

The first application of vibrational spectroscopy methods to characterize platelets in patients with cardiovascular disease

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Abstract

The task of the study was to assess the feasibility of assessing platelet characteristics of the healthy persons and the patients with CVD using Raman spectroscopy.

Methods. Two groups of subjects were examined - healthy volunteers (group 1, n=11) and patients after a acute myocardial infarction (MI) who received double anti-platelet therapy (group 2, n=9). All patients had a history of myocardial infarction, arterial hypertension, and were on antiplatelet therapy. Smoking patients were excluded from this study. All volunteers had signed informed consent which was approved by Independence Local Ethic Committee. Blood samples were collected into vacuum tubes containing EDTA and then were centrifuged at 60 g for 15 min to separate platelet-rich plasma. Platelets were finally collected by further centrifugation of the supernatant at 1500 g for 15 min. All the centrifugations were carried out at 4°C. After platelet preparation the samples were immediately taken to be examined by SERS spectroscopy. SERS spectra were obtained by Centaur U («NanoScanTechnology» LTD, Russia) Raman spectrometer, using the 532 DPSS Cobolt Samba laser with 45 mW power on sample. The optical scheme included Olympus BX 41 microscope with 100X (NA 0.9) objective. Spectrometer had a focal length of 284 mm with 1200 g/mm diffraction grating and was equipped with an Andor IDus 401 CCD camera with 1024 × 256 pixels. Spectrometer had spectral resolution of 2,5 cm⁻¹. The laser spot of 1 × 25 mm size was positioned at the platelets. Rayleigh scattering was eliminated by the notch filters. Due to plasmon resonance generation availability, rough titanium surfaces with gold nanoparticles (530 nm and 570 nm for gold nanoparticles and rough Ti surface respectively) were used to enhance Raman signal up to 103 times.

Averaged spectra from healthy and CVD patients are displayed on Figure 1. To determine the normal distribution for both groups (healthy and CVD samples), data were analyzed by the Student's t-test, and $p < 0.05$ was considered statistically significant with a 95% confidence interval (95%; Statistics) for the comparisons of mean Raman peaks. Spectral changes of amino acid tryptophan and amide groups were detected. The main vibrational bands are represented in Table 1. The main characteristic bands also are marked on Figure 1. The presented data include 400-1750 cm⁻¹ «fingerprint» spectral range. Main result obtained in the study was the identification of changes in spectral shifts and intensities for the evaluated groups of patients. In particular, differences were revealed in the spectra

of the region of disulfide bridges (S = S bonds) of the low-frequency region, tyrosine, tryptophan and amide groups.

Biography:

Vladimir Rafalskiy has completed his PhD at the age of 27 years from Smolensk State Medical University (Russia) and postdoctoral studies in the same University in 2004. He is the director of Clinical Study Center and professor at the Internal Diseases Department in Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation. He has published more than 150 papers and 15 monograph books.

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