The synovial fluid analysis by using Raman Scattering spectroscopy in order to elucidate the synovial joint pathology

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Abstract. In this paper, we present the results of experimental studies by using Raman-scattering spectroscopy method of synovial fluid that was obtained from the joint cavity during the surgery. Analyzing the composition of synovial fluid, it was identified that with the progress of degenerative-dystrophic process in synovial fluid of the affected joint, the gross amount of components increases at wave numbers: 1155 cm⁻¹ (hyaluronic acid (C-O, C-C)) and 1250 cm⁻¹ (Amide III). The entered optical numbers allow us to characterize synovial fluid (SF) in osteoarthrosis (OA). This specific Raman-scattering spectroscopy method may become a new diagnostic screening in order to elucidate the synovial joint pathology.

1. Introduction

The contained synovial fluid (SF) in the joint cavity being a biological medium is unique in biophysical and physical-chemical content and properties. It provides with the sliding of the bones' articular surfaces playing a damper function. Its damage causes a change in the cell composition SF [1]. SF or synovia is a sort of life activity indicator of the joint, which is formed and replenished by means of substances originating from the blood plasma and secreted covering layer of articular membrane - is typified as a boundary layer between stratum synoviale, cartilage and subchondral bone [2]. In light of this, for early and accurate diagnostics of joint disease and, thereby, for the more accurate treatment order of a patient, there is a necessity to carry out an analysis of synovial fluid.

Unfortunately, people conduct a study on synovial fluid very rare, because analysis' interpretation of results causes considerable difficulties. Standard diagnostics of synovial fluid is comprised of macropanel whereby we can define the volume, colour, fluid viscosity, turbidity and other external feature; cell counting; as well as microscopic analysis of native preparation; cytologic screening of the stained preparation [3].

Raman scattering spectroscopy method has certain advantages in comparison with the analytical methods that mentioned above. The most important of them are the simplicity of sample preparation, minimal invasiveness and a big amount of received information. Spreading this method in medicine can improve the results of surgical treatment of patients as well as to choose rational therapeutic approach.
for patients, and put in perspective the functional outcomes of surgical interferences that attempted to correct synovial joint pathology.

The aim of this work is to study the joint synovial fluid in the treatment of osteoarthrosis by using Raman scattering spectroscopy method.

2. Research materials

Patients' samples of SF were obtained by knee joint puncture with a disposable syringe. This procedure was performed according to the standard technic under aseptic conditions. 5-10 ml of normal saline solution were preliminarily injected in the joint in order to obtain a right amount of SF. The sampling was taken before diagnostic arthroscopy from patients without any obvious clinical signs of joint disease, the absence of which was confirmed (group 1). The patients with chronic damages of infra-articular structures before doing arthroscopy (group 2). Before doing knee arthroplasty in connection with the destructively-dystrophic osteoarthrosis disease at the IV stage (group 3).

The samples of synovial fluid were carried out using a stand that implements the SRS method. This stand includes a high-resolution digital spectrometer Shamrock sr-303i with a wavelength range of 200-1200 nm, with built-in cooling chamber DV420A-OE, a fiber-optics probe RPB-785 for Raman spectroscopy, combined with laser module LuxxMaster LML-785.0RB-04 with laser emission wavelength of 785 nm and with a line width of 0.2 nm. [4]

The processing of Raman spectra was carried out in the Wolfram Mathematica 9 program. The unknown spectrum was removed from noise during the processing by a smoothing median filter (5 points). We had been determining approximating straight line of an auto-fluorescent component in the picked interval 700-2200 cm\(^{-1}\) by using iterative algorithm (a fifth-degree polynom), then we subtracted this component, and as a result, we obtained the allocated Raman spectrum. The separation of the overlapped lines in the spectrum was carried out in the software environment of MagicPlotPro.

3. Results and discussion

The averaged Raman spectra of the SF samples of three groups are shown in the figure 1. The curves for better visibility are shifted vertically relative to each other for 2 relative units. The value of the intensities for the case of the 1155 cm\(^{-1}\) in figure 1 is difficult to compare, but it is used below in figure 2.

![Figure 1 - Raman spectra of the synovial fluid of the knee joint: I group - without pathology; II group - with chronic damage of infra-articular structures; III group - the stage 4 of osteoarthritis.](image-url)
In a comparative analysis of the spectra of SF samples, it turned out that in the case of chronic damage to the structures of the knee joint and destructively-dystrophic diseases in SF, components at 744 cm\(^{-1}\) (Protein (C-C-O), deformation twisting) \[5\], 948 cm\(^{-1}\) (secondary structure of protein (N-Ca-C stretching, \(\alpha\)-helix))\[6\], 1001 cm\(^{-1}\) (phenyl alanine E-ring) \[7\]. Whereas during the SF analysis of joints without any pathology such components were not determined. Also in the SF samples of the second and third groups, the content of the components progressively increased at wave numbers: 1155 cm\(^{-1}\) (Hyaluronic acid (C-O, C-C)) \[5\], 1206 cm\(^{-1}\) (Hyaluronic acid, phenylalanine) \[5\] and 1337 cm\(^{-1}\) (Hyaluronic acid, nucleic acids) \[5\], 1125 cm\(^{-1}\) (C-C, C-O stretching of glycosidic linkage) \[6\], 1250 cm\(^{-1}\) (Amide III)\[6\], 1442 cm\(^{-1}\) (CH2 / CH3 deformational twisting) \[7\].

Relatively constant components of SF in all three groups of samples were (C = O) lipids corresponding to a wave number of 1745 cm\(^{-1}\) \[8\]. This number used as a denominator in the input optical numbers (A1-6):

\[ A_i = \frac{I_i}{I_{1745}} \]

Where \(I_i\) – the intensity values at the corresponding wave numbers:

- \(i = 1\) – 1001 cm\(^{-1}\) (phenyl alanine E-ring);
- \(i = 2\) – 1125 cm\(^{-1}\) (C-C, C-O stretching of glycosidic linkage),
- \(i = 3\) – 1155 cm\(^{-1}\) (hyaluronic acid (C-O, C-C)),
- \(i = 4\) – 1206 cm\(^{-1}\) (hyaluronic acid, phenyl alanine),
- \(i = 5\) – 1250 cm\(^{-1}\) (Amide III),
- \(i = 6\) – 1442 cm\(^{-1}\) (CH2/CH3 deformational twisting).

Based on the obtained values of the optical numbers A1-6 there were constructed two-dimensional diagrams (Figure 2).

Figure 2 - Two-dimensional groups diagrams of samples' comparison of synovial fluid.

The two-dimensional diagrams show that all the optical numbers (A1-6) progressively increase due to intensity enhancement of the destructively dystrophic processes in joint from the first to the second
and third groups of the study samples. In such a way the number A1 changes its magnitude from 0.3-0.65 to 0.6-1.4, the number A2 changes its magnitude from 0.7-0.8 to 0.7-1.4, the numbers A5 and A6 change their magnitude from 0.8-0.9 to 0.9-1.5 and from 0.6-0.8 to 0.75-1.4. This makes perfect sense because SF from a transudation becomes an exudation during the involvement in the pathological process, which have an effect on its component composition - increase in the protein content. This phenomenon can be explained not only by the synovial membrane hyperpermeability during inflammatory state but also by the increase of g-globulins output by synovial cells.

We attribute this increase of the numbers A3 (from 0.7-0.8 to 0.7-1.3) and A4 (from 0.8-0.9 to 0.8-1.35) to increase of the synovial fluid viscosity along with a progressive increase of disruption product of an articular hyaline cartilage in the second and third groups of SF samples. The two-dimensional diagrams analysis showed that with the aid of the entered optical numbers it is possible to verify the degree of development of destructively dystrophic diseases of the knee joint for sure.

4. Conclusion

The component composition of synovial fluid can be identified in the setting of various pathologies of the joint by using Raman-scattering spectroscopy method.

In the case of destructively dystrophic diseases in the synovial fluid of the affected joint the amount of protein components and disruption product of the cartilaginous tissue increases - that can be judged by the growth of the wave numbers of 1001 cm\(^{-1}\) (phenyl alanine E-ring), 1155 cm\(^{-1}\) (hyaluronic acid (C-O, C-C)), 1206 cm\(^{-1}\) (hyaluronic acid, phenyl alanine), 1125 cm\(^{-1}\) (C-C, C-O stretching of glycosidic linkage), 1250 cm\(^{-1}\) (Amide III), 1442 cm\(^{-1}\) (CH2 / CH3 defromational twisting), as well as 744 cm\(^{-1}\) (protein (C-C)).

By using entered optical numbers, it is possible to verify the degree of destructively dystrophic diseases development of the knee joint, which may become a new diagnostic screening method in order to educe the synovial joint pathology.

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