Does infrared spectroscopy provide new details about the extracellular vesicles?

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Extracellular vesicles (EVs) have recently become the focus of scientific interest: it was recognized that cells release nanostructured lipid-bilayer-enclosed bodies, so called extracellular vesicles, which play a key role in intercellular communication. Despite their promising potential as early stage biomarkers of different pathological states or/and therapeutic delivery vehicles, the clinical use of EVs is hindered by the lack of proper and reliable isolation, determination and characterization methods and protocols.

Infrared spectroscopy (IR) is a label-free method that can detect changes of functional groups in molecules from biological samples, providing a **biochemical fingerprint**. Our research group was the first performing exploratory IR study on EVs. By detailed spectral analysis we identified changes in protein structures and the relative lipid content for different EV subpopulations (exosomes, microvesicles and apoptotic bodies) isolated form Jurkat T-cell line [1]. Based on selected amide and C-H stretching-band intensity ratios, we proposed a 'spectroscopic protein-to-lipid ratio' (P/L), which provides a useful index for EV characterisation. Using proper calibrations and known protein concentrations, spectroscopic P/L can serve for indirect quantification of total lipids, too [2]. Selected IR markers such as spectroscopic P/L, amide I/amide II ratio and α-helix/β-sheet ratio related to global protein secondary structure/orientation were used to monitor EVs formed and accumulated *in vitro* during the storage of red blood cells (RBCs). These IR markers alter in function of storage time and medium, and might be indicative for biochemical and morphological changes occurring in RBC concentrates.

Despite the wealthy information on structural changes of proteins, no detailed information is provided regarding the less expressed IR bands of the lipid bilayers enclosing the EVs. To get direct information on size/morphology of EVs, combination with other techniques are required.

Small-angle X-ray scattering (SAXS) is one such technique. It was found to be a powerful tool for quantifying the overall size and the bilayer profile of synthetic phospholipid vesicles [3]–[5]. However, because SAXS is only selective for the size and electron density contrast, scattering from objects of nearly the same size overlap, making the results difficult, if not outright impossible, to interpret. This is the case with EVs: the scattering from their protein content does not allow the quantification of the phospholipid bilayer profile. Furthermore, the common laboratory instruments suffer from the lack of X-ray intensity, making the study of inherently dilute EV samples prohibitively long. There are several possible solutions to these problems. Contrast variation techniques (e.g. solvent replacement for SAXS and deuteration for small-angle neutron scattering) can help suppressing the scattering of e.g. the protein part of the sample. Because SAXS is SI-traceable, i.e. able to give true sizes with well-

characterized uncertainty, it can be used for characterizing simpler systems (synthetic lipid vesicles or inorganic nanoparticles) to be used as reference systems for measurements which require external calibration (e.g. flow cytometry and various chromatographic techniques).

References

[1] J. Mihály, R. Deák, I. C. Szigyártó, A. Bóta, T. Beke-Somfai, and Z. Varga, "Characterization of extracellular vesicles by IR spectroscopy: Fast and simple classification based on amide and CH stretching vibrations," *Biochim. Biophys. Acta BBA - Biomembr.*, vol. 1859, no. 3, pp. 459–466, Mar. 2017.

[2] T. Visnovitz *et al.*, "An improved 96 well plate format lipid quantification assay for standardisation of experiments with extracellular vesicles," *J. Extracell. Vesicles*, vol. 8, no. 1, p. 1565263, Jan. 2019.

[3] Z. Varga *et al.*, "A Closer Look at the Structure of Sterically Stabilized Liposomes: A Small-Angle X-ray Scattering Study," *J. Phys. Chem. B*, vol. 114, no. 20, pp. 6850–6854, 2010.

[4] Z. Varga, A. Wacha, U. Vainio, J. Gummel, and A. Bóta, "Characterization of the PEG layer of sterically stabilized liposomes: a SAXS study," *Chem. Phys. Lipids*, vol. 165, no. 4, pp. 387–392, May 2012.

[5] Z. Varga, A. Wacha, and A. Bóta, "Osmotic shrinkage of sterically stabilized liposomes as revealed by time-resolved small-angle X-ray scattering," *J. Appl. Crystallogr.*, vol. 47, no. 1, pp. 35–40, Feb. 2014.