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Raman spectroscopy for evaluation of the bladder capsule after the lyophilization process

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In this work, the capsules of the bladder after the lyophilization process were studied by Raman spectroscopy. As a result of the conducted research, it was found that the lyophilization process carried out using the „LIOPLAST"technology does not significantly affect the composition and structure of the bladder capsule. The statistical analysis also showed that there were no significant spectral differences in the composition of the samples under study.

Keywords: raman spectroscopy, spectrum, statistical analysis, bladder, lyophilization.

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Introduction

Currently, the assessment of the composition of donor materials is an urgent task for their subsequent use in regenerative medicine [1]. The lyophilization process is one of the methods of preservation of biological products. However, it is not fully understood how much the structure of biomaterials changes after the lyophilization process. Optical research methods are widely used in solving various biomedical problems [2–4]. The Raman spectroscopy method is one of the most common optical methods among them [5–7].

In many studies [5–7], the authors demonstrate the Raman scattering as an effective tool with high accuracy for evaluating bladder tissues. For example, the authors of Ref. [5] present a clinical study of bladder biopsies for ex vivo tumor classification using a compact fiber probe-based Raman scanning system. The Raman spectroscopy method is used in Ref. [6] to study the microenvironment of bladder cancer cells. The authors of Ref. [7] applied the Raman spectroscopy to identify non-cancerous and tumor tissues of the bladder and determine their biochemical differences.

The aim of the study was to use the Raman spectroscopy to assess the relative composition of the bladder capsule after the lyophilization process.

Materials and research methods

Allogeneic bladder capsules of various donors with a size of $1.5 \times 1.5 \times 0.2$ cm were studied. After removal, some of the biomaterials were placed in saline solution with an antibiotic and stored for 3 days in a refrigerator at a temperature of $+4^\circ$C from the moment of sampling to the start of experiments. People of the same age and gender were the donors of the studied samples. The thickness of the studied samples was $0.4-0.7$ mm. Next, on the day of the study, the biomaterials were placed in distilled water. Another part of the biomaterials from the same donors was lyophilized using „LYOPLAST" technology (TU-9398-001-01963143-2004).

All samples were conditionally divided into 2 groups: Group 1 — freeze-dried bladder capsules and Group 2 — native bladder capsules from the same donors.

The Raman spectroscopy method was implemented using an experimental bench consisting of a RPB-785 Raman probe combined with a LuxxMasterLML-785.0RB-04 laser module (power up to 500 mW, wavelength 784.7 ± 0.05 nm) and a high-resolution ShamrockSR-303i digital spectrometer, providing a spectral resolution of 0.15 nm, with an integrated cooled camera DV420A-OE. The Raman spectra were analyzed in the range of $500-1800\,\mathrm{cm}^{-1}$. The spectra processing was performed in the Wolfram Mathematica 12 software environment and included removal of noise with a smoothing averaging filter for 7 points, polynomial approximation using an iterative algorithm of the fluorescent component of the spectrum and its subtraction, and obtaining the final RAMAN spectrum.

The informative value of the obtained Raman spectra was increased by performing a nonlinear regression analysis of the spectra consisting in their decomposition into spectral lines. The composition of spectral lines is determined based on literature analysis and multi-iterative modeling of Raman spectra.

Results and discussion

Fig. 1 shows the averaged Raman spectra of all the studied samples. They are represented by

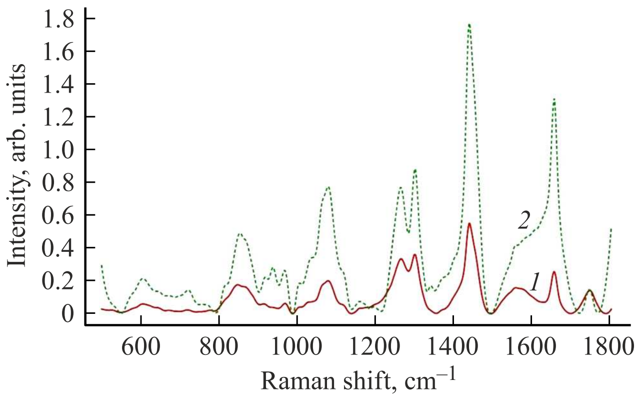

Figure 1. Averaged Raman spectra of the studied sample groups: *1* — freeze-dried bladder capsules, *2* — native bladder capsules from the same donors.

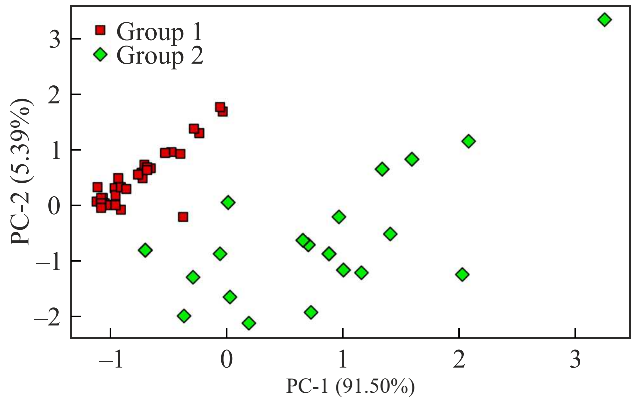

Figure 2. PCA analysis for the studied sample groups: 1 — freeze-dried bladder capsules, 2 — native bladder capsules from the same donors

the main lines $\sim 604\,cm^{-1}$ (*Nucleotide conformation*), $\sim 720\,cm^{-1}$ (*DNA*), $\sim 853\,cm^{-1}$ (*Ring breathing mode of tyrosine & C-C stretch of proline ring*), $\sim 936\,cm^{-1}$ (C-C *stretching in protein*), $\sim 966\,cm^{-1}$ (*Lipids*), $\sim 1077\,cm^{-1}$ (v(C-C) *phospholipids (lipid assignment)*), $\sim 1262\,cm^{-1}$ (*Amide III*), $\sim 1298\,cm^{-1}$ (CH_3, CH_2 *twisting*, *collagen*), $\sim 1438\,cm^{-1}$ (CH_2 *deformation*), $\sim 1555\,cm^{-1}$ (*Amide II*), $\sim 1654\,cm^{-1}$ (*Amide I, α-helix protein*), $\sim 1745\,cm^{-1}$ (*Phospholipids*).

The RS spectra of the studied groups are mostly similar, but differ in intensity, with the exception of the RS line $\sim 1745\,cm^{-1}$(*Phospholipids*). The intensity of the RS lines of native and lyophilized bladder capsules may differ due to various factors, including water content, structural changes, protein denaturation, chemical changes, instrumental variability, sample preparation, tissue type, lyophilization conditions, and storage conditions [8].

The amplitude of the intensity of the RS lines corresponding to DNA significantly decreases after lyophilization of the samples: $\sim 604\,cm^{-1}$ (*Nucleotide conformation*), $\sim 720\,cm^{-1}$ (*DNA*), which may indicate the destruction of cellular structures after the lyophilization process.

At the same time, the RS lines corresponding to the organic structure are preserved in the samples after lyophilization: $\sim 1262\,cm^{-1}$ (*Amide III*), $\sim 1298\,cm^{-1}$ (CH_3, CH_2 *twisting, collagen*), $\sim 1438\,cm^{-1}$ (CH_2 *deformation*), $\sim 1555\,cm^{-1}$ (*Amide II*), $\sim 1654\,cm^{-1}$ (*Amide I, α-helix protein*), $\sim 1745\,cm^{-1}$ (*Phospholipids*), which indicates the preservation of these structures after the lyophilization process.

The method of linear discriminant analysis in the RS-Tool program was chosen for the additional analysis of the studied samples.

Fig. 2 shows the results of the PCA analysis for the 2 studied groups of samples (in the SPSS Statistics program). Group 1 corresponds to the values $-0.5 < PC-2 < 2$ and $PC-1 < 0$, and group 2 occupies almost the entire specified range of PC-1 and PC-2.

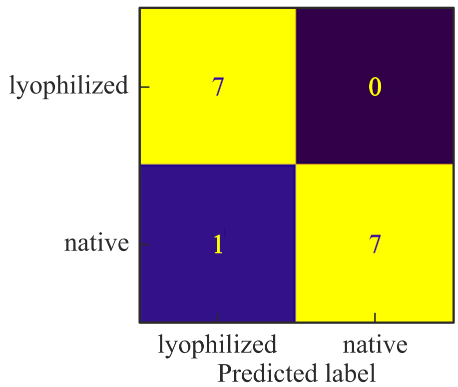

Figure 3. Matrix of solutions.

It can be seen from Fig. 2 that the changes in PC-1 and PC-2 are insignificant between the two study groups, which indicates a slight difference in the structure of the samples after the lyophilization process. The main spectral differences between the studied groups appear on the RS lines corresponding to the cellular structures in the samples.

The results of the group classification are shown in Fig. 3. It can be seen that the number of correctly classified values is approximately equal for each of the groups. The number of correctly classified values was 7 out of 8 for groups 1 and 2.

Conclusions

Using Raman spectroscopy, it was found that after lyophilization, cellular structures are destroyed (RS lines $604\,cm^{-1}$ (*Nucleotide conformation*) and $720\,cm^{-1}$ (*DNA*)). It is shown that the RS lines corresponding to the organic structure: $936\,cm^{-1}$ (C-C *stretching in protein*), $966\,cm^{-1}$ (*Lipids*), $1077\,cm^{-1}$ (v(C-C) *phospholipids (lipid assignment)*), $1262\,cm^{-1}$ (*Amide III*), $1298\,cm^{-1}$ (CH_3, CH_2 *twisting*, *collagen*), $1438\,cm^{-1}$ (CH_2 *deformation*), $1555\,cm^{-1}$ (*Amide II*), $1654\,cm^{-1}$

(*Amide I*, *alpha-helix protein*), 1745 cm^{-1} (*Phospholipids*) are preserved after lyophilization using the „LYOPLAST" technology, which indicates the absence of a significant effect on the organic structure of biomaterials.

The possibility of using Raman spectroscopy to evaluate the process of lyophilization of the bladder capsule is shown.

Compliance with ethical standards

The studies were performed according to Declaration of Helsinki, the protocol was approved by the Ethics Committee (extract from the protocol № 207 from a session of the Bioethics Committee of the Samara State Medical University dated 09.12.2020).

Conflict of interest

The authors declare that they have no conflict of interest.

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