Autofluorescent Characteristics of Different Stages of Dental Carious Lesions

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Abstract. Spectroscopic methods are a powerful tool in the study of pathological changes in biological tissues, allowing obtaining of morphological and biochemical information about their state. Fluorescence spectroscopy is distinguished by its high sensitivity and the ability to work with biological samples in real time, allowing both primary diagnosis of the studied biological objects and monitoring of pathological processes occurring there. Biological tissues such as teeth do not exhibit rapid metabolic activity, which can lead to a dramatic change in the content of fluorophores and tooth absorbers, which allows *in vitro* studies of their fluorescence properties.

This work presents the results of fluorescence characteristics evaluation of healthy dental tissues and of the various stages of caries development *in vitro,* using the excitation-emission matrix (EEM) technique for detection of emission in the range of 280-500 nm for excitation and 300-800 nm for the emission signal. A FluoroLog3 spectrofluorometer (HORIBA, JobinYvon, France) was used with a fiber optic module F-3000 (HORIBA Scientific, France) in EEM measurement mode. The main endogenous sources of fluorescent signal in healthy and carious dental structures had been identified. Various forms of collagen (type I, III and V) have a major contribution to the fluorescence signal of normal and precarious dental tissues. In the advanced stages of caries growth, accumulated porphyrins from the bacterial flora are responsible for the appearance of red–shifted fluorescence signal obtained.

Keywords: endogenous fluorescence, excitation-emission matrix, caries, collagen, porphyrins.

1. INTRODUCTION

Teeth are organs that play a major role in the mechanical fragmentation of food and are involved in the formation of certain sounds of articulate speech.

Dental caries is one of the most common diseases in human teeth. It affects all ages and is a widespread medical problem. Caries can affect the tooth enamel layer (superficial lesion) or the enamel and dentin at the same time (medium and deep cavities).

Initially, disturbances in the mineral exchange in the enamel, expressed in a decrease in the mineral composition and a violation of the prism structure, occur (precarious stage). In the second stage, bacterial decay of the enamel occurs, which gradually encompasses the structure of the dentin (carious stage) (Ando et al., 2001; Ratledge et al., 2001, Mortensen et al., 2018).

Any diagnostic procedure for the diagnosis of carious dental structure must be specific, valid, reliable and clinically proven. Early diagnosis of the carious lesion has assumed a particular importance because the ability to detect early lesions on reversible stage of their development offers many advantages, including opportunities for drug treatment and shortening of the time for therapy. Caries detection system should permit proper discrimination between healthy and diseased tooth structures. The availability of non-invasive, sensitive, quantitative and qualitative methods for clinical caries diagnosis could open up new possibilities for research and for applications in the clinical practice. Optical methods are very appropriate for such carious evaluation and

light-induced fluorescence is one of the appropriate tools for such clinical validation. (Uzunov et al., 2003)

The fluorescence spectroscopy is noninvasive, fast and reliable detection technique with high sensitivity to early small alterations in tissues morphological and biochemical properties. It could simplify the dentist's work as well because it can prevent the confusion between tooth demineralization and early carious enamel lesions. These two conditions look practically the same to the naked eye, but their fluorescence emission is situated in different wavelength range. (Rodrigues et al., 2017). Structural proteins fluorescence in den-tal tissues could be observed after illumination with UV or blue excitation as an emission signal in blue-green spectral region. (Lee. 2015; Ko et al., 2017; Abdel Gawad et al., 2019)

Carious lesions are caused by metabolically produced acids made by bacteria. According to previous studies, there are six main cariogenic bacteria, namely: *Streptococcus mutans, S. sanguis, Actinomyces viscosus, Prevotella intermedia, Lactobacillus acidophilus, and Candida albicans*. (Zhu et al., 2015) Those bacteria also have specific fluorescent properties that could be used as markers of their presence and accumulation in pathological areas of the teeth surface. Excitation in ultraviolet region lead to appearance of emission in 430-440 nm. (Zhu et al., 2015) Endogenous porphyrins in bacterial flora with their specific emission in the region of 635-710 nm are another significant indicator of carious mass accumulation. (Shi et al., 2001)

Combined studies of fluorescent properties of structural proteins in the dental tissues and the accumulated bacterial carious flora could allow extracting specific spectroscopic features with diagnostic significance allowing differentiation not only of carious lesion presence but to the stage of the carious lesions' development. In our study we used excitation-emission matrix development technique to evaluate fluorescence signal in a broad spectral range that allow covering main fluorescent sources in ultraviolet, visible and near-infrared spectral areas.

2. METHODS AND MATERIALS

The spectra of normal dental tissues are compared with those of several types of pathological entities (lesions), and the various features in the observed signals are classified according to the type and degree of development of the lesions. The influence of the contained fluorophores and absorbers in a given object on the type of fluorescence spectra obtained is analyzed.

In Table 1 are presented quantity and stage of development of carious teeth investigated *in vitro* using fluorescence technique of (EEM) excitation-emission matrix detection. Samples were obtained after their extraction due to reasons including caries and periodontal problems in the patients in the Faculty of Dental Medicine, MU-Sofia. All needed ethical issues for usage of human tissue samples including informed consent and ethical approval are received according best practice of FDM-MU-Sofia.

TABLE 1. Number of tooth samples investigated on different stage of carious lesion development.

#	Tooth condition	Number of samples
1	Healthy enamel	7
2	Healthy dentine	7
3	White spot lesion	4
4	Brown spot lesion	5
5	Superficial caries	4
6	Medium caries	3
7	Deep cavity	7

The healthy teeth were additionally demineralized with a 35% phosphoric acid (SAREMCO DENTAL, Saremco Microcid Etchant Gel) for 10 s, 30 s, 1 min, 5 min and 24 h to evaluate the influence of demineralization process only on the spectra detected. Fluorescence spectra from demineralized area were obtained after each procedure. The last demineralization led to a complete decay of the tooth enamel so that the dentine area was revealed.

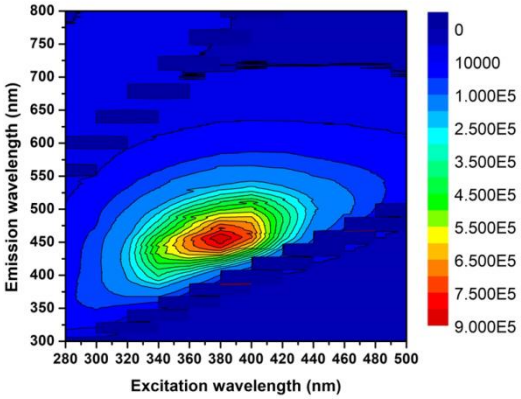
Spectrofluorometer FluoroLog 3 (HORIBA Jobin Yvon, France) was used for the fluorescence measurements of the surgically removed dental samples. This system excitation light source is a xenon lamp with output power of 300 W, performance range of 200-650 nm and PMT detector with performance range of 220-800 nm for fluorescence detec-tion. Since our samples vary in shape and dimensions, their fluorescence was investigated with additional fiber optic module F - 3000 (HORIBA Jobin Yvon, France), which allows sample measurements outside of the sample chamber. Measurements of the fluorescence signals of the different tissues obtained in EEMs were performed with applied excitation in 280-500 nm spectral region and emission observed between 300 nm and 800 nm.

3. RESULTS AND DISCUSSION

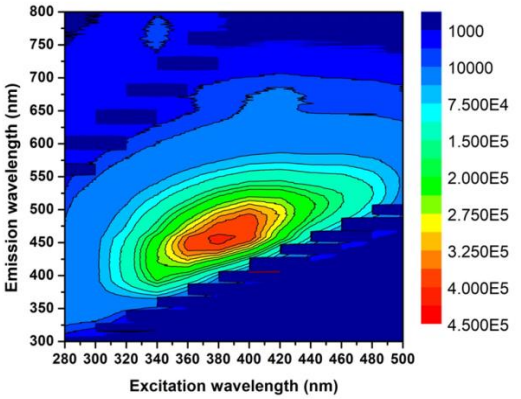
On Fig. 1 a, b and c are presented EEMs data for normal sound tooth, precarious and carious lesions.

Naturally, existing fluorophores in teeth tissues are mainly collagen molecules in enamel and dentine. Therefore the collagen type I, type III, type V and VI solely and collagen cross-links are the main sources of fluorescent signal in normal dental tissues. During the carious lesion development, the concentration of these structural proteins is reduced due to tissue demolition and the fluorescence signal related is faded with advance of caries growth. Pure collagen decrease is clearly pronounced with the decrement observed in the EEM islands for wavelengths' couples: excitation 360-380 nm and emission 400-450 nm, Fig.1.

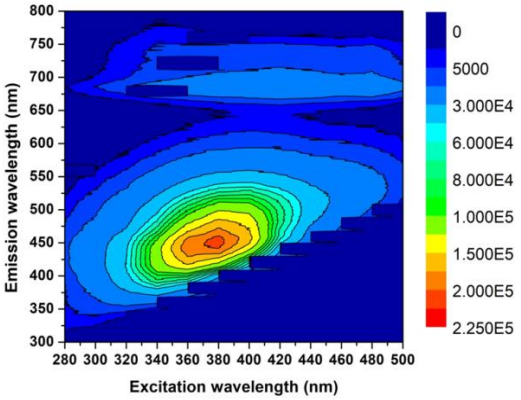
Such decrease is observed for collagen cross-links as well, with longer excitation (440 nm) and emission (500-540 nm), see Fig. 2. This decrease of the fluorescence signal of the main endogenous fluorophore – collagen is due to the demineralization and destruction of the structure of the dental matrix in general.



(a) Healthy tooth



(b) Precarious lesion



(c) Carious lesion

Fig. 1 Excitation – emission matrices of normal tooth enamel (a), precarious tooth lesion (b) and superficial caries (c) using excitation in the region of 280-500 nm and emission at the region of 300-800 nm. The excitation-emission matrices represented have fluorescence emission intensity scales referred as 4: 2: 1.

The formation of the fluorescence signal in the red spectral region correlating with the accumulating bacterial carious mass was observed as well. The level of this red emission signal corresponds to the stage of dental lesion development. On Fig. 1 it is pronounced as appearance of long-wavelength EEM islands in the region of 650-750 nm emission with broad excitation range for almost whole ultraviolet A and visible violet-blue spectral regions.

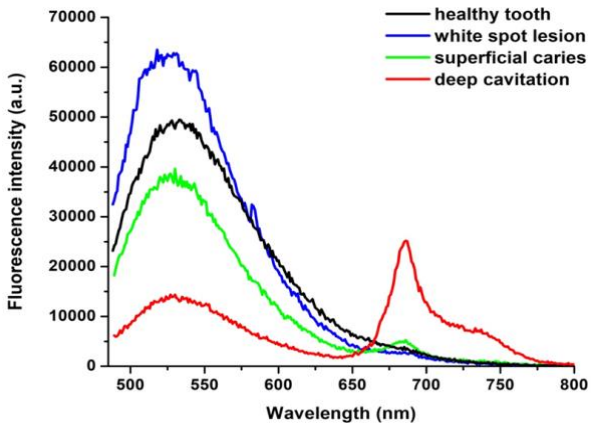


Fig. 2 Comparison of the fluorescence emission spectra of different stages of carious lesion development upon excitation of 440 nm – healthy, precarious lesion, superficial and deep carious lesions.

Red emission in the 650-800 nm region is addressed to endogenous porphyrins accumulated due to carious mass in the lesion developed, see on Fig. 2 cases of superficial caries and deep cavity lesions.

We applied detection method, using the autofluorescence of the sound teeth, precarious and carious lesions on the base of excitation emitting matrices, which shows a good performance both in detecting (and staging) the caries and in the detection of tooth enamel demineralization. On Fig. 3 are presented dynamic alterations in the intensities of maximum for collagen cross-links (at 490 nm) after excitation on 340 nm for different carious lesions and different level of tooth demineralization, corresponding to the carious cavities depths. Demineralization levels were mimicked using 35% phosphoric acid treatment of the sound teeth.

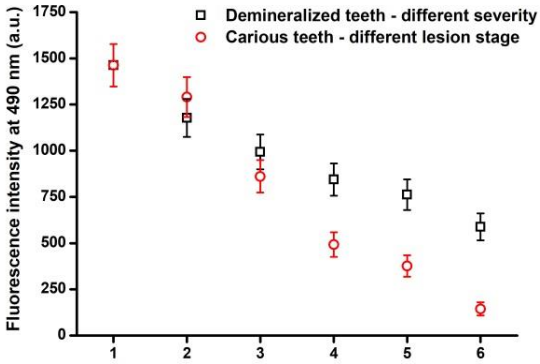


Fig. 3 Comparison of the intensity decrease in the cases of different stages of carious and demineralization lesions. The points presented are the mean values of the fluorescence intensity for each tooth lesion type with their standard deviation. 1 – sound tooth, 2 – white spot lesion and 10 s – demineralized lesion, 3 – brown spot lesion and 30 s – demineralized lesion, 4 – superficial cavity and 1 min – demineralized lesion, 5 – medium-depth cavity and 5 min – demineralized lesion, 6 – deep cavitation and 24 h – demineralized lesion.

TABLE 2. Diagnostic accuracy for different stages of caries fluorescence using excitation at 340 and 440 nm, relative to healthy teeth fluorescent spectra.

Carious stage	Excitation 340 nm	Excitation 440 nm
White spot lesion	81.8 %	87.1 %
Brown spot lesion	92.5 %	91.2 %
Superficial caries	93.6 %	93.8 %
Medium depth cavity	93.9 %	93.9 %
Deep cavitation	93.8 %	96.8 %

The results of the diagnostic accuracy for the excitation wavelengths 340 and 440 nm are presented in Table 2. Dimensionless values of the fluorescence emission intensities on 430 nm (collagen), 490 nm and 550 nm (collagen cross-links) and 650 nm (bacterial porphyrins) are used for discrimination algorithm develo-ped, as follow for excitation at 340 nm: $R_1=I_{430}/I_{490}$ and $R_2=I_{650}/I_{490;}$ for excitation at 440 nm: $R_3=I_{650}/I_{550}$. Diagnostic accuracy achieved is higher than 90% for all carious stages except white spot lesions, which is comparable and higher than the diagnostic accuracy obtained using standard clinical techniques for dental

caries evaluation, such as radiography and trans-illumination techniques. (Ratledge et al., 2001; Emami et al., 1996)

4. CONCLUSIONS

The information contained in the spectral shape changes, related to the content of intrinsic fluorophores, allows a more accurate differentiation between carious and demineralized teeth, as well between different stages of carious lesion growth. No absolute intensity determination is required in this situation, since a definite diagnosis could be established based on the fluorescence intensity changes at 430, 490, 550 and 650 nm emission maxima observed. On the basis of the results obtained in the present study, the potential is demonstrated of the light-induced fluorescence method for noninvasive early diagnosis in dentistry. This spectroscopic investigation could be applied in designing a simplified fluorescence-imaging device for detection and differentiation of the initial tooth caries from demineralized lesions and diagnostics of carious lesions stage of development.

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