The assessment of human skin biomatrixes using raman spectroscopy method

E V Timchenko¹, P E Timchenko¹, L T Volova², D A Dolgushkin², P Y Shalkovskaya¹, S V Pershutkina¹, I F Nefedova³

¹Samara National Research University, 443086 Russia, Samara, st. Moscow highway, 34.
²Institute of Experimental Medicine and Biotechnology (IEMB) Samara State Medical University, 443079 Russia, Samara, st. Gagarin, 20.

E-mail: laser-optics.timchenko@mail.ru

Abstract. There are presented the results of the analysis of the implants made of human skin by Raman scattering method. The main spectral distinctions of bioimplants by using various methods for their manufacture are shown at wavenumbers 1062 cm⁻¹, 1645 cm⁻¹, 1260 cm⁻¹, 850 cm⁻¹, 863 cm⁻¹, corresponding to components that are important for the quality of implant: glycosaminoglycans, amide type I, amide type III, asymmetrical association C-O-S of vibration of glycosaminoglycans GAGs, tyrosine and a C-C stretching of proline ring, ribose. Has been carried out two-dimensional analysis of optical coefficients providing an opportunity to control the quality of cutaneous implants in the process of manufacturing it, and detailed analysis of Raman scattering spectroscopy.

Keywords: Raman scattering spectroscopy, optical coefficients, cutaneous implant, quality control.

1. Introduction
Over the last years in different branches of medicine widely have been using the materials of biogenicity for tissue defects replacement. The full recovery of skin integument after thermal injury and information research for this purpose are the important problems of Combustiology. 60 thousand people die each year from burn injuries worldwide [1, 2]. Unfortunately, the optimum plastic materials for tissue defects replacement have not been found yet. The biotechnological methods for obtaining and using skin equivalents in clinical practice are actively developing. However, there is a problem of quality control of such implants.

In order for use the implants effectively for tissue defects replacement during the manufacturing process must be carefully removed all immunogenic factors that can lead to rejection of donor material [3, 4]. At the same time in the process of manufacturing of implants must maintain their native structure and composition. The methods used to achieve these goals are usually having the opposite effects: the extremely aggressive removal of immunogenic components can destroy the structure and composition of the tissue, while the more sparing techniques can save the enhanced immunogenicity of implants [5, 6].

For quality control of implants is used biochemical, cytological and histological research methods [7, 8, 9]. However, these methods are time-consuming and often lead to changes in the structure and composition of the material quality in the process of their analyzing.
On the current level of science and technology development to evaluate the implants made of human skin may be used physical and optical research methods that allow people to examine complex biological objects quickly without damaging their structure. Raman scattering spectroscopy (RS) [10, 11, 12] because of its noninvasiveness is widely used in biological objects for quality control of materials of tissue engineering [13, 14, 15]. It is known work [12], in which the Raman spectroscopy method RS is claimed to be the best method for analyzing the chemical composition of human skin. During the analysis, it was found that the absence of Raman peaks at wave numbers 717 cm\(^{-1}\), 1157 cm\(^{-1}\) and 1526 cm\(^{-1}\), indicates the absence of lipids in the horny layer of skin. Also in works [15, 16] was used Raman spectroscopy method to evaluate the collagen type I and glycosaminoglycans (GAGs) in composition of biomaterials, corresponding to lines 937 cm\(^{-1}\) and 1062 cm\(^{-1}\).

The mission is to use Raman spectroscopy to evaluate the quality of a person's skin implants manufactured in different ways.

2. Materials of research

As the objects of research were used 8 lyophilized bioimplants with preserved epidermis (the samples manufactured according to the protocol number 1), 8 lyophilized bioimplants without epidermis (the samples manufactured according to the protocol number 2), and 2 control samples of untreated human skin (Figure 1). Lyophilized implants were manufactured by the technology "Lioplast" (TU-9398-001-01963143-2004), which is based on the use of physical factors processing of biomaterials (ultrasonic, lyophilization) [16, 17].

![Figure 1](image)

**Figure 1.** Smears of the samples: a) a sample with the epidermis treated with the protocol number 1; b) the sample without the epidermis treated with the protocol number 2; c) A control sample of untreated human skin. H & E stain. The increase of 400.

The skin has typical histological structure in control samples: the epidermis was presented with 5 layers; dermis papillary and reticular layers. The samples manufactured according to the protocol №1, were keeping arrangement of layers of the epidermis; the cells of basal layer were more elongated, the nuclei were flattening assuming drop shape. In the cells of spinous and granular layers was found empty vacuoles on the place of the nuclei. Dermal papillae were smoothed. The dermis was represented as loose areolar connective tissue with fibers of different thickness. Under basal layer of the epidermis was observed focal changes in the form of lacunae of various sizes. On some fibers were located single cells. The sample manufactured according to the protocol №2, did not contain the epidermis. The boundary between the papillary and reticular layers of dermis has not been visualized. Dermis was presented with fragmented fibers of loose areolar connective tissue and contained capillary loop (pic. 1, a, b, c). Control of the qualitative composition of skin surface of implants was carried out using an experimental stand, including a high-resolution digital spectrometer Shamrock sr-303i with built-in cooling chamber DV420A-OE, a fiber optic probe the RPB-785 for Raman spectroscopy, combined with laser module LuxxMaster LML-785.0R -04 [16]. The stand has the following characteristics: scanning range of wavelengths from 190 nm to 1200 nm; time of exposure from 0.04 to 600s; camera resolution of 1024 *255 pixels, laser power 50-450 mW. Processing of the
spectra was carried out in the program Wolfram Mathematica 8. Error of method at determining of used coefficients was <7% (GOST 8.207-76).

3. Results and discussion
In Figure 2a presented average Roman spectrum obtained from the surface of lyophilized bioimplants and control skin samples. In view of the fact that the skin is a multi-component object, in the spectrum of which many lines overlap, it had to carry out the separation of the obtained spectra into spectral lines using deconvolution of Lorentz-Gauss functions in MagicPlotPro software environment (Figure 2b). Average value of the coefficient of determination of the resulting spectrum, generated by the spectral profiles from the original Raman spectrum for area 300-2199 cm$^{-1}$ was $R^2=0.98$, and average standard deviation of analysis $\sigma = 8.81$. Registered Raman bands correspond to oscillation modes, which are shown in Table 1.

![Figure 2. The results of research: A) Averaged Raman spectrum of bio-implants manufactured by Protocols 1, 2 and control samples; B) The software division lines in the spectrum.](image)

| Wavenumber, cm$^{-1}$ | Substance, oscillation |
|-----------------------|------------------------|
| 620                   | γC–C phenylalanine (protein) [18] |
| 815                   | RNA phosphodiester bands [18] |
| 850                   | Asymmetrical C-O-S association of vibration GAGs, tyrosine and C-C stretching of proline ring (protein) [18] |
| 863                   | Ribose vibration (RNA) [18] |
| 937                   | Glycosidic bond C-O-C vibration in the GAGs, C-C stretching of proline / valine (protein) [18] |
| 1003                  | Phenylalanine (C-C-aromatic ring) (protein) [11,14] |
| 1033                  | ν (C-H) Phenylalanine Breath (protein) [18] |
| 1062                  | Glycosaminoglycans (α- glycans) [18] |
| 1124                  | C-C stretching of lipids, C-H stretching of protein , CH и C-OH deformation [14] |
| 1196                  | CH3 lipid ring |
| 1202                  | Hydroxyproline, tyrosine [18] |
| 1260                  | Amide III [18] |
| 1383                  | NH2 vibration |
| 1410                  | ν(C-O) from COO (amino acids of glutamine acid) [18] |
| 1553                  | NH deformation, Amide II [18] |
| 1645                  | Amide I [18] |
Analysis of the spectra from the surface of skin implants, obtained by different methods showed differences between them at wavenumbers 1062 cm\(^{-1}\), 1645 cm\(^{-1}\), 1260 cm\(^{-1}\), 850 cm\(^{-1}\), 863 cm\(^{-1}\), corresponding to components that are important for the quality of implant: glycosaminoglycans, amide I, amide III, asymmetrical C-O-S association of vibration GAGs, tyrosine and C-C stretching of proline ring and ribose [17, 18, 19]. Therefore, these wavenumbers can be used as criteria for the quality of processing of skin bioimplants. Relatively permanent component in skin implants is an amide II [15, 16], corresponding to the wave number 1553 cm\(^{-1}\), so it was used as the denominator (I\(_{1553}\)) in the input optical coefficient (k):

\[
k = \frac{I_i}{I_{1553}},
\]

where I\(_i\) – the values of intensity on the wavenumbers for the analyzed components. Recovery properties of implants are identified by their structure and composition. Thus, the saved glycosaminoglycans, proline and hydroxyproline affect its architectonics and elasticity while the residual RNA cells are capable of eliciting a reaction of immunological response and rejection of the implants. The proposed coefficients allow evaluating these criteria. In the Figure 3 are presented two-dimensional diagrams of the input optical coefficients k.

**Figure 3.** Two-dimensional diagram of the optical input coefficients.

Analysis of two-dimensional diagrams in Figure 3 shows that the treated samples of skin implants manufactured according to the protocol №1 have values of optical coefficients I\(_{1645}\)/I\(_{1553}\)>0.49, and 0.73 < I\(_{1202}\)/I\(_{1553}\) < 0.81, and characterized by lower content of amide I and hydroxyproline than the sample manufactured according to the protocol №2. Quite the opposite, the content of glycosaminoglycan is greater than in the samples manufactured according to the protocol №2, which has the values of optical coefficient 0.91 < I\(_{1062}\)/I\(_{1553}\) < 1.13. Also Figure 3 shows that the value of the lines intensity of amide I and amide III in control sample has a maximum value and corresponds to optical coefficients 0.6 < I\(_{1645}\)/I\(_{1553}\) < 0.71, 1.1 < I\(_{1260}\)/I\(_{1553}\) < 1.34.

Based on the carried out analysis, diagrams were constructed relatively represented the maximum content of substances found by Raman spectroscopy method on the surface of samples of human skin bioimplants manufactured in different ways (Figure 4).
Figure 4. Diagrams substances as defined by the surface of the skin implants: a) a sample of the epidermis treated with the protocol number 1; b) the sample without the epidermis treated with the protocol number 2; c) A control sample of untreated human skin.

Analysis of the diagrams, shown in Figure 4, showed that with proposed coefficients and using Raman spectroscopy can be compared the relative content of the components of surfaces of the skin implants, also choose the optimal methods for their processing and control their effectiveness.

4. Conclusion
The spectra's analysis from the surface of skin implants obtained by different methods showed differences between them at wavenumbers 1062 cm\(^{-1}\), 1645 cm\(^{-1}\), 1260 cm\(^{-1}\), 850 cm\(^{-1}\), 863 cm\(^{-1}\), corresponding to components that are important for the quality of implant: glycosaminoglycans, amide I, amide III, asymmetrical C-O-S association of vibration GAGs, tyrosine and a C-C stretch ring proline, ribose.
Raman spectroscopy allows to carry out quick noninvasive control of the structure of the skin bioimplants; obtain an composition evaluation of components of surfaces bioactive matrixes; choose optimal bio-carriers and control the effectiveness of methods of their processing; optimize the creation of cellular-tissue products for regenerative medicine.

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