Characterization of EVs by Flow Cytometry: A Methodological Approach

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Flow cytometry is an advantageous tool for the analysis and characterization of extracellular vesicles (EVs) because of its robust statistical power and its multiparametric capabilities. The goal of nanoscale flow cytometry analysis and nanoscale sorting, termed Small Particle Flow Cytometry (SPFC), is to accurately represent the size distribution and scatter profiles of these vesicles. As with conventional flow cytometry, the suspended vesicles pass through a chamber and are hydrodynamically focused. As vesicles pass through, the laser light is refracted and scattered in all directions. Their scatter properties are measured by detectors; concurrently fluorescent dyes can be used to tag various properties of interest. Characterization of extracellular vesicles (EVs) is greatly impeded by several factors: their size (below 100nm), their overlapping size distribution, particle polydispersity, and an overall low refractive index (Orzoco, Van Der Pol). Because conventional flow cytometers are not equipped with necessary optical capacities to reach this lower threshold, instruments that are used for SPFC typically have specific adaptations. In light of these enhancements, there are a number of modifications to the conventional form of analysis that should be considered. Here we describe a comprehensive methodology for the set up and standardization of EV analysis using SPFC. Controls of different size ranges, fluorescent intensities, and materials can be used to set up distribution curves that are then used for instrument optimization and as a reference guide. Using these controls, FACS instruments can be primed for the detection, analysis and sorting of specific EV populations. This allows for cross platform comparison and the ability

to monitor both Quality Control (QC) and Quality Assurance (QA). The method here will describe the use of nanoparticles to optimize a flow cytometer for small particle detection. It will also outline the procedures necessary to recover EVs for downstream applications, particularly in a cardiac modality.