In vitro quantification of white spot enamel lesions adjacent to fixed orthodontic appliances using quantitative light-induced fluorescence and DIAGNOdent

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The aims of this in vitro study were 2-fold: 1) to evaluate two fluorescence methods (DIAGNOdent and QLF (quantitative light-induced fluorescence)) for quantification of white spot lesions adjacent to fixed orthodontic appliances; and 2) to determine the inter-observer agreement of the DIAGNOdent and QLF methods for quantification of incipient enamel lesions adjacent to fixed orthodontic appliances. Forty-one premolar teeth with visually sound smooth surfaces or visually white spot enamel lesions were included in the study. Orthodontic brackets were fixed adjacent to the lesions, thus simulating the position of fixed appliances during orthodontic treatment. All teeth were measured using both the DIAGNOdent and QLF methods. Of the 41 teeth, 20 smooth surfaces were randomly selected and analyzed by 4 operators using both DIAGNOdent and QLF. The teeth were sectioned into 300-µm-thick slices using a water-cooled diamond saw and the slices manually ground to 80–100 µm thickness. Histopathology and transverse microradiography were performed to provide the gold standards for verification of lesion depth and mineral loss, respectively. The Spearman rank correlation coefficients between lesion depth determined by histopathology and the DIAGNOdent and QLF were 0.76 and 0.82, respectively, whereas the Pearson correlation coefficients between mineral loss and the two methods were 0.64 and 0.84, respectively. Inter-observer agreement was found to be 0.80 and 0.93 for DIAGNOdent and QLF, respectively. In conclusion, QLF may be a suitable method for quantifying incipient carious lesions adjacent to fixed orthodontic appliances. □ *Caries detection; smooth surface caries; laser fluorescence; orthodontic bracket*

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Dental caries is the result of an imbalance between demineralization and remineralization of dental hard tissues, and the process is influenced by challenge and resistance factors. Enamel demineralization with white spot formation on the buccal surfaces of teeth is a common side effect of orthodontic treatment with a fixed appliance (1). Fixed orthodontic appliances render tooth-cleaning more difficult and increase the risk of plaque and food retention. This results in a rise in number of micro-organisms such as mutans streptococci and lactobacilli (2, 3), which are associated with the initiation of dental caries and further development of the carious lesion. Consequently, patients undergoing orthodontic treatment with fixed appliances are at increased risk for caries on buccal surfaces. Gorelick et al. (4) reported an increase in white spot lesions in 50% of patients after orthodontic treatment. The prevalence of smooth-surface lesions also increases during treatment (1, 5, 6).

After removal of fixed orthodontic appliances, when oral hygiene is facilitated, the process of superficial enamel demineralization can be arrested, while affected areas may even be remineralized (7). A sensitive and reliable method for detecting and monitoring early carious lesions would therefore have a significant effect on caries prevention in the case of orthodontic patients during and after treatment. To date, several advanced methods for the early detection and quantification of carious lesions have been introduced and tested, e.g. the QLF (quantitative laser/light-induced fluorescence) method (7–13), DIAGNOdent (14–19), and Electronic Caries Monitor (ECM) (20). In the present study, the QLF and DIAGNOdent methods were employed to quantify white spot lesions around orthodontic brackets in vitro.

DIAGNOdent was introduced in 1998 (21, 22) as a complement to conventional probing and bitewing radiography for the detection and quantification of occlusal surface carious lesions. The tooth surface is illuminated with laser light (wavelength of 655 nm), which is mainly absorbed by the bacterial by-products existing in the porous carious lesion. Some of this light is re-emitted as near-infrared fluorescent light. Changes in the tooth substance associated with the amount of bacterial by-products are reflected as an increase in the re-emitted fluorescent light and thus the difference between sound and decayed areas is recognized. It has been reported that the amount of fluorescence relates more closely to bacterial presence than to mineral content of the tooth (23, 24). It would be of interest to determine whether

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DIAGNOdent is sensitive enough to detect and quantify small incipient carious lesions on smooth surfaces adjacent to orthodontic brackets.

QLF is an optical method for detecting and quantifying enamel caries. It has been evaluated in many in vitro studies and in a few clinical studies since its introduction 20 years ago. Visible light within the blue–green region is used as the light source for detecting smooth-surface caries at an early stage. The mechanisms of the method have been described in detail previously (7–11, 25). When the tooth is illuminated with a regular light emerging from an arc lamp provided with an optical bandpass filter with a peak intensity of 370 nm, autofluorescence occurs in the yellow region of the light spectrum. This fluorescence may be observed through a yellow highpass filter that excludes the tooth-scattered light. When enamel demineralization takes place, minerals will be replaced mainly by water, which results in a reduction of light absorption by the enamel, and the intensity of the fluorescence will decrease consequently. The demineralized region will therefore appear darker than the surrounding sound tooth structure.

The aims of this in vitro study were: 1) to evaluate and compare two fluorescence methods (DIAGNOdent and QLF (quantitative light-induced fluorescence)) for quantification of white spot lesions adjacent to fixed orthodontic appliances; and 2) to study the reliability of the DIAGNOdent and QLF methods in terms of inter-observer agreement for quantification of incipient enamel lesions adjacent to fixed orthodontic appliances.

Materials and methods

Teeth

The material comprised 41 premolar teeth which had been extracted for orthodontic reasons. The teeth had visually sound approximal or buccal surfaces or had incipient lesions on these surfaces. After extraction, the teeth were immediately placed in thymol-saturated saline and stored under refrigeration for no longer than 1 month. The teeth were thoroughly rinsed under tap water and cleaned with a toothbrush. Approximal surfaces were included in the study material to simulate buccal surfaces due to difficulty in collecting initial incipient lesions on buccal surfaces. Orthodontic brackets (Ormco with metal composition of 8.6% Ni, 0.1% Mo, 69.7% Fe, 18.5% Cr and Si+) were cemented with regular orthodontic composite cement in contact with the carious lesions, thus simulating the position of brackets in the clinical situation. Care was taken to avoid excess cement along the bracket, close to the lesion. After bonding, each tooth was wrapped in a tissue, dampened with thymol saline, and kept individually in plastic cans under refrigeration.

QLF

The QLF device employed in this study consisted of a camera (Panasonic WV-KS 152) with non-coherent light from a xenon lamp as the light source combined with a filter system. Each tooth was dried with compressed air for about 5 s and the QLF image was captured with a focus on the lesion and the edge of the bracket base, which was adjacent to the lesion. Data from all 41 teeth were collected by one operator. Image capturing was performed under identical conditions in a dimly lit room. The records were stored in a computer and analyzed using custom-made software (QLF 1.98i, Inspektor Research System BV, Amsterdam, The Netherlands). Reconstruction of lesion areas was then performed as follows: the patch line used as reference for reconstruction of the dematerialized region is set along the sound tissue close to the lesion. One border of the reconstruction patch nearest to the bracket was excluded due to lack of sound tissue between lesion and bracket. A satisfactory reconstruction is obtained subjectively by looking at the reconstructed images. Mean fluorescence loss in the lesion (Δ F) was calculated and registered. The QLF images of each tooth surface were printed as reference images to facilitate measurements with DIAGNOdent later on.

DIAGNOdent

Before measurement with DIAGNOdent, a pilot study on five extracted teeth with orthodontic brackets was carried out. Individual calibration was done on a sound spot and measurement was performed on the metal bracket. It was found that the metal orthodontic bracket did not give any fluorescent signal and thus had no effect on the DIAGNOdent readings.

Each tooth was retrieved from the thymol-saturated saline, wiped with a paper tissue, and dried in air for approximately 5 s. The measurements with DIAGNOdent were performed using a probe with conical tip, recommended for occlusal surfaces, in order to reach the area of the lesion close to the bracket base. The device was calibrated against a ceramic standard before each measurement session. The standard value for each tooth was calibrated before each measurement by measuring at a sound site. The lesion area or the visually sound surfaces were carefully scanned with the probe by holding the tip in contact with the tooth surface and tilted around the measuring site in order to collect the fluorescence from all directions. The maximum readings and the corresponding measuring sites in the picture were registered to facilitate subsequent preparation of the tooth slice.

Observer agreement

Twenty teeth were randomly selected from the 41 teeth. Three other operators repeated the same measuring procedure with DIAGNOdent as described above and under identical conditions. For the QLF method, three sets of new images were acquired and analyzed by each of the operators. When the measurements were complete, inter-

observer agreement from four operators including the first operator was calculated for both methods.

Histopathological and microradiographic analyses

When the measurements with DIAGNOdent and QLF had been completed, the teeth were prepared for the histopathological examination and microradiographic analysis, which served as the gold standards for validation of lesion depth and mineral loss, respectively. The brackets were carefully debonded from the tooth surface with a bracket remover. Each tooth was embedded in methylmetacrylate and sectioned with a diamond saw perpendicular to the occlusal surface in a bucco-lingual or mesiodistal direction; this was done at the test sites indicated on the pictures. Tooth slices, approximately 300-μm thick, were obtained and examined under the microscope at ×16 magnification. A 3-point scale was used to stratify the sites according to the histopathological appearance of demineralization: 0 = sound; 1 = enamel caries limited to the outer half of enamel; 2 = caries extending into the inner half of the enamel, but not to the dentino-enamel junction (DEJ). The tooth slices were then manually ground to 80–100 μm thickness and analysed with transverse microradiography (TMR) according to the method described by de Josselin de Jong et al. (26). The microradiographic image of each tooth slice and an aluminum step wedge for calibration were recorded on holographic film (Kodak SO-253) exposed to Ni-filtered Cu Kα radiation at 20 kV and 55 mA, with an exposure time of 20 s. The film was developed in accordance with the manufacturer's instruction. Mineral content depth profiles were recorded with a microscope-densitometer-computer set-up equipped with a 'Ikegami CCD camera' and software for TMR 2000 (Inspektor Research Systems BV, Amsterdam, The Netherlands), using the densitometric values to calculate the mineral content according to the formula of Angmar et al. (27). The tracings were expressed as integrated mineral loss (ΔZ) using the established parameter definition (28). The mean integrated mineral loss from three scans was obtained for each specimen.

Data analysis

Inter-observer agreement

The inter-observer agreement between the four operators, using QLF for capturing and analysis and DIAGNOdent for measurements of lesions in connection with orthodontic brackets, was analysed by intra-class correlation coefficient (ICC).

Correlation between lesion depth and fluorescence methods

Validation was performed using histological analysis as the gold standard to determine lesion depth. Spearman's rank correlation coefficient was calculated between histology and readings from DIAGNOdent and QLF.

Correlation between mineral loss and fluorescence methods

The integrated mineral loss was calculated from TMR analysis. The correlation between the integrated mineral loss and the mean fluorescence loss determined by QLF and the DIAGNOdent readings was analyzed using Pearson's correlation coefficient.

Results

The result of the histopathological examination is presented in Table 1. Of all the specimens, 16 were diagnosed as sound and 25 as having enamel lesions. There were no dentinal caries included in the material. The box plot (Fig. 1A, B) demonstrate the relation between lesion depth determined by the histological examination and the DIAGNOdent readings and mean fluorescence loss in the lesion measured with QLF. Spearman's rank correlation coefficients were $r = 0.76$ and $r = 0.82$ for DIAGNOdent and QLF, respectively. For assessment of the correlation between mineral loss as measured with TMR and readings from DIAGNOdent and QLF, the correlation coefficients were 0.64 and 0.84, respectively (Figs 2 and 3). The intra-class correlation coefficient (ICC) values for inter-observer agreement were 0.80 and 0.93 for DIAGNOdent and QLF, respectively.

Discussion

It is widely recognized that progression of an incipient carious lesion can be arrested and remineralized. This knowledge has resulted in the development of several diagnostic techniques for early detection and quantification of carious lesions. Carious lesions on buccal tooth surfaces are frequently found in orthodontic patients who are under treatment with fixed appliances, both during treatment and after removal of brackets (1, 4–6).

In the present study, two fluorescence-based methods, QLF and DIAGNOdent, were applied for detection and quantification of natural enamel lesions adjacent to orthodontic brackets. Histopathology and transverse microradiography (TMR) were employed as the gold standard for validation of lesion depth and mineral loss.

Table 1. Results of histopathological examination

Teeth	Number	%
Sound	16	39
Lesions extended to the outer half of enamel	20	49
Lesions extended to the inner half of enamel	5	12
Total	41	100

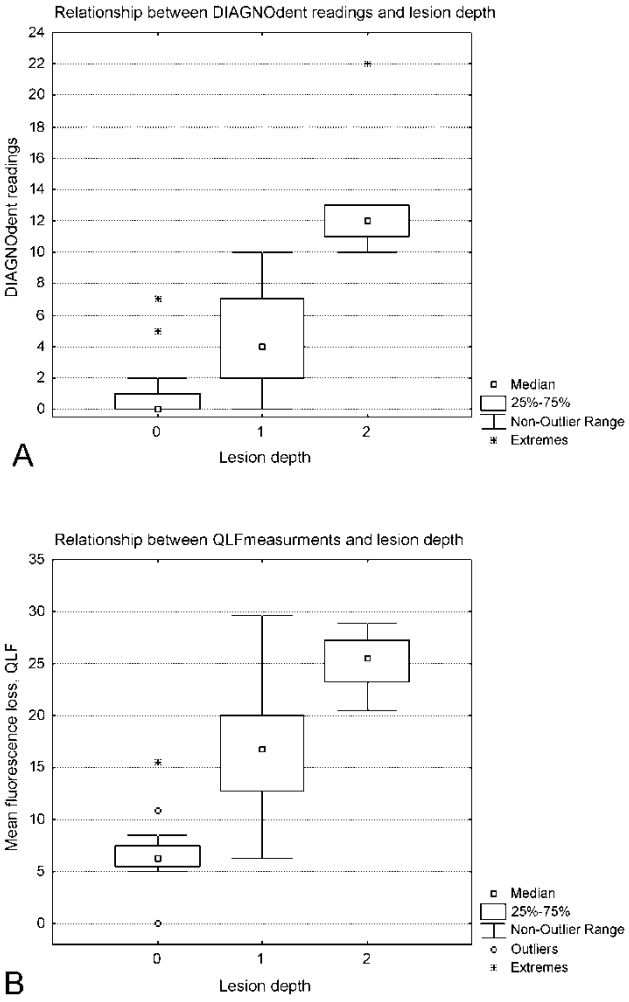

Fig. 1. A. Relationship between lesion depth determined by the histopathological examination and the DIAGNOdent readings. B. Relationship between lesion depth determined by the histopathological examination and mean fluorescence loss in the lesion registered with QLF.

The correlation analyses showed that the association between lesion depth and QLF readings was slightly stronger than the association between lesion depth and DIAGNOdent readings, 0.82 versus 0.76 (Fig. 1A, B). In Fig. 2, even though an *r*-value of 0.64 was obtained for DIAGNOdent based on this material, the data looked quite scattered compared with those of QLF, thus indicating that there was a weak correlation between DIAGNOdent readings and mineral loss in the lesions. Regarding mineral loss, the QLF data seemed to reflect the TMR assessments to a higher degree than the DIAGNOdent data (0.84 versus 0.64), implying that QLF is a better method for evaluating mineral loss in carious lesions in the enamel. This finding concurring with the results of other studies (7, 13, 29, 30) is not surprising, since the mechanism of the QLF method relates more to mineral changes than DIAGNOdent does.

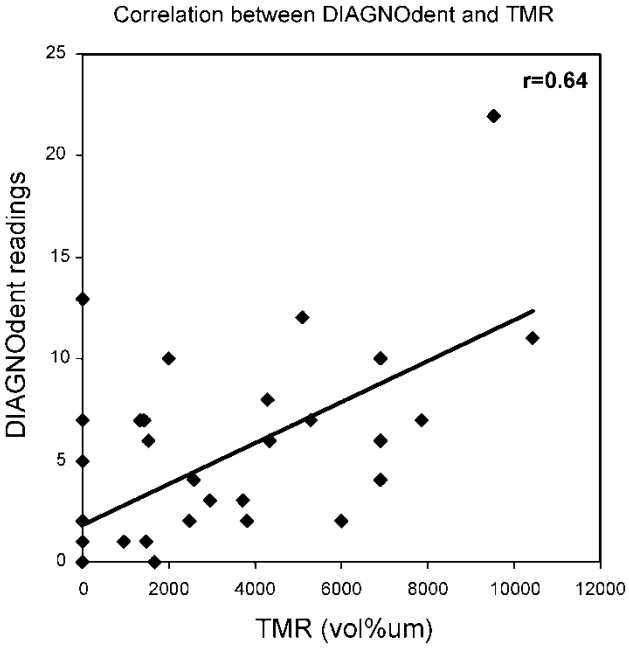

Fig. 2. Correlation between mineral loss determined with TMR and DIAGNOdent readings.

Figs 2 and 3 show a couple of observations with high QLF and DIAGNOdent readings in combination with no mineral loss according to TMR. This may result from several factors. One possibility could be a slight stain on the tooth surface, which could cause false-positive read-

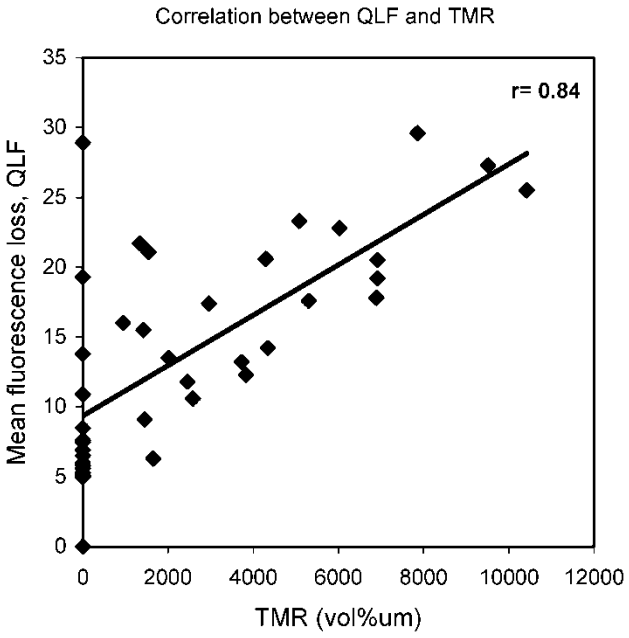

Fig. 3. Correlation between mineral loss determined with TMR and mean fluorescence loss in the lesion registered with QLF.

ings. It could also be due to the fact that the TMR data are representative of only a narrow region of the experimental area and the actual measuring spot might have been ground away during the tooth preparation, especially when the lesion was fairly small. Moreover, the measured fluorescence loss is a mean value over the whole area, while the mineral loss as measured with TMR represents the mineral loss in a thin tooth slice taken from this area. When plotting the linear regression line for QLF, if we had taken into consideration these specimens as false-positive and excluded them, the starting point of the regression line at the y-axis would have been close to 5%, which is a generally acceptable threshold for sound surface when applying the QLF method.

During the experiments, we found that the composite material used to cement orthodontic brackets generated some fluorescence, which was about −15% compared to the surrounding sound enamel using QLF. Therefore, for appropriate measurements, precautions should be taken to prevent excess cement along the bracket.

Reproducibility of the two methods applied in this study was assessed by intra-class correlation coefficient (ICC) for DIAGNOdent and QLF. The ICC values were 0.80 and 0.93, respectively. Combined with the good caries detection performance of these devices, the excellent reproducibility shown by QLF indicates that the method may be suitable for longitudinal monitoring of enamel lesions, which is in agreement with the results from studies on artificial enamel lesions of Benson et al. (31). QLF may thus also be useful for assessment of lesion status in patients at caries risk, such as orthodontic patients, and in evaluation of the outcome of preventive interventions.

However, for quantification on dentinal lesions, QLF may be less effective than DIAGNOdent, since it is suggested only for enamel lesions. Additionally, DIAGNOdent showed good reproducibility and the technique appears to be easier to use in dental practice and is readily transportable.

There are several factors which need to be considered before recommendations can be made for clinical application. 1) The material included in this study contained both approximal and buccal surface lesions. Approximal lesions usually have more absorbed material that might fluoresce than a typical lesion on a buccal surface and, thus, the readout from both devices might be overestimated based on our result. 2) Although the specimens included in the present study are natural incipient lesions, they are not characteristic orthodontic lesions. A typical orthodontic lesion usually spreads widely around the bracket, which makes it difficult to capture a QLF image with optimal light intensity in any part of the lesion. Furthermore, if the lesion is close to the gingiva and the bracket, reconstruction is more difficult due to insufficient sound tissue between lesion, bracket and gingiva. 3) Both methods have a number of potential sources of error, e.g. variation between operator performance, humidity of the tooth surfaces, the presence of plaque, calculus, stain, etc. (11, 15), and DIAGNOdent readings may be influenced by the storage media of the extracted teeth. So far, most of the available reports for both methods are laboratory studies, especially for the QLF method, and so more clinical studies need to be carried out.

Our results demonstrate that QLF is potentially useful in orthodontic practice to quantify and monitor lesions at an early stage and thus helpful in the decision either to stop treatment or to apply more preventive and therapeutic effort. In conclusion, compared with DIAGNOdent in vitro, QLF may be a useful method for the quantification and assessment of natural incipient enamel lesions around fixed orthodontic appliances.

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