The importance of the biomimetic composites components for recreating the optical properties and molecular composition of intact dental tissues.

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Abstract. The objective of this paper was to investigate whether it is possible to obtain biomimetic materials recreating the luminescent properties and molecular composition of intact dental tissues. Biomimetic materials were produced and their properties compared with native dental tissues. In addition, the overall contribution of the organic and non-organic components in the photoluminescence band was investigated. The results showed that it is possible to develop biomimetic materials with similar molecular composition and optical properties to native dental tissues for the early identification of dental caries.

1. Introduction
The discovery and detection of carious lesions is the starting point in dentistry. The benefits of the use of infrared, Raman and luminescent spectroscopy to differentiate intact and carious tooth enamel and dentin have been shown, but these methods do not provide information about the types of disturbance present in the tooth matrix. The present study investigates tooth and biomimetic composites (BC), demonstrating the importance of biomimetic composite components for recreating the optical properties and molecular composition of intact dental tissues.

First, several ionic components (L-arginine hydrochloride, L-histidine, L-lysine hydrochloride and hyaluronic acid) were introduced into the biocomposite via the synthesis of biomaterials using calcium hydroxyapatite. Based on the results of our previous studies in which other amino acids were used to study the molecular composition of the samples [1,2], it was necessary to use all of the selected ionic components together to recreate the molecular composition of intact dentin as in [3].

2. Materials and methods

2.1. Preparing dental tissue samples
Voronezh State University Ethics Committee approved of this study (approval number 001.027-2016).

In order to compare the characteristics of the native dental tissues and biomimetic materials modeling their composition, 10 samples of intact enamel and 10 samples of intact dentin were investigated. The samples (cuts) were extracted from clinically healthy teeth that were removed from...
patients at the age of 18-25 according to the orthodontic indications. The cuts were prepared in the following manner. Originally the teeth were washed in the running water, mechanically cleared of dental plaque, then their surface was dried using filter paper. After cleaning stage, the teeth were divided at a specialized setup with a diamond disk using water cooling into the plates with the thickness of up to 1 mm. Small rotation speed of the disk was used to make sure the samples were not overheated. The resulting microsections of the teeth were polished with the use of a diamond paste. Then the microsections of the teeth were once again washed and cleaned.

2.2. Preparing biomimetic samples
The inorganic component of the elaborated biocomposite is a nanocrystalline carbonate-substituted calcium hydroxyapatite (C-HAP). It was obtained with the use of an organic source of calcium (egg shell) according to the methods described previously [4]. The morphological arrangement of C-HAP synthesized using this technology is identical to the morphology of human hard dental tissue and is necessary to obtain biomimetic samples capable of supplying the natural biogenic C-HAP [5–7]. The organic component of the synthesized biocomposite is a mix of polar amino acids found in the enamel dental tubule, L-arginine hydrochloride, L-histidine, L-lysine hydrochloride and hyaluronic acid, with nanocrystalline C-HAP in an isotonic solution in a proportion typical of native human dental tissue. Note that the ratios of the organic and inorganic components were taken as 5/95, 25/75, 40/60, thus, approximately reproducing the composition of human tooth enamel and dentin.

2.3. Research methods
The diffractometer studies (X-ray diffraction using a Seifert XRD 3003 PTS-HR) and IR-spectroscopy data (diamond PLATINUM ATR, Vertex-70 (Bruker, Germany)), shows that the defined biocomposite composition used yielded results in good agreement with those in [1–3].

Luminescence spectra were obtained at the room temperature from the samples surface according to the technique described in [8] with the use of the unit supplied with TRIAX 550 monochromator and CCD detector. The laser output power was of 30 mW.

3. Results

3.1. IR-data
Figure 1 shows IR-spectra of synthetical materials and native dental tissue.

![Figure 1. The FTIR spectra of: (a) 1,2 - HAP (nanocrystalline), 3 - HAP (microcrystalline); (b) 1 - nanocrystalline HAP, 2 – BC 25/75; (c) 1- sound enamel, 2 -sound dentin, 3 - carious enamel.](image-url)
Figure 1a, shows the transmission IR-spectra of two nanocrystalline samples of C-HAP with different amounts of a carbonate anion (spectra 1, 2). The various CO\textsubscript{3} content can be seen from the comparison of vibration band intensities in the region 1400 – 1450 cm\textsuperscript{-1}, which are related to a B-type carbonate substitution in C-HAP. Figure 1a also presents the IR-spectrum of a microcrystalline HAP (curve 3). Figure 1b shows the transmission IR-spectrum of the biocomposite with the ratio of organic/non-organic component 25/75 (curve 2) and an IR-spectrum of the HAP used to synthesize it (curve 1). Figure 1 shows the IR-spectra of native dental tissues: 1 sound enamel, 2 sound dentin, 3 carious enamel. Note that the vibration bands from the amide groups in the biocomposite spectra in the range of 1400 – 1800 cm\textsuperscript{-1} are related to vibration modes of the organic component. These vibration modes are identical to similar vibration modes of native dental tissues, see figure 1c, curves 1, 2, 3. Comparison of the data shown in figures 1a and b with the results of figure 1c makes it possible to evaluate the correspondence of the molecular composition of the biocomposite to the composition of native tissue as well as to identify its structural disorganization in carious processes. It should be noted that all IR-spectra of biogenic samples are characterized by the redistribution of the intensity of the vibration band intensity in the range 960 – 1100 cm\textsuperscript{-1}.

3.2. Data of laser induced luminescence

The laser-induced luminescence data confirms the differences in the composition between the samples of micro- and nanocrystalline HAP and native dental tissues that have been identified using IR-spectroscopy. The study of the contribution of the organic and non-organic components in the photoluminescence band showed that certain contributions of different organic components are found in a specific region of the photoluminescence band. Note that this type of research has already been described in detail in [9].

The comparative analysis of all spectra figure 2a, b, c shows that synthetic HAP, biocomposite and biological materials have a wide luminescence band in the range of 540 – 620 nm, which agrees with published data for dental enamel and dentin. Ideally, biomimetic materials should be structurally arranged like enamel and tooth dentin, so the luminescence spectra of nanocrystalline HAP (different CO\textsubscript{3} concentration) - figure 1a, curve 1, 2 and microcrystalline HAP - figure 1a, curve 3 samples were compared to sound enamel - figure 1c, curve 1, 2, sound dentin - figure 1c, curve 3 and carious enamel - figure 1c, curve 3.

![Figure 2](image)

**Figure 2.** The luminescence spectra of: (a) 1,2 - HAP (nanocrystalline), 3 - HAP (microcrystalline); (b) 1 - nanocrystalline HAP, 2 - organic component of BC, 3 -- BC 25/75; (c) 1,2 - sound enamel, 3 - sound dentin, 4,5 - carious enamel.
4. Discussion

4.1. Features of IR-spectra of the transmission of the samples

Comparison of the IR-spectra of the HAP samples presented in figure 1 allows one to identify special features of the enamel associated with stoichiometry and periodic structure of the enamel hydroxyapatite. The size of hydroxyapatite nanocrystals is known to vary from 70 to 500 nm in one direction of growth in native dental tissue [4,10] and was confirmed by the redistribution of the band intensity (maxima) in the IR-spectra in the range from 960 to 1100 cm\(^{-1}\). Note that nanocrystalline HAP with a crystal length of 50 – 70 nm was used for the synthesis of the biocomposite sample. The IR-spectrum of the nanocrystalline HAP - curve 1, and the biocomposite with the ratio of organic/non-organic component of 25/75 are shown in figure 1b. At the same time in the IR-spectrum of dentin, the ratio of intensities in the range from 960 to 1100 cm\(^{-1}\) experiences changes and corresponds with the nanocrystalline HAP sample, with the entire spectrum being consistent with that of the biocomposite sample - figure 1a, curve 3. The same is observed in the IR-spectra of caries-damaged enamel - figure 1c, curve 3, but is caused by structural disorganization of the enamel tissue and a reduction in its crystallinity, which is consistent with the previous research [11].

Note that while comparing curve 1 in figure 1b (the biocomposite spectrum) and curves 1, 2, and 3 in figure 1c (native dental tissues), the position and intensity of vibratory modes from the organic component are found to correspond. This indicates that ionic components, L-arginine hydrochloride, L-histidine, L-lysine hydrochloride and hyaluronic acid, could be employed to recreate the molecular composition of enamel and dentin to develop biomimetic materials. In addition, as suggested by the IR-spectroscopy data, the effect of the organic component is less pronounced in biocomposites with a high level of HAP [3].

4.2. Features of laser-induced luminescence

The results of the analysis of the luminescence spectra - figures 2a, b, c show the importance of using C-HAP as the non-organic component as well as the organic component to develop materials for recreating the optical properties of human teeth.

Regarding hydroxyapatite, note that the change in the shape of the luminescence band from nano- to microcrystalline HAP - figure 2a, curves 1 & 3 appears to be caused by the structural perfection and dimensional weight of apatite. In the present report, a theoretical and experimental study was performed to compare the structure of tooth enamel and dentin microcrystals with the synthetic nanocrystalline HAP samples [11,12], indicating that the synthetic material and biogenic tissues are identical in size. Thus, note that luminescence of the healthy enamel behaves identically to HAP - figure 2c, curves 1, 2. The change in the shape of the maximum luminescence band for intact enamel due to its complex hierarchical structure. The luminescence spectrum 1 in figure 2c obtained from intact enamel is more consistent with the spectrum of microcrystalline HAP in figure 2a, curve 3, while spectrum 2 in figure 2c also obtained from enamel is consistent with the luminescence spectrum of nanocrystalline HAP - figure 2a, curve 1. Enamel in differently-oriented crystals and sizes is in agreement with the available data. However, with regard to the material structure and its effect on the photoluminescence spectrum, it is important to note that the data from [13] indicated that the maximum luminescence band of a defect-free hydroxyapatite is within another spectrum. Changes in the local atomic structure of HAP have a considerable effect on the position of the luminescence band as the shape and size of the HAP crystals remain the same. The study of the luminescence of nanocrystals and microspheres of the hydroxyapatite [14,15] indicates that the change in the position of the maximum luminescence is due not only to the shape and size of the HAP, but is related to defects in the HAP crystals (i.e., with the local atomic structure of the HAP). Hence, considering the composition of the apatite of tooth dentin as well as HAP obtained using the liquid-phase method, defects in the HAP structure have more effect on the shape and position of the luminescence band.
[13,16–18]. It should also be noted that the materials were synthesized with a microelement composition identical to that of enamel and dentin, having the essential structural B-defect (replacement of the PO₄ and CO₃ groups). It has been suggested that this defect is responsible for changes in the intensity of the luminescence band in such samples. In [19], there is much discussion of how many defects in the human dental enamel are associated with the replacement of HAP atoms by foreign ions, despite good crystallization of the HAP crystals of human dental enamel.

The analysis of the shape and features of the luminescence spectrum of the synthesized biocomposite - figure 2b, curve 3 allows one to identify the contribution of C-HAP - figure 2b, curve 1) as well as the introduced organic substances - figure 2b, curve 2. A detailed investigation of the resulting data indicates that the typical features in the luminescence spectra of native dentin - figure 2c, curve 3 are present in the spectra of native tissue and biomimetic materials, and that the highlighted areas - figure 2a, b, c can be used to identify similarities and differences between native tissues and synthesized biocomposite.

The comparative analysis of the spectra of native sound and caries tissue as well as that of the biocomposite - figure 2b, c shows that the luminescence largely depends on the type of structural defect and quantitative ratio of the organic and non-organic component in intact dental tissues. Changes in the regions of 550 – 575 and 600 – 625 nm for the biocomposite luminescence spectra suggest that the changes in luminescence of native tissue are due to structural disorganization and demineralization processes, including tooth organic transformation. Therefore, it can be assumed that the luminescence largely depends on the type of structural defect and quantitative ratio of the organic and non-organic component in intact dental tissues and less on the hierarchy.

5. Conclusion

The identified features in the IR and luminescence spectra in the HAP samples with varying stoichiometry and dimensionality, biocomposite and native human dental tissue can be employed to develop the technology for biomimetic materials with a similar molecular composition and optical properties to native dental tissues. These findings indicate that such research can assist in the development of a highly efficient method for the identification of dental caries at an early stage.

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