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Spectral Analysis of Structural Changes of the Heart Valves at Different Stages of Their Decellularization

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Abstract

Presents the results of applying the method of Raman spectroscopy for the qualitative analysis of the surfaces of the heart valve of sheep before and during their decellularization. Optical analysis showed that the implementation of decellularization valves reduces the content of glycosaminoglycans, proteins and lipids. Found that using specified optical coefficients, it is possible to control the efficiency of the process of decellularization heart valves.

Keywords: Raman spectroscopy, heart valve, optical coefficient, decellularization, spectral analysis.

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1. Introduction

The problem of treatment of diseases of the heart valves in people is one of the priority tasks of modern medicine. One of the most radical methods of treatment is valve replacement [1, 2]. Clinical cardiac surgery is the need to create new types of implants and improve their production technology. [3, 4].

The decellularization is one of the helper methods of tissue engineering heart valves. It is aimed at removing cells from tissue with preservation of the extracellular matrix and three-dimensional structure of the material [5, 6]. Some authors consider that reducing the antigenicity of the tissue during the process of decellularization must be as complete removal of cell components [7, 8].

To date, there is no universal method of decellularization heart valves. Also, there are no generally accepted methods of monitoring its effectiveness. With this purpose, the currently used histological, histochemical, biochemical and immunological methods. Their main drawback, along with the complexity and high cost, is the destruction

of analyzed samples [5-8]. Therefore, the search of optimal methods of analysis of the qualitative composition of heart valves in the process of decellularisation is an urgent task.

The method of Raman spectroscopy can be effective when evaluating the effectiveness of decellularization sample surface of the heart valves, as they are able to determine the content of the main components of the matrix and does not require destruction of the biological material when doing research [9-11].

Objective: using the method of Raman spectroscopy to analyze the qualitative composition of the surface of the heart valves before and after the implementation of their decellularization.

2. Materials and methods studies

As the material of the study used aortic valves in adult sheep. The decellularization of valves carried out according to the protocols [12, 13] in the modification at the Institute of experimental medicine and biotechnology, Samara state medical University. Allocated to phase 1 of decellularisation to enzyme treatment and phase 2 after it. Samples of biomaterials were stored before the study in phosphate-saline with added antibiotics at 4°C.

The spectral characteristics of the samples were studied by means of experimental stand that includes a high-resolution digital spectrometer Shamrock sr-303i with a built-in cooling chamber DV420A-OE, fiber optic probe for Raman spectroscopy RPB785, combined with the laser module LuxxMaster LML-785.0 RB-04 (with adjustable power up to 500 mW, wavelength 785 nm) [14].

3. The results of the research

Figure 1 shows averaged spectra of CU to surfaces of the aortic valves to decellularization (control) and spectra of the CU surfaces of the valves during execution of decellularization before (phase 1) and after (phase 2) enzyme treatment of samples.

When performing Raman spectroscopy of the surfaces of the valves before and in the process of decellularization received qualitatively the same CU strip.

As can be seen from Figure 1 in the process of the first stage of decellularization has been a reduction of intensities on the wave numbers 1062 cm^{-1} and 1440 cm^{-1} corresponding to OO-3 symmetric stretching of glycosaminoglycans and chondroitin-6-sulfate; proteins, lipids.

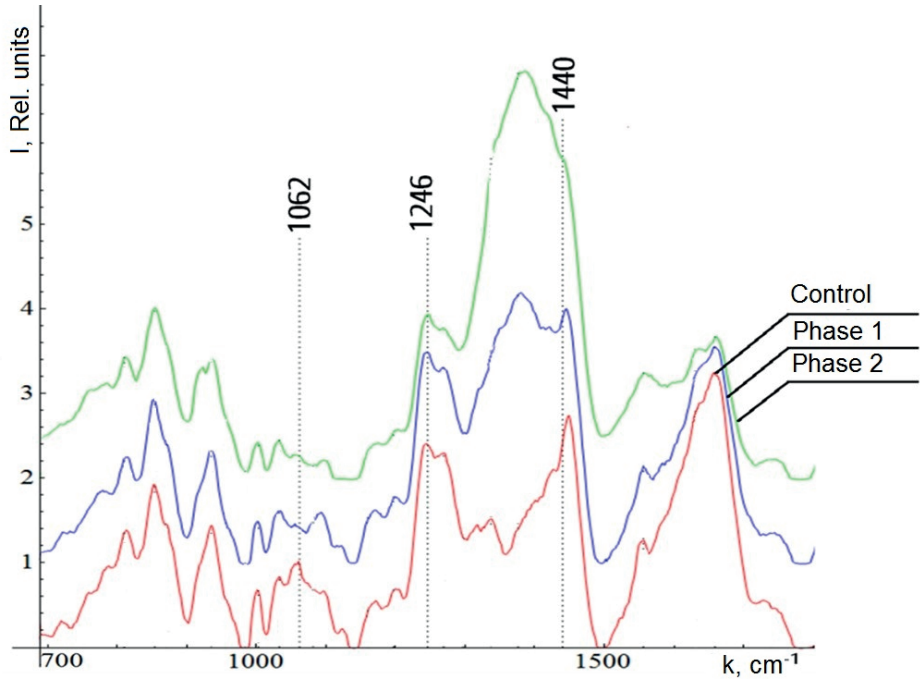

Figure 1: Averaged spectra of the surfaces of the aortic valve of the heart before and during their decellularization.

The relatively permanent component of the surfaces of the samples before and in the process of decellularization were amide III, the corresponding intensity line on the wave number 1246 cm^{-1} [15], so its value was used as the denominator in the calculation of the introduced optical factor *f*

$$f = \frac{I_i}{I_{1246}},$$

where Ii is the intensity value at a wave number of the analyzed component I_{1246} - value intensity at the wave number of amide III.

Figure 2 shows two-dimensional diagrams of the optical coefficients, reflecting the change in the composition of the main components of the surfaces of the aortic valve of the heart before and in the process of decellularisation at different stages.

Analysis of two-dimensional dependencies found that the execution phases of decellularization observed a gradual decrease in the optical coefficients I_{1062}/I_{1246} and I_{1440}/I_{1246} compared to the values obtained in the study of surfaces of intact aortic valve.

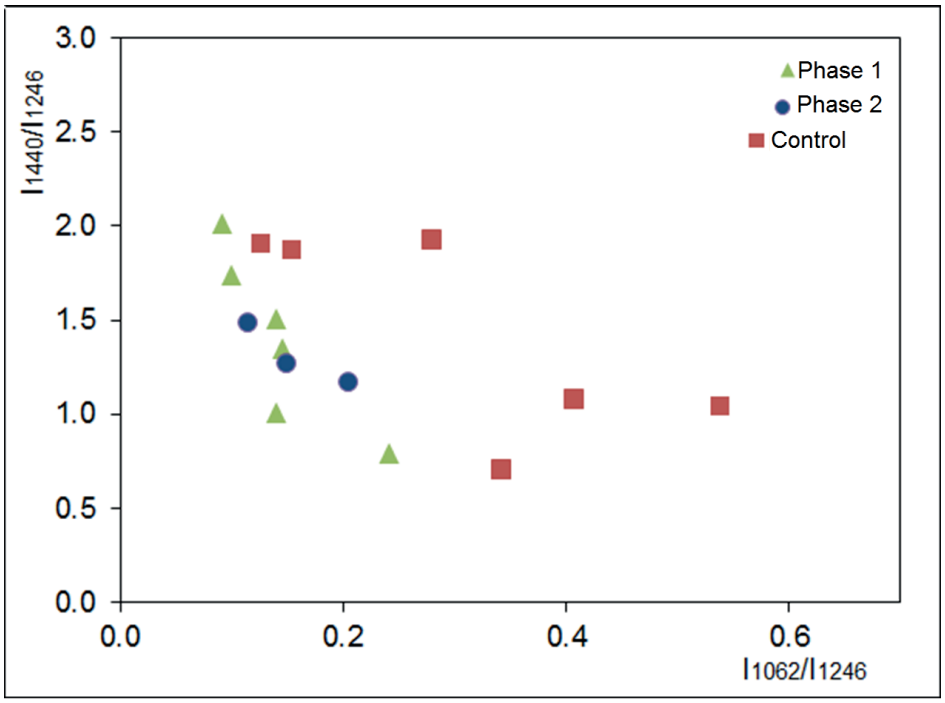

Figure 2: a two-Dimensional diagram introduced optical coefficients (control samples prior to the execution of decellularization, stage 1 – the samples before enzymatic treatment, phase 2 – samples after enzyme treatment).

4. Conclusion

In the study of the surfaces of the aortic valves before and in the process of decellularization using Raman spectroscopy it was found that after the first stage of decellularization has been a reduction of intensities on the wave numbers 1062 cm^{-1} and 1440 cm^{-1} corresponding to fosfodiesterasi communication RNA; OSO-3 symmetric stretching of glycosaminoglycans and chondroitin-6-sulfate; proteins and lipids.

With the introduction of optical coefficients and their two-dimensional analysis was the efficiency of the process of decellularization aortic valve, which is indirectly manifested by the reduction of the surface samples of lipids, proteins, glycosaminoglycans.

Using specified optical coefficients, it is possible to control the efficiency of the process of decellularization heart valves.

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