

Conference Paper

Analysis of Synovial Fluid for Study of Diseases of Joints Using the Method of Raman Spectroscopy

Timchenko P.E.¹, Timchenko E.V.¹, Volova L.T.², Dolgushkin D.A.²,
Yagofarova E.F.¹, and Markova M.D.¹

¹Samara National Research University, 34, Moskovskoye shosse, Samara, 443086, Russia

²Samara State Medical University, 89, Chapayevskaya St., Samara, 443099, Russia

Abstract

This paper presents the results of experimental studies using the synovial fluid Raman spectroscopy method, which was obtained from the joint cavity during the operation. By analyzing the composition of the synovial fluid, it was found that with the development of the degenerative-dystrophic process in the synovial fluid of the affected joint, the total number of components at wave numbers: 1155 cm^{-1} (Hyaluronic acid (C-O, C-C)) and 1250 cm^{-1} (Amide III). The introduced optical coefficients allow estimating the synovial fluid in osteoarthritis and further this method of Raman spectroscopy can become a new diagnostic screening for the detection of articular pathology.

Keywords: Raman spectroscopy, osteoarthrosis, synovial fluid

Corresponding Author:

E F Yagofarova

l_yagofarova@mail.ru

Received: 17 January 2018

Accepted: 25 March 2018

Published: 17 April 2018

Publishing services provided by
Knowledge E

© Timchenko P.E. et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the PhysBioSymp17 Conference Committee.

1. Introduction

The synovial fluid contained in the joint cavity is a biological medium unique in biophysical, physicochemical properties and composition, ensuring the sliding of the articular surfaces of bones playing a damper function, its damage causes a change in the cellular composition of the synovial fluid [1]. synovial fluid or synovia is a kind of indicator of the vital activity of the joint, which is formed and replenished by substances coming from the blood plasma and secreted by the membrane of the joint membrane, is considered as a boundary layer between the synovial membrane, cartilage and subchondral bone [2]. Therefore, for the timely and accurate diagnosis of joint disease and, as a consequence, the more accurate appointment of subsequent treatment of the patient is the analysis of synovial fluid.

Unfortunately, the study of synovial fluid is relatively rare; interpretation of the results of analysis causes considerable difficulties. Standard diagnosis of synovial fluid includes macroscopic analysis by which the volume, color, viscosity, turbidity and other

OPEN ACCESS

external signs are determined, cell counts are taken, as well as microscopic analysis of the native preparation, cytological examination of the colored preparation [3].

At present, optical methods of research have become widely used for the study of bioobjects: backward scattering spectroscopy [4], microscopic analysis [5, 6], and Raman spectroscopy [7, 8]. Raman spectroscopy has certain advantages over other methods. The most important of them are the simplicity of sample preparation, minimally invasive and large amount of information received. The spread of this method in medicine can improve the results of surgical treatment of patients, allowing reasonably select the rational tactics of treatment of patients, as well as objectively evaluate the functional outcomes of surgical interventions undertaken to correct the pathology of the joints.

The authors of the articles [9, 10], conducted studies of the structure of the synovial fluid and evaluated the content of their protein components.

The aim of this work is to study the joint synovial fluid in case of osteoarthritis with the help of the Raman spectroscopy method.

2. Materials and methods of research

Studies were performed on samples of synovial fluid obtained from the articular knee bag, by puncturing the knee joint with a disposable syringe. Differences in Raman spectra were determined for samples of synovial fluid in the diagnosis of osteoarthritis. Samples were divided into two groups: 1 - conditionally healthy (control samples), 2 - developed osteoarthritis.

Samples of synovial fluid were investigated using a stand that implements the Raman spectroscopy method. The method includes a high-resolution digital spectrometer Shamrock sr-303i with a spectral range of 200-1200 nm, with a built-in cooled camera DV420A-OE, a fiber-optic probe RPB-785 for Raman spectroscopy, combined with a LuxxMaster LML-785.oRB-o4 laser module with a length a wave of laser radiation of 785 nm and a line width of 0.2 nm [11, 12].

Processing of Raman spectra was carried out in the program Wolfram Mathematica 9.

3. Results and discussion

The average Raman spectra of the synovial fluid are shown in Fig. 2. The decoding of the main lines is given in Table 1.

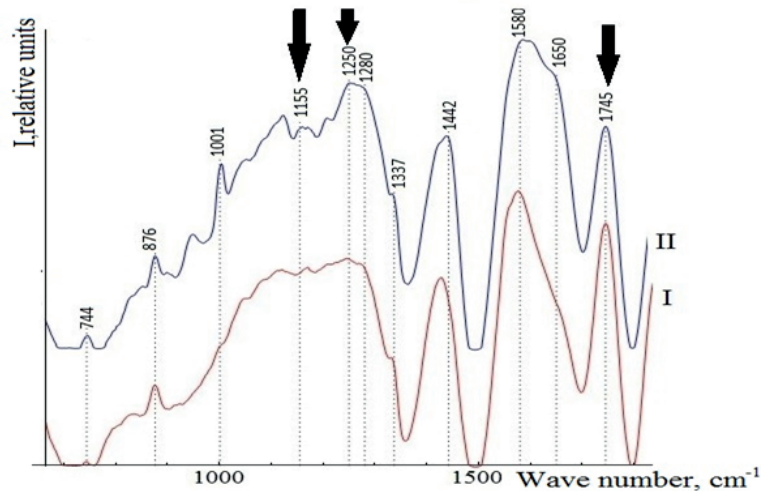


Figure 1: Averaged spectra of Raman scattering of synovial fluid: I - Conditionally healthy; II -Developed osteoarthritis.

TABLE 1: Interpretation of Raman spectra of synovial fluid.

CM ⁻¹	Substance, oscillation
744	Protein (C-C-O) [13 7]
876	Protein (C-C deformation) [14 8]
1001	Respiratory ring of phenylalanine [15 9]
1155	Hyaluronic acid (C-O, C-C) [13 7]
1250	Amide III [16 10]
1280	Amide III [13 7]
1337	Hyaluronic acid [13 7], nucleic acids
1442	CH ₂ / CH ₃ deformation twisting [17 11]
1580	Amide I [13 7]
1650	Amide I [17 11]
1745	(C = O) Lipids [18 12]

The increase in the content of hyaluronic acid at a wave number of 1155 cm⁻¹ (Hyaluronic acid (C-O, C-C)) was revealed on the spectra of the conditionally healthy specimens of synovial fluid in comparison with the developed osteoarthritis (Fig. 2) (Figure 2), the presence of which provides the viscosity of the synovial fluid, lubricating effect and absorption of shock impulses in the joint [19], as well as an increase in the Amide III content at a wave number of 1250 cm⁻¹ resulting from the wear of the cartilaginous tissue, the subchondral bone [20].

The relatively constant component of the synovial fluid is (C = O) lipids corresponding to a wave number of 1745 cm⁻¹, so it was used as a denominator in the introduced optical coefficients, which will be used to record the changes that occur in the synovial fluid in osteoarthritis.

1. optical coefficient K - showing the ratio of hyaluronic acid to assess the visco-elastic properties of the synovial fluid:

$$K = \frac{I_{1155}}{I_{1745}}$$

2. Optical coefficient L - showing the ratio of the amide component of the synovial fluid to assess the degree of wear of the cartilage:

$$L = \frac{I_{1250}}{I_{1745}}$$

where I_i are the intensity values at the corresponding wave numbers.

Based on the values of the obtained optical coefficients K and L, a two-dimensional diagram was constructed (Figure 2).

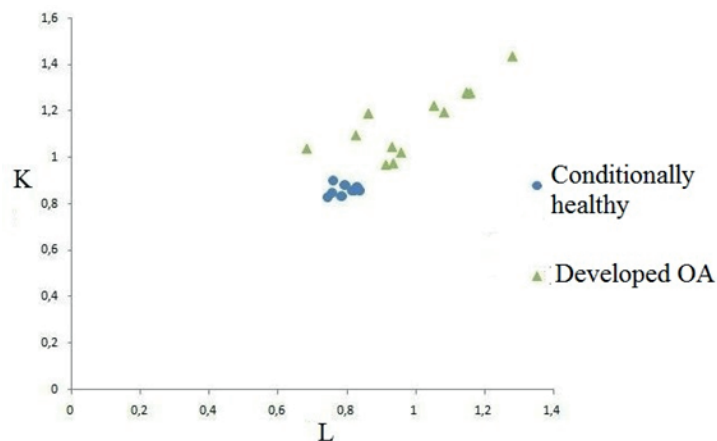


Figure 2: The two-dimensional diagram of experiments with synovial fluid.

From the two-dimensional diagram it can be seen that all the selected coefficients grow with osteoarthritis. coefficient L (Amide III) in the range of 0.7-0.9. In the process of destruction, the cartilaginous tissue loses its ability to function normally and the decay products of the cartilaginous tissue contribute to a change in the composition of the synovia, which is expressed in the growth of these coefficients.

The analyzed samples are not pure cases of osteoarthritis, but osteoarthrosis combined with joint injuries. Probably, the increase in the coefficient K, which is located in the region of 0.9-1.3 and is associated with hyaluronic acid, is caused by the reaction of the body, which manifests itself in an attempt to reduce the load on the damaged joint surface by increasing the viscosity of the synovial fluid by increasing the concentration of hyaluronic acid.

The analysis of the two-dimensional diagram showed that with the help of the introduced optical coefficients it is possible to reliably verify the development of osteoarthrosis.

4. Conclusions

As a result of the Raman spectroscopy analysis, the component composition of the test substance can be identified.

With the development of the degenerative-dystrophic process in the synovial fluid of the affected joint, the total number of components at wave numbers increases: 1155 (Hyaluronic acid (C-O, C-C)) and 1250 cm^{-1} (Amide III).

The use of the proposed optical coefficients characterizing the synovial fluid in osteoarthritis with the help of Raman spectroscopy may later become a new diagnostic screening for the detection of joint pathology.

The work was supported by the Ministry of Education and Science of the Russian Federation.

Acknowledgments

The reported study was funded by RFBR according to the research project № 17-44-630343 p_a

References

- [1] Yu.M. Chernyakova, E.A. Semyentovskaya. Synovial fluid: composition, properties, laboratory methods of research - 2005, - №2 (in Russian)
- [2] Nesterenko S.A. The syndrome of disturbance of the patellar dysplastic genesis equilibrium from the position of the concept of multifactoriality // Orthopedics, traumatology and prosthetics 2000, No. 3, p. 17-19 (in Russian)
- [3] Methods of clinical laboratory research: a textbook / Ed. V.S. Kamyshnikova. 3rd ed., Moscow: MEDpress-inform, 2009, 752 p. (in Russian)
- [4] V.P. Zakharov, O.N. Makurina, E.V. Timchenko, P.E. Timchenko, S.P. Kotova, Valliylov Ecological monitoring of megapolis on the basis of differential backscattering control of the wood culture // Laser Physics, 2009, 19 (6), pp. 1366-1372 10.1134 / S1054660X0905 (in Russian)
- [5] Zaharov V.P., Timchenko E.V., Timchenko P.E., Zolotuhina A.D., Alembekov S.V. Alteration of hydrosphere optical properties by synthetic active compounds // Computer Optics, 2011 35 (2), pp. 238-242 (in Russian)
- [6] Timchenko P.E., Zakharov V.P., Volova L.T., Boltovskay V.V., Timchenko E.V. Diagnostics of bone implantat and control of their process osteointegration with

- of method confocal microscopy // *Computer Optics*, 2011 35 (2), pp. 183-187
- [7] Karen A. Esmonde-White, Gurjit S. Mandair, FarhangRaaii, Jon A. Jacobson, Bruce S. Miller, Andrew G. Urquhart, Blake J. Roessler, and Michael D. Morris. Raman Spectroscopy of Synovial Fluid as a Tool for Diagnosing Osteoarthritis / *J Biomed Opt.* 2009; 14 (3)
- [8] AlizKunstar, Anne M. Leferink, and A. A. van Apeldoorn Label-free Raman monitoring of extracellular matrix formation in three-dimensional polymeric scaffolds / *J R SocInterface* 2013
- [9] Karen A. Esmonde-White, Gurjit S. Mandair, FarhangRaaii, Jon A. Jacobson, Bruce S. Miller, Andrew G. Urquhart, Blake J. Roessler, and Michael D. Morris. Raman Spectroscopy of Synovial Fluid as a Tool for Diagnosing Osteoarthritis / *J Biomed Opt.* 2009; 14 (3)
- [10] AlizKunstar, Anne M. Leferink, and A. A. van Apeldoorn Label-free Raman monitoring of the extracellular matrix formation in three-dimensional polymeric scaffolds / *J R SocInterface* 2013
- [11] E.V. Timchenko, N.V. Tregub, L.A. Taskina, E. A. Selezneva, P.E. Timchenko Optical methods for control of hydrogen influence on plants // *Proc. of SPIE Vol. 9221, Remote Sensing and Modeling of Ecosystems for Sustainability XI*, 2014, pp. 922108 doi: 10.1117 / 12.2061357
- [12] E.V. Timchenko, R.E. Timchenko, N.V. Tregub, L.A. Taskina, E.A. Selezneva Mapping of the Samara city by definition of areas with hydrogen degassing using Raman spectroscopy // *Proc. of SPIE*, 2014, Vol. 9448, No. 94480K doi: 10.1117 / 12.2179510 (in Russian)
- [13] Karen A. Esmonde-White. RAMAN SPECTROSCOPY DETECTION OF MOLECULAR CHANGES ASSOCIATED WITH OSTEOARTHRITIS 2009, p. 212.
- [14] Karen A. Esmonde-White, Gurjit S. Mandair, FarhangRaaii, Blake J. Roessler, Michael D. Morris. Raman Spectroscopy of Dried Synovial Fluid Droplets as a Rapid Diagnostic for Knee Joint Damage. *Biomedical Optical Spectroscopy*, edited by Anita 2008, Vol. 6853 doi: 10.1117 / 12.770360
- [15] Karen A. Esmonde-White, Gurjit S. Mandair, Francis W.L. Esmonde-White, FarhangRaaii, Blake J. Roessler, Michael D. Morris. Osteoarthritis Screening using Raman Spectroscopy of Dried Human Synovial Fluid Drops. *Optics in Bone Biology and Diagnostics* 2009, Vol. 7166 doi: 10.1117 / 12.810057
- [16] David I. Ellis, David P. Cowcher, Lorna Ashton, Steve O'Hagan And Royston Goodacre. "Illuminating disease and enlightening biomedicine: Raman spectroscopy as a diagnostic tool". *Analyst* 2013, V. 138, No. 14, July 21, pp. 3847-4204

- [17] Karen A. Esmonde-White, Gurjit S. Mandair, FarhangRaaii, Jon A. Jacobson, Bruce S. Miller, Andrew G. Urquhart, Blake J. Roessler, and Michael D. Morris. Raman Spectroscopy of Synovial Fluid as a Tool for Diagnosing Osteoarthritis. *J BiomedOpt* 2009; 14 (3): 034013. doi: 10.1117 / 1.3130338
- [18] Stetsyura, I. Yu. Remotely Displaceable Sensors Based on the Effect of Giant Raman Scattering of Light for In vitro Studies. diss. Cand. fiz-mat. sciences. / Stetsyura Inna Yuryevna - Saratov, 2016. 134 p.
- [19] E.S. Spirkina, E.L. Matveeva, A.G. Hasanov. Comparative characteristics of the biochemical composition of the synovial fluid of the knee and elbow joints of a person. *Clinical medicine*. -2013, No. 2 (90) Part 1. p. 87 89. (in Russian)
- [20] Panzera D., Kirk T.B., Anamalay R.V. // Intern. Tribology Conference AUSTRIB'94. Perth, Australia, 5-8 Dec. 1994. - p.407-414