Search for biomarkers in chemically and UV-induced NETosis

Frank Laboratory of Neutron Physics

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Raman spectroscopy is a powerful diagnostic tool in life sciences based on vibrational spectroscopy that can detect a specific chemical fingerprint of biomolecules.

NETs: **Neutrophil extracellular traps (NETs) are produced by neutrophilic granulocytes and consist of decondensed chromatin decorated with antimicrobial peptides. They defend the organism against intruders and are released upon various stimuli, including ultraviolet (UV) radiation. It's well known that extended exposure of the skin to UV leads to its damage and loss of protective properties. Many cells of the immune system, including neutrophils, are involved in the photoaging process [1]. NETosis activation and release is a dynamic process that can come in two forms, suicidal and vital NETosis. NETs might also have a deleterious effect on the host, because the extracellular exposure of histone complexes could play a role during the development of autoimmune diseases, like systemic lupus erythematosus [2]. NETs could also play a role in inflammatory diseases, as NETs could be identified in preeclampsia [3].**

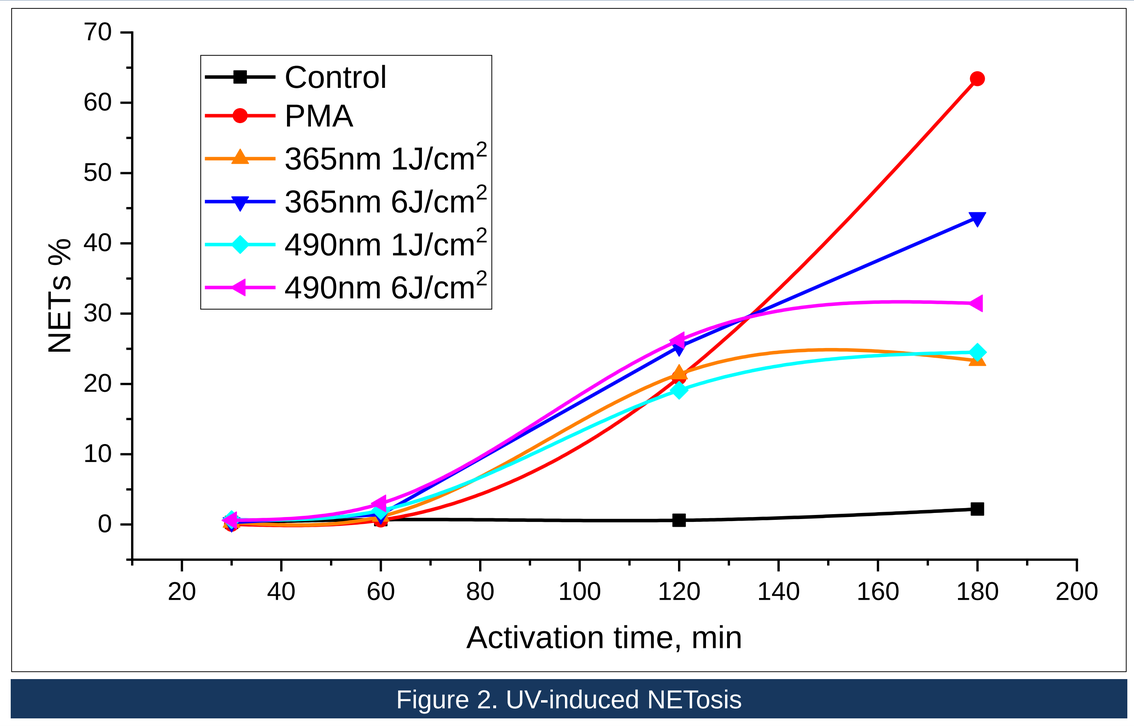

Figure 2. UV-induced NETosis

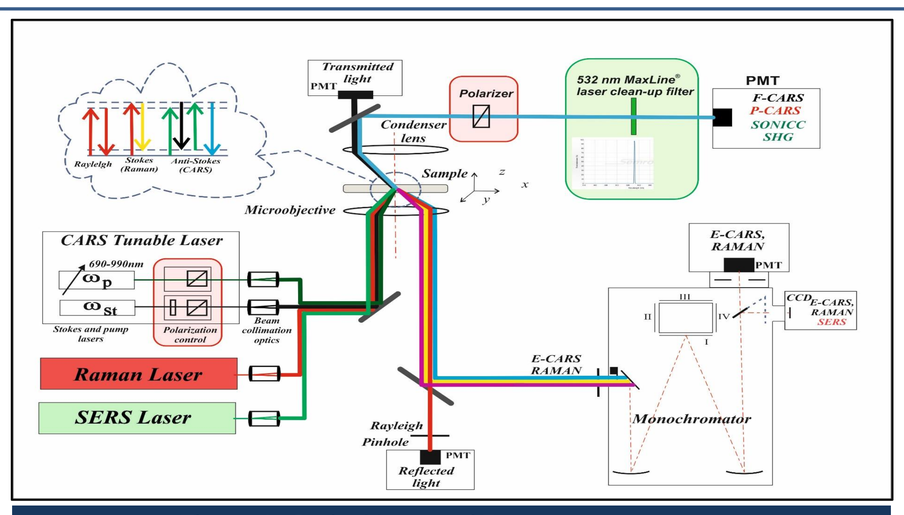

Figure 1. Optical layout of Raman spectrometer

Spectral biomarker: **The main goal of this study was to reveal a possible spectral biomarker in neutrophil activation by measuring and comparing the Raman spectra of UV-induced/PMA activated and non-activated neutrophil granulocytes.**

The low-frequency Raman spectrum of PMA-activated (180 min) neutrophils contains distinctive citrulline peaks (Fig. 3), which indicates on the PAD4 mechanism, in which the process of histone citrullination is observed, while the NOX-dependent signaling pathway is realized under the UV radiation.

Neutrophils activation: **In continuation of the initiated study on the activation of neutrophils by two approaches: biological (bacterial) and chemical (PMA) stimuli, we came over to examine the ability of neutrophils to realize NETs under the UVA (315-400 nm) irradiation. Human neutrophils were isolated from the whole blood obtained from healthy voluntary donor. Neutrophils unexposed to UV radiation were used as a negative control, while the positive control was represented by cells unexposed to UV radiation but stimulated with PMA (50 nM). We applied sensitive Raman spectroscopy (Fig. 1) and succeeded to register citrulline Raman band evaluation during the first hour of neutrophil cells activation [4]. In the novel set of the experiments we started to apply UVA radiation in a dose-depended manner (Fig. 2).**

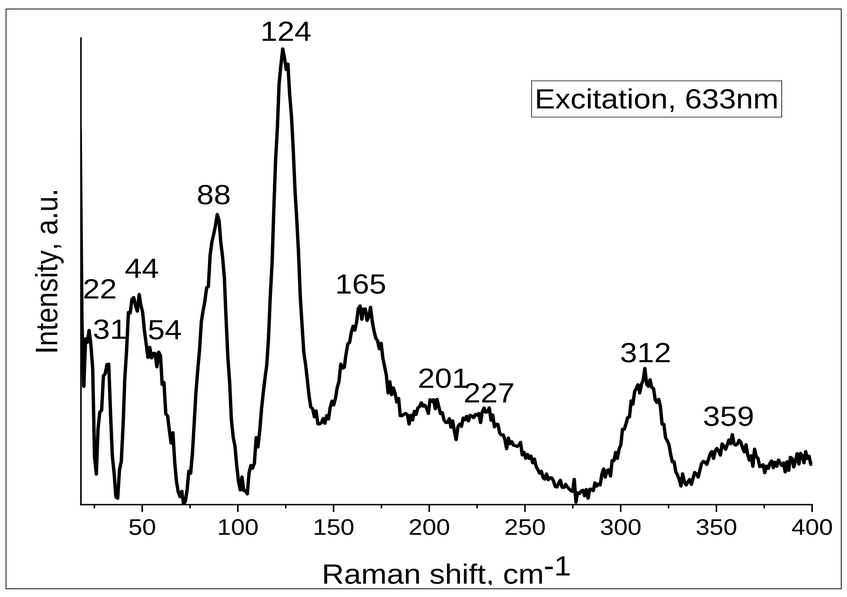

Figure 3. Low frequency Raman spectra of chemically activated neutrophils

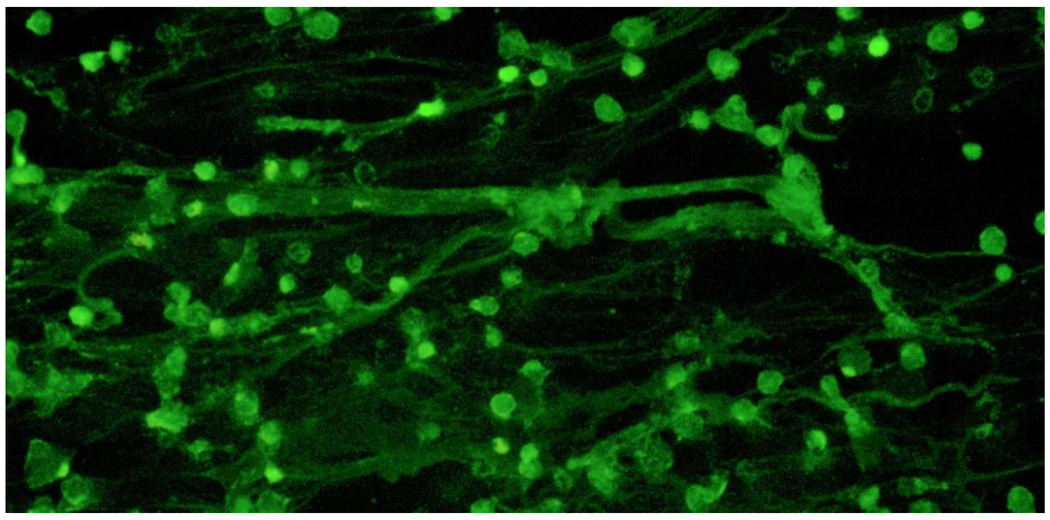

Figure 4. Immunofluorescence imaging of cells in the form of cloud-like-spread

Conclusion: **Data analysis was done implementing immunofluorescence microscopy (Fig. 4) and Raman spectroscopy. It was revealed that UV-induced activation undoubtedly leads to the formation of netotic cells in the form of cloud-like-spread in the observed immunofluorescence imaging. Nevertheless, in contrast to NETosis activation with the calcium ionophore A23, the citrulline peak in Raman spectra has not observed. It is an evidence of the NOX-depended signaling pathway under the UV radiation applied. This research is in progress, including study of low frequency range of Raman spectra of DNA backbone vibrations for the netotic cells.**

References:

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3. Gupta A.K., Hasler P., Holzgreve W., Gebhardt S., Hahn S. (November 2005). "Induction of neutrophil extracellular DNA lattices by placental microparticles and IL-8 and their presence in preeclampsia".
4. Arzumanyan Grigory, Gur'ev Aleksandr, Mamatkulov Kahramon , et. al (2020) , JRS "Micro Raman spectroscopy for NETosis detection".