Application of Raman spectroscopy to assess the articular surface after performing chondroplasty in rabbits

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Abstract. This paper presents the results of expanded analysis after the experimental researches with the samples femoral bones of rabbits in distal epiphysis area using the Raman spectroscopy method. It has been found that values of the optical ratios are characterized by reduction of Raman bands during transition from the intact cartilage zone to the PFP plastic region on the wave numbers 956 cm⁻¹ (PO₃⁻⁴ (symmetric stretching phosphate oscillation)), 1069 cm⁻¹ (CO₃²⁻ (C–O planar stretching vibration)) and relatively constant coefficients on the wavenumbers 852 cm⁻¹ (Proline), 1250 cm⁻¹ (Amid III), 1587 cm⁻¹ (Amid II) and 1660 cm⁻¹ (Amid I), and also on the 1745 cm⁻¹ ((C=O) Lipids).

1. Introduction

The issue of rehabilitation of full-layer defects of the articular surface is one of the most important in modern medicine. Keeping of the damages leads to increase in the number of destructive process and emergence of osteoarthritis. The most used method to compensate the defects is their plastic with using the biological or artificial materials [1].

At present there are no precise and unmistakable methods for treatment of full-thickness damage in the articular cartilage. Shortage of the blood supply and low metabolism are the cause that a complete reparative regeneration is non-likely only with little size and depth of defects [2]. That became a reason of innumerable researches devoted to finding methods for recovering of a damaged tissue, among them based on the use of regenerative medicine technologies. Among them, the use of autologous and allogeneic chondrocytes on various types of scaffolds is known [3].

The method of a screening assessment of the newly formed regenerate quality after chondroplasty hasn’t been found yet. Most often, this requires a biopsy and a long microscopic study with the preparation of the biomaterial.

One of the most widely used screening methods is Raman spectroscopy (Raman). This method allows to observe the component composition of biomaterials. Raman spectroscopy has a number of advantages, such as ease of sample preparation, minimally invasive, a large amount of information [6, 7].
For example, the article [8] gives assessment of polarized Raman spectroscopy usage in the study of biochemical compositions and changes in the explant orientation of a affected porcine cartilage. Also, it was found that the method of polarized Raman spectroscopy has the potential for the diagnosis and detection of early cartilage damage at the molecular level.

The objective of the study is evaluation the possible usage of Raman spectroscopy method to characterize the areas of the articular surface after chondroplasty in the experiment in rabbits.

2. Materials and methods

The objects of the study were samples of distal epiphysis of the femur of rabbits. The animals had been operated in a variety of ways of plasty after the creation of two full-layer osteochondral defects of the articular surface of the femoral condyles during the experimental researches. Demineralized spongiosis "Lioplast" ® (Russia, Samara) was used as a plasty material coupled with cultures of chondrocytes with platelet-rich plasma (PRP) and material having only plasty PRP.

In the dynamics the animals were removed from the experiment. The animals had been operated in order to bioply of distal epiphasis of the femur with the further macro-and microscopic study of the articular surface, also using Raman method.

The surfaces of the newly formed regenerates of both condyles, intact hyaline cartilage and the surfaces which borderline with the newly formed regenerates were studied in the samples.

The samples were analyzed using experimental setup, realizing method Raman spectroscopy. Processing of the obtained Raman spectra was performed in Wolfram Mathematica [9, 10].

3. Analysis

Figure 1 show spectrum obtained in the research of femoral bones of rabbits in distal epiphysis area, 1 month after the replacement of PRP defects. The main spectral differences of the plasty zone, boundary zone and intact cartilage are observed on the bands at 956 cm\(^{-1}\) (PO\(_3\)\(^{-4}\), symmetrical valence oscillation of phosphate), 1004 cm\(^{-1}\) (breathing ring of phenylalanine), 1069 cm\(^{-1}\) (valence vibrations of CO\(_3\)\(^{2-}\)).

At the same time, collagen, which are the main structural components of connective tissue and correspond to the intensities of the lines at the wave numbers 1660 cm\(^{-1}\) (amide I), 1587 cm\(^{-1}\) (amide II), 1250 cm\(^{-1}\) (amide III) and 1270 cm\(^{-1}\) (amide III), does not change significantly in the study of the three zones of the articular surface. The analysis of the Raman spectroscopy bands is presented in the table 1.

| Wavenumbers, cm\(^{-1}\) | Assignments |
|------------------------|-------------|
| 852                    | C=C, Proline [11] |
| 956                    | PO\(^{3-4}\) Symmetrical valence oscillation of phosphate [12] |
| 1004                   | Breathing ring of phenylalanine [13, 14] |
| 1069                   | Valence oscillation CO\(_3\)\(^{2-}\) [14] |
| 1250                   | Amid III [15, 16] |
| 1270                   | Amid III, CH2 oscillation of glycine and proline [16] |
| 1445                   | (CH\(_2\))/(CH\(_3\)) collagen and other proteins [11, 17] |
| 1587                   | Amid II (C - N, C-C strength) [11] |
| 1660                   | Amid I, C-C valence oscillation [17] |
| 1745                   | V(C=O) Lipids [18] |

After processing in Wolfram Mathematica, the spectra can still contain contours with an unresolved internal structure, which is caused by the overlapping of adjacent lines. In order to be able to analyze
them, the spectra decomposition shown in figure 1 was carried out on the line in MagicPlotPro 2.7.2 program. Deconvolution was carried out by preparing a template of trial Gauss functions of individual peaks (the width and position of the peak in the template is fixed), followed by applying it to the experimental spectrum to find the amplitude of individual bands.
Figure 1. Raman spectra of articular surface of femoral bones of rabbit in distal epiphysis area, 1 month after the replacement of PRP defects: (a) area plasty of the defect I; (b) area plasty of the defect II; (c) area of intact cartilage.

The results of the spectra deconvolution were used in further analysis. Coefficients were introduced to assess the quality of the surface of the newly formed regenerate. Amide I corresponding to the line intensity at 1660 cm\(^{-1}\) was used as a denominator in the introduced coefficients:

\[
K_n = \frac{I_n}{I_{1660}}
\] (1)

where \(I_n\) – the intensity values at the corresponding wavenumbers
n = 1 – 852 cm\(^{-1}\) (C-C Proline);
n = 2 – 956 cm\(^{-1}\) (PO\(_4^3\) Symmetric stretching vibrations of the phosphate);
n = 3 – 1250 cm\(^{-1}\) (Amide III);
n = 4 – 1587 cm\(^{-1}\) (Amide II (C-C stretching vibration));
n = 5 – 1745 cm\(^{-1}\) ((C=O) Lipids).

Also, the coefficient \(A\), which determined the ratio of mineral components, was used:

\[
A = \frac{I_{956}}{I_{1063}}
\] (2)

where \(I_{1069}\) - is the intensity values at wavenumber 1069 cm\(^{-1}\) (Carbonate CO\(_3^{2-}\)).

Figure 2 shows two-dimensional (2-D) diagrams of the input coefficients, which allow us to identify each of the studied areas of the samples.
Figure 2. Two-dimensional diagrams of coefficients for different areas of articular surface of femoral bones of rabbit in distal epiphysis, 1 month after the replacement PRP.

For zone of intact cartilage values of the optical coefficients were: 2,2<A<3,3; 4<K2<7. The surface of these areas was characterized by an increase in the intensity of the peaks at the wave numbers (PO$_3^{-4}$ (symmetric stretching vibrations of the phosphate)) and CO$_2$-$3$ (C-O planar valence). For areas plasty PRP they are: 1 < K2 < 3; 0,9 < A <1,6.

The two-dimensional PRP diagrams also show that the values of the introduction of the coefficients corresponding to Proline, amide II, amide III and lipids vary slightly, which corresponds to 0,6<K1<1,25; 1,5<K3<2,4; 1,4<K4<2,4; 0,8<K5<1,5.

Analysis of two-dimensional diagrams showed that with the help of the introduced coefficients it is possible to reliably verify the zone of intact articular surface and the zone of newly formed after chondroplasty regenerates.

4. Conclusion
Deconvolution of the spectra by selecting the spectral profile allows conduct advanced component analysis of the studied zones of a biological object. It is found that the values of the optical coefficients are characterized by a decrease in the peaks during the transition from the intact cartilage zone to the plasty region of the PRP at the wave numbers 956 cm$^{-1}$ (PO$_3^{-4}$ (symmetric stretching vibrations of the phosphate)) and 1069 cm$^{-1}$ (CO$_2$-$3$ (C-O planar valence)).

The input optical coefficients allow verify reliably areas of intact cartilage and newly formed regenerates.

Spectral analysis of the surface of intact articular cartilage, plasty areas of PRP, after 1 month, and the border zone showed their differences. This may confirm indirectly that the newly formed regenerate is non-organotypical.

Thus, Raman spectroscopy method can determine a non-compliance of the surface quality of the newly formed regenerate with intact hyaline cartilage even before the time-consuming microscopic research at the stage of primary biopsy of the material.
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