Testing of Raman spectroscopy method for assessment of skin implants

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Abstract. Results of studies of testing of Raman spectroscopy (RS) method for assessment of skin implants are presented. As objects of study were used samples of rat’s skin material. The main spectral differences of implants using various types of their processing appear at wavenumbers 1062 cm⁻¹, 1645 cm⁻¹, 1553 cm⁻¹, 851 cm⁻¹, 863 cm⁻¹, 814 cm⁻¹ and 1410 cm⁻¹. Optical coefficients for assessment of skin implants were introduced. The research results are confirmed by morphological analysis.

1. Introduction
Due to the high prevalence of thermal injuries and high mortality is still relevant problem of improving the results of treating patients with burn wounds [1]. The problem of restoring humans’ defects of skin material is one of the most important problems of the modern world. [2]

For treatment of skin tissue burn defects are used different biological materials. Active development of biotechnologies resulted in the creation of entirely new regenerative technologies that allow to recover biological tissues, called biomatrix. For today, there is still no optimal method of their processing and control of effectiveness.

In studying the effectiveness of the method of manufacturing biomatrix is worth considering several major factors affecting the quality of the resulting implant. These include immunogenic properties of biomatrix caused by epidermal cells and endothelial cells in the dermis. The processed tissues must not contain cells, including cell components: cytoplasm and nucleuses. Their presence in biomatrix can promote a violation of cell biocompatibility of invitro and cause side effects in invivo conditions during subsequent processing. [3]

In the manufacture of skin biomatrix the treatment of samples is carried out by different methods [2]. However, the processing may be followed by a decrease of glycosaminoglycan’s, proteoglycans and glycoproteins, which are part of biomatrix’s intercellular substance that can lead to implant rejection [4].

At the same time, the loss of glycosaminoglycans leads to loss of turgor (elasticity) of material [5], which can also adversely affect the engraftment of implant. Therefore, complex assessment of processing of skin biomatrixes is a highly urgent problem. There are number of methods to control the quality of biomatrixes, these include biochemical analysis
(cytological [6, 7], histological [8]). However, this analysis is very time consuming and destructive [6]. Therefore, for assessment of skin biomatrixes it is necessary to use optical methods of control that are not destructive.

Raman spectroscopy [9, 10, 11] due to its simplicity, efficiency and non-invasiveness is suitable for quality control of results of tissue engineering [12, 13, 14]. In article [11] was used Raman spectroscopy method, as the optimal method for studying biological tissues. In the article were studied healthy and with tumor mass skin samples of rat mammary. Analysis of the principal components of matrix was carried out in 900 - 1790 cm\(^{-1}\) range of wavenumbers, typical for collagen, proteins, lipids, DNA, and amide I. The authors of work established that cancerous skin tissues have a higher protein concentration than the normal ones. In article [14] this method was applied to assess rat’s skin carcinoma and normal skin. Carried out analysis revealed that significant changes between tissues appear in the 1200-1600 cm\(^{-1}\) range, Amide III band including, δCH\(_2\), amide II, and in the range of wavenumbers above 2,800 cm\(^{-1}\) for CH\(_2\), CH\(_3\) and OH stretchings.

Main changes in spectra were observed in bands of collagen corresponding to line 1328 cm\(^{-1}\), changing of which reveals the presence of pathology of skin.

Objective of the work - to carry out studies on the possibility of using Raman spectroscopy method for assessing skin implants.

2. Materials of research

As objects of study were used samples of rat’s skin material subjected to 2 different types of processing: thermochemical treatment with incomplete epithelialization and chemical treatment of the skin tissue using "Lioplast"® (TY-9398-001-01963143-2004) technology.

Control of processing of skin matrix was carried out using an experimental stand shown in Fig. 1, which includes a high-resolution digital spectrometer Shamrock sr-303i with a built-in cooling chamber DV420A-OE, a fiber-optic probe for Raman spectroscopy (RS) RPB-785, combined with laser module LuxxMaster LML-785. 0RB-04 and coordinated with the laser Raman probe.

![Figure 1. Experimental stand that implements RS method: 1 – the studied object; 2 - Raman Probe RPB785; 3 - spectrometer Shamrock sr-303i; 4 - Built-in cooling chamber DV420A-OE; 5 - Laser Module LuxxMasterRamanBoxx; 6 - power supply of the laser module; 7 - computer; 8, 9, 10 - Information electric cables; 11 - coordinate table]
Built in broadband filter probe 2 is designed to isolate radiation in the studied spectral range, which is then sent via fiber to the spectrometer 3 with an integrated camera 4. The coordinate table 11 with stepper motor allows to scan the sample dimensionally with steps 1-1, 5 mm. Processing of the spectra was carried out in WolframMathematica 8 program. As a result of test experiments was selected laser power of 100 mW.
Also was calculated error of the method, which amounted 10, 1%.

3. Results and discussion
Fig. 2 shows averaged Raman spectra of skin implants samples. Registered Raman bands correspond to vibration modes, which are represented in the table 1.

![Figure 2. Averaged Raman spectra of skin implants. №1, 2 - protocols of skin biomatrixes (1-chemical treatment, 2 – thermochemical treatment)](image)

| Wavenumber cm⁻¹ | Fragment oscillation                                      |
|-----------------|-----------------------------------------------------------|
| 863, 975        | Vibration ribose (RNA)                                    |
| 1062            | Glycosaminoglycan’s (α-glycan’s)                          |
| 1202            | Hydroxyproline, tyrosine                                 |
| 1260, 1268, 1271| Amide III (collagen)                                     |
| 1410, 1412, 1415| v (C-O) from COO (amino acid glutamic acid)               |
| 1434, 1446      | δCH2, δCH3, C-H vibrations (proteins and lipids)         |
| 1553, 1556- 1557| NH deformation Amide II                                  |
| 1645            | Amide I                                                   |

Fig. 2 shows that the Raman spectra of all samples are similar in nature. However, at wavenumbers 1062 cm⁻¹, 1645 cm⁻¹, 1553 cm⁻¹, 863 cm⁻¹ and 1410 cm⁻¹ corresponding to glycosaminoglycan’s, collagen type I, collagen type II, ribose (RNA) and epithelium [15, 16, 17], there are differences between the samples of skin implants. These components determine the quality of implants. Therefore, these wavenumbers can be used as control criteria of skin biomatrixes.
Relatively constant in skin implants is collagen III [14] corresponding to wavenumber 1260 cm\(^{-1}\), so it was used as denominator in introduced optical coefficient:

\[
k = \frac{I_i}{I_{1260}},
\]

where \(I_i\) - intensity values at wavenumbers 1062 cm\(^{-1}\), 1645 cm\(^{-1}\), 1553 cm\(^{-1}\), 850 cm\(^{-1}\), 863 cm\(^{-1}\), 1202 cm\(^{-1}\) and 1410 cm\(^{-1}\) corresponding to glycosaminoglycans, collagen type I, type II collagen, glycosaminoglycans, ribose (RNA) hydroxyproline and epithelium 1260 cm\(^{-1}\) - a line corresponding to collagen III.

Further, Fig. 3 shows the two-dimensional diagrams of introduced optical coefficients.

![Figure 3](image)

**Figure 3.** Two-dimensional diagrams of introduced optical coefficients for rat’s skin implants

Analysis of two-dimensional diagrams (Fig. 3) showed that the content of glycosaminoglycans (GAGs), corresponding to wavenumber 1062 cm\(^{-1}\), more in implants made by protocol №2. This is due to the fact that when using this type of treatment, a chemical treatment is weaker, preserving GAG, compared to chemical treatment by protocol №1. However, when using the treatment protocol №2 in skin implant has a residual epithelium (optical coefficient \(I_{1410} / I_{1260}\) to 0, 8), the presence of which is expressed by the values of intensity of Raman lines on the wave of 1410 cm\(^{-1}\) (Fig. 2). This is due to the fact that with this type of processing, implants were not completely epithelialized.

Implants, made by protocol №1 contain cells, as evidenced by the value of optical coefficient \(I_{863} / I_{1260}\) to 0, 63. Fig. 3 also shows that the optical coefficients vary depending on the types of implants manufacturing.
Based on the carried out analysis was constructed histogram comparing different types of treatment in the manufacture of skin biomatrixes and shown morphological analysis of skin implants made according to protocols (Fig. 4).

Using morphological analysis it was established that samples of skin tissue, made by protocol №2, have the structure of tissue with maintaining its main components, including hair follicles with the remnants of hair shaft. Implants which made by protocol №1 contain cellular components. Also experimentally was found, that using a thermo-chemical treatment in the manufacture protocols of skin implants, their collagen structure is destroyed (collagen I), which is caused by a decrease of optical coefficient $I_{1645}/I_{1260}$ to 0, 35.

Given analysis showed that introduced criteria allow to assess skin implants made by various types of treatment. Thus, Raman spectroscopy method can be used for assessing skin implants.

4. Conclusions
Features of Raman spectrum for skin biomatrix of rats with different protocols of manufacturing were obtained. It was established that main differences appear at wavenumbers 1062 cm$^{-1}$, 1645 cm$^{-1}$, 1553 cm$^{-1}$, 863 cm$^{-1}$ and 1410 cm$^{-1}$ corresponding to glycosaminoglycans, collagen type I, collagen type II, ribose (RNA) and epithelium. It is also experimentally found that using thermochemical treatment in protocols of manufacturing of skin implants their collagen structure is destroyed (primarily collagen I).

Were introduced criteria allowing to assess skin implants made by various protocols, that allows to continue using Raman spectroscopy method for assessing skin implants.
The research results are confirmed by morphological analysis.

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