Ascites-derived EVs – An isolation approach using free-flow electrophoresis

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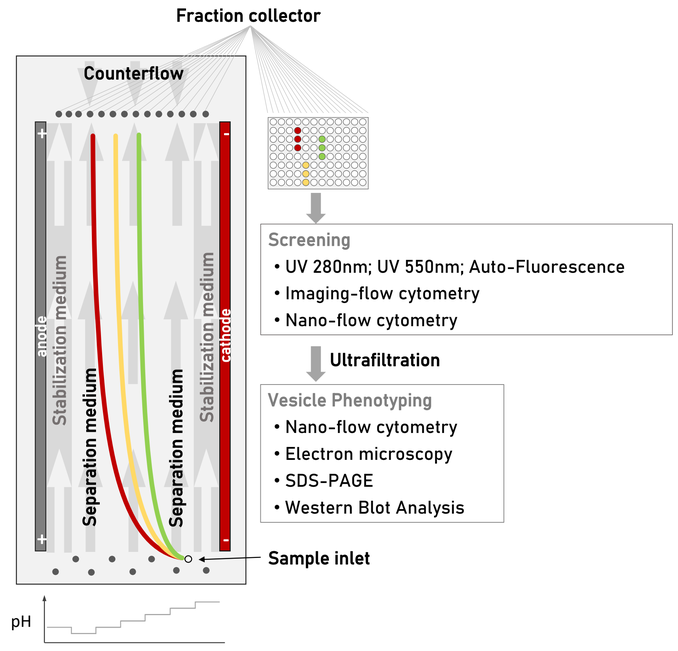
Background

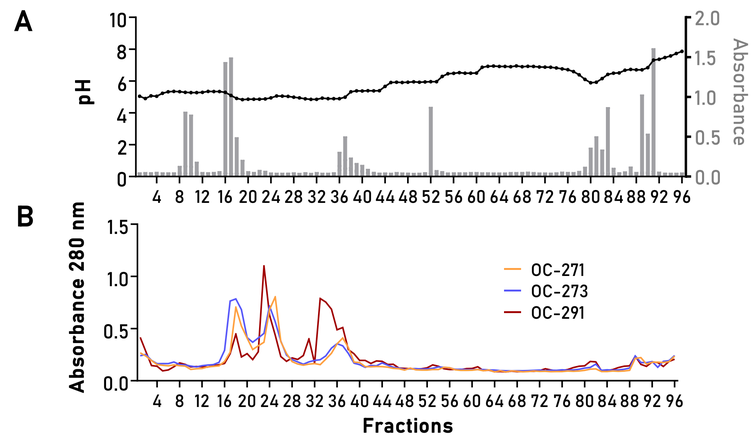
Although extracellular vesicles (EVs) have been characterized to some extent and a wide variety of purification techniques have been described, the separation of EVs from other extracellular particles remains challenging, especially for primary bioliquids such as ascites or blood plasma.

As a novel approach for purifying EVs from primary biofluids, we used free-flow electrophoresis (FFE), a well-established matrix-free, liquid-based, highly versatile technology for separating analytes according to their net charge.

In FFE, buffers with different pH values are introduced into a separation chamber via vertically arranged inlets at the bottom of the chamber, creating a continuous laminar flow of distinctly defined pH zones along the longitudinal axis. After sample injection, the sample is transported by the laminar flow of the aqueous medium. Subsequently, a high voltage perpendicular to the laminar flow direction is applied, which results in the deflection of the respective analytes in the laminar film according to their mobility and their isoelectric point, depending on the pH values of the buffers introduced. Once the samples have passed through the separating chamber, samples are adjusted to a neutral pH using an appropriate counterflow and finally collected through a 96-outlet tubing into a 96-well plate.



FFE enables secretome separation

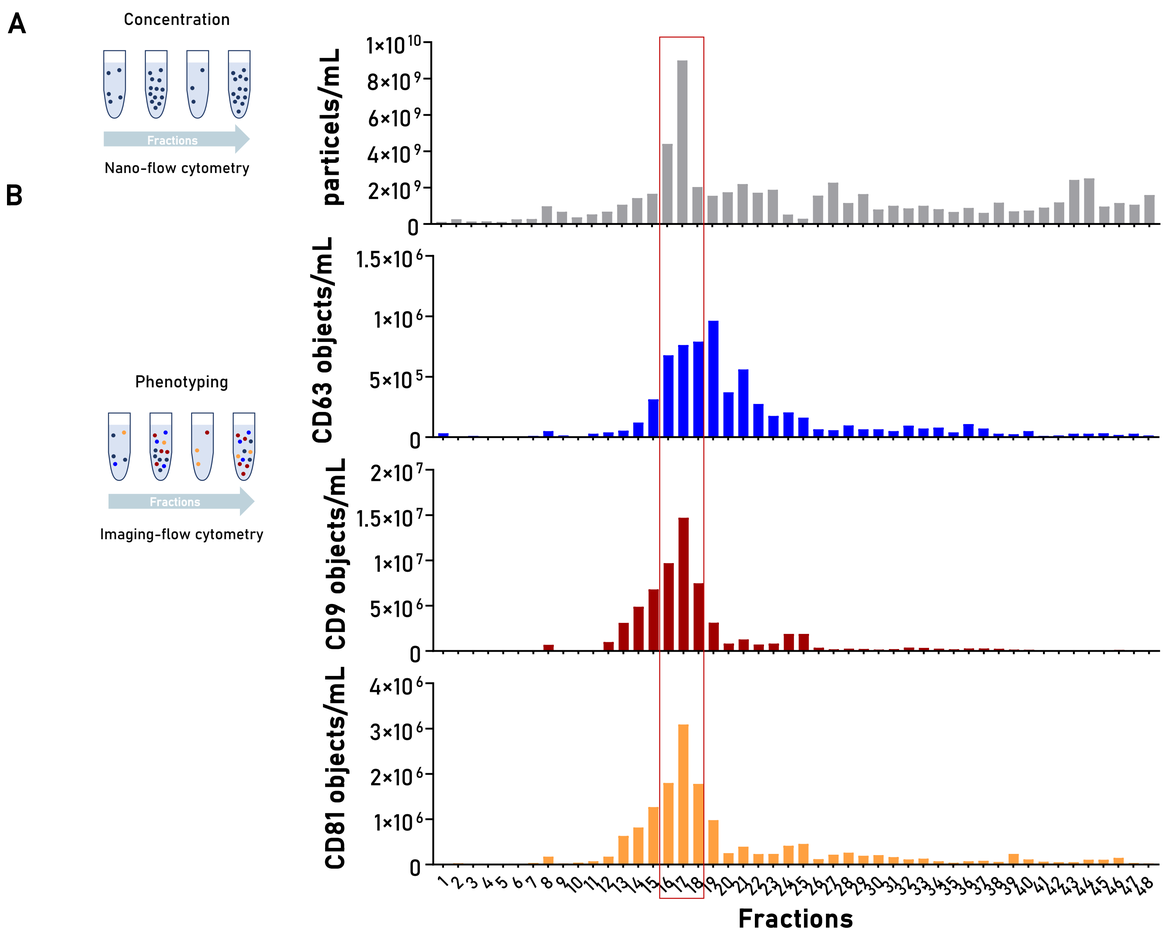


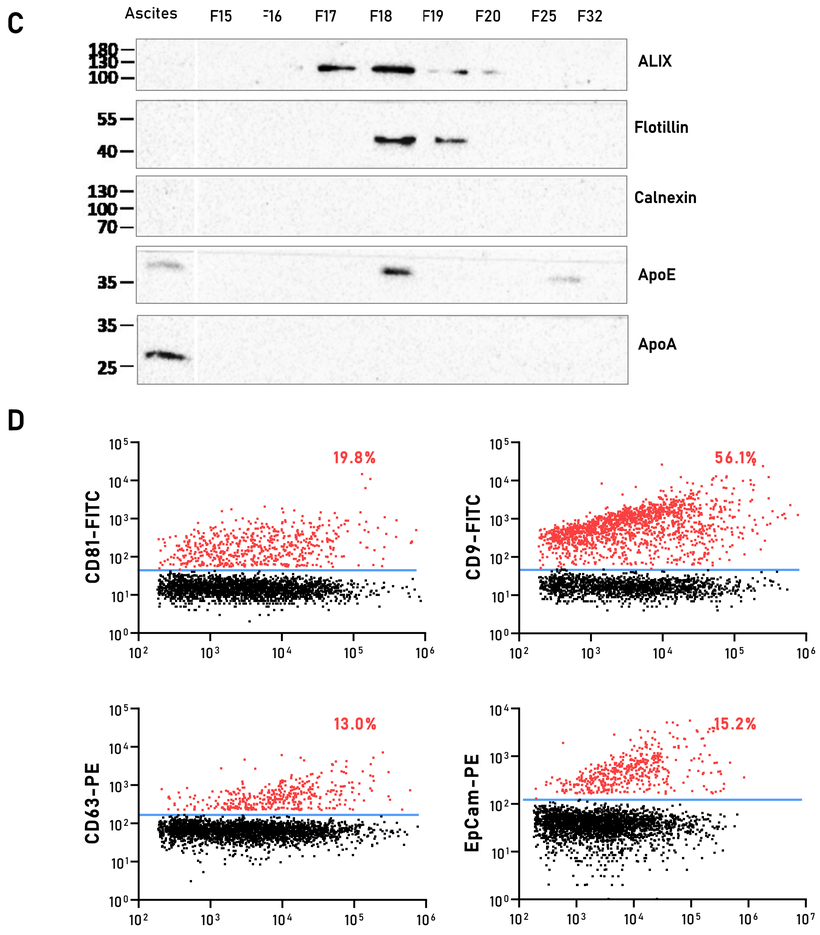
FFE efficiently separates the ascites secretome

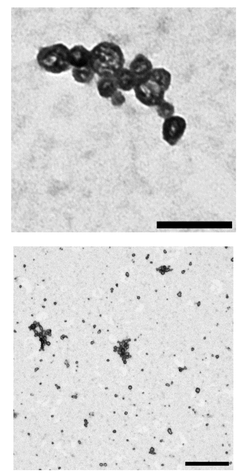
A) A stable pH gradient is achieved after flushing the FFE system with the corresponding buffers.

B) Pherogramms of applied and FFE-processed ascites samples from three different patients.

FFE facilitates the enrichment of *bona fide* EVs from human ascites



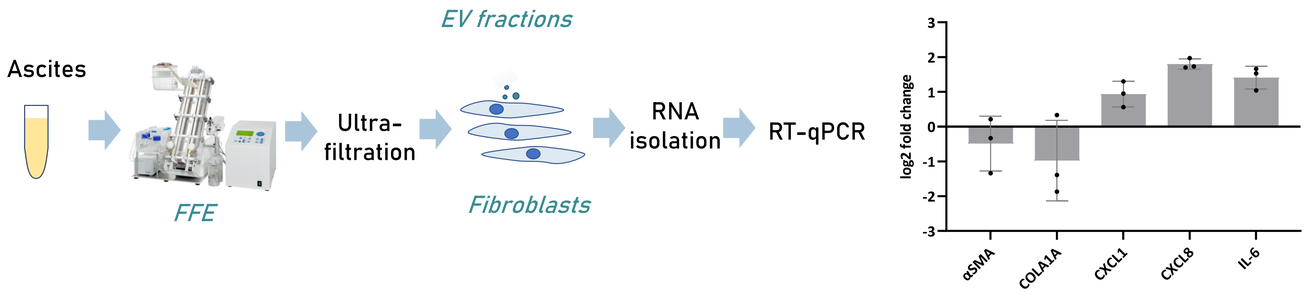




Single-vesicle phenotyping FFE-EV preparations

A) Particle concentration of the first 48 fractions was determined by nano-flow cytometry (nFC). Concentrations show a relatively high particle concentration in fractions 16-18. **B)** FFE fractions were analyzed by imaging-flow cytometry (IFCM) concerning their phenotypic properties of CD63, CD9, and CD81. **C)** Western blot analyses for EV markers and putative contaminants. **D)** Single-vesicle phenotyping using nano-flow cytometry using antibodies directed against the three tetraspanins CD9, CD63, and CD81, respectively, and in addition against EpCAM. **E)** EVs were further validated using electron microscopy.

FFE-EV preparations modulate fibroblast inflammatory markers



Fibroblasts were incubated with ascites-derived EVs and relative expression levels from indicated genes were determined by RT-qPCR comparing mock-treated (PBS) and EV-treated cells.

Conclusions

- "Novel" method for purification of extracelluar vesicles from biofluids
  - *ascites, plasma, serum, urine, duodenal fluid, …*
- Full secretome analysis
- Highly reproducible
- Protocols can be individually designed
- Mid- and high- throughput of samples (up to 240 samples per 12h running time)
- Can be used as a semi-analytical tool (charge-dependent isolation)
- Purification of distinct subpopulations

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Acknowledgements

Conflict of interest: Gerhard Weber CEO and founder of FFE Service GmbH, the manufacturer of the FFE instrument.