EXTRA CELLULAR VESICLES SEPARATION AND BIOPHYSICAL CHARACTERIZATION

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Key Words: Nanoparticle tracking analysis, cryo-tomography, heparin affinity chromatography, restricted access chromatography, multi-angle light scattering.

Cells bud off up to 10-fold of their biomass in form of extracellular vesicles. The term ‘extracellular vesicles’ refers to a heterogeneous population of vesicular bodies of cellular origin that derive either from the endosomal compartment (exosomes) or as a result of shedding from the plasma membrane (microvesicles, oncosomes and apoptotic bodies). Extracellular vesicles carry a variety of cargo, including RNA, proteins, lipids and DNA, which can be taken up by other cells, both in the direct vicinity of the source cell and at distant sites in the body via biofluids, and stimulate a variety of phenotypic responses. These functions of extracellular vesicles are not necessary when single cells are produced in a bioreactor for the plain purpose of biomass generation. The fact that cells in in-vitro culture release that many extracellular vesicles is completely neglected by research in the life science and biochemical engineering community. In addition, a lot of chromatin is present in cell culture supernatant. Due to the presence of chromatin the particle count is always overestimated due to the similar size between chromatin and extra cellular vesicles.

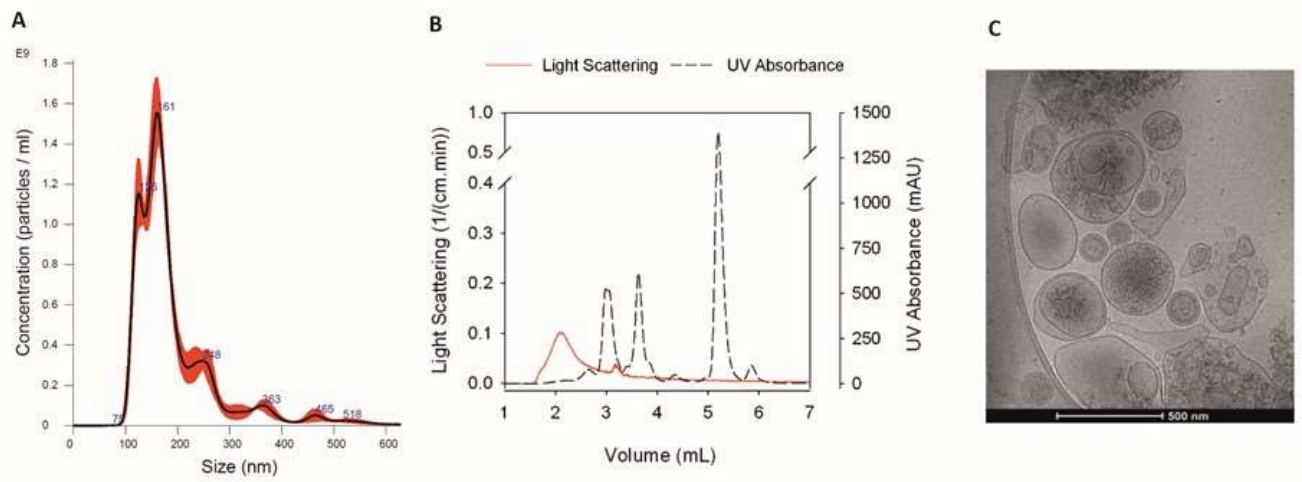

Figure 1 Comparison of Nanoparticle tracking analysis (a), HPLC-SEC (B) and high resolution electron microscopy(C) for detection and quantification of EVs demonstrating the challenge of identification and quantification.

Currently extra cellular vesicles (EV) are often determined by biochemical analysis using antibodies against certain target proteins. After ultracentrifugation of the clarified cell culture supernatant, the resuspended pellet is subject to Westernblot analysis or after disintegration of the vesicles to ELISA using the respective antibodies to marker proteins. The tetraspanins such as CD63, CD81, CD82, CD53, and CD37 are used and protein of Endosomal Sorting Complex Required for Transport (ESCRT) machinery as the prime targets, but nowadays the EXOCARTA (http://www.exocarta.org/) database is available for selection of the appropriate marker proteins. It has been shown that high speed centrifugation may alter the shape of EVs and lead to aggregation also with other particles. Often the clarified supernatant is subjected to analysis of the target proteins, but this is even more prone to misidentification.
We have developed a strategy to characterize and isolate extra cellular vesicles. The cell culture supernatant of a supernatant containing a viral vector or a virus is filtered by 0.8 µm filter then loaded on a restricted access chromatography (Capto Core) and the flow through is processed by heparin affinity chromatography Capto Heparin using a linear salt gradient. The separation and characterization of extra cellular vesicles is demonstrated using an insect cell culture overexpressing virus-like particles. The process qualifies either for in-depth characterization, but also for large scale processing of extra cellular vesicles.