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Raman Spectroscopy for Analysis of Implants from the Dura Mater

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Abstract

In this paper we present results of the comparative evaluation of the structural properties of the dura mater specimens (DM), manufactured using the "Lioplast" technology, used in the clinic in the field of atrophic processes in multiple gums recessions, using the Raman spectroscopy (RS) method. The introduced coefficients and a two-dimensional analysis that showed that the processing retains the main components and removes DNA / RNA, which increases the quality that provides access to quality materials in the treatment of multiple gum recessions. It was found that the main differences appear at wavenumbers of 835 cm^{-1} (tyrosine), 855 cm^{-1} (proline), 940 and 1167 cm^{-1} (GAGs, CSPGs), 1240 cm^{-1} (amide III), 1560 cm^{-1} (amide II) and 1447 cm^{-1} (lipids and proteins). It is shown that Raman spectroscopy can be used to evaluate implants from the dura mater.

Keywords: Raman spectroscopy, dura mater, biomaterial, spectral analysis.

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1. Introduction

Restoration of tissues of atrophied gums with adentia is a very urgent problem [1]. Also, with the transplantation of native implants, there is a risk of immune rejection of the implant and an increase in the recovery time of the patient [2]. Therefore, the main problem of tissue engineering is the development and quality control of materials that can restore or replace this or that function of damaged tissues and organs.

Since the 20th century, many surgical techniques and their modifications have been proposed to close gum recessions: the method of coronally displaced flap in combination with a free connective tissue graft, envelope technique for closing single recessions, microsurgical tunneling techniques to eliminate multiple recessions. Multiple gum recessions occur more frequently in clinical practice than single [3].

The quality, bioplastic, integration properties of the resulting allogenic material are predetermined by the completeness of the removal of cellular components for better material integration and reducing the risk of implant rejection and the preservation of

the extracellular matrix (EM), whose main components are collagen, glycosaminoglycans, proteoglycans [4, 5]. At present, the quality of bioimplants can be assessed in vitro using a set of morphological, morphometric, biochemical and optical methods of investigation, including Raman spectroscopy [4, 6].

There are many methods to control the quality of bioimplant treatment, including histological, molecular and biochemical analysis. These methods allow qualitatively and quantitatively to assess the structural composition of the biomatrix, however this analysis is destructive and a considerable amount of time is required to output the results of the histological and cytological analysis. Therefore, to evaluate the composition of biological tissues, it is necessary to use optical control methods that are non-invasive. One of the most common optical methods for evaluating the quality of a bioimplant is Raman spectroscopy [6].

Raman spectroscopy allows real-time non-destructive, quantitative and qualitative analysis of the composition of biological objects and provides information on the molecular structure with high spatial resolution. In article [4] it is shown that this method is used for the investigation of spinal cord injuries. Raman spectra showed distinct differences between damaged and healthy spinal cord. Presumably, these differences are associated with cell death, demyelination, and changes in extracellular matrix composition, such as an increase in the relative concentration of proteoglycans (456-541 cm^{-1}) and hyaluronic acid at the lesion where the glial scar is formed. In addition, the analysis showed the presence of carbonyl-containing compounds, presumably lipid oxidation products (1398-1519 cm^{-1}), and acid catalyzed hydrolysis of glycosaminoglycan fragments (1114-1167 cm^{-1}). Eventually, the observation points to the possibility of using the RS to study the progression of lesions and to study possible ex vivo treatment methods and to monitor possible therapeutic procedures in the spinal cord in vivo.

In [6], the use of RS for the identification of spinal nerves in operations of minimally invasive spinal surgery is described, based on quantitative spectral analysis of the main components: proteins, lipids, and DNA. The results confirmed the possibility of identification of the spinal cord and spinal nerve with the help of RS.

The aim of this study: evaluation of implants from the dura mater with Raman spectroscopy.

2. Materials and methods of research

The subjects of the study were dura mater (DM) samples measuring 10*10 mm. All samples were divided into 3 groups: 1 group - lyophilized, processed according to the "Lioplast" ® technology after radiation irradiation (sterile); 2 group - before sterilization (non-sterile) and group 3 - native samples.

The process of obtaining implants from the allogeneic dura mater with the technology "Lioplast" ® consists of several stages: special ultrasonic cleaning from antigenic structures for primary sterilization of the material and viral inactivation, lyophilization, sterilization by the radiation method. The use of chemical factors in the manufacture of used in the minimum amount to reduce allergic reactions and complications.

As the main method of bioimplant analysis, the Raman spectroscopy method was implemented using an experimental stand including a high-resolution digital spectrometer Shamrock sr-303i combined with a LuxxMaster LML-785.0RB-04 laser module (power up to 500 mW, wavelength 785 nm) and built-in cooled DV420A-OE camera providing a spectral resolution of 0.15 nm (spectral range 200-1200 nm). The spectra were taken from each side of the sample at five different points [7, 8].

3. Results and discussion

Figure 1 shows the characteristic averaged Raman spectra of groups of samples of the dura mater. Differences are manifested in the RS lines 814 cm^{-1}, 1002 cm^{-1}, 1101 cm^{1}, 1167 cm^{-1}, 1240 cm^{-1}, 1447 cm^{-1} and 1560 cm^{-1}, corresponding to molecular vibrations of glycosaminoglycans, proteoglycans, phenyl assignment, deoxyribose (B, Z-marker), lipids and proteins, amide III and amide II (C-N-H valence) (N-H deformation vibration) [4, 6, 9 - 13].

It can be seen from Figure 1 that during processing, the line at the wave number of 1167 cm^{-1} is retained in the samples, which indicates the preservation of glycosaminoglycans and proteoglycans (GAGs, CSPGs) during processing that play an important role in implant engraftment. The intensity of the line at the 1447 cm^{-1} wavenumber corresponding to the relative concentration of lipids and proteins also preserved in processed implants.

The collagen component, in addition to the Raman lines of proline and hydroxyproline, is represented by amide groups III (in the region 1230-1289 cm^{-1}), amide II (in the range 1555-1565 cm^{-1}) and amide I (in the range 1655-1675 cm^{-1}), as well as a 1030 cm^{-1} Raman line corresponding to the CH_2-CH_3 vibrations of phenylalanine.

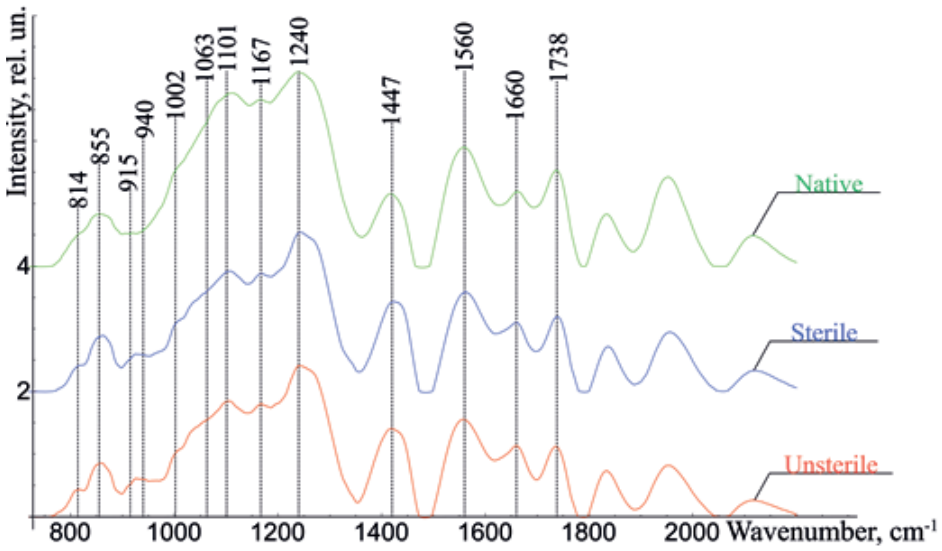

Figure 1: Averaged Raman spectra for samples of the dura mater.

It should be noted that in all samples of bone bioimplants manufactured using the Lioplast technology, there was no high intensity at the wave number of 1738 cm^{-1}, corresponding to cholesterol and phospholipids (C = O valence). The use of low-frequency ultrasound at the stages of bioimplant treatment made it possible to achieve maximum removal of lipids and bone marrow elements, which was confirmed in the spectral characteristics of the surfaces of the bioimplant samples studied.

Since the test samples have a multicomponent composition, analysis of the Raman lines corresponding to lipids, amides and GAG's without additional mathematical processing is difficult. Therefore, using the spectral contour selection method in the MagicPlotPro 2.7.2 software environment, a spectral curve analysis based on its least squares fit was performed, followed by expansion into spectral lines.

To assessment of the component composition of the bioimplant surface on the basis of the dura mater, we introduced relative coefficients. Relatively constant component in the investigated samples of the dura mater was the amide I [4] corresponding to the wave number of 1660 cm^{-1}, therefore it was used as a denominator (I_{1660}) in the introduced coefficients:

$$k_1 = \frac{I_{1447}}{I_{1660}}, k_2 = \frac{I_{1167}}{I_{1660}},$$

where I_i are the intensity values at the wave numbers of the analyzed components.

The ratio I_{1447} / I_{1660} determines the relative concentration of lipids and proteins in the sample.

The optical coefficient I_{1167} / I_{1660} is used to assess the safety of the extracellular matrix and reflects the relative concentration of glycosaminoglycans and proteoglycans GAGs, CSPGs.

Figure 2 shows a two-dimensional diagram of the input coefficients, which shows the differences between samples of different groups.

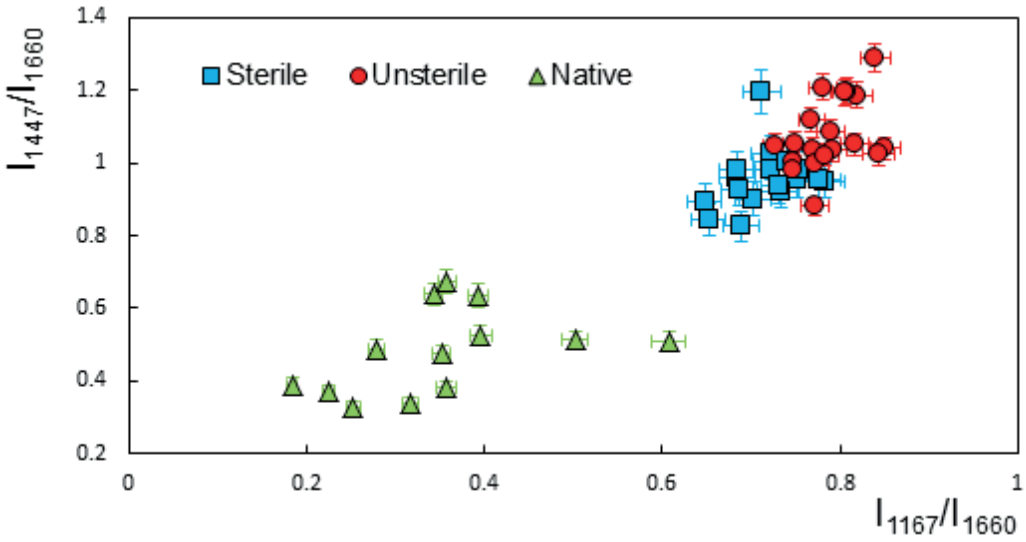

Figure 2: Two-dimensional diagrams of the introduced coefficients.

It can be seen from the analysis of Figure 2 that the values of the optical coefficient reflecting the relative concentration of glycosaminoglycans and proteoglycans I_{1167} / I_{1660} vary within the limits of $0.1 < I_{1167} / I_{1660} < 0.6$ for native and $0.6 < I_{1167} / I_{1660} < 0.9$ for samples treated with Lioplast (Figure 2), which reflects an increase in the relative concentration of GAGs, CSPGs with respect to secondary collagen structures (amide I) in the manufacturing process and indicates the preservation and production of a qualitative extracellular matrix. For non-sterile samples, the values obtained were higher.

Together, both coefficients allow differentiating native and processed samples. For sterile and non-sterile samples, close values of lipid concentration are characteristic.

Thus, when using spectral analysis of Raman spectra for the evaluation of implants made on the basis of the dura mater, it is shown that during their processing components that negatively affect their quality are removed, while the required level of the extracellular matrix remains.

4. Conclusions

Specific features of Raman spectra for samples of the allogeneic dura mater obtained by different methods have been established. The main differences appear at wave numbers 835 cm^{-1} (tyrosine), 855 cm^{-1} (proline), 940 and 1167 cm^{-1} (GAGs, CSPGs), 1240 cm^{-1} (amide III), 1560 cm^{-1} (amide II) and 1447 cm^{-1} (lipids and proteins).

A two-dimensional analysis of the introduced optical coefficients makes it possible to evaluate bio-implants based on the dura mater, manufactured according to various protocols. It was shown that during their processing components that negatively affect their quality are removed, while the required level of extracellular matrix is retained.

Acknowledgments

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