RAMAN SPECTROSCOPY FOR EVALUATION OF THE BLADDER CAPSULE AFTER THE LYOPHILIZATION PROCESS

E.V. Timchenko[1,2*], P.E. Timchenko[1,2], L.T. Volova[2], O.O. Frolov[1], E.S. Semibratova[1]

[1] Samara National Research University, 34, Moskovskoye shosse, Samara, 443086, Russia

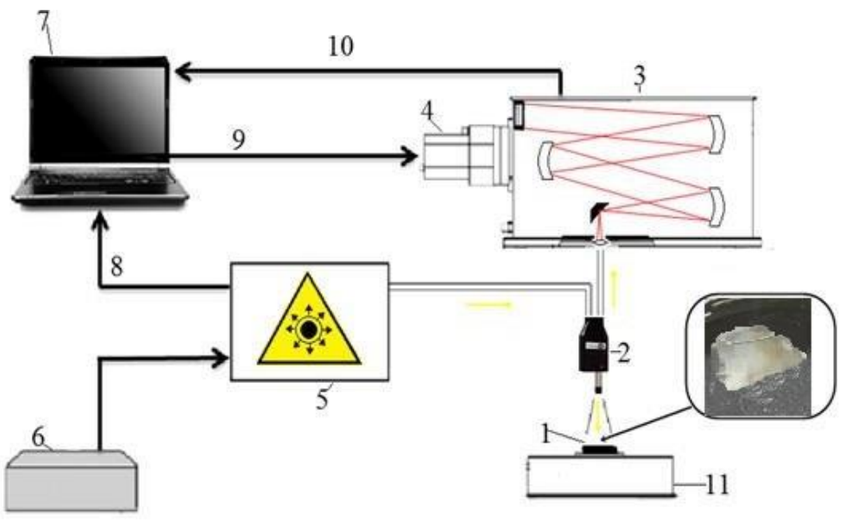
[2] Samara State Medical University, 89, Chapaevskaya Street, Samara, 443099, Russia

RELEVANCE

Currently, an important task is to assess the composition of donor materials for their subsequent use in the field of regenerative medicine. One of the ways to preserve biological products is the lyophilization process. However, it is still not fully understood how the structure of biomaterials changes after this procedure. Optical research methods are widely used to solve various problems in the field of biomedicine.

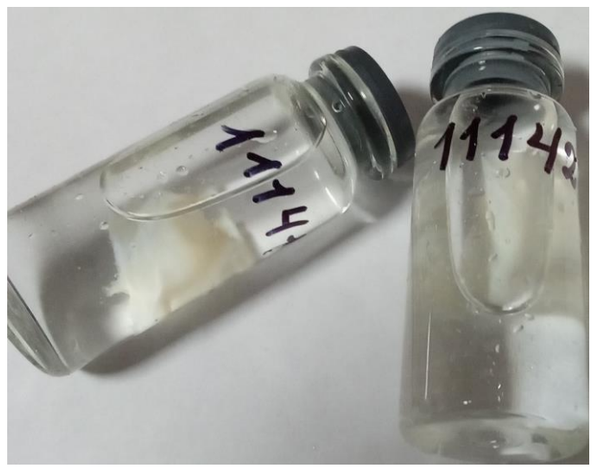
MATERIALS AND METHODS OF RESEARCH

Allogeneic capsules of the bladder of various donors, measuring 1,5x1,5 cm ± 2mm, were selected as objects of research. After removal, part of the biomaterials was placed in a saline solution with an antibiotic and stored at a temperature of t = +4 °. On the day of the study, one part of the biomaterials was immersed in distilled water, while the other part, belonging to the same donors, underwent the lyophilization process using the "LIOPLAST" technology (TU-9398-001-01963143-2004).



Raman Spectroscopy experimental stand:
1 – subject;
2 – Raman probe RPB785;
3 – spectrometer Shamrock sr-303i;
4 – built-in cooling camera DV420A-OE;
5 – laser module LuxxMaster Raman Boxx;
6 – laser module power source;
7 – computer;
8,9,10 – information electrical cables;
11 – coordinate table.

The studied samples



RESULTES OF RESEARCH

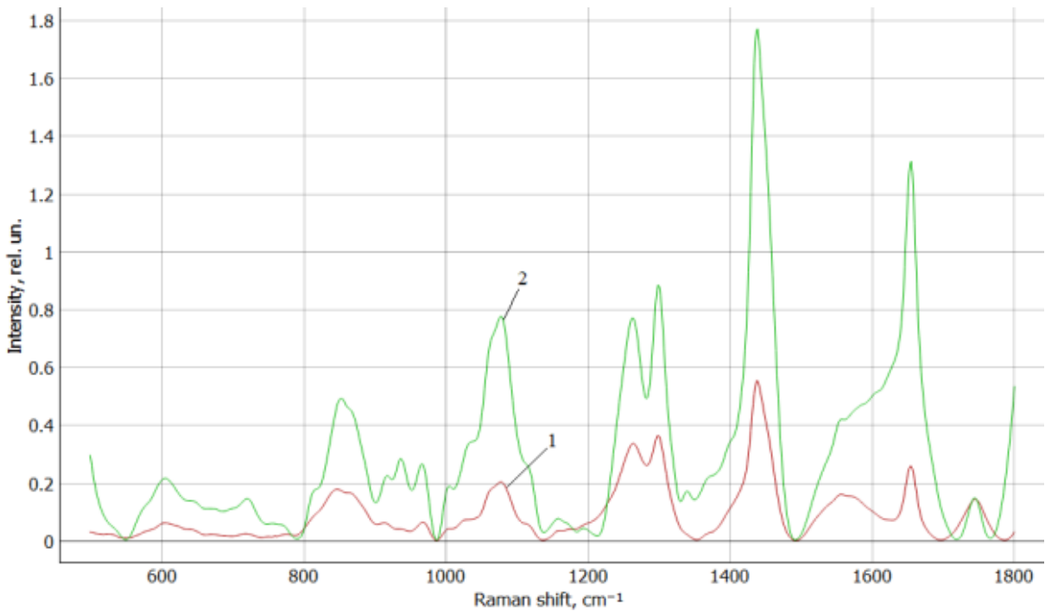


Figure 1. Averaged Raman spectra of the studied sample groups: 1 – lyophilized bladder capsules (30 Raman spectra); 2 – native bladder capsules of the same donors (20 spectra)

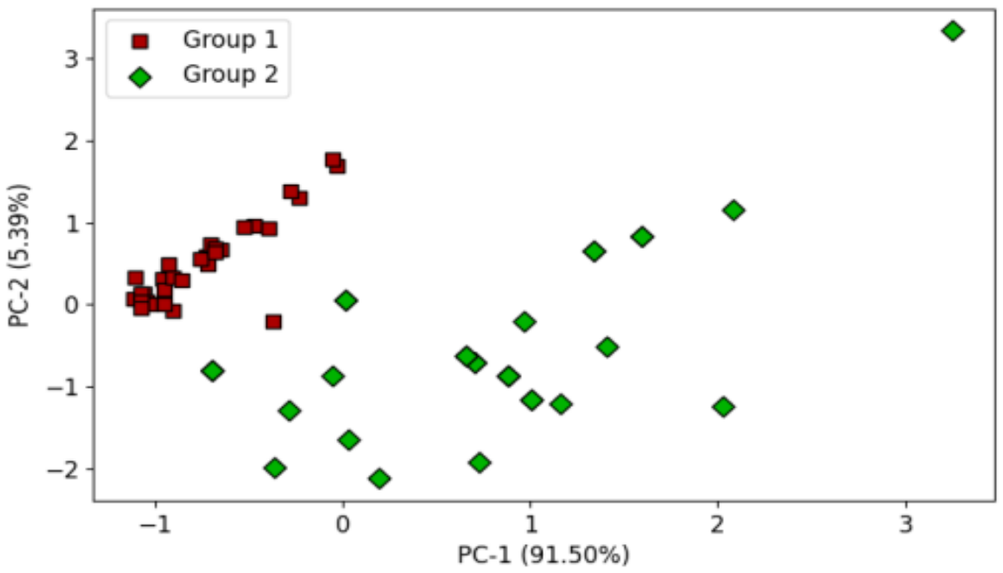


Figure 2. Linear discriminant PCA-analysis for 2 groups of samples

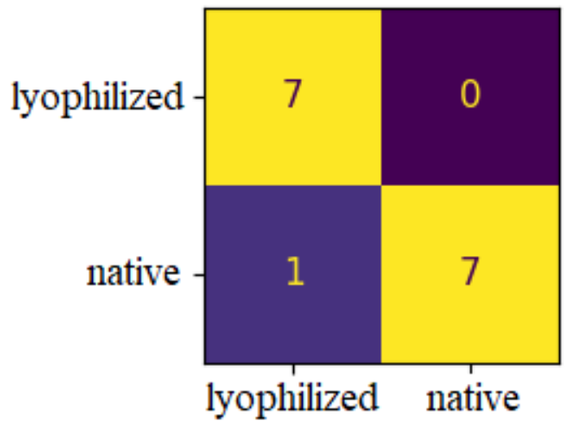


Figure 3. The decision Matrix

CONCLUSION

Using the method of Raman spectroscopy, it was found that after the lyophilization process, cellular structures are destroyed (RAMAN lines 604 cm^{-1} (Nucleotide conformation) and 720 cm^{-1} (DNA)). It is shown that the RAMAN lines corresponding to the organic structure are 936 cm^{-1} (C-C stretching in protein), 966 cm^{-1} (Lipids), 1077 cm^{-1} (ν(C-C) or ν(C-O), 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, α-helix protein), 1745 cm^{-1} (Phospholipids) are preserved after the lyophilization process, this indicates that the lyophilization process using the "LIOPLAST" technology does not have a significant effect on the organic structure of biomaterials.

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