Application of two fluorescence methods for detection and quantification of smooth surface carious lesions

Abdulaziz Saad Aljehani

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Application of two fluorescence methods for quantification of smooth surface caries

OPPONENT:
Professor Jukka Meurman, Department of Cariology, Institute of Dentistry, University of Helsinki, Helsinki, Finland

EXAMINING COMMITTEE:
Professor Björn Ögaard, Department of Orthodontics, Faculty of Dentistry, University of Oslo, Oslo, Norway
Professor Jan Huggare, Department of Orthodontics, Institute of Odontology, Karolinska Institutet, Huddinge, Sweden
Associate professor Solveig Fure, Department of Cariology, Faculty of Odontology, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

SUPERVISORS:
Dr. Xie-Qi Shi, Department of Cariology and Endodontology, Institute of Odontology, Karolinska Institutet, Sweden
Professor Birgit Angmar-Månsson, Department of Cariology and Endodontology, Institute of Odontology, Karolinska Institutet, Sweden

Author’s address:
Dr. Abdulaziz Aljehani
1) Karolinska Institutet
   Institute of Odontology
   Dept. of Cariology and Endodontology
   P.O. Box 4064
   141 04 Huddinge
   SWEDEN
   E-mail: Aljehani.Abdulaziz@ki.se

2) Security Force Hospital
   Dept. of Dentistry
   P.O. Box 5966
   Makkah City
   Saudi Arabia
   E-mail: Abdulaziz.Aljehani@hotmail.com

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Dedicated to my family who made significant contributions throughout the process of bringing this thesis to completion

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Abstract

Early diagnosis of carious lesions is a key factor in prevention and management of dental caries. With non-invasive, quantitative diagnostic methods it should be possible to detect lesions at an initial stage and subsequently monitor lesion changes over time during which preventive measures are introduced. Conventional methods are inadequate for a modern approach to clinical caries management. The need for objective and quantitative methods for detecting and monitoring progression of carious lesions is therefore obvious.

Aims

The present thesis is aimed at evaluating two fluorescence devices, DIAGNOdent and QLF for detection and quantification of carious lesions on smooth surfaces under both in vitro and in vivo conditions, with special reference to applications in orthodontic patients.

Material and methods

Validity of DIAGNOdent and QLF: (papers I, IV)

The validity of two fluorescence methods, DIAGNOdent and QLF, was evaluated for the purpose of caries detection and quantification on non-cavitated carious lesions on smooth surfaces. In paper I, the performance of these fluorescence methods was validated by microradiographic and histopathological analyses in order to correlate the two methods with mineral loss and lesion depth. In paper IV, the DIAGNOdent and the DIAGNOdent pen were validated by histopathological analysis in terms of Spearman’s rank correlation coefficient.

Reliability of DIAGNOdent and QLF: (papers I, II, IV)

The reliability of DIAGNOdent and QLF in terms of observer agreement on quantification of enamel caries on smooth surfaces was evaluated and compared under in vitro conditions employing Intra-class correlation coefficient (ICC) (paper I, IV). The inter- and intra-observer agreement of the DIAGNOdent method was also tested under in vivo conditions (paper II).

Application of DIAGNOdent as a monitoring tool for evaluation of preventive programs: (paper III)

The capacity of DIAGNOdent for monitoring caries progression was evaluated in a clinical study where twelve subjects with 127 test teeth were randomly assigned to two
caries preventive treatment groups. The lesion changes over a 12 months period were followed by DIAGNOdent. The mean changes of the DIAGNOdent values for the two treatment groups were analyzed by three-way ANOVA.

Results
In the in vitro studies on comparisons between the fluorescence methods with respect to the correlation with lesion depth, the correlation coefficients for DIAGNOdent and QLF were 0.76 and 0.82, respectively (paper I), and 0.55 and 0.52 for DIAGNOdent and DIAGNOdent pen, respectively (paper IV). The QLF method showed higher correlation with mineral loss, 0.84, compared to 0.64 for DIAGNOdent. The ICC values showed excellent intra-examiner agreement, and good inter-examiner agreement under both in vitro and in vivo conditions. For longitudinal quantification, there was a significant difference in the DIAGNOdent readings between the first and the final evaluation, p=0.025. However, the difference between the two treatment groups regarding the changes of DIAGNOdent values over time was not statistically significant, p=0.87.

Conclusions
It was concluded that the applied fluorescence methods are reliable and valid for quantification of natural carious lesions on smooth surfaces. The results suggested that it might be feasible to use DIAGNOdent for longitudinal monitoring of carious lesions on smooth surfaces. QLF and DIAGNOdent may aid clinical decision making in caries susceptible individuals, such as orthodontic patients.
List of papers included in the thesis

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals:


Introduction

During the past few decades, major changes have been observed not only in the prevalence of dental caries, but also in the distribution and pattern of the disease in the population (Marthaler, 1990, 2004). Most notably, the rate of lesion progression through the teeth has slowed. The chronic nature of the disease is reflected in slow lesion progression. This offers a window of opportunity for intervention to reverse the loss of mineral or arrest lesion progression, before the development of irreversible damage to the dental hard tissues. In clinical dental practice, the decrease in the rate of lesion progression has led to modification of thresholds for restorative intervention and a change towards a less invasive approach to management of the disease. The changes in prevalence and progression of dental caries may be attributed to the widespread use of fluoride containing dentifrices in combination with changes of diet and oral hygiene (Bratthall et al., 1996; Hänsel Petersson et al., 1996).

Smooth surface carious lesions are frequently found among patients with high caries activity, and in orthodontic patients after removal of fixed orthodontic appliances. On removal of the bands and brackets at the end of active orthodontic treatment, clinical examination discloses the presence of these lesions, which may range in severity from incipient, non-cavitated to advanced lesions. While minor lesions may be aesthetically disturbing, advanced lesions may require restorative treatment (Basdra et al., 1996; Gorton and Featherstone, 2003).

The prevalence of white spot lesions in patients who get orthodontic treatment is in the range of 50-96% (Gorelick et al., 1982; Øgaard et al., 1988; Gorton and Featherstone 2003; Boersma et al., 2005). This high prevalence is attributed to the presence of brackets, arch wires, ligatures and other orthodontic auxiliaries that complicate conventional oral hygiene measures, which in turn leads to increase the risk for plaque and food retention. With the increase in plaque retention, there is also an increase in the number of micro-organisms such as mutans streptococci and lactobacilli (Lundström and Krasse, 1987a,b; Chang et al., 1999), which are associated with the initiation and further development of dental caries.

Enamel demineralization and white spot lesions occur during and sometimes remain after orthodontic treatment (O’Reilly and Featherstone, 1985, Årtun and Brobakken, 1986). Studies have reported that visible white spot lesions can develop as quickly as
within only one month after the fitting of a fixed bonded orthodontic appliance (Øgaard et al., 1988, Melrose et al., 1996). This highlights the need for caries risk assessment at the beginning of treatment. Clearly, the best approach during orthodontic treatment is to prevent lesions occurring. It has been concluded that fluoride preparations, oral hygiene instruction and dietary control have the greatest effect on reducing demineralization (Millet et al., 1999).

On the basis of these current concepts of the disease process, lesion detection and early intervention, the primary goals of modern clinical management of caries are: to inhibit the initiation of new lesions, to arrest the progression of established lesions and to enhance the natural process of lesion repair by remineralization (Featherstone, 2004). A further goal, reflecting the reality of decision-making in general dental practice, is to recognize irreversible loss of tooth structure early, and to restore it using minimally invasive therapy.

It is clear that key factors in implementing treatment based on these principles are the accurate detection of carious lesions and quantification of lesion progression over time. Interpretation of these data, as part of the overall diagnosis, allows the clinician flexibility in selecting intervention appropriate to the individual patient.

**Conventional methods for quantification of smooth surface carious lesions**

For several decades, the accepted method for detecting carious lesions in patients, as well as in clinical trials, has been a combination of clinical examination (visual-tactile, light, mirror, and probing) and bitewing radiographs. Lesions on buccal and lingual surfaces are usually detected solely by visual inspection. The appearance of a suspected lesion is judged by its color, white or brown, lesion reflectance, shiny or dull, and surface texture, rough or smooth. Visual inspection is based on subjective evaluations, which may lead to large diagnostic variations among different examiners. Furthermore, this method is qualitative, which restricts its applications, such as for monitoring lesion development and controlling the effectiveness of preventive procedures.

The use of the dental explorer to probe enamel is no longer recommended because probing was shown to potentially damage the enamel surface of subsurface carious lesions and even cause cavitation, thereby increasing the rate of progression of a carious lesion (Ekstrand et al., 1987; van Dorp et al., 1988).
Thus it is necessary and important to look for and test new objective and quantitative methods in order to aid clinicians in detecting and monitoring progression of carious lesions on smooth surfaces.

**Laser fluorescence methods for caries detection and quantification**

As a complement to conventional methods, several new diagnostic techniques for the purpose of detection and monitoring progression of early carious lesions have been introduced and tested. Among these, two laser fluorescence methods: quantitative light-induced fluorescence method, QLF (de Josselin de Jong *et al.*, 1995; Emami *et al.*, 1996; Al-Khateeb *et al.*, 1997, 1998; Tranaeus *et al.*, 2001a,b), and DIAGNOdent (Shi *et al.*, 2000, 2001a,b; Pinelli *et al.*, 2002; Staudt *et al.*, 2004; Mendes *et al.*, 2005) have generated a great deal of research interest in the early detection of carious lesions on occlusal and smooth surfaces.

**The near infrared fluorescence method - DIAGNOdent**

Fluorescence is a well-known phenomenon in science and technology. In simple terms, light at one wavelength (excitation wavelength) is absorbed by a substance and emitted as a second longer wavelength (emission wavelength). The auto fluorescence phenomenon of dental hard tissues has been known for a very long time (Benedict, 1928). The cause of baseline fluorescence in sound teeth is still unclear. It might be the result of combining the inorganic matrix with absorbing organic molecules (Hibst *et al.*, 2001). Red light, as well as infrared fluorescence radiation, is less absorbed and scattered by enamel than light of shorter wavelengths, so that it penetrates the tooth more deeply (Hibst *et al.*, 2001). It is therefore possible to measure fluorescence from underlying carious dentin.

Hibst and Gall (1998) found that red light induced fluorescence could differentiate between sound and carious tooth tissue. Fluorescence spectroscopic investigations revealed considerable contrast between sound and carious tooth tissues when excited by red light with a wavelength of 655 nm (Hibst *et al.*, 2001). Fluorescence was found to be more intense in carious tissue compared with sound tissue (Figure 1).
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Fig. 1. Fluorescence spectra of sound and carious tooth areas when excited by red light, 655 nm, (Lussi et al., J Dent Res 2004).

Based on the above mentioned fact, a laser–based instrument, DIAGNOdent (KaVo, Biberach, Germany), for the detection and quantification of carious lesions was introduced in 1998 (König et al. 1998; Hibst and Gall, 1998). The main unit of DIAGNOdent is connected to a hand probe by a cable, with descendant and ascendant optical glass fibers. The probe comes with 2 attachments, one with the tapered tip “A”, intended for occlusal surfaces, and the other with the flat tip “B”, intended for smooth surfaces (Figure 2).

Fig. 2. DIAGNOdent (original version)
The DIAGNOdent unit contains a laser diode (655 nm, modulated, 1 mW peak power) as the excitation light source, and a photo diode combined with a band pass filter (transmission > 680 nm) as the detector. Laser light is generated by the main unit and transmitted through the excitation optical fiber to the tip of the hand piece. Once the tip is in contact with a tooth surface, the laser energy penetrates the tooth surface and is absorbed by the surrounding tooth material, and fluorescence within the infra-red spectrum occurs. The emitted fluorescence, as well as backscattered ambient light, is collected by the tip and carried back to a photo diode detector in the main unit via the detection fibers. The band pass filter absorbs the backscattered excitation and other short wavelength ambient light and transmits the long-wavelength fluorescence radiation. To eliminate the long-wavelength ambient light also passing through the filter, the laser diode is modulated, and only light showing the same modulation characteristic is registered by the main unit and displayed as nominal values ranging from 0 to 99, where 0 indicates minimum and 99 maximum fluorescence. The principle of DIAGNOdent is presented in Figure 3.

Fig. 3. The DIAGNOdent principle (Lussi et al., J Dent Res 2004).

Hibst and Gall (1998) reported that carious tissue emits stronger fluorescence than sound tissue in the red and infrared part of the spectrum (\(\lambda = 655\) nm). Thus, the fluorescence from a carious region, greater than that from sound tissue, is expressed as a higher numerical readout by the device. The exact mechanism of detection has not been
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fully clarified; however, one assumption is that the device measures the fluorescence of bacterial disintegrated products within carious tissues, possibly porphyrins (Hibst et al., 2001).

Recently a new version of DIAGNOdent, the DIAGNOdent pen, was introduced. This new device is based on the same principle as the original DIAGNOdent. The main unit measures 21 cm with two sapphire tips, wedge shaped and tapered shaped. The thickness of the wedge shaped tip is only 0.4 mm with a width of 1.1 mm in order to improve the access to proximal surfaces whereas the thickness of the tapered shaped tip is 0.7 mm. The device is cordless and handy to operate (Figure 4).

Fig. 4. The DIAGNOdent pen

The DIAGNOdent device has been studied extensively both in vivo and in vitro for detection of carious lesions for occlusal and smooth surface caries detection (for a review see Bader and Shugars, 2004).


Recently DIAGNOdent has been tested in vitro for quantification of lesions adjacent to fixed orthodontic appliances (Staudt et al., 2004). The results suggested that the DIAGNOdent method might be appropriate for early detection and assessment of white spot lesions in orthodontic patients. To our knowledge there are to date no published studies evaluating the clinical application of DIAGNOdent for detection and monitoring of white spot lesions on smooth surfaces in orthodontic patients following fixed appliance therapy.

To achieve the maximum extension of the lesion volume, one must tilt the instrument around the measuring site with continuous rotation of the probe tip around its axis during the measuring period. This ensures that the tip picks up fluorescence from all
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directions. There are several factors that need to be taken into consideration when using DIAGNOdent. The manufacturer recommends that all measurements should be made at room temperature of about 22º C and the tooth surfaces should be dry. Calibration of the device against the ceramic standard before the measurement session is important to ensure accurate measurements over an extended period of time (Karlsson et al., 2004). It is also essential to perform the measurements on a clean tooth because the instrument is very sensitive to the presence of stain, deposits, calculus, as well as certain types of composite filling material, and remnants of pastes, which may produce fluorescence and therefore, cause false-positive readings (Lussi et al., 1999, 2005; Shi et al., 2000). Further, potential sources of error should be considered such as storage media for extracted teeth, which may affect the DIAGNOdent values and make it difficult to directly apply cut-off values obtained from in vitro studies to values under clinical conditions (Shi et al., 2001b).

QLF: Quantitative laser/light-induced fluorescence

In the beginning of 1980s laser light was used to induce auto fluorescence of enamel, and was employed to develop a sensitive, non-destructive diagnostic method for detection of enamel demineralization and dental caries (Bjelkhagen et al., 1982). Since then this method has been developed, validated and applied to in vitro, in situ and in vivo studies (Hafström-Björkman et al., 1992; de Josselin, de Jong et al., 1995; Emami et al., 1996, Al-Khateeb et al., 1997a,b 1998; Tranaeus et al., 2001a).

The QLF equipment comprises a light source that generates non-coherent light from a xenon lamp provided with an optical band pass filter with a peak intensity of 370 nm in order to produce violet-blue light. A CCD based digital camera and a PC are also included in the equipment. When violet-blue light illuminates the tooth surface, a digital fluorescent image is captured by the camera, transferred to the computer and displayed on the monitor simultaneously. Data are collected, stored, and analyzed by custom-made software (Figure 5).

The QLF method is based on the principle that mineral loss, caused by carious destruction of tooth enamel, can be detected and measured as a decrease in fluorescence intensity when exposed to violet-blue light. The fluorescence of a carious lesion viewed by QLF is lower than that of sound tissue and thus appears as a dark area in a QLF.
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image. Several effects may contribute to a decreased fluorescence of incipient carious lesions; the most probable are the following: firstly, light scattering in a lesion, which is much stronger than in sound enamel (Angmar-Månsson and ten Bosch, 2001), causes the light path in the lesion to be shorter than in sound enamel, thus the absorption per volume unit is small in the lesion and the fluorescence will become low. Secondly, light scattering in the lesion acts as a barrier, blocking excitation light from reaching the underlying fluorescent dentine, and also as a barrier to fluorescence light from dentine from reaching the tooth surface.

Fig. 5. The QLF principle (Stookey, Dent Clin North Am 2005)

There is a relation between mineral loss and fluorescent radiance, i.e. decreasing fluorescence with increasing mineral loss (Emami et al., 1996; Al-Khateeb et al., 1997 a, b). To enable calculation of loss of fluorescence in the carious lesion, the fluorescent radiance of sound tissue at the lesion site is reconstructed by interpolation from the radiance of the sound tissue surrounding the lesion (de Josselin de Jong et al., 1995). The difference between the measured values and the reconstructed values gives the resulting fluorescence loss in the lesion.

Studies have shown that QLF is a sensitive method for detecting and monitoring carious lesions of smooth surfaces (for review see Stookey, 2005). With application of the QLF method, clinical studies intended to assess the effectiveness of caries preventive measures took less time and were hence cheaper. The QLF not only provides quantitative data but also displays the fluorescence image of the tooth on the monitor and this is highly instructive for demonstrating lesion progression and/or regression to
the patient with no danger to operator or patient. However, a quantitative evaluation can only take place if a variety of confounding factors are controlled. As the surface of the tooth or the lesion may be covered by dental plaque, calculus, and/or extrinsic discolorations, professional cleaning prior to QLF recording is essential. The effect of dehydration is a potential source of error for *in vitro* studies using QLF. There are also other reasons for measurement errors, including examiner effects, subject effects and imperfect calibration. The daylight or office light may influence the quality of the QLF image. The method does not differentiate between active and arrested carious lesions or between carious lesions and developmental defects with lower mineral content. If gingivitis exists, gum bleeding and/or an increased sulcus fluid flow rate may interfere with both the capture of fluorescence images and the following analysis. It was noted that the orientation of the camera when capturing the QLF image was important in visualizing different aspects of the tooth surface (Benson *et al.*, 2003). The equipment is still expensive, but will probably become less expensive in the future.
Aims

General aim

The present thesis aimed at evaluating two fluorescence devices, DIAGNOdent and Quantitative Light-Induced Fluorescence method (QLF) for detection and quantification of carious lesions on smooth surfaces in vitro and in vivo.

Specific aims

1. To validate the DIAGNOdent method in vitro and compare it with Quantitative Light-induced Fluorescence (QLF) to enable quantification of white spot lesions adjacent to orthodontic brackets (Paper I).
2. To compare the reliability of DIAGNOdent with the QLF method in terms of inter-observer agreement of in vitro quantification of enamel lesions adjacent to orthodontic brackets (Paper I).
3. To evaluate the reliability of DIAGNOdent in terms of intra- and inter-observer agreement, enabling quantification of white spot lesions in orthodontic patients subsequent to fixed appliance therapy (Paper II).
4. To monitor changes in incipient carious lesions that had been formed around orthodontic bands and brackets in vivo, using DIAGNOdent (Paper III).
5. To evaluate the effect of two preventive programs on regression of incipient carious lesions that had been formed in vivo around orthodontic bands and brackets during orthodontic treatment (Paper III).
6. To compare the validity and reliability of the newly introduced DIAGNOdent pen with those of the original DIAGNOdent device used for in vitro quantification of carious lesions on smooth surfaces (Paper IV).
Materials and methods

All four studies were approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (124/02, 60/03, and 564/03).

Materials:

Extracted teeth

The material used in the in vitro studies is summarized in Table 1.

In Papers I and IV, the material comprised premolar teeth that had been extracted for orthodontic reasons. The teeth were thoroughly rinsed under tap water and cleaned with a toothbrush.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Teeth</th>
<th>Visual appearance</th>
<th>Examined site</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>41</td>
<td>visually sound; incipient caries</td>
<td>smooth surface around bracket margin</td>
<td>DIAGNOdent QLF</td>
</tr>
<tr>
<td>IV</td>
<td>52</td>
<td>visually sound; non-cavitated carious lesions</td>
<td>smooth surface</td>
<td>DIAGNOdent pen</td>
</tr>
</tbody>
</table>

*Table 1. Study material*

In paper I, 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 simulating the position of brackets under clinical situation as demonstrated in Figure 6.

Precaution was taken to avoid excess cement along the bracket, close to the lesion. The teeth in Papers I, and IV were numbered and stored under refrigeration in thymol-saturated saline, in individual plastic containers.
Fig. 6. Tooth specimens cemented with orthodontic bracket under ordinary light

Patients
In Papers II, 137 test teeth from 13 patients with ages between 13 and 17 years were recruited from King Abdulaziz Hospital, Makkah, Saudi Arabia. The subjects were selected from orthodontic patients who had recently completed full fixed appliance therapy with an average treatment time of two years.

In Paper III, 127 test teeth from 12 patients exhibiting white spot lesions on the buccal surfaces were obtained from 12 subjects ranging in ages from 13 to 17 years.

Diagnostic methods:
Quantitative light-induced Fluorescence: Paper I

The QLF device (QLF 1.98i, Inspektor Research System BV, Amsterdam, the Netherlands) 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. A schematic drawing of the experimental setup is presented in Figure 7. QLF images of 41 smooth surfaces were captured with focus on the lesion and the edge of the bracket base. Reconstruction of the sound fluorescence of the lesion areas was then performed as follows; the patch line used as reference for reconstruction of the demineralized region was 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 was obtained by visual evaluation of the reconstructed images. Mean fluorescence loss in the lesion (Δ F) was calculated and registered. The QLF images of each tooth surface were printed out as reference images to facilitate measurements with DIAGNODent later on.
Before starting the measurements with DIAGNOdent, each measuring site was documented with either a digital photograph (Papers II, III, IV) or with a QLF image (Paper I). As recommended by the manufacturer, prior to every measurement session, each DIAGNOdent instrument was calibrated against its own supplied ceramic standard. First the standard value for each tooth was calibrated by measuring a sound site on the buccal surface. The lesion area or the visually sound surfaces were carefully scanned with the probe by holding the tip in contact with tooth surface and tilting the tip 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 the follow-up measurement or/and subsequent preparation of the tooth slice for histopathological analysis. Measuring details are listed in Table 2 for the four studies.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Condition</th>
<th>Tip</th>
<th>Measuring time</th>
<th>Humidity</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>in vitro</em></td>
<td>conical</td>
<td>10 s</td>
<td>dry 5 s</td>
<td>thymol</td>
</tr>
<tr>
<td>II</td>
<td><em>in vivo</em></td>
<td>flat</td>
<td>10 s</td>
<td>dry 5 s</td>
<td>oral cavity</td>
</tr>
<tr>
<td>III</td>
<td><em>in vivo</em></td>
<td>flat</td>
<td>10 s</td>
<td>dry 5 s</td>
<td>oral cavity</td>
</tr>
<tr>
<td>IV</td>
<td><em>in vitro</em></td>
<td>flat</td>
<td>10 s</td>
<td>dry 5 s</td>
<td>thymol</td>
</tr>
</tbody>
</table>

*Table 2. Measuring details*
Figure 8 illustrates the experimental setup of the DIAGNOdent measurement (Papers I, IV) and a clinical picture of white spot lesions (Papers II, III) after removal of orthodontic brackets.

**Fig. 8.** a. Experimental setup of the DIAGNOdent measurement; b. An example of a lesion in studies II, III, after removal of orthodontic brackets

Before the measurement with DIAGNOdent in Paper I, a pilot study on 5 extracted teeth with orthodontic brackets was carried out to test whether the bracket emits fluorescence when using DIAGNOdent. 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.

**Visual inspection: Papers II, III**

Visual examination was included in the clinical investigations, *i.e.* Papers II and III. Three examiners, two cariologists and one orthodontist, worked together to localize a lesion and to perform caries assessment. In case of disagreement, consensus was reached. Clinical caries assessment was performed by the same observers with the help of mouth mirrors and blunt probes under clinical lighting, according to the modified Ekstrand criteria and classification presented in Table 3 (Ekstrand *et al.*, 1997). The inclusion criterion was the presence of a white spot lesion on a smooth surface adjacent to the site of the orthodontic band or bracket.
<table>
<thead>
<tr>
<th>Code</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No or slight change in enamel translucency after prolonged air drying.</td>
</tr>
<tr>
<td>1</td>
<td>Opacity (white) hardly visible on the wet surface, but distinctly visible after air-drying</td>
</tr>
<tr>
<td>2</td>
<td>Opacity (white) distinctly visible without air-drying.</td>
</tr>
<tr>
<td>3</td>
<td>Localised enamel breakdown in opaque or discolored enamel and or greyish discoloration from the underlying dentine.</td>
</tr>
</tbody>
</table>

Table 3. Visual inspection criteria (modified Ekstrand criteria)

Validation of the diagnostic methods: (Papers I, IV)

The *in vitro* performance of the two laser fluorescence methods was validated by histopathological analysis (Papers I, IV) and transverse micro radiography (Paper I) to determine the lesion depth and mineral loss within the lesions, respectively.

**Histopathological analyses**

At the end of the experiments in Papers I and IV the teeth were prepared for histopathological examination, which served as gold standard in the validation of lesion depth. Each tooth was embedded in methyl-metacrylate and sectioned with a water-cooled diamond saw perpendicular to the occlusal surface in a bucco-lingual or mesio-distal direction at the test sites indicated on the pictures for the DIAGNOdent measurements. Tooth slices, approximately 300 µm thick, were obtained and examined under microscope at x16 magnification. The following 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). 3 = caries penetrating the DEJ but limited to the outer half of the dentin; 4 = caries involving the inner half of the dentin.

**Microradiographic analyses**

For the validation of mineral loss in Paper I, quantitative transverse micro radiography (TMR) was employed to analyze the correlation between the mineral loss in the lesions on one hand, and the QLF and DIAGNOdent reading on other. After histological
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analysis, the tooth slices of 300 µm thickness, were manually ground to 80-100 µm thickness and analysed with transverse micro radiography (TMR) according to the method described by de Josselin de Jong et al. (1987) for microradiographic analysis. 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 according to 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. (1963). The tracings were expressed as integrated mineral loss (ΔZ), using the established parameter definition (Arends and ten Bosch, 1992). The mean integrated mineral loss from three scans was obtained for each specimen.

Reliability of the diagnostic methods: (Papers I, II, IV)

Once the measuring procedures of both methods had been established and carried out by the first operator, the measurements were repeated after a few days by the same and/or another investigator. Detailed information such as the number of teeth and observers involved in each study is listed in Table 4. For studying the reproducibility of the QLF method (paper I) 3 sets of new images were acquired and analyzed by each operator, respectively. To determine the reliability of DIAGNOdent (Papers II, IV), the pre-selected measuring sites documented by digital photographs were scanned under identical conditions. The DIAGNOdent reading was noted and the corresponding site was registered on the photographs. After a 1-week interval, the same measuring procedures were repeated by the same observers. The observers again took independent readings of the same sites using the photographs as a guide. No records of the DIAGNOdent readings from the first session were available during the second measurement session. On conclusion of all measurements with the diagnostic methods, intra-and inter-observer agreements were analysed.
Application of DIAGNOdent as a monitoring tool for evaluation of preventive programs: (Paper III)

Following completed orthodontic therapy the twelve subjects with 127 test teeth exhibiting white spot lesions on the buccal surfaces were randomly divided into two treatment groups. The experimental group received professional teeth cleaning combined with oral hygiene instruction, while the comparison group received oral hygiene instruction only. The pre-selected measuring sites were measured by DIAGNOdent and at baseline 3, 6, 9, and 12 months. The numerical readings given by the DIAGNOdent were recorded in a case report form (CRF). No records of the DIAGNOdent readings from the previous measurement sessions were available at the follow up measurement sessions.

Statistical analyses

Validity: Correlation coefficient

Spearman’s Rank correlation coefficient was performed to analyze the correlation between the gold standard for lesion depth determined by histopathology and the readings of the diagnostic methods in Papers I, IV. The correlation between the integrated mineral loss and the mean fluorescence loss determined by QLF and the DIAGNOdent readings (Paper I), as well as the correlation between DIAGNOdent and the DIAGNOdent pen (Paper IV) was analyzed using Pearson’s correlation coefficient. Mean changes of the DIAGNOdent values during five visits over a period of one year for the two treatment groups in Paper III were analyzed with a three-way ANOVA with repeated measures of two factors. The within factors are time (five time points) and

<table>
<thead>
<tr>
<th>Paper</th>
<th>Condition</th>
<th>Samples</th>
<th>Pre-training</th>
<th>Observers</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>in vitro</td>
<td>20</td>
<td>no</td>
<td>4</td>
<td>inter-observer</td>
</tr>
<tr>
<td>II</td>
<td>in vivo</td>
<td>137</td>
<td>yes</td>
<td>3</td>
<td>intra-and inter-observer</td>
</tr>
<tr>
<td>IV</td>
<td>in vitro</td>
<td>52</td>
<td>yes</td>
<td>2</td>
<td>intra-and inter-observer</td>
</tr>
</tbody>
</table>

Table 4. Reliability studies
Application of two fluorescence methods for quantification of smooth surface caries

quadrant (four levels) and the between factor is treatment group (OHI, and OHI plus PTC). Statistically significant difference was accepted at P < 0.05.

**Reliability**: inter-and intra-observer agreement

Intra-class correlation coefficient (ICC) was used to evaluate the level of agreement between and within observers in the two measurement sessions for both *in vitro* and *in vivo* comparisons.

**Results**

*Validity of the diagnostic methods*

The distribution of teeth with respect to lesion depth in the *in vitro* studies is listed in Table 5.

<table>
<thead>
<tr>
<th>Lesion Depth</th>
<th>Paper I</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Lesions involving the outer half of enamel</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Lesions involving the inner half of enamel</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>Lesions involving the outer half of dentin</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Lesions involving the inner half of dentin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 5.** Results from histopathology: number (n) and percentage (%) of sound and carious teeth.

In the *in vitro* studies with comparison between the fluorescence methods and lesion depth, Spearman’s rank correlation coefficient for DIAGNOdent and QLF were 0.76 and 0.82, respectively, whereas the Pearson’s correlation coefficient between mineral loss as measured with TMR and readings from the two methods were 0.64 and 0.84, respectively (Paper I).

In Paper IV, the correlations with histology were 0.55 and 0.52 for DIAGNOdent and the DIAGNOdent pen, respectively, whereas the Pearson’s correlation coefficient between DIAGNOdent and the DIAGNOdent pen at the first and second measurement sessions were 0.92 and 0.96, respectively.
Reliability of the diagnostic methods

In Paper I, the inter-observer agreement between the four operators on measurements of enamel carious lesions in connection with orthodontic brackets were 0.80 and 0.93 for DIAGNOdent and QLF, respectively.

In Paper IV, the ICC values for intra-observer agreements were excellent for both DIAGNOdent and the DIAGNOdent pen, 0.98, whereas the inter-observer agreements were relatively lower, 0.70 and 0.68, for the DIAGNOdent and DIAGNOdent pen, respectively.

In Paper II, under in vivo conditions, the reliability of DIAGNOdent was excellent in terms of intra-examiner agreement, 0.95, and the inter-examiner agreement was good, 0.69 and 0.82 for the first and second measurements, respectively.

Evaluation of preventive programs using DIAGNOdent as a monitoring tool

In Paper III, for longitudinal quantification of carious lesions on smooth surfaces, there was a significant difference in the DIAGNOdent readings between the first and the final evaluation, p=0.025. However, the difference between the two treatment groups regarding changes of the DIAGNOdent values over time was not statistically significant, p=0.87.

Spearman’s rank correlation coefficient between the visual scores and the DIAGNOdent readings was 0.63. The differences between the visual scores of the baseline and those of the final examination were not statically significant, p=0.8.
Discussion

Appropriate diagnosis of carious lesions at their earliest stages is a major factor in prevention and management of dental caries. Carious lesions on smooth surfaces are usually visually detected first as white spot lesions. However, visual inspection alone is inadequate to monitor changes of a lesion over time. Thus objective and quantitative diagnostic methods would improve the potential for appropriate clinical caries management.

The studies on which this thesis is based were designed to evaluate the application of fluorescence methods in order to aid the quantification of smooth surface carious lesions. The methods were evaluated with special reference to their application to the teeth of orthodontic patients, where the risk of developing carious lesions is high. The applications of these methods were not only in detection and quantification of lesions on smooth surfaces, but also for monitoring lesion changes following preventive intervention. The reliability of the two fluorescence methods, in quantification of smooth surface caries in terms of agreement within and between operators, was also evaluated.

In the present thesis the primary aim was to evaluate the two fluorescence devices, DIAGNOdent and the quantitative light-induced fluorescence method (QLF) used in in vitro detection and quantification of carious lesions on smooth surfaces adjacent to fixed orthodontic brackets simulating clinical situations. The methods were validated by two well established methods as gold standard; histopathology and transverse microradiography, used here in the determination of lesion depth and mineral loss. The correlation analyses showed that the associations between the two fluorescence methods and lesion depth were comparable (0.82 and 0.76), indicating good correlations with gold standard. Similar correlation with lesion depth for the two methods was also found in earlier studies (Shi et al., 2001a, b). When validated by TMR for mineral loss the QLF method had higher correlation coefficient (r=0.84) than the DIAGNOdent (r=0.64). This implies that QLF is a better method for evaluating mineral changes in the enamel lesions, a finding which is in agreement with the results from previous studies (Al-Khateeb et al., 1998, Shi et al., 2001a, Benson et al., 2003, Pretty et al., 2003). The higher correlation with TMR for the QLF method could be expected since the
mechanisms behind the function of the QLF method are more directly related to the
changes in mineral content, whereas in the DIAGNOdent method the increase of
fluorescence due to the presence of caries is related to bacterial metabolites, rather than
to crystalline disintegration (Hibst and Paulus, 1999).
However, DIAGNOdent compared well with QLF in respect of correlation with lesion
depth for quantification of natural carious lesions on smooth surfaces, while QLF
showed closer correlation with mineral changes, and is therefore the method of
preference for scientific purposes. The QLF method has many merits, however, because
of its hard- and software components it is rather an expensive technique. For dentists
who are not familiar with image reconstruction software it may be cumbersome in the
conventional dental setting. Furthermore, another major disadvantage of QLF is related
to technical limitations of the method, namely the technique can not be applied to any
white spot lesion on the smooth surface in the orthodontic patient, e.g. if the lesion is
situated close to the gingiva and/or to the orthodontic bracket, the reconstruction of the
sound fluorescence becomes rather difficult due to insufficient sound tissue between
lesion, bracket and gingiva. A less expensive, more compact device such as
DIAGNOdent, would probably find greater acceptance by clinicians to use in dental
practice for longitudinal monitoring of lesion response to preventive measures in their
caries risk patients.
For clarification, in the present thesis, the DIAGNOdent method was selected for
further investigations in possible application for scientific purpose, such as evaluation
of preventive programs. In this context, the DIAGNOdent method was applied as a
monitoring tool to evaluate the effect of two preventive programs on incipient carious
lesions, which had been formed in vivo around orthodontic bands and brackets during
orthodontic treatment. As mentioned before an important feature of a diagnostic method
is to provide consistent and standardized measurements between and within observers.
In Paper II, the reliability of DIAGNOdent was evaluated in orthodontic patients under
clinical conditions, in order to determine whether the results of the in vitro study (Paper
I) discussed above were reproducible, and with respect to possibly affecting variables
under clinical conditions. Excellent intra-examiner agreement, 0.96, was found with in
vivo quantification of white spot lesions on smooth surfaces, which is in good
agreement with previous reports by Shi et al., (2001b) under in vitro conditions, and
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under in vivo conditions (Pinelli et al., 2002). The inter-observer agreement of 0.75 is in general agreement with the findings of Paper I, and with those from a recent in vitro study on smooth surface caries (Pinelli et al., 2002). However, less experienced operators would probably need increased training to obtain the necessary skills in the technique. These findings of high reliability of DIAGNOdent indicate consistent performance. Thus, the method may be suitable for longitudinal monitoring of carious lesions and thus aid clinical decision making with respect to management of orthodontically induced smooth surface lesions, provided that DIAGNOdent is sensitive enough to detect small changes in carious lesions over time.

To test the applicability of DIAGNOdent for monitoring caries progression, DIAGNOdent was applied to in vivo longitudinal assessment of white spot lesions over a period of one year. During this year the effect of two intervention programs intended to arrest or reverse lesion progression were compared using the DIAGNOdent registrations (Paper III). All the lesions were also examined by conventional visual inspection during the de-bonding visit and 12 months thereafter. The differences between the visual scores of the baseline and those at the final examination were not statistically significant, p=0.8. In other words, the progression/regression of the white spot lesions during a period of one year could not be registered by means of visual inspection. A similar finding was reported in a previous study (Heinrich-Weltzien et al., 2005) which demonstrated a weakness in visual inspection for detection of smooth surface initial carious lesions. Although improvements in visual inspection with new scoring systems seem promising (Ekstrand et al. 1997; Nyvad et al., 1999; Fyffe et al., 2000), further clinical validation is still necessary.

Unlike the findings from visual inspection, the DIAGNOdent readings showed statistically significant differences between the first and the final evaluations (p=0.025). Generally, the mean DIAGNOdent values for all teeth had decreased by the final evaluation, reflecting regression of the carious lesions. DIAGNOdent thus offered possible means of detecting and quantifying minor changes that had occurred during the study period of one year. These findings confirm the results from a previous study applying the QLF method (Al-Khateeb et al., 1998).
In Paper III, none of the lesions had fully recovered after one year. The white spot lesions formed around the brackets during orthodontic treatment might possibly be remineralized over a relatively long period of time. As was reported in a previous study (Øgaard et al., 1989), white spot lesions were found to be present 5 years after the completion of orthodontic treatment.

Fluoride per se can not prevent caries development if the cariogenic challenge is unchanged (Holmen et al., 1986). Good oral hygiene and improved dietary habits are essential prerequisites to initiate the healing process of the white spot lesions (Årtun and Thylstrup, 1986, 1989; Holmen et al., 1987). Following these principles, no excessive topical fluoride in addition to the recommended fluoride toothpaste was supplied (Paper III). The degree of remineralization was associated with a reduction of the cariogenic and microbial challenge by oral hygiene instruction and professional teeth cleaning. However, in our study (Paper III), there was no statistically significant difference between the effects of the two preventive programs regarding changes of the DIAGNOdent values over time. The combination of professional teeth cleaning and oral hygiene instruction had the similar efficacy as oral hygiene instruction only on promoting re-mineralization of white spot lesions. However, it should be further explored whether long term or more frequent application of professional tooth cleaning might have a significant effect on the re-mineralization of white spot lesions.

In Paper IV, the performance of two different DIAGNOdent devices for quantification of smooth surface carious lesions were compared. The results showed that both DIAGNOdent and the DIAGNOdent pen had excellent intra-observer agreements and moderate inter-observer agreements. The conformity on the device level was also tested where excellent agreement was found between the two generations of DIAGNOdent device. Although the DIAGNOdent pen performed equally well as DIAGNOdent for quantification of carious lesions on smooth surfaces using histology as gold standard, more in vitro and in vivo studies with larger samples need to be carried out before any recommendation can be given with regard to the validity of the device. Possible application of the DIAGNOdent pen for scientific purposes, such as evaluation of preventive programs, and to what extent the changes of a lesion can be reliably detected needs however, to be further explored under clinical conditions.
Recommendations for clinical practice based on the present results

Our studies have shown promising results for quantification of carious lesions by DIAGNOdent and QLF on smooth surfaces. Thus, both methods have potential clinical application for detecting carious lesions at an early stage and for quantifying the disease process. Furthermore, DIAGNOdent could be a useful method for monitoring the outcome of caries preventive programs in susceptible individuals, such as orthodontic patients. However, some factors may influence and affect the DIAGNOdent readings obtained under a clinical setting. For optimal application, the following factors should be considered:

1. In order to register accurate data for each lesion in longitudinal studies, the DIAGNOdent measurements should be performed preferably by the same operator since the intra-observer agreement of the method is higher than the inter-observer agreement. If several operators have to be involved, pre-training and calibration between the operators seems to be important (Paper IV). In addition, frequent calibration of the DIAGNOdent device against a ceramic standard and sound tooth surface is necessary (Karlsson et al., 2004; Braun et al., 2005).

2. The tooth surface should be cleaned before measurement because the instrument can be influenced by the presence of deposits; plaque, calculus, toothpastes, and prophylaxis pastes (Shi et al., 2000; Mendes et al. 2004; Lussi et al., 2005).

3. DIAGNOdent measurement is extremely sensitive to stains, even slight discoloration (Shi et al., 2000; Bamzahim et al., 2002; Francescut and Lussi, 2003). Therefore, DIAGNOdent readings of discolored surfaces should be interpreted with caution. It is important to be aware that the signal may be over stimulated when applying DIAGNOdent on stained sites.

4. It is important to point out that the recommended cut-off values based on in vitro studies can only be used as a guide when making decision. The clinical assessment should include all available information, such as the patient's case history, oral hygiene, fluoride experience and dietary habits, as well as perceived caries activity and the status of the tooth surface. If properly applied and correctly interpreted, DIAGNOdent would improve caries detection and quantification and, thereby, the selection of proper treatment.
Possible clinical applications of QLF and DIAGNOdent in future

Most of the available reports on QLF and DIAGNOdent are laboratory studies, and accordingly more clinical studies based on relatively larger samples need to be carried out.

There is little information on the use of DIAGNOdent or QLF in teeth with enamel fluorosis or hypomineralization. Such teeth might influence the fluorescence intensity measured by the QLF and the DIAGNOdent methods. Therefore, further research is required to validate this fact.

To our knowledge, there are no studies published evaluating the DIAGNOdent system for longitudinal quantification of changes in white spots lesions, which have developed adjacent to fixed orthodontic appliances during the treatment period. With the new DIAGNOdent pen system, the finer tip would enable better access for measuring carious lesions around the bracket. Further investigations are warranted into the application of DIAGNOdent to aid clinical quantification of incipient carious lesions adjacent to fixed orthodontic appliances.

DIAGNOdent may be a very helpful aid for caries detection under circumstances where bitewing radiographs are not applicable, such as dental examination for patients with fixed orthodontic appliance, or in epidemiological studies.

Conclusions

Based on the four studies of the thesis, it may be conclude that:

1. The fluorescence methods, QLF and DIAGNOdent, are comparable for quantification of the lesion depth of natural enamel lesions on smooth surfaces adjacent to fixed orthodontic appliances

2. For studying the mineral changes in enamel carious lesions on smooth surfaces, QLF may be the preferable method before DIAGNOdent due to its higher correlation coefficients found in Paper I.

3. Moderate validity and good reproducibility of the DIAGNOdent method would enable longitudinal monitoring of the caries process and evaluation of the outcome of preventive measures.
4. The new DIAGNodent pen seems to be as effective as the original DIAGNodent device regarding its validity and reliability for quantification of smooth surface caries.
Abdulaziz Aljehani

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References


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