Detection of intraoral lesions using a fluorescence camera

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ABSTRACT
Optical methods for the detection of carious lesions, calculus and plaque have the advantage of being minimally invasive. The use of endogeneous fluorescence markers like porphyrins could simplify the application of fluorescence techniques in the dental practice. It is known that porphyrins are produced by some of the bacterial species that are present in the oral cavity. Since porphyrins have an excitation band at about 400nm they have the potential to be used as fluorescent markers of locations in the oral cavity where the production of bacteria is out of the limits of healthy regions. Further, modern and efficient GaN-based semiconductor diodes emit light in this spectral range and thus make the implementation of fluorescence sensors with excitation at this wavelength easy.

Carious lesions, calculus and plaque have been measured using a self build fluorescence camera using GaN-diodes for illumination at 405nm. Further, emission spectra under this excitation were recorded. For the latter purpose freshly extracted teeth were used. It has been found that already in the case of an initial carious lesion red porphyrin-fluorescence is emitted whereas it is absent in healthy enamel. In already brown coloured carious lesions the emission bands of porphyrin are present but the observed overall fluorescence intensity is lower, probably due to the absorption of the fluorescence by the carious defect itself. In dental calculus, dental plaque and subgingival concrements porphyrin originated luminescence was found as well. Since in these cases the emission spectra differ slightly it can be concluded that they originate from different types of porphyrins and thus also from different bacteria. These results show that this fluorescence technique can be a promising method to diagnose carious lesions, calculus and plaque.

Keyword List: fluorescence, porphyrin, bacteria, caries, calculus, plaque, camera, imaging, emission spectrum

1. INTRODUCTION
In dentistry non invasive methods that can diagnose early stages of diseases like caries or periodontitis are desirable. Such diseases are started by the infection of the oral cavity with specific bacteria. The next step is that these bacteria accumulate and grow on specific intraoral areas in the form of plaque. If the plaque is not removed secondary effects like demineralization or bone loss can follow. There are methods like i.e. x-ray radiography that are only sensitive to these secondary effects and not to the primary effect of the bacteriological equilibrium being out of order at specific intraoral areas. However, methods that are instantaneously able to detect the bacteriological concentration and show up sites where the concentrations are out of healthy limits would allow a much earlier diagnoses of the coming intraoral diseases. With this information these sites could be treated and secondary effects caused by bacteria minimized. Some methods like i.e. the strip mutans test allow already to get an information about the global average concentration of such bacteria. However, this test gives no spatial resolution to identify specific areas with increased accumulation of these bacteria. Other methods are the growth of bacterial cultures or DNA sequencation and amplification after the acquisition of bacterial agglomerates from specific areas using a paper tip. Especially the latter methods has the advantage of being able to identify the bacterial species. However, they can not be used to inspect routinely all intraoral areas. The traditional method of optical inspection has the advantage of high spatial resolution but is i.e. lacking the sensitivity to detect very early carious lesions, because it visualizes only the late secondary effect of demineralization. Further there is the possibility to use staining solutions to visualize plaque with the slight disadvantage that the stains remain for some time.

Obviously a method of choice should have the following characteristics. It should be instantaneous, work with sufficient spatial resolution, give signals that correlate with the concentrations of pathogen key bacteria, allow an easy documentation of the situation, should be sufficiently low in price and fast in use in order to be employed routinely.
In principal a camera based fluorescence method would have the potential to fulfill the requirements above if there would exist intrinsic fluorescent molecules that are produced by the relevant bacteria. Such molecules can be the porphyrins, which are known to be produced at least by some bacteria. It can be excited efficiently in the so called Soret band at about 400nm and with lower efficiency in four Q-bands having their maxima between about 500 and 630nm. These porphyrin molecules fluoresce mainly in the red and near IR. The molecular structure of the porphyrin molecules is shown in Fig. 1. Depending on the side groups of the various porphyrin derivates the maxima of the excitation and luminescence bands can be shifted. Further it is known that the exact positions of the maxima are shifted with the type of solvent. In the following thus such a fluorescent camera based method to identify plaque, calculus, subgingival concrements and carious lesions under the excitation in the Soret band of the porphyrins is investigated. Further, emission spectra under the excitation in the Q-band at 405nm are reported for these sites and compared to the emission spectra of some bacteria in vitro under the same excitation.

2. EXPERIMENTAL

For the studies an intraoral camera of the type Vistacam (Dürr Dental, Germany) has been modified by exchanging the white LEDs of the camera by blue LEDs emitting at 405nm an optical power of 60mW. Further an optical long pass filter has been introduced into the beam path in front of the CCD-sensor to cut down the excitation light below 495nm. To acquire images by the camera software of the type DBSWIN (Dürr Dental, Germany) has been used. As a result of the digitization of the video signal the images were composed of 720x576 pixel with 3x8bit intensities of the RGB-channels. Some of the images were recorded with automatic white balance off (Fig. 2 and 5) the others with white balance on in the video processing. In order to collect conventional intraoral images an unmodified intraoral camera of type Vistacam (Dürr Dental, Germany) has been used.

The local emission spectra have been investigated using a spectrometer of the type HR4000 (Ocean Optics, Netherlands) that has been coupled by a fiberoptical cable to the site. To cut down the excitation light below 495nm an optical long pass filter has been introduced into the beam path in front of the spectrometer.

3. RESULTS

3.1. Caries lesions

Typical images of uncavitated caries defects taken with the intraoral camera and the fluorescence camera are shown in Fig. 2. The beginning demineralization of the enamel can be observed in the intraoral image by surfaces resembling unglazed china or chalk (arrow). It is well known that this demineralization is caused by plaque that lowered the pH for some time under a critical value of about 5.5 and etched the enamel surface. The resulting surfaces are called white spot lesions. They can be remineralized in order to convert them back to sound enamel. The fluorescence image of the same intraoral region shows at the position of the white spot lesion red luminescence. As a consequence of the green luminescence of sound enamel the “red” white spot lesion can be much easier observed in the fluorescence image than in the normal intraoral image on the left. Therefore the dentist has a higher sensitivity to observe the white spot lesion in the fluorescence image compared to the standard intraoral image. Hence a dentist could treat and remineralize the affected sites earlier if he uses the fluorescence camera. Further it should be noted, that the fluorescence image shows red luminescence also on the right side of the white spot of the ordinary intraoral image, where no white spot has yet developed. One can speculate that the demineralization is not strong enough in this region to become visible in the ordinary intraoral image.

It is interesting to investigate the fluorescence spectra of the enamel and the white spot lesion. They are shown in Fig. 7. The fluorescence of the intact enamel decreases monotonically in intensity from the green to the red spectral region with increasing wavelength. The sharp edge on the short wavelength side at about 500nm is caused by the cut off of the
optical long pass filter. The fluorescence spectrum at the white spot lesion (Caries 1) shows a similar decrease of the intensity from the green to the red spectral region superimposed by two dominant emission peaks at about 640 and 700nm. These peaks in the emission spectrum are very similar to the emission spectra of porphyrins. Therefore it is very probable that the fluorophore that is excited at 405nm is a porphyrin that has been produced by bacteria and accumulated at the tooth surface. It is interesting to investigate, whether all white spot lesions have the same emission spectrum. Therefore a caries lesion of another patient was examined. The fluorescence spectrum of this lesion is shown as 'Caries 2' in Fig. 7. In this spectrum also porphyrin fluorescence is observed. However, compared to the spectrum 'Caries 1' the maxima of the emission are shifted about 10nm to shorter wavelength. It is known that different types of porphyrins have slightly different emission spectra. Thus one can speculate that this shift is caused by different bacteria with different metabolisms that have produced different types of porphyrin. But since it is known that the emission spectrum of a specific porphyrin changes with the type of solvent the shift could also be interpreted as being due to differences in the local environments of the molecule (pH-value, composition of saliva and bacterial film etc.).

A similar situation can be found in a single tooth having caries lesions of different progression as shown in Fig. 3. Here the initial caries lesion shows emission peaks shifted to longer wavelength compared to the peaks of the much more progressed caries lesion. Thus also in this case one can speculate that in the differently progressed caries lesions different bacteria are dominant that produce different types of porphyrin. Further it should be noted that the absolute luminescence intensity in the more progressed caries lesion has been significantly lower than in the initial caries lesion. This can be explained by a significant absorption of the porphyrin luminescence in the brown coloured lesion. Therefore the absolute porphyrin luminescence intensity is not a good measure for the progression of carious defects. However, the normalized emission spectra in Fig. 3 show that the emission spectrum of the much more progressed caries lesion has larger intensity contributions in the red range of the visible spectrum than the initial lesion. Hence the ratio of the luminescence intensities in the red and the green part of the visible spectrum is a much better indicator of the activity of the defect than the amount of the red luminescence intensity itself.

### 3.2. Supragingival plaque

Since the development of caries starts with the accumulation of plaque on the tooth surfaces the fluorescent emission of plaque has also been investigated. In Fig. 4 the emission spectra of plaque and a typical image taken with the fluorescence camera are shown. It can be seen that the emission spectra of the plaque of Patient 1 is quite similar to the spectrum of the initial caries as shown in Fig. 3. Again emission bands similar to the emission of porphyrins are observed. But the emission spectrum of the plaque of patient 2 differs from the one of patient 1 and shows similarities with the emission spectrum of initial caries in Fig. 3. However, in the case of the plaque of patient 2 an additional band at about 590nm is present. This large spectral shift can not be explained by a different local environment. It is more likely that there is a different bacterium present in the plaque of patient 2 that produces in its metabolism a different fluorophore that is probably another type of porphyrin. Further measurements of the emission spectra for other patients showed still different emission spectra of the plaque than for patient 1 or patient 2. Accordingly the composition of the fluorophores in the plaque differs from patient to patient. This reflects a large variety in the composition of plaque by different bacterial species producing different fluorophores or porphyrins.

### 3.3. Dental calculus

As shown in Fig. 3 strong fluorescence emission in the red spectral range can also be observed from dental calculus under the excitation at 405nm. The emission spectrum, as shown in Fig. 7, is very similar to the emission spectra of caries lesions. Accordingly it is also in the case of dental calculus very probable that the fluorophor is a porphyrin. This porphyrin has been produced by bacteria that have been present in or on the surface of the calculus and has been accumulated there.

### 3.4. Subgingival concrements

Like dental calculus also subgingival concrements on root surfaces emit enhanced red luminescence when excited with light of a wavelength of 405nm as shown in Fig. 6. Again the emission spectrum shows the fingerprint of porphyrins with a band at about 640nm and a another broader band at about 700nm. Also the emission band that has been observed for the plaque of Patient 2 as shown in Fig. 4 at about 590nm is present. Similar to the emission spectrum of enamel as shown in Fig. 4 and 7 the emission spectrum of the root surface without concrement decreases with increasing wavelength above 520nm. The cut off below 500nm is caused by the optical long pass filter in front of the spectrometer. Similar spectra but with missing band at 590nm have been obtained on the concrements of other root surfaces. This indicates that different bacterial species with different emission spectra accumulate on and in the concrements.

### 3.5. Dental bacteria in vitro

There is a large variety of bacteria in the oral cavity. Some specific types are known to have a high correlation with a dental disease. I.e. Streptococcus Mutans, Streptococcus Sobrinus and Lactobacillus have a high correlation i.e. with
dental caries. For i.e. the bacteria Actinobacillus Actinomycetemcomitans, Porphyromonas Gingivalis, Bacteroides Forsythius, Prevotella Intermedia and Fusobacterium Nucleatum a correlation to the periodontal disease exist. Therefore some specific bacteria have been grown on agar, removed from their substrate and spectroscopically investigated upon excitation with light of 405nm on a glass slide. The emission spectra are shown in Fig. 7 on the right side. It can be seen, that the porphyrin related luminescence with a band at about 640nm and another broader band at about 700nm can be clearly observed for Actinobacillus Actinomycetemcomitans and Fusobacterium Nucleatum that are correlated with the periodontal disease. Porphyromonas Gingivalis which is also correlated with the periodontal disease shows only a very low band at about 625nm. The bacterium Streptococcus Mutans that is correlated with dental caries shows emission bands at about 580nm and 640nm of somewhat lower intensity than Actinobacillus Actinomycetemcomitans and Fusobacterium Nucleatum. Interesting is that for this bacterium the ratio of the red to green luminescence is significantly higher than for enamel. Thus the ratio of the red to green luminescence as suggested earlier can be a much better indicator for dental caries than the red intensity itself. For the Lactobacillae no significant luminescence has been found.

4. CONCLUSIONS

It has been shown in the previous section exemplary that plaque, carious lesions, dental calculus and subgingival concrements emit red light under the excitation with light of 405nm. The band structure of the emission spectra, however, is complex and differs often from one area to another. On the other hand enamel, dentin and healthy root surfaces without concrements show a fluorescence that decreases significantly with increasing wavelength from the green at about 510nm to the red. Further it has been found that at least some of the relevant bacteria that are correlated with dental diseases show enhanced red fluorescence. Thus the described fluorescent camera is probably a very good tool to distinguish healthy dental surfaces from infected sites where the bacterial concentration is out of balance or healthy limits. The fluorescence camera has the advantage that large areas of dental surfaces can be inspected with high spatial resolution within seconds, which is impossible i.e. with the Diagnodent (Kavo) that uses only a fibreoptic probe to inspect fluorescence intensities in the IR point by point by moving the probe. Due to this high spatial resolution it is possible to identify even small sites with this bacterial imbalance within short time and to store and document them on a PC. For the detection of these sites it is advantageous to compare the ratio of the red to green fluorescence at these sites with the ratio of uninfected sites. It is especially of an advantage, that for the examples shown above the sensitivity is that high that already the plaque formation can be diagnosed, from which later on the dental disease could follow. Thus it seems also appropriate to use the fluorescence camera to identify the hygienic status of patients. Additionally the images with the luminescent plaque can be used to motivate the patients to enhance their activities in the removal of plaque. Further, after removal of the plaque from the surfaces of the teeth caries lesions in an early stage can be diagnosed with the camera and care can be taken to remineralize the defects. However, studies that prove the correlation of dental diseases like caries with the output of the fluorescence camera over a large number of patients are necessary. For the Diagnodent system, that excites porphyrins in the long wavelength Q-band at 655nm and measures the emission intensity above 680nm such studies already have been made for dentin caries and show in general a high specificity and sensitivity in comparison with histology, microradiography or clinical opening. It should be noted here that the excitation process of the porphyrin is more efficient in the Soret band that is used in the fluorescence camera than in the Q-bands at longer wavelength that is used by the Diagnodent system. Further the output power of the excitation light is in the case of the fluorescence camera 60mW whereas the optical output power of the Diagnodent system is below 1 mW.

With a miniaturized camera head the fluorescence camera also has the potential to identify harmful subgingival plaque in the tooth pocket. This is supported by the fact that it has been found that at least Actinobacillus Actinomycetemcomitans and Fusobacterium Nucleatum which are correlated with the periodontal disease show enhanced red fluorescent compared with the healthy root surface. From the point of the application it is also interesting to point out that the excitation light with the wavelength of 405nm has a penetration depth of at least millimeters into healthy enamel and dentin. Thus the excitation light can excite fluorophores that are not directly on the surface of the tooth but more inside the volume of the tooth. In addition enamel and dentin show low absorption to emitted red light. Therefore it is possible to excite and collect with the fluorescent camera signals from points that have a distance of the order of millimeters from the surface. Due to light scattering in the enamel and dentin the emission signals of such deeper points are spatially unsharp and diffuse. Nevertheless there is a chance to find defects or lesions inside the tooth that are not directly located on the surface of the tooth like it is i.e. the case for secondary caries between fillings and the enamel or dentin of for defects on directly inaccessible approximal surfaces. At one patient for example it was possible to identify a caries lesion below the enamel
of an incisor with the fluorescence camera. The same lesion was impossible to identify by the usual visual inspection because the caries lesion started from the approximal side between the incisors. However, upon mechanical probing the enamel over the cavity was broken and the cavity became visible.

The red light emission originates from fluorophores that are produced by the metabolism of some intraoral bacteria. Since the emission spectra show similarities with the emission spectra of porphyrins it is reasonable to conclude that the relevant fluorophores are porphyrins. However, there is in principal a large variety of porphyrins with different side groups can be produced. Therefore it would be interesting to identify the actually produced type of porphyrin for each bacterial species that gives rise the emission of red light. Some of the bacteria that give rise to the red fluorescence have been identified. But there is a much larger variety of bacteria present in the oral cavity. Therefore additional effort is needed in future to identify more of the bacterial species that show red fluorescence upon excitation in the Soret band of the porphyrins. Of special interest are here bacteria like Streptococcus Sobrinus, Actinomyces Odontologica, Actinomyces Naeslundi that are associated with caries and Bacteroides Forsythus and Prevotella Intermedia that are associated with periodontitis.

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6. COLOUR FIGURES

Figure 2: Images of a caries defect obtained with an intraoral camera (left) and with the fluorescence camera (right).

Figure 3: Images of a freshly extracted tooth taken with the intraoral camera (top left) and the fluorescence camera (bottom left) and emission spectra (right) of the indicated caries lesions.

Figure 4: Emission spectra of plaque (left) and intraoral image of plaque taken with the fluorescence camera (right).
Figure 5: Images of the front teeth obtained with an intraoral camera (left) and with the fluorescence camera (right). Calculus shows up in the fluorescent image in red.

Figure 6: Emission spectra of a root surface of a tooth in a region without and with concrement (left) and image of the root taken with the fluorescence camera (right). The arrows indicate roughly the positions of the spectral measurements.

Figure 7: Emission spectra of the caries defect shown in Fig. 2, intact enamel and calculus shown in Fig. 3 (left) and some bacteria as grown on agar (right).