Spectral analysis of juvenile dentin biomaterials

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Abstract. The experiments in optical evaluation of biomaterials taken from juvenile dentin have been made using the Raman spectroscopy method. It was shown that juvenile teeth lose their mineral components and preserve organic components in the process of demineralization with the use of hydrochloric acid.

1. Introduction
Currently, a lot of biomaterials are used for osteogenesis in dental surgery. The quality of biomaterials is defined by full regeneration of bone tissues and is provided by optimal conditions for regenerative processes such as no reaction of immunological rejection, biodegradation ability [1]. Biomaterials based of juvenile dentin that can be less rejected by the cells of recipient compared to brefomatrix are affordable alternative to it. Unlike brefobone used for allogenic plasty, demineralized dentin as a non-vascular tissue does not have antigenic properties; therefore, protein incompatibility of the tissues is less.

Thus, the work [2] shows that using dentin as augmentat practically in every form has great potential. The work reveals that demineralized dentin matrix is engaged in the process of osteoinduction faster than the mineralized one. The growth factors and proteins that take part in matrix mineralization provide substitutive resorption of dentin subsequently substituted by native bone tissue. The related antigenic composition of dentin protein component does not provoke immune response and is fully engaged in the process of bone remodeling.

The evaluation of demineralization of biomaterials taken from juvenile dentin is an urgent task as the quality of produced biomaterial has direct impact on its rejection.

One of the simplest methods of biomaterial quality evaluation is the Raman spectroscopy method that provides information about components of bone minerals and matrix collagen [3].

The aim of the study is evaluation of biomaterials received from juvenile dentin using the Raman spectroscopy method.

2. Materials and methods
The subject of the study was dentin taken from healthy juvenile teeth pretreated mechanically. All samples were cut in two equal parts and divided in two main groups depending on the stages of their treatment: the 1st group – mineralized biomaterials from juvenile dentin, 2nd group – demineralized biomaterials from juvenile dentin. Demineralization was made using hydrochloric acid solution of stage
1, 2 of normality according to "LYOPLAST" technology [technical specification TU-9398-001-01963143-2004].

![Figure 1. Demineralized samples.](image)

The main method of analysis of biomaterials based on juvenile dentin was the Raman spectroscopy method implemented by the experimental stand including the Raman probe RPB-785 (focal length of 7.5 mm), combined with the laser module LuxxMaster LML-785.0RB-04 (power up to 500 mW, wavelength of 784.7 ± 0.05 nm) and the high-resolution digital spectrometer Shamrock sr-303i providing spectral resolution of 0.15 nm with the build in cooling camera DV420A-OE (spectral range of 200-1200 nm) [4].

The analysis of the spectra was made using the software MagicPlotPro and with the use of the method of linear discriminant analysis (LDA) in the software IBMSPSS Statistics. The spectra were averaged using the mathematical software Mathematica 8 [5].

3. Analysis

Figure 2 shows the average Raman spectra of the two groups of samples: the juvenile teeth before and after demineralization. The main differences can be seen in the Raman lines of 588 cm\(^{-1}\), 919 cm\(^{-1}\), 956 cm\(^{-1}\), 1003 cm\(^{-1}\), 1036 cm\(^{-1}\), 1070 cm\(^{-1}\), 1242 cm\(^{-1}\), 1270 cm\(^{-1}\), 1426 cm\(^{-1}\), 1449 cm\(^{-1}\), 1665 cm\(^{-1}\). The reduction of intensity of the lines of 588 cm\(^{-1}\) (\(\nu_4\) PO\(_4\)), 956 cm\(^{-1}\) (PO\(_4\) from apatite), 1036 cm\(^{-1}\) (\(\nu_3\) PO\(_4\)) and 1070 cm\(^{-1}\) (\(\nu_1\) CO\(_3\)) indicate the destruction of mineral components in the process of demineralization. While the organic components preserve in the process of demineralization, as evidenced by the lines of 854 cm\(^{-1}\), 919 cm\(^{-1}\) and 1270 cm\(^{-1}\) (Collagen), 1242 cm\(^{-1}\) (Amide III), 1426 cm\(^{-1}\) (Deoxyribose, (B,Z-marker)), 1449 cm\(^{-1}\) (Collagen (CH\(_2\))) and 1665 cm\(^{-1}\) (Amide I from peptide bonds) [6-8].

The line of 919 cm\(^{-1}\) (C-C stretch of proline ring/glucose/lactic acid) is of particular interest. This line partially corresponds to lactic acid that in interaction with hydrochloric acid forms chloropropane acid relating to organic compounds.
Figure 2. The average Raman spectra of the juvenile dentin samples: 1 – the samples before demineralization, 2 – the samples after demineralization.

To make the received Raman spectra more informative nonlinear regressive analysis of the spectra was made including their spectral line decomposition. Figure 3 shows the results of decomposition of spectral contour on the sum of distribution of the Gaussian lines.

Figure 3. Spectral contour decomposition of the samples of enamel and dentin.

The average value of the coefficient of determination of the result spectrum on the initial one in the range of 800-1780 cm\(^{-1}\) was R\(^2\) = 0.99, the relative error of spectral line intensity evaluation a was less than 6\%, the average standard deviation of the coordinate of the line x\(0\) was 0.8 cm\(^{-1}\), the average standard deviation of the width of the Gaussian line (HWHM) dx was 1.8 cm\(^{-1}\).
For relative quantitative analysis of component composition of hydroxyapatite the ratios of the Raman lines intensities to Amide I line intensity were used.

The method of linear discriminant analysis in the software IBM SPSS Statistics was chosen for further analysis of spectral lines of the researched subjects received after decomposition.

![Figure 4. The chart of values of linear discriminant function.](image)

Figure 4 shows the results of LDA comparing of the two groups of samples. 72 spectra of juvenile teeth (40 demineralized and 32 mineralized) were analyzed. The discriminant function LD-1 describes the 100 % of dispersion. The positive values of LD-1 characterize the Raman spectra of mineralized materials. The areas of the groups do not have intersections.

![Figure 5. The values of factor structure coefficients.](image)
Figure 5 shows the coefficients of the factor structure matrix that have physical meaning of correlation between variables in the model and discriminating function. The higher the modulus value of LD-1 for variable, the more it defines the difference in the discriminant model between the groups of samples.

The discrimination adequacy of the method is characterized by the value of AUC = 1 that indicates the great quality of the diagnostic tool. The standard error SE was 0%, the confidence interval of AUC is in the range of 1–1. The optimal cut-off point of the presented algorithm determined according to the balance between sensitivity and specificity was 0.5. The sensitivity and specificity of the diagnostic model in this cut-off point were 100 %.

4. Conclusions
The main spectral differences between demineralized biomaterial and juvenile dentin compared to the mineralized one were found as a result of the study. It was shown that mineral components are destructed in the process of demineralization, as evidenced by significant reduction of intensity of the lines of 956 cm\(^{-1}\) (PO\(_4\) from apatite) and 1070 cm\(^{-1}\) (\(\nu_1\) CO\(_3\)), and organic components are preserved, as evidenced by the lines of 854 cm\(^{-1}\) (Collagen), 1242 cm\(^{-1}\) (Amide III), 1665 cm\(^{-1}\) (Amide I from peptide bonds).

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