RAMAN SPECTROSCOPY FOR EVALUATION OF DECELLULARIZED GRAFTS IN BURN INJURY TREATMENT

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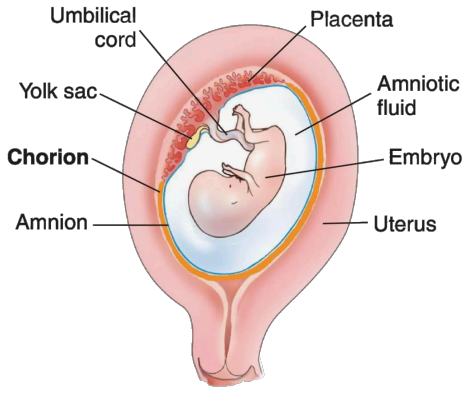
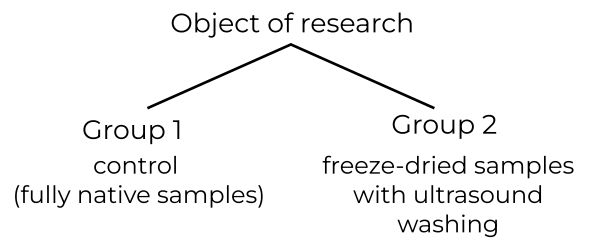
The purpose of the research: *he use of Raman spectroscopy to evaluate decellularized amnion-based grafts during their ultrasonic cleaning treatment.*

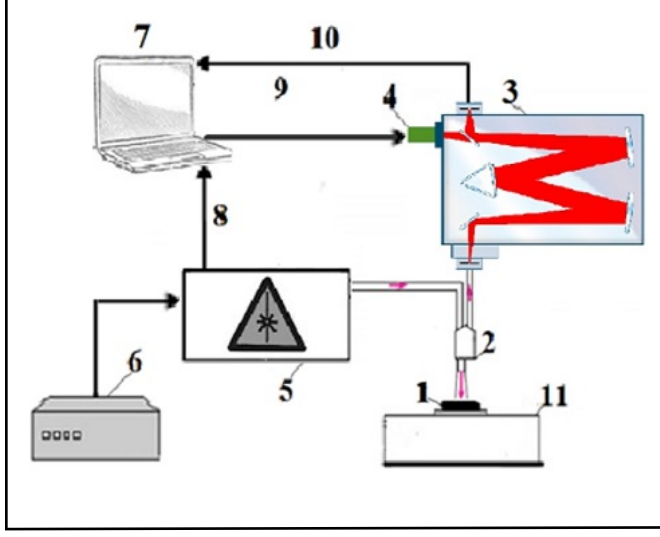
The object of research: *The object of research: a decellurized amniotic membrane, which is manufactured using a patented technology - RF Patent No. 2835347.*





The amniotic membrane is washed in running water from blood clots, then the biomaterial is treated in an ultrasonic bath with a frequency of 35 ± 10 % kHz, after which the bioimplants are placed in a chamber in which a vacuum is created with a residual pressure of 1-2 Pa, and after washing in 0.9% NaCl saline solution, freezing at a temperature of -60 ° C, lyophilized.

Fig.1. The method of constructing the experiment



1 - object;
2 - KR probe RPB785;
3 - LuxxMasterRamanBoxx laser module;
4 - power supply of the laser module;
5 - Shamrock SR-303i spectrometer;
6 - Built-in cooling chamber DV420A-OE;
7 - computer;
8, 9, 10 - information electrical cables;
11 - optical table

Fig. 2. Experimental stand

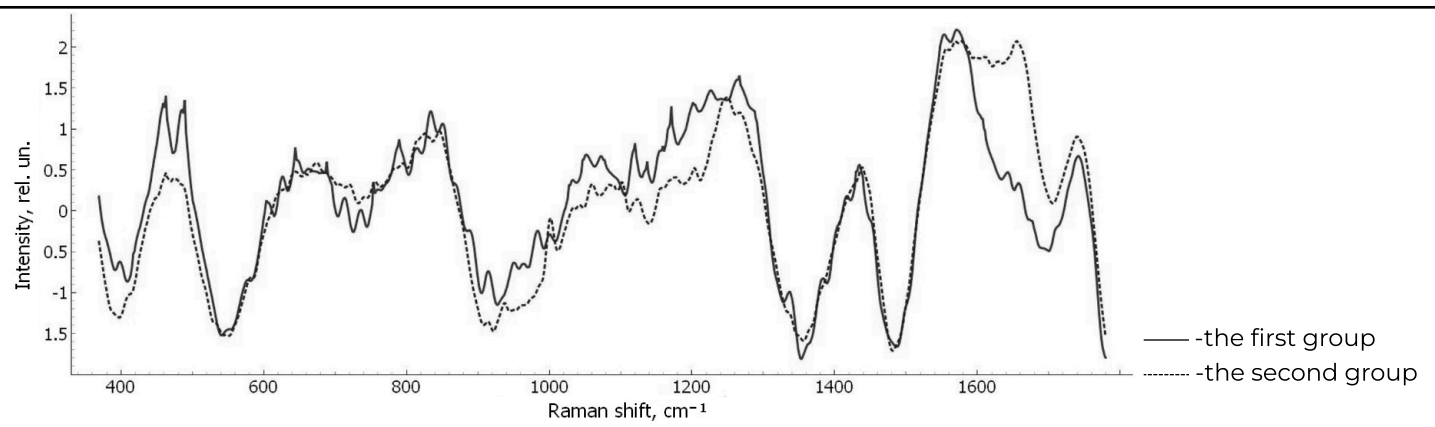


Figure 3 shows that the main spectral differences between the studied samples are observed on the following Raman lines: ~1650 cm-1 (Amid I), ~1240 cm-1 (Amid III), ~1100-375 cm-1 (Several bands of moderate intensity, belonging to amide III and other groups (proteins)), ~600-800 cm-1 (Nucleotide conformation), ~481 cm-1 (DNA), ~447 cm-1 (Ring torsion of phenyl)
Analysis of Figure 3 shows that after ultrasonic treatment, there is an increase in the intensity of the Raman line in the region of ~1650 cm-1 (Amid I) and a slight decrease in the intensity of the RAMAN line in the region of ~1240 cm-1 (Amid III) and ~1100-375 cm-1 (proteins). These changes are probably due to the fact that ultrasound treatment has some effect on the amide structures, which are triggers for the formation of a vascular network (angionesis).
There is also a decrease in the intensity of RAMAN lines in the range of ~600-800 cm-1 (Nucleotide conformation) and ~481 cm-1 (DNA) compared with control samples, which indicates a decrease in the antigenicity of the samples after ultrasound treatment.

Fig. 3. The averaged Raman spectra of the studied groups.

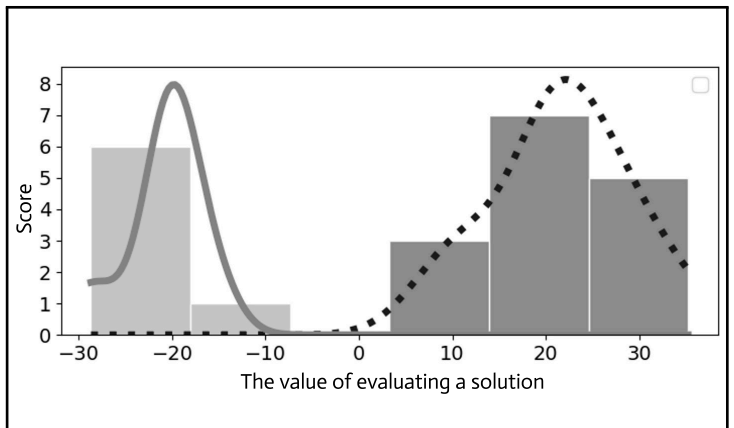
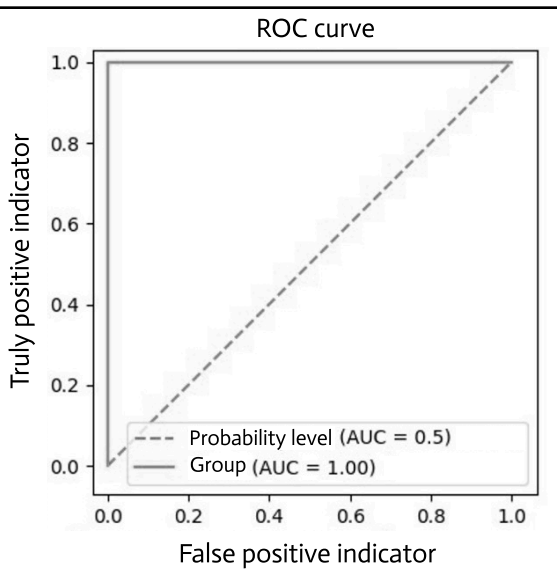


Figure 4 clearly demonstrates how the LDA model separates the control group and the group after ultrasound treatment based on the crucial values. The clear separation between the distributions confirms the high accuracy of the model and the effectiveness of the selected features for classification.

Fig. 4.Distribution of model solutions for each group



The ROC curve in Figure 5 demonstrates the classification quality of the LDA model. The area under the curve (AUC) is 1.0, which indicates an ideal separation of the groups. The curve deviates significantly from the level of random guessing (AUC = 0.5), which confirms the high efficiency of the model.

Fig. 5. The ROC curve

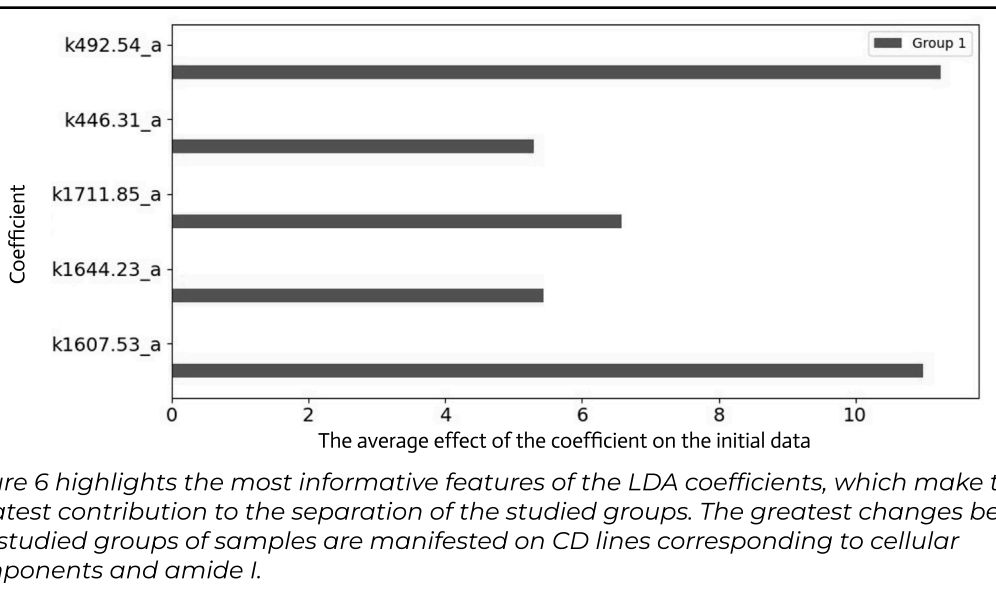


Figure 6 highlights the most informative features of the LDA coefficients, which make the greatest contribution to the separation of the studied groups. The greatest changes between the studied groups of samples are manifested on CD lines corresponding to cellular components and amide I.

Fig. 6. The absolute average values of the features for the most significant variables of the classifier model.

Conclusions*: As a result of the conducted studies, spectral differences were established between the native group of the studied objects and the group of objects treated with ultrasound, which are manifested on the following Raman lines: ~1650 cm-1 (Amid I), ~1240 cm-1 (Amid III), ~1100-1375 cm-1 (Several bands of moderate intensity, belonging to amide III and other groups (proteins)), ~600 800 cm 1 (Nucleotide conformation), ~481 cm-1 (DNA), ~447 cm-1 (Ring torsion of phenyl). These changes are due to a decrease in the antigenicity of the samples after ultrasound treatment, as well as changes in amide structures due to angionesis.*

It has also been established that ultrasonic treatment, using the patented LYOPLAST technique, ensures the preservation of the structure of the studied biomaterials.

Detailed mathematical processing using statistical methods of analysis and ROC analysis showed high efficiency of separation between the studied groups. It is shown that using Raman spectroscopy, it is possible to perform rapid analysis of the composition of decellularized biomaterials in their manufacture with specified properties for certain tasks.