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Research Article

Assessment of caries activity using the Calcivis® Caries Activity Imaging System

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Abstract. Objective: An adequate diagnosis of dental caries means not only assessment of the change and the spread of change in the lesion, but also making a decision concerning its possible activity. Caries activity is primarily assessed using visual-tactile criteria that help estimate the probability of a carious lesion to progress. The aim of the present study was to test the capability of a new approach to ascertain lesion activity by means of bioluminescence (Calcivis® Caries Activity Imaging System, Calcivis) in-vitro. Subjects and Methods: 46 extracted permanent posterior teeth were included in the study (30 occlusal surfaces, 16 smooth surfaces). The investigation sites were classified by two examiners using ICDAS and NYVAD criteria for lesion activity (activity yes/no) and consensus score of each site was determined as reference value. The sites were photographed using a prototype Calcivis System and the images were evaluated by both examiners for the presence of activity (bioluminescence, blue spots at the investigation sites). Correlation of methods was calculated using Spearman's rank correlation coefficient (rs). Agreement between methods was assessed by kappa statistics. ROC curves were created for Calcivis and both visual methods and the areas under the Curve (AUC) were compared (α=0.05). Results: Significant positive correlation was found between Calcivis and the visual detection methods: rs ICDAS=1.0, rs Nyvad criteria=0.776 (p<0.001). Kappa-values were: Calcivis/ICDAS=1.0, Calcivis/Nyvad=0.78. AUC for Calcivis was 1.0 (ICDAS as reference value) and 0.89 (Nyvad criteria as reference value).No significant differences were observed between the AUCs (p=0.30). Conclusion: The in-vitro use of the bioluminescence method showed good agreement with visual findings in assessment of the activity of a carious lesion in the area of occlusal and smooth surfaces.

Keywords: Dentistry; caries activity; occlusal surfaces; smooth surfaces; Calcivis; bioluminescence

1. Introduction

Current data show that caries in permanent teeth is one of the most widespread diseases in the world [1], although a decline in caries prevalence has been reported in many European countries [2]. More and more often, a change in the characteristic of dental caries away from dentin cavities and toward non-cavitated initial enamel lesions can be observed [3]. The early detection of these lesions now occupies a place of great importance in dentistry. Apart from the timely identification of carious changes, making a decision on the possible therapies is equally important. The decision for preventive or operative care of the teeth is not made exclusively on the basis of the extent of the caries. Especially in the case of initial lesions, the activity of the change in the caries plays a part in the treatment decision [4, 5]. Lesions that are active but not yet cavitated can well be arrested using suitable methods (including fluorides). This avoids restorative treatment in favour of maintaining the hard tooth tissue.

When caries occurs, the less resistant interprismatic enamel is dissolved first. The organic acids formed by bacteria can now diffuse deeper into the watery shell around the apatite crystals, owing to the concentration gradient, and dissolve out additional ions (such as calcium, hydroxyl and phosphate ions). These reach the surface through the widened pores along the concentration gradient and then get into the plaque. In the beginning, once the noxious agents that produce the caries are eliminated, minerals in the saliva can cause an active lesion to regress into an inactive, arrested lesion, or appropriate preventive measures can support such a development. An arrested lesion exhibits a smooth, shiny, hard, partially brown surface. The discolorations can be explained by exogenous pigments being deposited from tobacco, for instance, or tea, coffee, and food during remineralization. If preventive measures are not used, demineralization progresses into the dentin [6].

Usually the teeth are given a detailed examination using primarily visual detection methods that ideally can define all gradations of dental caries, from enamel lesions up to manifest dentin caries. With regard to lesion activity, not only a single factor might determine whether a carious lesion is active or arrested (inactive) [7-9]. The presence of plaque, the localization of the lesion, the clinical appearance, the presence or lack of a shiny surface, and tactile characteristics (roughness) are used as predictors to evaluate the change. The criteria of Nyvad et al. [9] are well suited, especially for assessment the activity of a lesion. This classification is aimed at the presence of plaque on caries predilection sites, among other things. If the teeth are cleaned prior to visual examination, the criteria of the "International Caries Detection and Assessment System" (ICDAS) can be used to define the extent and activity of the caries [5, 10].

An innovative approach for detecting caries activity is the Calcivis® Caries Activity Imaging System (Calcivis Ltd, Edinburgh, UK, hereinafter without the ® sign). It enables the activity of a carious lesion to be determined using a digital camera. The process proceeds as follows: a patented photosensitive protein is applied to the tooth surface by means of the Calcivis System. In the case of active lesions, free calcium ions dissolve out of the hard tooth tissue and are found unbound on the surface of the lesion. The photoprotein bonds these calcium ions and emits blue light. This light signal – called bioluminescence – is proportional to the freely available calcium on the carious surface. This process is simultaneous with the digital image taken by the camera head, so that images of the surface can be taken (with and without bioluminescence). When these images are superimposed, it is possible to determine whether a lesion is active (blue areas). Initial studies show the potential of this method for the digital detection of active lesions [11, 12].

At present, there are only a few studies available that take activity into account [7, 13, 14] but no published studies on the use of the Calcivis. Therefore the aim of the present in-vitro study was to investigate the ability of the Calcivis System for activity assessment. The following null hypothesis (H0) was the focus of the examinations: the findings determined by visual criteria do not correlate positively with the results obtained using the Calcivis System. The corresponding alternative hypothesis (H1) was: the findings determined by the visual criteria correlate positively with the results obtained using the Calcivis System.

Furthermore, the diagnostic accuracy of the Calcivis System was to be ascertained in relation to the visual detection of caries activity.

2. Materials and Methods

2.1. Preparing for the study and visual classification. The study was independently reviewed and approved by the ethics committee of the Medical faculty of Philipps-University, Marburg (approval number 64/15) and was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. Prior to the study, a power calculation was performed using G*Power 3 [15]. In expectation of a medium correlation, $\mu = 0.05$ and a power of 0.9, a sample size of 36 teeth was ascertained for a power of 0.9. A dropout rate of 10% was added on, so that a total of 40 teeth were planned to include in the study.

Extracted teeth exhibiting initial or moderate carious lesion on the occlusal or smooth surfaces were included in the study. Teeth with developmental defects or dental fluorosis were excluded, as were teeth with obvious dentin caries or extremely decayed teeth.

After extraction, the teeth were stored in a 0.001% sodium azide solution and then cleaned with a small rotating brush and a cleaning paste (Clinpro Prophy Paste, 3M ESPE, Seefeld, Germany). The remaining paste was removed with a multifunctional syringe using water and air. Immediately thereafter, the teeth were stored in water. First, the carious lesion on each tooth was selected. Then the teeth were bedded in silicone for later reposition (Silaplast Futur, Detax GmbH, Ettlingen, Germany).

The visual classification was performed by two examiners (AJM and LK) by means of consensus decision according to the two criteria described below. The extent of the lesion (caries) was estimated and the activity determined:

2.1.1. Nyvad et al. criteria [9], (called NYVAD in the following): Code 1 = Active caries (intact surface), surface of enamel is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved gently across the surface; generally covered with plaque. No clinically detectable loss of substance. Smooth surface: Caries lesion typically located close to gingival margin. Fissure/pit: Intact fissure morphology; lesion extending along the walls of the fissure;

2 = Active caries (surface discontinuity), same criteria as score 1. Localized surface defect (microcavity) in enamel

only. No undermined enamel or softened floor detectable with the explorer. 3 = Active caries (cavity), Enamel/dentin cavity easily visible with the naked eye; surface of cavity feels soft or leathery on gentle probing. There may or may not be pulpal involvement.

4 = Inactive caries (intact surface), Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface. No clinically detectable loss of substance. Smooth surface: Caries lesion typically located at some distance from gingival margin. Fissure/pit: Intact fissure morphology; lesion extending along the walls of the fissure.

5 = Inactive caries (surface discontinuity), same criteria as score 4. Localized surface defect (microcavity) in enamel only. No undermined enamel or softened floor detectable with the explorer. 6 = Inactive caries (cavity), Enamel/dentin cavity easily visible with the naked eye; surface of cavity may be shiny and feels hard on probing with gentle pressure. No pulpal involvement.

7 = Filling (sound surface)

8 = Filling + active caries; Caries lesion may be cavitated or non-cavitated.

9 = Filling + inactive caries; Caries lesion may be cavitated or non-cavitated.

2.1.2. B: International Caries Detection and Assessment System (ICDAS) [10]: Code 1 = First visual change in enamel: opacity or discoloration (white or brown) after prolonged air drying, which is not seen on a wet surface;

2 = Distinct visual change in enamel: opacity or discoloration distinctly visible when wet, lesion must still be visible when dry;

3 = Localized enamel breakdown due to caries with no visible dentine or underlying shadow: opacity or discoloration wider than the natural fissure/fossa when wet and after prolonged air drying;

4 = Underlying dark shadow from dentine;

5 = Distinct cavity with visible dentine: visual evidence of demineralization and dentine exposed; 6 = Extensive distinct cavity with visible dentine and more than half of the surface involved.

For the ICDAS codes, the characteristics of an active lesion are defined as followed [10]:

ICDAS Codes 1, 2, 3: Surface of enamel is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved gently across the surface. Lesion is in a plaque stagnation area, i.e.: pits and fissures, near the gingival and approximal surface below the contact point;

ICDAS Code 4: Probably active;

ICDAS Codes 5, 6: Cavity feels soft or leathery on gently probing the dentin.

The characteristics of an inactive lesion are:

Codes 1, 2, 3: Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface. For smooth surfaces, the caries lesion is typically located at some distance from the gingival margin;

ICDAS Codes 5, 6: Cavity may be shiny and feels hard on gently probing the dentin.

2.2. Examinations using the bioluminescence method.
Images of the investigation sites were taken using the Calcivis System according to the manufacturer's specifications. To do so, the following procedure was used: Immediately before the measurement, protein available in powdered in a sterile packed container was mixed with distilled water in a prescribed concentration and drawn up into an applicator by means of a needle. This applicator was inserted into the Calcivis handpiece, which simultaneously functions as a camera. The camera was connected to a laptop and the Calcivis program started. After recording the tooth data, the tooth to be examined and the camera head were inserted into a darkened box and the photographic process was started. To this end, the activation button on the handpiece was pressed, which applied a defined amount of the protein (concentration 50 μg/ml) directly to the tooth surface being examined. Immediately after this procedure (duration 185 ms), the image of the tooth surface was displayed on the screen (Figure 1b and 1d). This image was then used to evaluate the presence of activity (blue areas at the investigation site).

2.3. Statistical analysis.
SPSS (15.0) and MedCalc (version 15.6.1) were used for the statistical evaluation. The agreement between the findings (visual vs. bioluminescence) was determined using the kappa statistic. In order to investigate the correlation between the findings, the results were represented by means of crosstabs and the correlation analyzed using the chi-squared test ($X2$ test) and Spearman's rank correlation coefficient. To determine the diagnostic accuracy of the Calcivis, receiver operation characteristic curves (ROC) were constructed and the areas under the curves compared [16]. The significance level was $\alpha = 0.05$.

3. Results

A total of 46 extracted posterior teeth (35 molars, 11 premolars) with one investigation site each were included in the study. The investigation sites were on the occlusal surface of 30 teeth and on the smooth surfaces of 16 teeth. Table 1 summarizes the distribution of the caries findings according to NYVAD/ICDAS. The correlation was significantly positive ($X2$ test, p = 0.004).

Using the visual criteria, 41 investigation sites were classified as active lesions and five investigation sites as inactive caries. One investigation site showed no agreement between
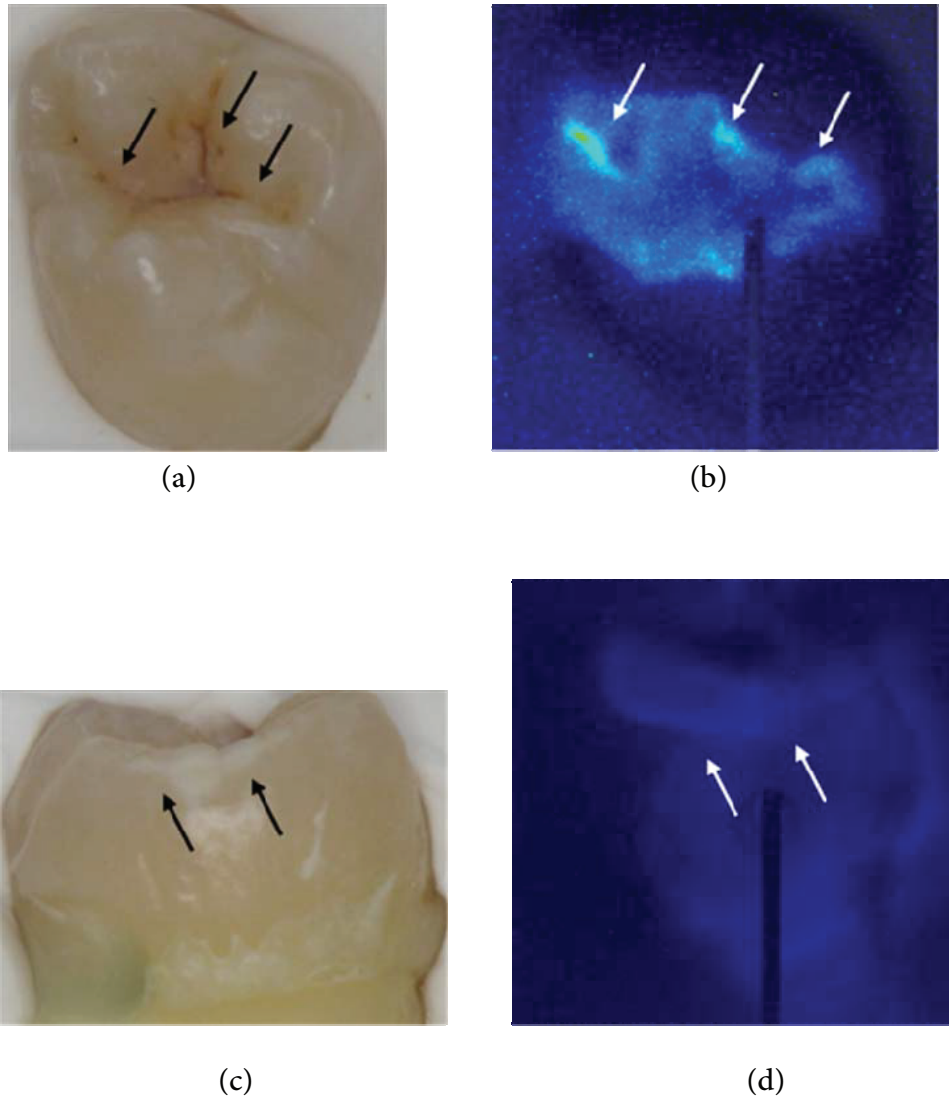
(a)

(b)

(c)

(d)

Figure 1: Examples of study teeth (arrows on the investigation sites): a) Molar with active occlusal lesion, and b) corresponding Calcivis image, c) Molar with active lesion on the smooth surface, and d) corresponding Calcivis image.

Table 1: Cross tabulation of caries scores according to Nyvad- und ICDAS criteria for all teeth.

ICDAS	Nyvad						
	0	1	2	3	4	5	N total
0	0	0	0	0	0	0	0
1	1	11	1	0	2	0	15
2	0	11	3	0	1	0	15
3	0	0	7	1	0	1	9
4	0	1	5	1	0	0	7
N total	1	23	16	2	3	1	46

NYVAD and ICDAS criteria with respect to the activity of the lesion (Table 2). The kappa value for the agreement between the NYVAD and ICDAS criteria (activity) was 0.78 for all teeth. The correlation was significantly positive ($X2$ test, p < 0.001). For all investigation sites the kappa values for the agreement of the findings with regard to lesion activity (yes/no) compared to Calcivis were: NYVAD-Calcivis = 0.78 and ICDAS-Calcivis = 1.0.

The correlation of the visual bioluminescence method was significantly positive (p < 0.001, two- sided), and where rs: NYVAD-Calcivis = 0.776 and rs: ICDAS-Calcivis = 1.0. Thus the alternative hypothesis was confirmed.

The visual findings with respect to activity were first used as the reference value to determine the diagnostic accuracy. In Figure 2 shows the ROC curves are presented. The corresponding areas under the ROC-curve (AUC), specificity and sensitivity of the Calcivis method are summarized in
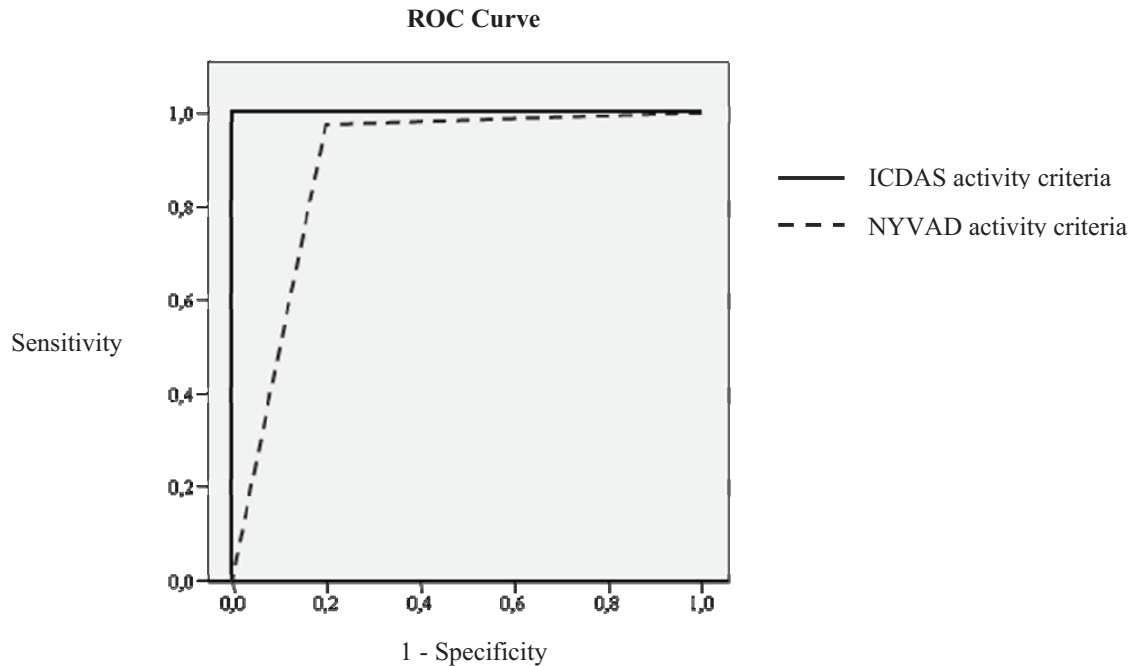
Figure 2: Receiver operating characteristic (ROC)-curves for the Calcivis using visual activity criteria as reference value.

Table 2: Cross tabulation of activity assessment according to Nyvad- und ICDAS criteria and Calcivis findings. Agreements in the findings are marked in bold.

Calcivis activity	Nyvad activity		N total	ICDAS activity		N total
	No	Yes		No	Yes	
No	**4**	1	5	**5**	0	5
Yes	1	**40**	41	0	**41**	41
N total	5	41	46	5	41	46

table 3. There were no significant differences between the Areas under the curve (p = 0.30).

4. Discussion

The present study is the first to evaluate the suitability of the newly launched Calcivis System for determining the activity of a carious lesion compared to visual detection methods. In the process, the already well-established Nyvad and ICDAS criteria were used. Typically, examiners assess caries activity by using visual-tactile criteria that help them estimate the probability or tendency of a carious lesion to progress. No single factor is exclusively valid for defining activity [8]. The visual methods used in the present study describe the characteristics of the lesion primarily with regard to colour, shininess, surface roughness, and consistency. The NYVAD criteria are also aimed at detecting the presence of plaque on caries predilection sites. Since the teeth in the present study were cleaned before detection, a potential accumulation of plaque on the investigation sites was assumed whenever

the NYVAD criteria were applied. This deviation concerns only one investigation site where the NYVAD criteria pointed to activity, but not the ICDAS definition (Table 2). This could probably have caused misinterpretations when these criteria were used, which would then be reflected in the results regarding the specificity and sensitivity of Calcivis as measured by the visual methods (Table 3). Nonetheless the differences were not statistically significant.

For the clinical assessment of active lesions, there were also studies on using impression materials that are capable of indicating the production of lactic acid on the tooth surface by means of a color change (3M ESPE: Clinpro Cario Diagnosis, Full Arch Lactic Acid Locator) [14]. It must be noted that this product is not yet commercially available everywhere.

In order to weight the various factors for determining lesion activity, Ekstrand et al. [14] developed a decision tree that takes account not only of the ICDAS code, but also the color and position of the lesion on the tooth, as well as the surface condition. Systems of this kind can be helpful for making a definitive treatment decision, mainly

Table 3: Area under the ROC-Kurve (AUC), specificity and sensitivity of Calcivis for assessment of lesion activity using the visual findings as reference.

Reference	AUC (95% confidence interval)	Standard error	Specificity (%)	Sensitivity (%)
Nyvad activity	0.89 (0.68-1.0)	0.11	80	97.6
ICDAS activity	1.0 (1.0-1.0)	0.00	100	100

in a clinical setting. The fact must not be left unmentioned that the tendency of gingiva to bleed can also be used as a predictor of the activity of lesions close to the gingival cuff [17]. However, this characteristic cannot be used as a factor in an in-vitro study.

As already described, an increase in initial lesions is being observed despite the general trend toward caries decline. Among other things, this is related to the ability to detect these changes with more certainty and more frequency thanks to improved caries detection methods. Here in particular, it is therefore that much more important to distinguish whether the lesions are active and could transition to dentin caries if given inadequate treatment or none at all. If an increasing number of active initial lesions are found in a patient, this could point to enhanced caries risk [4, 5]. Apart from the dentist's treatment planning, this is also important to the patient in view of the additional costs to be expected for health services.

Visual methods are not capable of digitally representing the activity of a lesion. In this sense, presenting oral findings objectively and documenting them can be a good way to improve communication with patients and enhance their motivation. The new Calcivis camera system objectively records and displays carious lesions with regard to their activity. Hence it is possible to provide a digital documentation and follow-up of the level of activity of the caries in order to enhance patients' amenability to preventive or minimally invasive therapies. Another advantage of digital documentation is that the findings are readily available to be compared to current findings at recall sessions. This can also improve quality assurance in the dentist's office.

In this in-vitro study Calcivis showed good correlation and high agreement with visual criteria regarding lesion activity assessment. Hence the bioluminescence can be used in detecting the activity of a carious legion in the area of occlusal and smooth surfaces. As a clinical relevance the method can be valuable in assessment of active caries lesions and thus in identification of patients at high caries risk.

Acknowledgement

Conflict of interest

The authors (Anahita Jablonski-Momeni and Lukas Kneib) declare that they have no conflict of interest

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