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On-Chip Depletion-Zone Isotachophoresis of Exosomes: A Solution to Overcome the Purity Limitations of Current Techniques

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Summary

This paper introduces a novel microfluidics-based technology capable of implementing on-chip isotachophoresis using one single electrolyte for the concentration and separation of essential sources of biomarkers, such as exosomes. Exosomes are extracellular vesicles produced by every cell that transport nucleic acids, proteins, lipids, and metabolites. They play a crucial role in cell-cell communication in a disease condition and normal metabolic processes in healthy individuals [1]. By leveraging the depletion-zone isotachophoresis (dzITP) principle [2], we have employed a method that replaces the trailing electrolyte with an ion-depleted zone, creating a barrier that anions cannot cross. Negatively charged analytes trapped between the ion-depleted zone and the leading electrolyte are, therefore, concentrated, depending on their electrophoretic mobility, in bands that occupy different positions in the microfluidic channel, thus mirroring the capabilities of classical isotachophoresis. The practical application of this technology is demonstrated through the concentration and separation of exosomes derived from cell cultures and blood plasma, implemented with silicon/glass-based microchips. Interestingly, although the exosome samples used in this study are pre-purified by density- and size-based techniques (e.g., tangential flow filtration and ultracentrifugation), the dzITP-based chip is capable of further separating extracellular vesicles from contaminants such as proteins or residues of cleaved extracellular vesicles. Thus, we demonstrate that dzITP can potentially obtain purer samples than currently routinely used techniques. The method described allows for on-chip isotachophoresis by simply preparing the solution of exosomes in a buffer that already contains the leading electrolyte. No other solution is required to operate the microchip. In this study, the buffer conditions are fine-tuned (e.g., ionic strength) to achieve the highest concentration of exosomes in the isolated band of interest. Following the assessment of the bandwidth of the exosomes, around a million-fold concentration is achieved in the device, in line with comparable devices described in the literature [3].

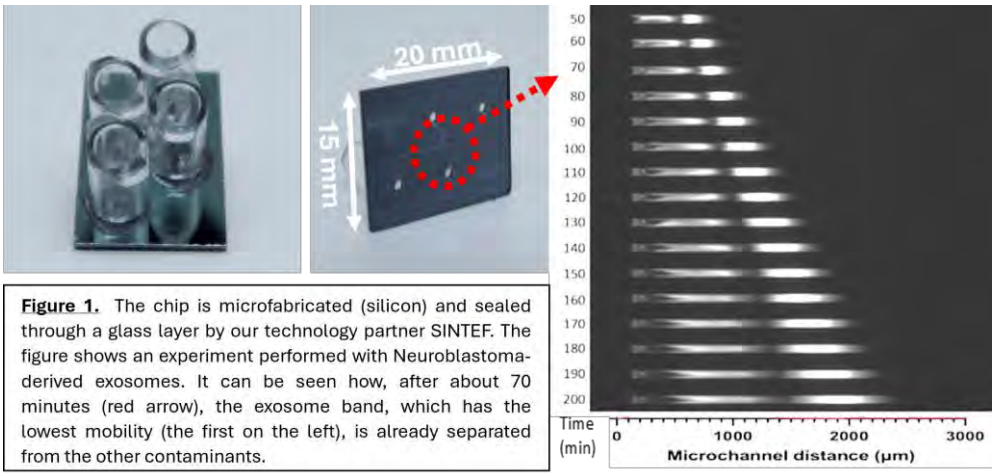

Figure 1. The chip is microfabricated (silicon) and sealed through a glass layer by our technology partner SINTEF. The figure shows an experiment performed with Neuroblastoma-derived exosomes. It can be seen how, after about 70 minutes (red arrow), the exosome band, which has the lowest mobility (the first on the left), is already separated from the other contaminants.

References

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[3] Y.-C. Wang, A.L. Stevens, J. Han, Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter, Anal. Chem. 77 (2005) 4293–4299.