CHARAKTERISIERUNG VON EXOSOMEN AUS BLUTZELLEN MITTELS NANO ELECTROSPRAY GAS-PHASE ELECTROPHORETIC MOBILITY MOLECULAR ANALYSIS (NES-GEMMA)

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Extrazelluläre Vesikel

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Brief summary:
Extracellular vesicles (EVs) are released by cells upon activation or stress and are present in all body fluids. They have recently emerged as versatile mediators of intercellular communication and as potentially rich reservoirs of clinical biomarkers. They are involved in a multiplicity of physiological processes, such as the regulation of the vascular function, and there is ample evidence for their roles in various pathological settings, such as cancer, inflammation and thrombosis. Channeling the properties of EVs towards therapeutic application and patient care is dependent on technological progress in analytical approaches.
The complexity of biological fluids along with the heterogeneity of EVs challenges their isolation and characterization. The most commonly applied separation protocols, such as ultracentrifugation and density gradient centrifugation, are hampered by uncontrolled loss of vesicles and co-isolation of contaminants, such as cellular debris, protein aggregates, lipoproteins, or nucleic acids. Flow cytometry has found widespread application for the characterization of EVs, but EVs smaller than 200 nm, representing a high percentage of all vesicles present in blood, are not detectable with this approach. Other methods, such as nanoparticle tracking analysis are capable of detecting vesicles down to a size of 10 nm, but are not able to differentiate them from non-vesicular material, such as protein aggregates.
Here, we will investigate the application of Nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analysis (nES-GEMMA) for the characterization of extracellular vesicles, in particular exosomes, from complex biological matrices, such as human blood or plasma. We aim to set up standardized protocols for the isolation of highly pure EV fractions and their characterization with nES-GEMMA. We will apply nES GEMMA to quantify exosomes, which is superior to indirect quantification based on protein concentration as used to date, and we will assess for the first time whether protein and lipid content are qualitatively influenced by the size of extracellular vesicles by collecting size separated vesicles via an electrostatic nanometer aerosol sampler. Finally, we will monitor eventual modifications in the composition of vesicles obtained from physiological and different pathological settings. As a first application in clinical samples, nES-GEMMA will be used in combination with conventional flow cytometry and nanoparticle tracking analysis to quantify and characterize extracellular vesicles from the plasma of healthy donors and sepsis patients.

Keywords:
Gas- and liquid-phase electrophoresis