Free Flow Electrophoresis allows preparation of extracellular vesicles with high purity

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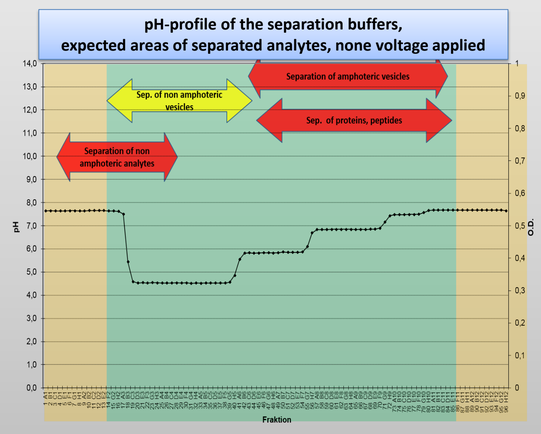
Abstract

Currently, it remains a challenge to prepare extracellular vesicles (EVs) to high purity, especially those from body liquids, such as plasma. Neither fractionation by density nor by size alone is sufficient to separate EVs from all contaminants, e.g. high and low density lipoprotein (HDL/LDL) and other material. 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 and low recovery.

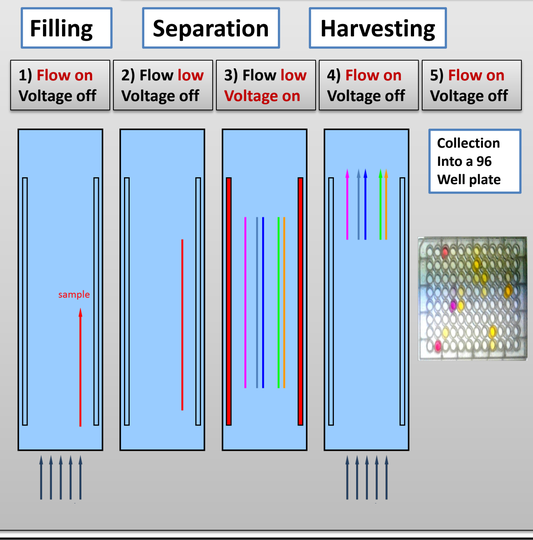
Free Flow Electrophoresis (FFE) is a well-established (semi-) preparative method to separate analytes with inherent difference of charge density and/or difference of pI-value.

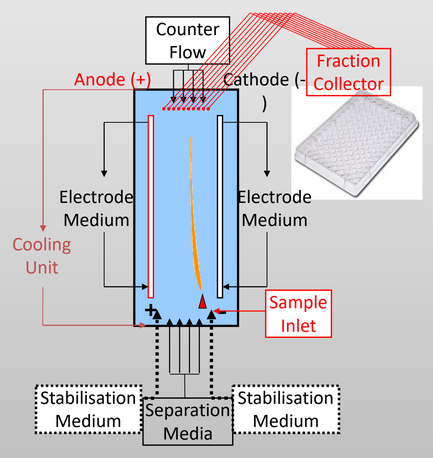
A **Free Flow Interval Zone Electrophoresis (FF-IZE)** method has been developed for the purification and isolation of EVs as well DNA and RNA from human plasma samples, using a set of buffer media of different pH-values ranging from pH 8.0 to pH 4.8. Upon processing supernatants of mesenchymal stem/stromal cells (MSCs) cultured in the presence of 10% human platelet lysate (hPL), EVs are recovered in a small number of FFE fractions lacking most proteins of the conditioned media. Currently, we characterize the identified fractions in more detail. Prepared EVs are quantified by Nano Particle Tracking Analysis (NTA) and imaging flow cytometry. Furthermore, the presence of several EV markers and the absence of contaminants are analyzed by Western Blot and mass spectrometry-based proteomics. Our obtained data demonstrate, FFE is a feasible and quick method to highly purify EVs in an accurate manner.

General IZE-pH protocol

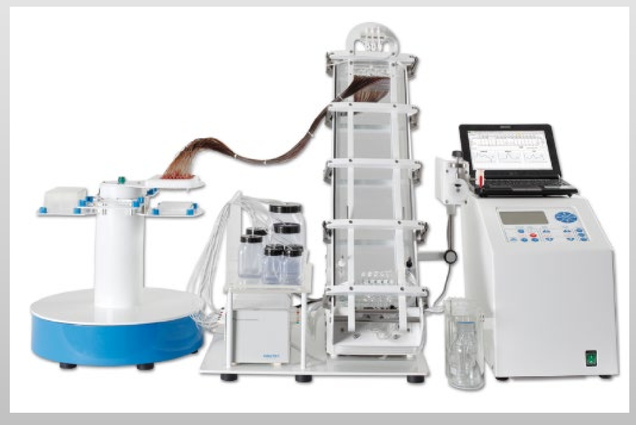


Principle of iZE-FFE = interval Zone Free Flow Electrophoresis





Free Flow Electrophoresis Instrument



Experimental procedure

Conditioned MSC media were harvested in six independent experiments to obtain samples for 6 biological replicates. Samples of the conditioned media were separated in three independent runs. To show reproducibility of the FFE, 9 technical replicates were performed.

Samples

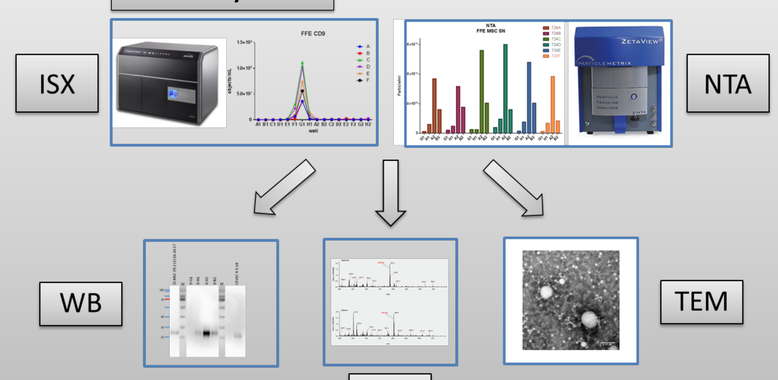
Samples
→ Conditioned MSC medium produced independently on different days
→ 6 biological replicates

Sample preparations
→ 3 times separated by FFE (Run1-3) on 3 subsequent days
→ 9 technical replicates

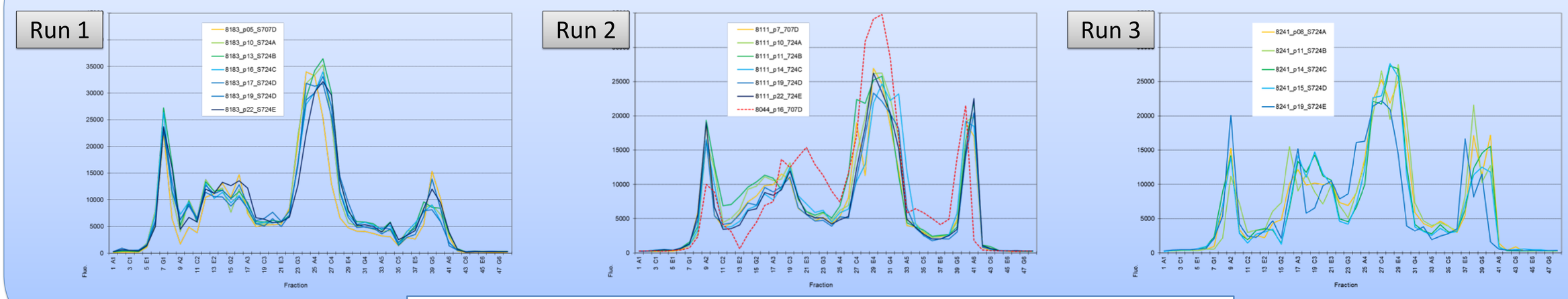
	Sample	MSC-CM
1	724A	70.2
2	724B	70.2
3	724C	85.2
4	724D	84
5	724E	88
6	707d	70.2

Separation

Analysis

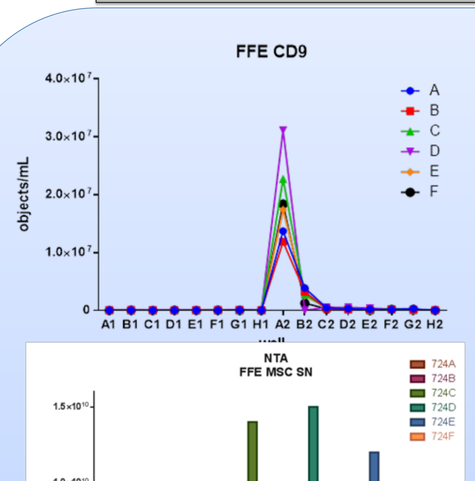


Separation monitored by Pherograms

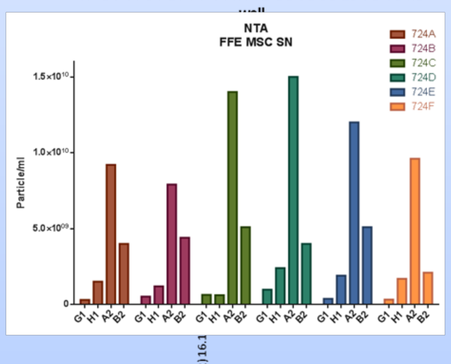


The separation is highly reproducible the overlay of the six samples are similar in each run

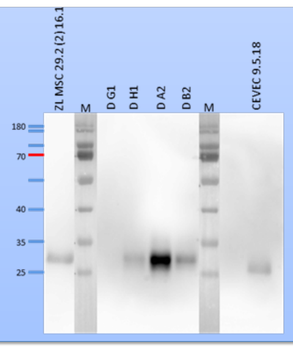
Analysis by ISX, NTA, WB



According to the FCM Amnis image stream analysis CD9 positive EVs are reproducibly enriched in fraction A2

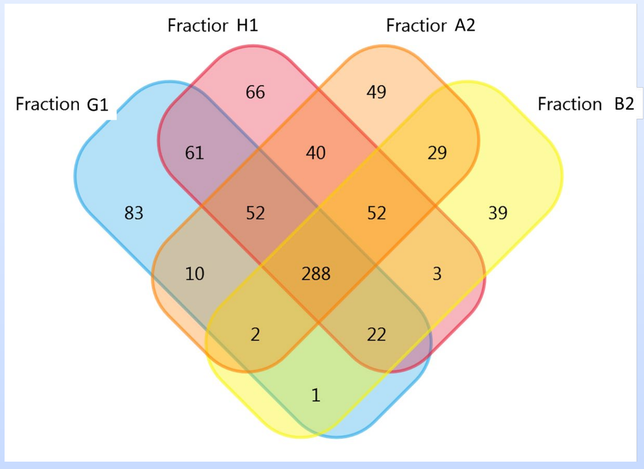


According to NTA most recordable particles are reproducibly enriched in fraction A2



WB analysis recovered the EV specific antigen CD9 abundantly in fraction A2

Analysis by Proteomics



The Venn diagram shows huge overlaps of the proteins detected in all of the four FFE fractions investigated. Several EV markers have been found to be enriched in fraction A2

Fraktion G1

CD	peptides
CD5 antigen-like	5

Fraktion H1

CD	peptides
CD5 antigen-like	11
CD9 antigen	2
CD226 antigen	1
CD44 antigen	1

Fraktion A2

CD	peptides
CD5 antigen-like	18
CD9 antigen	4
CD166 antigen	2
CD226 antigen	1
CD44 antigen	1

Fraktion B2

CD	peptides
CD5 antigen-like	10
CD44 antigen	1

Quantitatively the most CD markers were enriched in fraction A2

Conclusion

- FFE is a reproducible technique to isolate EVs from protein rich supernatants
- The purity of EVs reaches a high grade
- Contaminants like serum albumin are still recovered in reduced quantities by proteomic profiling
- The FFE separation depends on the quality of the sample to be processed: serum free samples result in higher EV purities than serum or hPL containing samples (data not shown)

Outlook

- FFE is a method that can be adapted to given requirements; conditions and buffers for example can be optimized to increase yields and/or purities
- One of the next goals is to use FFE to separate different EV subtypes from each other finally becoming able to comprehensively study the heterogeneity of EVs in given preparations.

Conflict of interest:

G. Weber, is CEO, C. Reiter and M. Meckel are employees of FFE Service GmbH, the manufacturer of the FFE instrument