Exosome purification with CIMmultus™ EV-1 Advanced Composite Column

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INTRODUCTION

Exosomes fulfill a critical role as communicators among cells, with targeting and message content depending on their surface receptors and payload. This makes them obvious candidates for an extensive range of diagnostic and therapeutic applications. This creates a need for a fast, robust and scalable purification procedure.

Their lipid envelopes make them vulnerable to multiple sources of stress. This restricts the range of operating conditions and affects the choice of processing media. Most porous-particle based chromatography media are optimized for proteins that are 5–50 times smaller than exosomes. Exosomes are too large to enter the pores which restricts the binding to the outer surface of the media. Beside capacity limitation, exosomes are easily damaged by shear stress. Flow inside the packed bed chromatography media creates eddies which can disrupt their lipid envelopes.

CIMmultus™ monolithic columns are designed to meet the special fractionation needs of very large biologics like exosomes. Monoliths support 10–100 times higher binding capacity than porous particle chromatography media and 2–3 times higher product recovery for large biologics. Flow rates are 10–50 times higher compared to traditional packed bed columns, with no compromise to capacity or separation performance. The internal flow through monoliths is laminar which eliminates the turbulent shear stress.

We show examples of exosome purification from cell culture with CORNERSTONE Exosome Process Development Pack and analysis of exosomal vesicle populations by Image stream flow cytometry.

MATERIALS AND METHODS

- Experimental work was performed with a HEK293T cell culture harvested in serum free media, obtained from FiberCell, Frederick, MD
- Purified exosome fractions were prepared with CORNERSTONE Exosome Process Development Pack incl. Kryptonase™ and CIMmultus™ EV Advanced Composite Column, obtained from BIA Separations
- Samples were analyzed with Image stream flow cytometry on ImageStreamX MkII instrument (ISX; Amnis/Millipore Sigma). Fluorescence-conjugated antibodies were used to label exosomal surface markers. Method was optimised for the detection of small EVs.

Figure 1. Flow distribution in traditional chromatography media (left) compared to monoliths (right). Shear in chromatography is created by eddies (vortices) that form in the spaces between the particles of packed bed columns. Lipid-enveloped biologics are prone to damage by shear forces. Monolithic chromatography media do not produce turbulent shear stress because flow through the channels is exclusively laminar.

PURIFICATION PROCESS

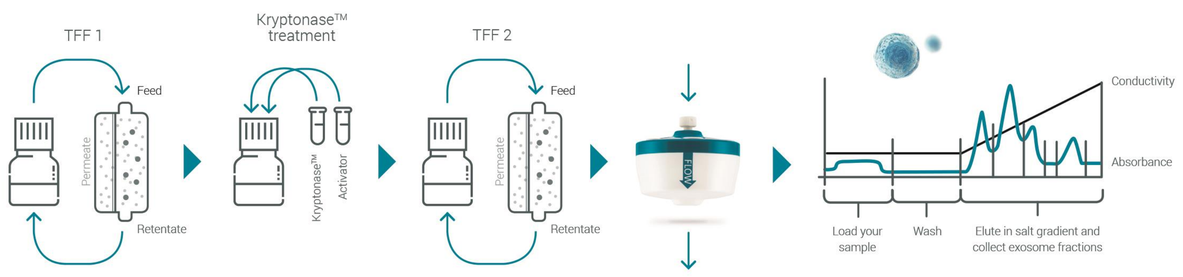

Figure 2. Schematic overview of the purification process. Clarified cell culture harvest was pretreated by TFF with integrated Kryptonase™ (Representative result of this step is shown in Fig. 4). Partially purified sample was loaded to CIMmultus™ EV column. Exosomes were eluted with salt gradient. Example of a purified fraction is shown in Fig. 5.

RESULTS

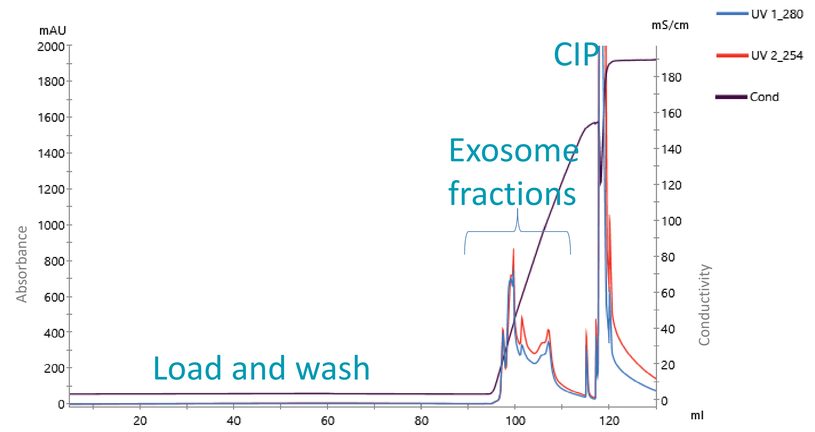

Figure 3. Concentrating and polishing exosomes with CIMmultus™ EV further reduces contamination by host cell proteins and DNA. 55 mL of partially purified cell culture from HEK293 cells was loaded to CIMmultus™ EV- 1mL column and eluted in desired volume.

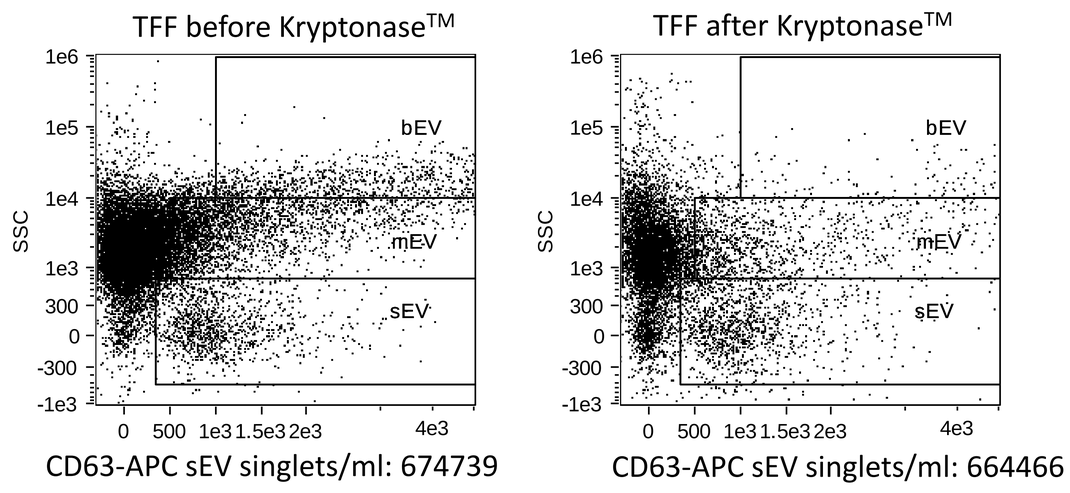

Figure 4. Image stream flow cytometry (ISX) of extracellular vesicles (EVs), before and after Kryptonase™ treatment. Visualization of single vesicles, stained by antigen coupled flourochromes bound to exosomal surface marker proteins. ISX was used for quantification by counting the marker positive EVs. According to their size, sEVs corresponding to exosomes and smaller microvesicles, were reflected in the sEV plot area that correlates with a size up to 200 nm. The plots illustrate the removal of chromatin aggregates by comparison of TFF-treated HEK293 cell culture before and after the treatment with Kryptonase™. The Aggregates in the size of bEV and mEV are markedly reduced after Kryptonase™ treatment, while the exosome population remains stable.

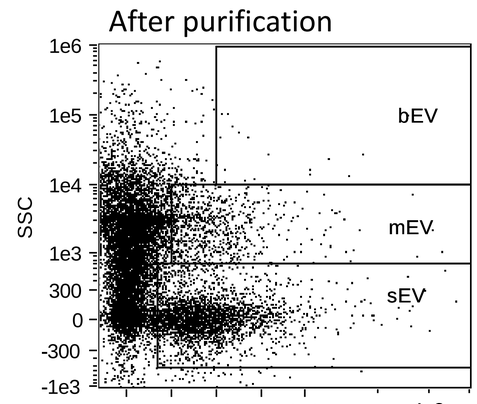

Figure 5. Image stream flow cytometry (ISX) of extracellular vesicles (EVs) after purification. Clarified HEK293 cell culture supernatant was processed by the CORNERSTONE Exosome Process Development Pack leading to concentrated high purity extracellular vesicles accumulated in few fractions. Note the strong concentration in the sEV area and the reduction of non-exosomal content regarding the remaining areas (bEV, mEV).

CONCLUSIONS

- Poster presents new approach to supporting exosome purification process of quality research-grade exosomes from cell cultures;
- Monoliths are ideally suited for processing large unstable products like exosomes: laminar flow creates low shear environment and large channels support high binding capacity;
- Image stream flow cytometry illustrates successful isolation of exosome population and reduction of aggregates and non-exosomal vesicles.

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