Detailed spectral analysis of decellularized skin implants

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Abstract. The results of detailed analysis of donor skin implants using Raman spectroscopy method are presented. Fourier-deconvolution method was used to separate overlapping spectrum lines and to improve its informativeness. Based on the processed spectra were introduced coefficients that represent changes in relative concentration of implant components, which determines the quality of implants. It was established that Raman spectroscopy method can be used in assessment of skin implants.

1. Introduction

Restoration of human skin tissue defects is one of the most important problems of the modern world. [1]

Biomaterials play a crucial role in implantology: maintain the shape and structure of tissues, provide mechanical stability and recovery of integrity of their functions [2]. Use of donor biomatrixes is limited by their immunogenic properties caused by epidermal cells and endothelial cells in tissue. During its implantation into an organism the process can be associated with the occurrence of undesirable effects such as disruption of homeostasis, possibility of implant rejection, its poor integration. Therefore, during the manufacturing of skin implants, tissues treated by decellularization method. This process can be followed by decrease of content of key components of biomatrix: glycosaminoglycans (GAGs) and proteins compared to native samples, which can lead to deterioration of integration during its implantation. At the same time the loss of glycosaminoglycans leads to the loss of tissue elasticity [3], which can also adversely affect the implant engraftment.

Thus, the actual problem of implantology is assessment of biomatrix treatment.

At the present level of development of science and technology optical methods can be applied for the assessment of human skin implants due to its simplicity, efficiency and noninvasiveness. Raman spectroscopy (RS) [6,7,8] finds wide application for quality control of materials in tissue engineering [9,10,11]. Authors of the work [8] used Raman spectroscopy method to study the chemical composition of human skin. The analysis found that the absence of peaks in the spectrum at specific wavenumbers confirms the absence of certain components in human skin. In works [11,12] was used Raman spectroscopy method for assessing the content of key skin components - amide type I and glycosaminoglycans (GAGs) and their corresponding wavenumbers.

At the same time implants, made from human skin, are multicomponent biomatrix, including in its composition a number of components, whose spectra may overlap each other, which, in turn, makes the joint spectrum less informative without special treatment. Thus, to highlight certain spectral lines it is necessary to apply methods of spectral decomposition of profile to highlight each individual component, which is part of skin [4].
One of these methods is method of Fourier-deconvolution. It provides a complete separation of components [4], when precisely known a testing function. However, in practice to improve the resolution of spectrum it can be used without knowledge of form of lines, using various testing functions. The advantage of this method - the consistency of selected components and their parameters when selecting various testing functions that indicates the reliability of the results obtained. As test functions are often occur Gaussian or Lorentz functions or their convolutions.

Detailed analysis can also be carried out using the method of higher derivatives. For this method does not required data about shape of elementary band like in Fourier-deconvolution [5].

Purpose of the work is to study the skin implants using method of Raman spectroscopy with the use of spectral decomposition of profile method.

2. Materials of research
As the objects of study were used allogenic treated skin and control sample (untreated human skin). Chemical treatment of biological tissues was carried out using the decellularization technology from Heinrich-Heine University (Germany) [13].

Control of the qualitative composition of skin surface of implant was carried out using an experimental stand, including a high-resolution digital spectrometer Shamrock sr-303i with built-in cooling chamber DV420A-0E, fiber-optic probe RPB-785 for Raman spectroscopy, combined with laser module LuxxMaster LML-785.0RB-04, which is described in detail in [14]. The stand has the following characteristics: scanning range of wavelengths from 190 nm to 1200 nm; the exposure time from 0.04 to 600s; camera resolution of 1024 * 255 pixels, 50-450 mW laser power with a wavelength of 785 nm.

3. Results and discussion
Spectra were recorded in the range of 500-2500 cm$^{-1}$. Processing of spectra was carried out in the Mathematica’8 software environment and was smoothing the noises with median filter, polynomial approximation of the fluorescent component in spectrum and subtracting it and obtaining the processed RS spectrum.

Using deconvolution of Lorentz-Gauss functions in MagicPlot Pro software environment were carried out spectral decomposition of profile into spectral lines (fig. 1). In view of the plenty of lines in spectrum and to improve stability of solution, treatment was carried out separately in spectral areas 300-746, 746-1356, 1356-1789 и 1789-2199 cm$^{-1}$.

Average value of the coefficient of determination of the resulting spectrum, generated by the spectral profiles from the original Raman spectrum for 300-2199 cm$^{-1}$ areas, and average standard deviation of analysis are given in table 1.

| RS area (cm$^{-1}$) | Average value of the coefficient of determination ($R^2$) | The average standard deviation of analysis ($\sigma$) |
|---------------------|-----------------------------|-----------------------------|
| 300-746             | 0.9998                      | 0.5365                      |
| 746-1356            | 0.9998                      | 0.2249                      |
| 1356-1789           | 0.9987                      | 0.6121                      |
| 1789-2199           | 0.9994                      | 0.2658                      |

The degree of treatment of skin implants is determined by the absence of immunogenic cells, such as epithelium, RNA, and preservation of glycosaminoglycan and amide groups in their composition, in this regard, the most interesting for the study is 746-1356 cm$^{-1}$ area, which includes the wave lines we are interested in.
Figure 1. The characteristic spectral profiles for areas:
(a) 300-746 cm$^{-1}$, (b) 746-1356 cm$^{-1}$, (c) 1356-1789 cm$^{-1}$, (d) 1789-2199 cm$^{-1}$

When studying the spectra the greatest interest have intensities at the wavenumbers 1062 cm$^{-1}$, 1645 cm$^{-1}$, 1260 cm$^{-1}$, 814 cm$^{-1}$, 1202 cm$^{-1}$, corresponding to components that are important for the quality of implant: glycosaminoglycans, amide type I, amide type III, asymmetric C-O-S bond oscillations of glycosaminoglycans, RNA and tyrosine, hydroxyproline [14,15,16].

The regenerative properties of implants are determined by their structure and composition. Thus, saved glycosaminoglycans, proline and hydroxyproline affect its structure and elasticity, and the residual RNA and cells are capable of causing the immunologic response of recipient and rejection of implants. The proposed coefficients allow evaluating these parameters. Relatively permanent component in skin implants is amide II [11,12] corresponding to the wavenumber 1553 cm$^{-1}$, so it was used as denominator ($I_{1553}$) in the introduced optical coefficients ($k$):

$$k = \frac{I_i}{I_{1553}},$$

where $I_i$ – intensity values at wavenumbers for analyzed components.

Figure 2 shows the two-dimensional diagrams of the introduced optical coefficients $k$. Clearly seen the difference between the control and treated samples.
The value of line intensities of amide I and amide III is greater in control sample compared to the sample treated. It is seen that the decellularization process of biomatrixes can reduce the content level of immunogenic cells, such as RNA and DNA, which, in turn, is represented on the change of the optical coefficient $I_{814}/I_{1553}$, while maintaining the immutability of the quantitative content of glycosaminoglycans (GAGs), as can be seen in Figure 2 from relation $I_{1062}/I_{1553}$. Thus, when using a detailed analysis of the Raman spectra for the assessment of skin implants it is shown that during their processing components, adversely affecting their engraftment, are removed, at the same time retained the necessary level of glycosaminoglycans, proline and hydroxyproline.

4. Conclusion
Deconvolution of spectra using the Gauss-Lorentz functions allows carrying out component structural analysis of Raman spectra. When studying the spectra the greatest interest have intensities at the wavenumbers 1062 cm$^{-1}$, 1645 cm$^{-1}$, 1260 cm$^{-1}$, 814 cm$^{-1}$, 1202 cm$^{-1}$, corresponding to components that are important for the quality of implant: glycosaminoglycans, amide type I, amide type III, asymmetric C-O-S bond vibrations of glycosaminoglycans, RNA and tyrosine, hydroxyproline. Raman spectroscopy can be used to assess skin implants.

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