Optical and electrophoresis methods for macrophage derived extracellular vesicles characterization

Clinical

Olesia Gololobova (olgol@email.unc.edu), UNC Chapel Hill, Nanomedicined Characterization Core Facility, **Marina Sokolsky-Papkov**, University of North Carolina at Chapel Hill, **Alexander Kabanov**, University of North Carolina at Chapel Hill

Extracellular vesicles (EVs) are native cell-derived nanovesicles which have attracted considerable attention in medicine and drug delivery. They effectively home to disease sites, including primary tumors, brain, lungs, etc. However, their separation and analysis can be challenging. Therefore, there is a critical need to develop robust, simple, reliable, and reproducible methods to isolate, quantify and characterize EVs in clinically appropriate amounts.

IC21 macrophages were cultured in normal and dense (bioreactor) conditions. EVs were separated from culture media by differential ultracentrifugation and yield and purity were compared.

EV size distribution, concentration and particles phenotype were analyzed by nanoparticle tracking analysis (NTA). Electrophoretic mobility of single particles was tracked to calculate zeta potential of individual vesicles. Protein/particle, the indicator of the purity of the isolated EVs, ratio was calculated based on the standard BSA protein assay. Extracellular vesicles protein markers expression was measured using capillary electrophoresis. Particles morphology was analyzed by transmission electron microscopy (TEM).

Nearly 4 times more particles were isolated from bioreactors. These vesicles were smaller than EVs isolated from flasks (115.1 ± 4.5 nm vs. 128.7 ± 11.3), while no difference in charge and shape/phenotype were observed. Notably, electrophoresis Simple Western blot revealed that bioreactor EVs had 4 times higher abundant of HSP90 and twice lower expression of CD9. Taken together, bioreactor EVs may can provide higher amount of vesicles but will require further analysis due to presence of smaller particles, with sizes below NTA detection limits and lower protein/particle ratio.

Macrophages cells grown in bioreactors can produce more extracellular vesicles, but purity and quality of the particles need to be carefully characterized. Single-particles optical methods and markers characterization by capillary electrophoresis are good initial techniques for EVs analysis to assess purity and quality of the formulations.