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ORIGINAL ARTICLE

Fluorescence diagnostics of dental erosion

Fluorescencyjna diagnostyka erozji zębów

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Abstract

In this article the prospects of applying the method of laser-induced fluorescence to the diagnostics of dental erosion at the initial stages has been investigated. It was found that at the initial stages of erosion development, the fluorescence intensity of eroded enamel was considerably higher than that of the intact enamel (no less than by 50%). Absorption spectrum of the affected enamel was observed to differ significantly from absorption spectrum of the intact enamel, as the localization of the main peaks changed significantly. To prove this hypothesis, investigations were performed in vivo on 60 patients at the early stage of erosion according to the preliminary clinical examinations. Fluorescence spectra of dental enamel were obtained from the intact and eroded areas of a tooth. Fluorescence spectrum from the intact area of a tooth was used as an indicator of spectral changes caused by pathological processes. These investigations were carried out using the method of LIF (laser-induced fluorescence). Also, the analysis of IR reflection spectra of dental enamel was performed for the extracted teeth in vitro. This made it possible to reveal deviations in stoichiometry of the chemical components forming the composition of dental enamel. The obtained results are very important for the investigation of mechanism of dental hard tissues fluorescence as well as for the prospective practical application of the method of laser-induced fluorescence to the diagnostics of dental erosion at initial stages.

Streszczenie

W tym artykule omawiamy możliwości zastosowania zjawiska fluorescencji wzbudzanej laserem (LIF) w diagnostyce erozji zębów w jej wczesnym stadium rozwoju. Stwierdzono, że we wczesnej fazie rozwoju erozji intensywność fluorescencji zerodowanego szkliwa była znacznie wyższa w porównaniu ze szkliwem prawidłowym (nie mniej niż o 50%). Spektrum absorbcji zmienionego szkliwa znacznie różniło się od wyników dla zdrowego szkliwa ze względu na znaczną zmianę w lokalizacji głównych punktów szczytowych. Dla udowodnienia tej hipotezy, przeprowadzono badania in vivo na 60 pacjentach we wczesnym stadium erozji, którą wstępnie zdiagnozowano na podstawie badania klinicznego. Spektra fluorescencji szkliwa otrzymano na podstawie badania obszarów zdrowych i zerodowanych zęba. Spektrum obszarów zdrowych posłużyło za wyznacznik zmian spektralnych wywołanych przez procesy patologiczne. Badania przeprowadzono za pomocą metody LIF (laser-induced fluorescence). Również badanie szkliwa w podczerwieni przeprowadzono na usuniętych zębach in vitro. Dzięki temu ujawniono odchylenia w stechiometrii poszczególnych składników chemicznych składających się na strukturę szkliwa. Otrzymane wyniki sa kluczowe dla badania mechanizmu fluorescencji twardych tkanek zęba; są również krokiem w kierunku praktycznego zastosowania metody LIF w diagnostyce erozji zęba w jej wczesnych stadiach.

KEYWORDS:
laser-induced fluorescence, dental erosion, early diagnostics, IR spectroscopy

HASŁA INDEKSOWE:
LIF, erozja zęba, wczesna diagnostyka, spektroskopia w podczerwieni

Introduction

In addition to caries, that is one of the most widely-spread dental diseases in humans, dental diseases which are known to be connected with non-carious lesions of dental tissues. Many of these lesions are caused by regressive changes in the structure and chemical composition of both dental enamel and dentine. Regressive changes represent a multifactorial, multi-parametric condition, which results in a loss of hard dental tissues and is not connected with the products of bacterial vital activity. One of the causes of the regressive changes is erosion.

The earliest stages of dental erosion in dentistry are usually ignored since the insignificant loss of the tooth surface material is considered a normal and inevitable process of everyday life which is within the normal range and does not require any intervention. However, it is well known that if no preventive measures are undertaken, this disease can later result in destruction of enamel and dentine, the changes in appearance and shape of teeth due to established conditions. Consequently, the diagnostics of the early stages of teeth erosion is very important. Moreover, the remineralization of demineralized enamel at the early stage of erosion is quite possible.

Methods for evaluation of dental erosion have already been established,[1-5] among them: contact and contactless profilometry, registration of enamel hardness profile, microradiography, optical coherent tomography, laser-induced fluorescence, reflection and scattering spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy and various chemical methods of analysis of calcium ions and phosphates.

Microhardness investigations were performed in the works of *Fosse* et al.,[6] *Tsui* and *Pharr*[7], *Arends* and *ten Bosch*[8], and those of nanohardness were made in the works of *Finke* et al.[9] and *Barbour* and *Rees*.[3] Nevertheless, developing of instruments that can quantitatively determine small changes in the surface such as early demineralization and erosive softening in natural conditions is a very complicated challenge. In the case of registration of micro- and nanohardness profile in dental enamel, the devices should be capable of measuring the surface of the natural surface of enamel with the internal curvature. As for the later stages of erosion, it is required to estimate the natural surface of dentine with its persistent demineralized organic matrix. Moreover, in order to estimate the losses of small amounts of mineral components, it is necessary to design a super-precise positioning system since the coatings are often inhomogeneous. A precise measuring of the above mentioned and some other properties of tooth surfaces, such as surface roughness, inside the oral cavity requires a lot of work on its development.[10,11]

Chemical analysis is widely applied to the study of kinetics and thermodynamics of dental enamel and dissolution of calcium hydroxyapatite by measuring the concentration of calcium and phosphates. Analysis for calcium is usually performed with the use of atomic-absorption spectroscopy while concentration of phosphates is made with spectrophotometry of the stained phosphate complex.[3] In order to detect hydroxyl ions, the pH-testing technique is also applied.[3] Nevertheless, a serious limitation of these methods is a complexity in recording the effect of interfering released ions.

Methods of the optical spectroscopy for diagnostics of erosion *in vivo* seem to be quite prospective, that is, luminescence spectroscopy and scattering spectroscopy. Surface of a tooth susceptible to erosion is rough and porous, hence, it will scatter more light than the unaffected tissue.[12] In their works, *Pretty* et al.[13] and *Elton* et al.[14] showed the efficiency of laser-induced fluorescence (LIF) technique for the registration of early erosion *in vitro*. However, it was also noted that for checking this method *in vivo* a great amount of work is still required. Methods for the estimation of early and later stages of erosion were discussed in numerous works.[2-5,15] In the research by *Thomas*[12] it was found that the enamel destroyed due to demineralization resulted in the increase of intensity of the fluorescence signal all over the spectral range. Similar behaviour was found in the works of *Ando* et al.[16] and *Van der Veen* and *ten Bosch*[17] during the investigations of demineralization in the dental samples *in vitro*. As opposed to these facts, in the work by *Borisova* et al.,[18] a decrease of fluorescence intensity was observed in the process of demine-

ralization. Difficulties in the estimation of erosion with the luminescence spectroscopy technique and inconsistency of the obtained results as well as their interpretation are mainly caused by insufficient understanding of the mechanism of fluorescence of hard dental tissues, both intact and eroded.

This work is aimed at the study of fluorescence mechanism at the initial stages of erosion of human teeth and the possibility of the diagnostics of dental enamel erosion by laser-induced fluorescence (LIF) technique *in vivo*.

Materials and methods

investigations were performed *in vivo* on sixty patients at the early stage of erosion according to the preliminary clinical examinations.

Fluorescence spectra were recorded with the use of the patented device designed on the basis of fiber-optic spectrometer a USB4000-VIS-NIR (Ocean Optics), connected to the computer.[19] Probing area of the tooth was determined by the area of the waveguide and was equal to 0.28 mm^2. Laser diode emitting at the wavelength of 445 nm was used as a source of fluorescence excitation. Radiation power density was not more than 20 mW/cm^2. Measurements were performed inside a shaded room with no sources of scattered light. Fluorescence spectra of dental enamel were obtained from the intact and eroded areas of a tooth. No less than 10 fluorescence spectra were registered from each area of each investigated tooth, and after that, all of the spectra were averaged. In cases when the intact area of the affected tooth was absent, the reference spectrum was obtained from the intact area of an equivalent tooth. This is very important because our investigations of the intact teeth indicate the dependence of fluorescence spectra both on the anatomic area of a tooth and on the tooth type in the upper and lower jaw.[20,30-35] Fluorescence spectrum from the intact area of a tooth was used as an indicator of spectral changes caused by pathological processes. Before performing the luminescence investigations, the patients underwent the procedure of professional oral hygiene and they were recommended to use toothpaste that did not make any considerable contribution into the registered signal.

IR reflection spectra were registered with the use of IR-Fourier spectrometer Nicolet 6700 produced by Thermo Scientific (CIF) Company. IR spectrometer Nicolet 6700 is a powerful universal device of research type providing maximum resolution and stability of the analysis results. Since the analysis of IR reflection spectra of dental enamel was performed for the extracted teeth *in vitro*, in order to obtain reliable results there was a special attachment in the kit of spectrometer intended for the measurement of diffuse reflection spectra. This made it possible to increase accuracy of determining the shape of spectral lines and to reveal deviations in stoichiometry of the chemical components forming the composition of dental enamel.

Results

Figure 1 represents fluorescence spectra of the intact enamel in different anatomic areas and of the enamel affected by erosion in the precervical area in the initial stage, averaged for all of the patients.

Figure 1 shows that under excitation with irradiation at the wavelength of 445 nm fluorescence spectra of intact and affected enamel differ considerably in their intensity. For example, at the initial stage of erosion development, intensity of fluorescence of affected enamel is significantly higher than that of the intact one (at least by 50%). From Fig. 1 it can also be concluded that fluorescence spectrum of the intact enamel in different anatomic areas as well as of the eroded enamel represents rather broad multi-component band with the maximum at 526 nm. In addition, the shape of fluorescence spectrum of the affected enamel coincides with the shapes of fluorescence spectra of the intact enamel for all the anatomic areas: precervical, equator and incisal edge.

IR absorption spectra of the intact and eroded enamel are presented in Figure 2.

Figure 2 reveals that the absorption spectrum of the eroded enamel considerably changes as compared with the intact enamel. For example, the eroded enamel demonstrates a narrow intensive peak at 1103 cm^{-1}. The band with a peak at 1037 cm^{-1} is typical of stoichiometric calcium hydroxyapatite crystal. In the spectrum of intact enamel absorption the band appears at approximately 1623

cm^{-1}. The eroded enamel did not show any clearly expressed bands within the absorption area of vibrations of spiral-formed structure of Amide I (1687, 1652 и 1623 cm^{-1}). Instead, a monotonous absorption is observed within this spectral range.

Discussion

Understanding the mechanism of fluorescence of hard dental tissues, both affected by pathological processes and intact ones, is extremely important for the interpretation of the obtained results, as well as for elucidation of possible reasons for the observed opposite tendencies in the spectral behaviour of fluorescence of enamel susceptible to erosion,[19] because no considerable spectral modifications have been observed at the early stages of erosion. Finding the solution to this problem is impossible without the investigation of the influence of organic and inorganic phases of hard dental tissues on the overall fluorescence signal. The same is true for the chemical composition and the structure of the intact and eroded hard tissues; mutual influence of different hard dental tissues on the overall fluorescence signal as well as on the optical properties of intact and eroded hard tissues.

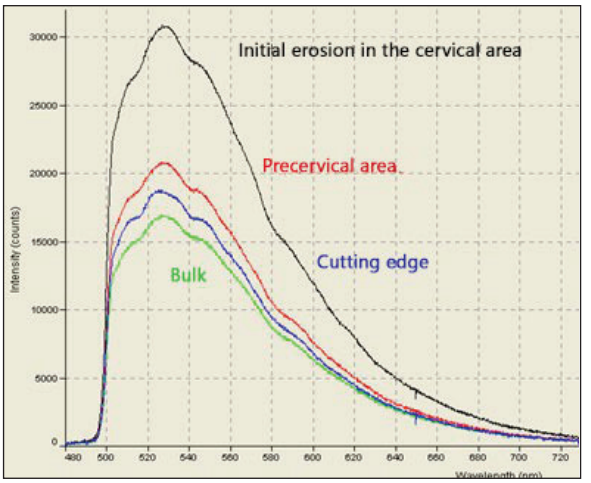

Fig. 1. *Fluorescence spectra of enamel: 1 – in the precervical area affected by erosion at the initial stage; 2 – in the intact precervical area; 3 – in the equator area; 4 – in the area of the incisal edge.*

Dental erosion is known to be identified as a chemical process proceeding in the aqueous phase that is not sufficiently saturated with dental mineral. As a result, different acids that are not connected with the vital activity of bacteria dissolve enamel and dentine.[21] In this situation corrosive acids can be of internal as well as of external origin. Internal acid is the acid formed in the stomach, while external acids are provided by the beverages and foodstuffs, for example, from nonalcoholic beverages, fruit juices and the like.[23,24]

Tooth enamel consists mainly of hydroxyapatite (HAp) $Ca_{10}(PO_4)_6(OH)_2$, but it can also contain impurity ions such as calcium carbonates and fluorides with a substitution in the anion sublattice as well as substitutional impurities of magnesium and sodium type with substitution in the cation sublattice. The share of these impurities varies

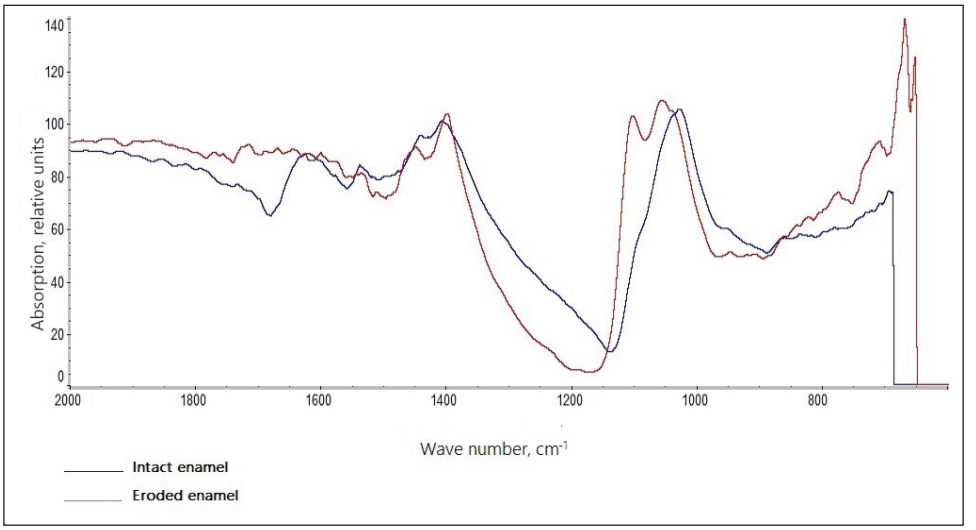

Fig. 2. *IR absorption spectra of the samples of the intact tooth enamel and the enamel of a tooth affected by erosion.*

for different patients. Moreover, it can vary from tooth to tooth. Such substitutions in the crystal lattice of dental enamel, especially substitution of phosphate ions by carbonate ions make minerals of the dental tissue partly soluble in weak acids as compared with HAp.

Solubility of many minerals, such as sodium chloride and similar ones, depends little on the pH factor. However, HAp solubility increases almost by an order of magnitude if pH is reduced by 1. Thus, for pH = 7, HAp solubility in water is approximately 30 mg/l, while at pH = 4 it is about 30 g/l. In order to explain this fact two reasons are considered. The first one implies that in a solution with low pH an excess of H^+ ions results in removal of hydroxyl OH^- ions from the composition of mineral (HAp). Since the production of the ion concentrations $[H^+][OH^-]$ remains constant for water, in the acidic solutions with the increase of $[H^+]$, the concentration of hydroxyl ions will decrease.

Second reason implies that phosphate ions in saliva and in dental deposit are present in four different forms: H_3PO_4, $H_2PO_4^-$, $H_2PO_4^{2-}$ and PO_4^{3-} and the ratio between them changes depending on the pH value in the oral cavity. According to *Dawes*,[25] the influence of hydrogen ion concentration (acidity of environment) on the concentration of phosphate ions of PO_4^{3-} type is the most important factor. Therefore, it turns out that the peracidity of solutions does not in fact have an influence on the content of Ca ions in HAp, but, on the contrary, noticeably decreases concentration of phosphate and hydroxyl ions. On the other hand, it can be noted that, in accordance with *Dawes*,[25] regular application of fluoride-containing toothpastes and gels for tooth brushing may result in wash-out of calcium from HAp crystals, which can also contribute to destruction of tooth enamel, i.e. to its erosion.

Saliva and fluid circulating in the area of dental deposit are usually oversaturated solutions relative to the tooth enamel, since pH value in these areas is higher than the critical one; therefore, human tooth enamel does not dissolve in saliva or in the area of dental deposit.[25] However, under exposure of dental enamel to the acidic environment (fruit juices, carbonated beverages) phosphate ions and hydroxyl groups are removed from Hap, which is the main component of enamel. Anyhow, stoichiometric composition of HAp is violated in time resulting in a progressive destruction of the enamel crystallites. It can be detected in IR absorption spectra of eroded tooth enamel (see Fig. 2).

Indeed, unlike intact enamel, for eroded enamel an intensive peak in the range of 1103 cm^{-1} is observed, that is characteristic for transition compounds of calcium with phosphate ion, such as tricalcium phosphate, oxycalcium phosphate and so on. Complicated spectral structure in the range of wavenumbers from 1000 to 1100 cm^{-1}, unlike clearly expressed band at 1037 cm^{-1}, characteristic of stoichiometric HAp in the intact enamel, means that several different components of calcium phosphates appear in the eroded enamel.

In the range of 1750 – 1670 cm^{-1} the enamel with erosion is characterized by a greater absorption as compared with the intact enamel. Absorption range at 1700 – 1600 cm^{-1} corresponds to the vibrations of Amide I; it is of a complicated shape and comprises three bands at 1687, 1652 and 1623 cm^{-1}. Each of these bands is related to specific vibrations of helical structure of Amide I. In the spectrum of intact enamel, there appears an absorption band near 1623 cm^{-1}. As for eroded enamel, no expressed bands are observed. Instead, a monotonous absorption is observed in this spectral range. Peak of absorption band for Amide II is observed in the range of 1544 cm^{-1} and its intensity is approximately the same for the intact enamel as well as for the enamel with erosion.[26] Due to a rather strong absorption of IR radiation by amid groups, it should be noted that eroded tooth enamel is characterized by relatively high content of organic components and intensive demineralization process. These results are in line with the fact that intact tooth enamel is characterized by relatively low content of proteins.[27,28]

Washing the phosphate ions and hydroxyl groups out of HAp composition under erosion may result in the formation of calcium carbonate ions due to its interaction with carbon dioxide that is present in the atmosphere. For carbonate-substituted HAp of A type absorption peaks are located at 1545 and 1460 cm^{-1}. However, for the intact and the eroded

enamel intensities of these peaks proved to be approximately equal. For carbonate-substituted HAp of B type, peaks are arranged at 1466, 1455 and 1422 cm^{-1}. Absorption band at 1414 cm^{-1} in the intact enamel corresponds to the stretching vibrations of COO^- ions and n_3 vibrations of CO_3^{2-}. For eroded enamel, the intensity value of this peak was a little bit greater and the peak itself was narrower. Comparison of the spectra shows that the absorption curve in the range of carbonate peaks for the eroded enamel is lower than for the intact enamel; their shape is more expressed and the sharpest decrease is observed in the range of 1545 cm^{-1}, which is typical of the carbonate-substituted HAp of A type. At the same time, no similar decrease of peak intensities in the range of 1415 and 1450 cm^{-1} is observed. This indicates a conservation of amide bonds in the eroded enamel in comparison with the intact enamel.

In the work by *Thomas*[12] erosion of teeth *in vitro* was studied with the use of optical methods. It was shown that, depending on the time of exposure to acid, i.e., with the development of enamel erosion, intensity of enamel fluorescence increased. Besides, fluorescence intensity increased due to the contribution of fluorescence in the range of 440 nm, which is characteristic of dentin, while intensity of fluorescence for enamel with a peak at 490 nm, on the contrary, decreased. It was found out that at the initial stage of erosion spectral shape of demineralized enamel fluorescence was similar to that of the intact enamel, but, while erosion developed, fluorescence spectrum became more and more similar to the spectrum of dentine. Such spectral behavior is, on the one hand, connected with the destruction of prism structure in calcium hydroxyapatite in tooth enamel as demineralization progresses, and, consequently, with the decrease of the share of enamel in total fluorescence spectrum. On the other hand, dentine is characterized by a higher content of organic phase, the fluorescence of which is more intense in the range of 400-500 nm, considering that dentine is not affected at the early stages of erosion.

Moreover, demineralization of enamel results in rougher and more porous surface, so, enamel affected by erosion, scatters more light than the intact one.[28,12] In the work by *Thomas*[12] it was found that the coefficient of the diffuse reflection increased with the process of enamel demineralization though the shape of reflection spectra remained the same. The change of scattering properties during demineralization is connected with destruction of the enamel waveguide structure, thus increasing intensity of the diffuse reflection. However, the highest reflection is observed in the long-wave range, while in a cyan range, strong absorption was noted.

Nevertheless, in the study by *Borisova* et al.[18] a decrease of fluorescence intensity was observed during demineralization of enamel. Complexity of the estimation of erosion with luminescence spectroscopy, inconsistency of the obtained results, as well as their interpretation are mainly due to the insufficient understanding of the fluorescence mechanism in hard dental tissues, both intact and affected by erosion.

Fluorescence of hard dental tissues was shown to have different nature.[30-34] It involves the influence of organic (first of all, structural elements of collagen) and inorganic phases (impurity crystals of calcium hydroxyapatite). It was also shown that the fluorescence intensity of tooth enamel depended on the anatomic area and on the tooth type. These results were primarily connected with the influence of adjacent tissues like DEJ (dentine-enamel junction) and dentine on the fluorescence spectrum as well as with a dependence of the optical properties of enamel on the anatomic area. Besides, the most intense fluorescence is typical of the multi-level structure of DEJ, while enamel is characterized by the lowest intensity. DEJ itself proved to demonstrate rather low fluorescence signal while conchoidal layers of dentine and enamel adjacent to DEJ show quite intensive fluorescence. Contribution of the fluorescent DEJ and dentine is of the highest value in the precervical area where thickness of enamel is the smallest. Therefore, fluorescence intensity of the intact enamel in the precervical area is higher than in the region of a tooth equator or incisal edge due to the glow in DEJ and dentine (see Fig. 1).

An increase of fluorescence intensity in the eroded enamel in the early stage was revealed

in this study as well. Nevertheless, no considerable spectral transformations were observed (see Fig.1). Enamel thickness is the lowest in the precervical region, so the fluorescence intensity here is the highest. At the initial stage of demineralization, light scattering increases; however, along with this, thickness of enamel decreases much more. It indicates the presence of two competing processes: the first one reduces and the second enhances the intensity of the exciting radiation transferred to DEJ and dentine. If the pathology is localized in the precervical region, the second process dominates, thus resulting in the enhancement of fluorescence intensity at the early stages of erosion.

The decrease of fluorescence intensity in eroded enamel, observed in *Borisova* et al.,[18] may be conditioned by the region of pathology localization. Indeed, if erosion affects, for example, the region of incisal edge, fluorescence intensity can be lower than that of the intact enamel. This is because enamel thickness in the region of the incisal edge is considerably higher than in the precervical area. With the development of erosion, enamel is gradually destroyed, coefficient of diffusive reflection enhances within the range of fluorescence excitation and consequently, the contribution of the most intensively fluorescent tissues – DEJ and dentine – to the overall fluorescence signal decreases. Also, a decreasing share of the endogenous phosphors having organic (first of all structural element of collagen) and inorganic nature (impurity calcium HAps) in the total fluorescence spectrum under erosion should be taken into account. In fact, under enamel demineralization considerable changes of chemical and mineral composition can be observed (see Fig. 2). Eroded enamel is characterized by a decrease in carbonate-substituted crystals of calcium hydroxyapatite as well as a considerable reconfiguration of organic phase (amide groups). Since impurity crystals of calcium hydroxyapatite, first of all, carbonate-substituted crystals in hard dental tissues, as well as structural elements of collagen, play the role of endogenous phosphors, it seems reasonable to consider them responsible for the observed decrease in fluorescence intensity of eroded enamel at the region of the incisal edge.

At the later stages of erosion, a decrease of fluorescence intensity should be expected, as in this case DEJ and dentine are becoming involved in this process.

Conclusion

Analysis of the obtained results indicates prospects of LIF technique application for the early diagnostics of erosion. Nevertheless, during the development of a reliable diagnostic device capable of detecting erosion at the early stages with LIF technique, it is necessary to take into account several aspects. First, it is the mechanism of fluorescence not only in the affected regions but also in the intact areas of hard dental tissues. Second, it is necessary to consider specific features of morphological structure, chemical and mineral composition of hard tissues in the demineralization area depending on the stage of progression of this kind of pathology. Third, multi-spectral processing of the information requires applying contemporary mathematical methods such as neural network recognition algorithms, algorithms realizing support vector machine method[35] and others.

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