Optical and liquid biopsy of patients with chronic kidney diseases and heart failure

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Introduction

In modern world practice, promising diagnostic methods are emerging, such as "optical biopsy" and "liquid biopsy", which are used for specific diseases biomarkers detection in biological tissues and fluids. Optical methods have the potential to overcome the limitations of traditional methods of clinical analysis. One of the most promising methods of optical analysis (and optical biopsy) is a Raman spectroscopy, which can contribute to understanding of molecular basis of diseases and creation of new bioanalytical tools for the diagnosis of diseases. Since each type of biological tissue and biofluid has an individual molecular composition and, thus, a unique spectral profile resulting from the transition of a molecule from one vibrational-rotational state to another, a set of such individual states of functional groups of nucleic acids, proteins, lipids and carbohydrates makes it possible to characterize component composition of tissues, which ultimately makes it possible to isolate disease markers.

Along with the use of optical biopsy methods, it is possible to apply a supersensitive technique for analyzing biofluids based on surface-enhanced Raman spectroscopy, which will be most effective for detecting low concentrations of disease markers in biological fluids. In the last decade, the development of nanotechnology has led to the creation of promising tools for solving new problems in the study of various human diseases, which is especially important for effective and targeted treatment and a deeper fundamental understanding of the biochemistry of diseases.

In this study we demonstrate application of conventional Raman spectroscopy for the analysis of skin and application of SERS for serum analysis to determine the presence of kidney and heart diseases.

Methods

The study of skin optical biopsy was performed for three groups of subjects: the target group consisting of 85 hemodialysis patients with kidney failure (90 spectra series), the adult control group constituted by 40 healthy volunteers (80 spectra) without systemic diseases and the young control group constituted by 84 healthy volunteers (168 spectra) without systemic diseases. Stimulation of the collected spectra was performed by the laser module (LuxxMaster LML-785.0RB-04, PD-LD, New Jersey) with the central wavelength of 785 nm. The Raman probe (RPB785, InPhotonics, Massachusetts) is able to focus the exciting radiation, as well as to collect and filter the scattered radiation. The focal length of the utilized Raman probe was 7.5 mm with the distance between the tested skin sample and the output lens of the Raman probe of 7 mm. The

collected signal was decomposed into a spectrum using a portable spectrometer (QE65Pro, Ocean optics, Florida).

In SERS analysis of blood the collected samples were placed in sterile tubes. Between sampling and direct recording of spectral characteristics, the samples were stored at -14 ° C. The experimental setup for blood liquid biopsy includes a spectrometric system (EnSpectr R785, Spektr-M, Chernogolovka, Russia) and a microscope (ADF U300, ADF, China). Focusing the exciting radiation and collecting the scattered radiation were implemented using 50x Objective LMPlan. The stimulation of collected spectra was performed by the laser module with central wavelength 785 nm. A yellow-green sol with a silver concentration of 0.05-0.1 g/l was obtained by reduction from an aqueous solution of silver nitrate with sodium citrate at a temperature of 95 ° C for 10 minutes. For SERS testing, a 1/1 silver colloid is added to the serum sample. Initial serum samples and samples of serum solutions with silver sol in a volume of 6 μl are applied to aluminum foil and dried for 60 minutes at room temperature.

In this study, the in vitro analysis of human serum was performed for 205 subjects, including 69 healthy subjects and 61 patients with chronic heart failure (CHF). Analyzed groups separation based on deep learning was implemented using a separate one-dimensional convolutional neural network (CNN). The choice of the CNN architecture for recognition of the current SERS dataset consisted of several consecutive stages. At the first stage, the verified CNN configurations and advanced deep learning practices based on CNN were examined. Analysis of the work by other research teams has shown that the following CNN configurations are characterized by their possible abilities to recognize Raman spectra: sequential CNNs, CNNs containing the Inception module, CNNs with residual connections, ensemble CNNs, CNNs based on a combination of convolutional layers with recurrent layers.

Results and discussion

Application of Raman spectroscopy to investigate the forearm skin has yielded the accuracy of 0.96, sensitivity of 0.94 and specificity of 0.99 in terms of identifying the target subjects with kidney failure. The autofluorescence analysis in the near infrared region identified the patients with kidney failure among healthy volunteers of the same age group with specificity, sensitivity, and accuracy of 0.91, 0.84, and 0.88, respectively. When classifying subjects by the presence of kidney failure using the PLS-DA method, the most informative Raman spectral bands are 1315 to 1330, 1450 to 1460, 1700 to 1800 cm^{-1}. In general, the performed study demonstrates that for *in vivo* skin analysis, the conventional Raman spectroscopy can provide the basis for cost-effective and accurate detection of kidney failure and associated metabolic changes in the skin.

The results of the SERS data for CHF demonstrates that CNN significantly outperforms standard methods of analysis as projection on latent structures and allows for detection of CHF with 95-100% accuracy. By means of multivariate analysis, the informative spectral bands associated with the CHF during disease progression were identified. In addition, the analysis of the correlation between the serum spectral characteristics and urea, creatinine has made it possible to determine the spectral bands correlated with levels of creatinine and urea into the complex spectral characteristics of serum. In general, the reported approach may form the basis for monitoring the health status of CHF patients and find application in studying other pathological conditions of the human body.

Conclusion

Raman-based optical and liquid biopsy may be promising in non-communicable diseases identification, as it provides fast and rapid diagnosis.

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