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Characterization of extracellular vesicles by IR spectroscopy: fast and simple classification based on amide and C-H stretching vibrations

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Abstract

Extracellular vesicles isolated by differential centrifugation from Jurkat T-cell line were investigated by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). Amide and C-H stretching band intensity ratios calculated from IR bands, characteristic of protein and lipid components, proved to be distinctive for the different extracellular vesicle subpopulations. This proposed 'spectroscopic protein-to-lipid ratio', combined with the outlined spectrum-analysis protocol is valid also for low sample concentrations (0.15-0.05 mg/ml total protein content) and can carry information about the presence of other non-vesicular formations such as aggregated proteins, lipoproteins and immune complexes. Detailed analysis of IR data reveals compositional changes of extracellular vesicles subpopulations: second derivative spectra suggest changes in protein composition from parent cell towards exosomes favoring proteins with β-turns and unordered motifs at the expense of intermolecular β-sheet structures. The IR-based protein-to-lipid assessment protocol was tested also for red blood cell derived microvesicles for which similar values were obtained. The potential applicability of this technique for fast and efficient characterization of vesicular components is high as the investigated samples require no further preparations and all the different molecular species can be determined in the same sample. The results indicate that ATR-FTIR measurements provide a simple and reproducible method for the screening of extracellular vesicle preparations. It is hoped that this sophisticated technique will have further impact in extracellular vesicle research.

Keywords: exosome; microvesicle; extracellular vesicles; ATR-FTIR spectroscopy; spectroscopic protein-to-lipid ratio; erythrocyte ghost membrane

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