Working with Primary Cancers: TFF+SEC for High-Efficiency EV Isolation

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Introduction

Extracellular vesicles (EVs) are investigated thorougly for next-generation cancer theranostics. In this scope, researchers dominantly focus on EVs from immortalized cancer cell lines to understand tumor biology and to manipulate it. Yet, reviews emphasize that such cell lines diverge in characteristics from primary cancers, as a combined result of several factors such as prolonged culture adaptation, loss of tumor subpopulations, and lack of three-dimensional architecture or stromal interactions [1]. As this can limit the predictive value of preclinical findings, it is a barrier for translational research. On the contrary to such limitations, primary models, such as tumors directly obtained from patients, can offer several advantages to capture clinically relevant information, especially for precision oncology where patient-specific features are of consideration [2].

In this application note, we evaluate tangential flow filtration (TFF) in combination with size exclusion chromatography (SEC) for processing primary cancer cell lines. By following the MISEV guidelines for the characterization of primary cancer cell-derived EVs, we demonstrate the feasibility of downstream analyses, which would enable access to precision cancer diagnostics and therapeutics studies.

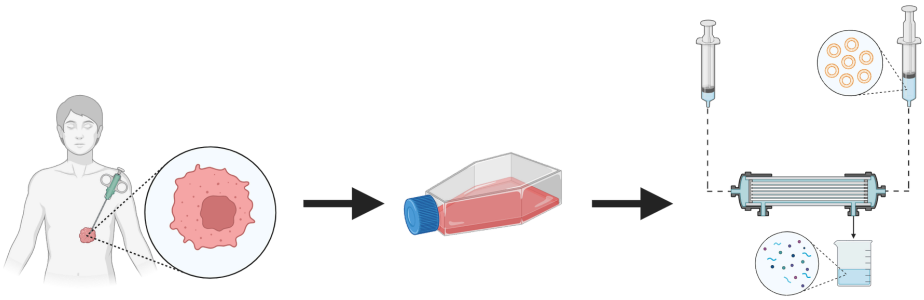

Figure 1: Schematic description of the study workflow.

Materials and Methods

The study was designed using 30 ml of supernatant primary pancreas cells supplied by CTIBIOTECH (https://www.ctibiotech.com/). Supernatant was sequentially processed according to HansaBioMed SOPs. Accordingly, sequential isolation was performed using TFF cartridges with 150-200nm and 50nm pore sizes (Product codes: HBM-TFF-MV and HBM-TFF-EVs-S) to enrich and concentrate small EVs. Followingly, this subpopulation was purified with SEC columns (Product code: HBM-PEV-5). As part of the characterization protocol, total particle number and size distribution were measured by NTA. Total protein amount was quantified by BCA analysis. CD9, CD63, and CD81 tetraspanin expression levels were detected with ELISA CD63 coated plates through a double-sandwich assay.

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Results

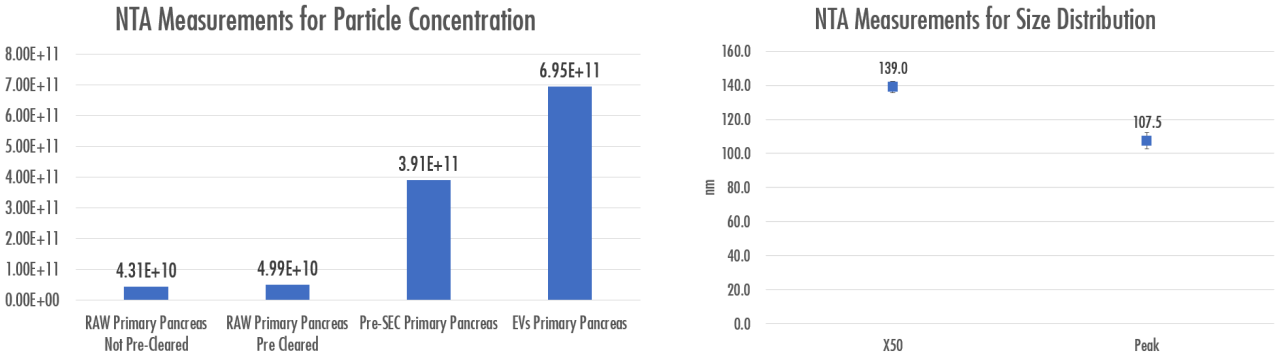

Figure 1: NTA measurements performed with Zetaview Evolution by Particle Metrix to evaluate concentration and size distribution of the raw material and the isolate

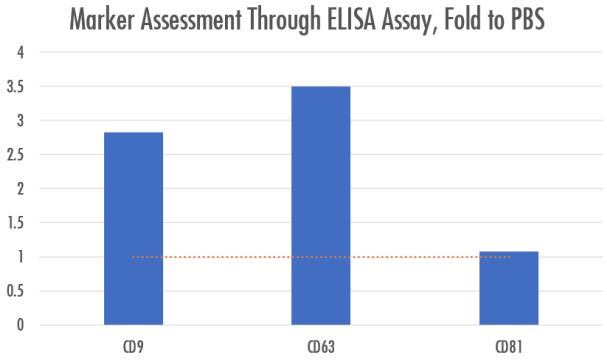

Figure 2: Double sandwich ELISA-based assessment of canonical EV markers

Conclusion

TFF is a versatile method that can work with any source of EVs. In this study, the results demonstrate that HansaBioMed's optimized protocol for EV isolation thorugh TFF and SEC can be utilized for purifying EVs from primary cancer cells with high yield. For this purpose, the primary cancer cell line provided by CTIBIOTECH (https://www.ctibiotech.com/) forms a suitable starting material with xeno-free culturing, minimizing external affects to downstream analysis. Taken together, TFF-based EV isolation from primary cancer cell lines enables the researchers to perform cancer studies with higher biological relevance.

References

[1] Mun, S., Lee, H. J., & Kim, P. (2024). Rebuilding the microenvironment of primary tumors in humans: a focus on stroma. Experimental & Molecular Medicine, 56(3), 527–548. https://doi.org/10.1038/s12276-024-01191-5

[2] Mann, B., Artz, N., Darawsheh, R., Kram, D. E., Hingtgen, S., & Satterlee, A. B. (2025). Opportunities and challenges for patient-derived models of brain tumors in functional precision medicine. Npj Precision Oncology, 9(1), 47. https://doi.org/10.1038/s41698-025-00832-w

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