P-02-Fractioning by Free Flow Electrophoresis (FFE) followed by Imaging Flow Cytometry analysis displays EV charge density heterogeneities within human plasma and cell culture supernatants

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Abstract

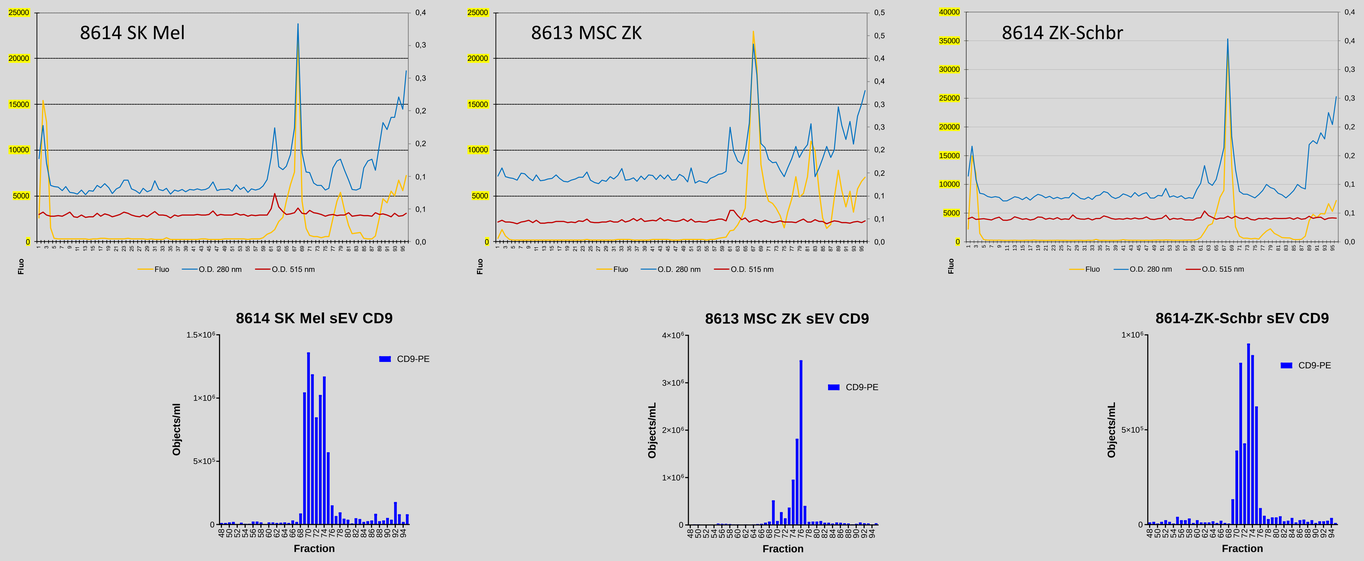
FFE allows quick separation and preparation of biological analytes including cellular organelles from various biological liquids according to their outer electric charges. Performing subsequent imaging flow cytometry (IFCM) analyses we demonstrate that also extracellular vesicles (EVs) can be effectively and quickly fractioned by FFE. Interestingly, EVs prepared from cell culture supernatants on average reveal higher negative charges than EVs from human plasma of healthy donors or melanoma patients, respectively.
Overall, applying the established and optimized EV separation protocols FFE provides a fast and feasible method for EV fractioning for appropriate down-stream analysis including IFCM. In its current form FFE allows fractioning of approx. 100 samples per working day.

Introduction

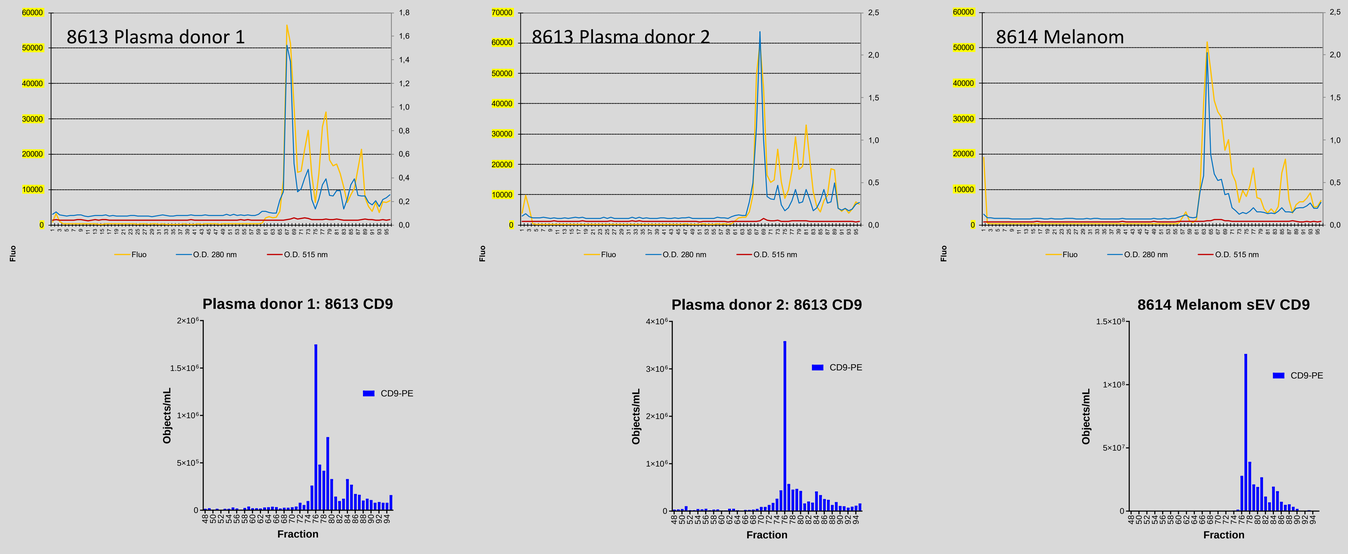
Despite the increasing interest in extracellular vesicles (EVs), obtaining EVs in high purity remains a tedious process. Neither fractionation by density nor by size alone is sufficient to separate EVs from most contaminants including lipoproteins. For now, a time-consuming combination of two methods (density and size separation) is required to enrich EVs to high purity at the expense of time.

Free Flow Electrophoresis (FFE) is a traditional method allowing separation of a multitude of different analytes according to their capabilities to migrate in an electric field through buffers with defined pH values. After separation the original sample contents are eluted in up to 96 different fractions, often allowing recovery of selected and highly purified analytes in distinct fractions. Technical details are provided on our homepage

Fractioning of EVs from cell culture supernatants



Fractioning of EVs from human plasma samples



Results

Applying the optimized EV separation protocol FFE can fraction undiluted cell culture supernatants as well as plasma with a processing rate of up to 1.5 ml/h. FFE-fractions of 150 µl as typically required for IFCM analyses can be obtained within 2 min and are collected in a 96-microtiter plate. Following a 2 min rinsing procedure for the removal of residual sample aliquots, the next sample can be processed. IFCM analyses confirm the power of FFE in separating different EV subpopulations and also uncovers differences in the EV composition within the plasma of healthy donors and melanoma patients, with significantly higher EV concentrations detected melanoma patient plasma than that of healthy donors.

Conclusion/ Outlook

FFE can be considered as an ideal platform for the dissection of the heterogeneity in given EV populations, both within primary body liquids including plasma and cell culture supernatants. Revealing different EV compositions in the plasma of healthy donors and melanoma patients, FFE may provide an ideal platform for the sample preparation for the subsequent biomarker discovery. Its usability as high-through-put EV preparation platform is warranted by its short processing times and the fact that FFE can be fully automatized to handle up to 120 microtiter plates and if desired process two different biological samples in parallel.

Acknowledgements

Conflict of interest:

G. Weber, is CEO, M. Meckel is employee of FFE Service GmbH, the manufacturer of the FFE instrument