Analysis of tooth tissues using Raman spectroscopy

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Annotation. The results of experimental studies of healthy tooth tissue and tooth tissues during caries disease are presented. Features of Raman spectrum of tooth tissues during caries disease are obtained: the main changes are detected at wavenumbers 956 cm⁻¹, 1069 cm⁻¹, corresponding to phosphates, and 1241 cm⁻¹, 1660 cm⁻¹, corresponding to collagen III and collagen I, respectively. Were introduced criteria allowing to detect caries and to identify weakening of tooth tissues, preceding the caries. The reliability of research results is confirmed by scanning electron microscopy.

1. Introduction

In dentistry a significant share occupy problems that associated with diseases and lesions of tooth tissues. The most common of them is caries, which is irreversible microbial disease that affects calcined tooth structure. [12]. Initially, it affects enamel and remains asymptomatic until it reaches the dentin and pulp. [3] At the same time it is known that the first signs of dental caries, which lead to a weakening of tooth tissue and can prevent its development, is very difficult to diagnose.

At present, are widely used non-destructive optical methods for assessing teeth. Among them is widespread Raman spectroscopy (RS) method, which is simple and requires no sample preparation. [4] So in [5-6] was assessed parallel-polarized and cross-polarized (polarization direction of return perpendicular to the polarization direction of transmission) Raman spectra at 959 cm⁻¹ intensity. In this article concluded that these introduced ratios of polarization can be used to assess the degree of demineralization.

In the study of authors [7] was shown that using Raman spectroscopy it is possible to assess mineral component of tooth tissue. In this work the carious areas of enamel are compared with healthy areas of the tooth at wavenumbers 960 and 880 cm⁻¹ corresponding to (PO₄)³⁻, and (CO₃)²⁻, respectively, since these peaks clearly shows the difference between healthy and carious areas of tooth. It also was experimentally proved that the enamel has a high concentration of mineral substances.

The author of work [8] studied the structural features of hard tooth tissues during cariosity, during non-carious lesion at the example of a generalized pathological abrasion of teeth. Also were studied pulpless teeth using infrared Raman spectroscopy. Was studied ratio of CO₃/PO₄, which shows a decrease of crystallinity by substituting phosphate-ion with carbonate-ion and mineral/organic matrix. It was established that the greatest ratio of CO₃/PO₄ observed in samples of enamel and dentin of pulpless teeth.

In dentistry, there are a number of devices for diagnosis of dental diseases. For example, the scanner ORTHOPHOS XG 3D, which has a high resolution (160/100µm.), which allows an accurate diagnosis...
of caries sequelas and to determine effective methods and tactics of canals treatment, but when examining by device there is irradiation of both patient and physician. [9] Apparatus KaVo DIAGNOdent, on the basis of fluorescence method allows to detect caries in the early stages, however, its significant disadvantage is a false triggering, for example, a reaction to dental plaque [10]. And device SIROInspect, also on the basis of fluorescence method can quickly and easily detect caries lesions during excavation process, but the accuracy depends on the doctor’s professionalism [11].

The objective of this work is application of Raman spectroscopy method to diagnose caries and weakening of tooth tissues in its early stages.

2. Materials and methods
As objects of the study were used 11 samples of large and small molars of patients with a diagnosis of "caries" (K02). Raman spectroscopy method was implemented using experimental stand, previously described in [12]. Measurements were carried out on the specified lines in Figure 1 at power in healthy area of 300 mW and 100 mW in carious area and exposure time of 20 seconds. The error of Raman spectroscopy method amounted to 3%.

For a comparative analysis of chemical composition was used a scanning electron microscope (SEM) JED – 2300 AnalysisStation(Japan). As a studied object were different parts of tooth areas (enamel, dentin, cement), both in normal and in case of caries.

![Figure 1](image.png)

**Figure 1.** Studied tooth: a tooth-section - 1 line - dentin, enamel 2,3 line (see from the bottom upwards) – dentin, enamel. There is a carious lesion in dentin and enamel in the tooth’s bulbous portion.

3. The results of research
Fig. 2 presents characteristic Raman spectra of tooth tissues obtained as a result of studies in lesioned and healthy areas of tooth.
Figure 2. Raman spectra of healthy samples: cement (1), dentin (2), enamel (3) and lesioned by caries cement (4), dentin (5), enamel (6).

From Figure 2 we can observe spectral differences between various tissues of healthy teeth (1-3 notation), for example, dentin, compared with other tooth tissues, is characterized by gaining the line at wavenumber 1069 cm$^{-1}$, corresponding to oscillations of $\nu_1$ ($\text{CO}_3^2$) substitution B-type where this group replaces phosphate and has a lower hardness [13]. Also in dentin are appear lines 1271 cm$^{-1}$ and 1660 cm$^{-1}$, corresponding to collagen III and collagen I, respectively. The almost complete lack of these lines in enamel is explained by the fact that enamel is the hardest tissue in the human body due to the high content of inorganic substances (to 97%), namely hydroxyapatite.

Mineral component of tooth is characterized by vibrational modes $\nu_1$, $\nu_3$ and $\nu_4$ ($\text{PO}_4^{3-}$) in hydroxyapatite molecule, Raman lines of which were registered at wavenumbers 581, 647, 955, 1038 and 1169 cm$^{-1}$ [14,15], and by vibration modes of $\nu_1$ ($\text{CO}_3^{2-}$) substitutions B-type, where ($\text{PO}_4^{3-}$) is substituted by ($\text{CO}_3^{2-}$), and A-type [15], where OH$^-$ is substituted by ($\text{CO}_3^{2-}$) at 874, 1067, 1100 and 1435 cm$^{-1}$ wavenumbers, respectively. The collagen component of Raman lines in addition to proline and hydroxyproline is represented by groups of amide III (in 1241-1271 cm$^{-1}$ range) [14,16], amide II (in 1520-1580 cm$^{-1}$ range) [14] and amide I (in 1630-1680 cm$^{-1}$ range) [14,16].

As can be seen from the figure during lesions of tooth tissues with caries (designations 4-6) the main changes are observed in decrease of lines at 431, 581, 956, 1041 cm$^{-1}$ wavenumbers, corresponding to phosphates and 1660 cm$^{-1}$ corresponding to collagen I.

For samples with an initial stage of caries and a weakened mineral component spectra are transient. Initial changes in spectrum primarily associated with both the remineralization of hard tooth tissues and the loss of collagen I.

For diagnosis of caries development process and weakening of hard tooth tissues (enamel, dentin, cement, pulp) were introduced following coefficients, constructed two-dimensional dependences, shown in Figure 3:

$$D = \frac{I_{956}}{I_{1069}}$$
$$K = \frac{I_{1660}}{I_{1241}}$$

where $I_i$ - intensity of line at the wavenumber;

D - coefficient proportional to the change of content of calcium carbonate-ions in tooth tissue (substitution of ions ($\text{PO}_4^{3-}$) to ($\text{CO}_3^{2-}$), B type);

K - coefficient characterizing collagen I loss compared to collagen III.
Fig. 3 shows a two-dimensional dependence of coefficients in different areas of tooth tissue. The figure shows that caries is characterized by a decrease of optical coefficients, due to a decrease of ions (PO₄)³⁻ and collagen I in tooth tissues. In pathologic processes such as caries, there is a shift towards the replacement of B-type. Loss of collagen I is most clearly visible for dentin, as in cement and enamel it’s initially small. Thus, Two-dimensional dependences in Fig. 3 allow to determine not only the type of tooth tissue, but also the presence of pathologic changes.

As a control method of analysis was used raster electron microscopy (REM). Fig. presents the microphotographs of the surface structure of healthy and carious tooth tissue samples. According to REM data about element composition (Fig 4) the caries samples are characterised by the reduced content of phosphorus and calcium and increased content of carbon and nitrogen, which confirms the obtained results and the reliability of the introduced coefficients.

**4. Conclusion**

Features of Raman spectrum for healthy and carious tooth tissues were obtained. It is established that caries is characterised by changing of spectrum at 956 cm⁻¹, 1069 cm⁻¹ wavenumbers, corresponding to phosphate, and 1241 cm⁻¹, 1660 cm⁻¹, corresponding to collagen III and collagen I.
Two-dimensional analysis of introduced optical coefficients D and K, which allows to diagnose the beginning of development of caries in hard tooth tissues, were carried out. It is established that caries is characterised by decreasing of coefficients due to decreasing of \((\text{PO}_4)^{3-}\) ions and collagen I in tooth tissue. Reliability of research results is confirmed by scanning electron microscopy.

The work was supported by the Ministry of Education and Science

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