was established as the gold standard; this was carried out under a stereomicroscope (magnification ×40), and the same scores: 0 (no caries), 1 (caries in the enamel) and 2 (caries in the dentin) were used.

Both methods were assessed for reproducibility and accuracy. Cohen's unweighted kappa was used to assess the intra-examiner and inter-examiner reproducibility. Sensitivity, specificity, accuracy and area under the receiver operating characteristic (ROC) curve for both methods were determined at the D_1 threshold (considering as disease both the gold-standard scores 1 and 2) and the D_2 threshold (considering as disease only gold-standard score 2), in vitro as well as in situ. We used the McNemar test to compare the sensitivity,

Table 1 In vitro and in situ unweighted kappa values for intra-examiner and inter-examiner reproducibility of visual inspection and LF device

Method	Condition	Intra-examiner	Inter-examiner
Visual	In vitro	0.69	0.61
	In situ	0.48	0.62
LF	In vitro	0.55	0.43
	In situ	0.36	0.64

specificity and accuracy between the methods and the conditions. The significance level was set at $P<0.05$.

Results

Histological examination revealed that of the 64 occlusal sites, eight were caries free (score 0), 38 had caries in the enamel (score 1) and 18 had caries in the dentin (score 2).

Reproducibilities are shown in Table 1. For both examiners, the highest intra-examiner values were observed for the visual inspection when in vitro and in situ evaluations were individually compared. The inter-examiner reproducibility varied from 0.61 to 0.64, except for the in vitro assessment using the LF device, which presented a lower value (0.43).

Table 2 shows the values of sensitivity, specificity, accuracy and area under the ROC curve (A_z) of both methods, under both conditions. The methods presented high specificity at the D_1 threshold, with no statistically significant difference. In vitro evaluations showed the highest values of sensitivity and accuracy for both methods when compared to the in situ evaluations at D_1 and D_2 thresholds, except for LF at the D_2 threshold regarding accuracy. The specificity of LF in vitro was not different from that in situ at D_1, whereas the specificity of visual inspection in vitro was higher than in situ at D_1. In addition, the accuracy of both visual inspection and LF in vitro were higher than in situ at D_1 and D_2, except for LF at the D_2 threshold.

For both methods, the results of sensitivity (at D_1 and D_2) and accuracy (at D_1) showed significant differences between in vitro and in situ conditions. However, sensitivity (at D_1 and D_2), specificity and accuracy (both at D_1) of the methods were not significantly different when the same condition was considered.

Discussion

Visual inspection and radiographic examination have been commonly used in clinical practice, but they are able to detect caries lesions only at an advanced stage [5]. The subjective nature of visual inspection has encouraged the development of auxiliary methods in daily practice [2, 16]. Among these methods, the laser fluorescence device (DIAGNOdent) provides a quantifiable, non-invasive method that has been widely disseminated [5, 8, 21, 24, 28, 29].

An ideal diagnostic method should have high sensitivity and high specificity [30] and should also be reliable, with good intra and inter-examiner reproducibility [31]. Reproducibility means the closeness of agreement between the results of measurements carried out under changed conditions of measurement. An accurate study requires the investigation of its reproducibility, indicating that it is reliable and may be validated.

In our study, for both visual and LF assessments, the intra-examiner kappa values decreased for the in situ condition when compared to the in vitro condition (Table 1), and this could be attributed to the greater difficulty in the inspection of the teeth and in the placing of the site on the palatal portion of the orthodontic appliances [19]. Our results are different from the findings of Costa et al. [16], who observed a higher intra-examiner agreement for the LF device than for the visual inspection. This could be due to the fact that the LF device provides a quantitative method, whereas visual inspection involves subjective aspects such

Table 2 Sensitivity, specificity, accuracy and area under the ROC curve (A_z) of visual inspection and LF device, under both conditions

Method	Condition	Sensitivity		Specificity		Accuracy		A_z	
		D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2
Visual	In vitro	0.63^{a}	$0.33^{a,c}$	1.00^{a}	0.95^{a}	0.68^{a}	0.78^{a}	0.817^{a}	0.796^{a}
	In situ	0.54^{b}	0.11^{b}	0.88^{a}	0.96^{a}	0.58^{b}	0.72^{a}	$0.710^{a,b}$	$0.708^{a,b}$
LF	In vitro	0.72^{a}	$0.42^{a,c}$	1.00^{a}	0.65^{b}	0.76^{a}	0.59^{b}	0.862^{a}	0.659^{b}
	In situ	0.55^{b}	$0.22^{a,b,c}$	1.00^{a}	0.87^{b}	0.61^{b}	0.69^{b}	0.777^{a}	0.662^{b}

D_1: 0=sound; 1–2=decayed

D_2: 0–1=sound; 2=decayed

Within columns, significant differences are represented by different superscript letters (a, b and c; McNemar test, $P<0.05$)

as background knowledge and the examiner's individual clinical experience [5, 32].

Regarding inter-examiner agreement, Klimm et al. [33] conducted in vitro studies and found values similar to ours for both visual inspection and LF assessments. In a recent in vitro study, lower LF inter-examiner reproducibility was observed [8]. The difference between the different results found in the literature can be attributed to a number of factors, such as the place of examination, lighting conditions, storage method of the teeth, the great variation in the values shown by the LF device, and the experience of the examiners in handling and calibrating the device.

Thus, this study attempted to neutralize such factors by standardization of the place and lighting employed for the assessments. However, it should be pointed out that volunteers reported different pressures from the LF device's probe when applied by different examiners during assessment of the sites, which may have led to different values. Even though a mild pressure is recommended, this criterion is subjective [16]. Thus, in the in situ analysis, the patients were questioned about the pressure applied and this was then controlled; it may be suggested that this aspect may have contributed to the higher LF inter-examiner reproducibility in the in situ condition than in the in vitro assessments.

Concerning sensitivity and specificity, in an in vitro study Rodrigues et al. [5] found values at the dentin thresholds similar to those presented in Table 2 for visual inspection, although they used the International Caries Detection and Assessment System (ICDAS) II criteria for this examination. For the LF device, higher sensitivity was found than for the visual inspection, although no statistically significant difference was observed, which was in agreement with the meta-analysis from Bader and Shugars [3]. However, at the D_2 threshold in vitro, visual inspection fared better than the LF device did, in agreement with the findings of Rodrigues et al. [17].

The results obtained for LF in situ were comparable to those of Verdonschot et al. [21], who observed lower sensitivity values than those for specificity. Chong et al. [7] found sensitivity values of 0.49, whereas specificity was lower (0.67) than in our results. On the other hand, in an in vivo study, Lussi et al. [34] reported high sensitivity values, indicating the laser as an excellent method for detection of caries in pits and fissures.

In our study the reduction in the sensitivity values for the LF device used in situ might have been due either to the difficulty in placing the point on the exact site or to the difficulty in performing the rotation movements, due to the position of each tooth on the orthodontic appliance [19, 34]. The presence of saliva should also be taken into account. Investigations conducted by Lussi et al. [24] and Shi et al. [19] revealed that the presence of humidity on the enamel surface interferes with the diagnosis of lesions; however, these authors did not observe significant changes in the diagnostic performance of the device. This could be also stated when the accuracy values were analyzed. For both methods the values were statistically higher in vitro than in situ, except for LF at the D_2 threshold. The area under the ROC curve also decreased for the in situ assessments, except for LF at D_2, which increased, with no statically significant difference. This type of analysis does not consider a stipulated cutoff limit for the calculation.

Conclusion

It can be concluded that visual inspection and the LF device showed different performances when in vitro and in situ conditions were compared. This means that caution must be exercised in extrapolating in vitro results to the clinical situation. Therefore, further in situ and in vivo studies are necessary, in order to validate these methods under clinical situations. Therefore, the null hypothesis was rejected.

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