Removal of host cell DNA and other contaminants from exosome preparations

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INTRODUCTION

Removal of host cell DNA is essential for all human-injectable biologics. This poster shows a method for achieving low host cell levels in preparations of exosomes. Purified exosome samples were prepared with anion exchange chromatography (AEC) and pre-treated with tangetial flow filtration (TFF) and nuclease treatment. Results are compared with an experimental control using TFF and size exclusion chromatohraphy (SEC).

The steps in purification process are illustrated by analytical size exclusion chromatography (SEC) on PATfix HPLC system with in-line UV, MALS and fluorescence detectors and by staining with Picogreen reagent. This technique visualizes sample composition by size, UV, light scattering and fluorescent properties.

MATERIALS AND METHODS

Preparative work:

- Experimental work was performed with HEK293T culture media by FiberCell Inc., Frederick MD and BM MSC media by Rooster Bio Inc., Frederick, MD.
- Diafiltration of culture media was performed on KrosFlo® KR2i TFF system (Repligen).
- Kryptonase™ treatment was done according to the Cornerstone Exosome Process Development Pack protocol (BIA Separations).
- Anion exchange chromatography was performed using CIMmultus™ EV (BIA Separations) on Akta Pure preparative chromatgraphy system (GE Healthcare).

Analytics:

- Analytical chromatography methods were performed on a PATfix™ HPLC, model LPG, with 50 mm path-length UV cell (BIA Separations, Slovenia); equipped with in-line UV, fluorescence and MALS detector: Dawn Heleos 365-H multi angle light scattering detector (Wyatt Technology, USA).
- Analytical size exclusion chromatography (SEC) was performed on a TSKgel™ G4000SWxl column (Tosoh Biosciences, USA).
- The PicoGreen® dsDNA Quantitation Reagent (Invitrogen) was used for detection of dsDNA by fluorescence detector (in-line) and for dsDNA quantification (off-line).

RESULTS

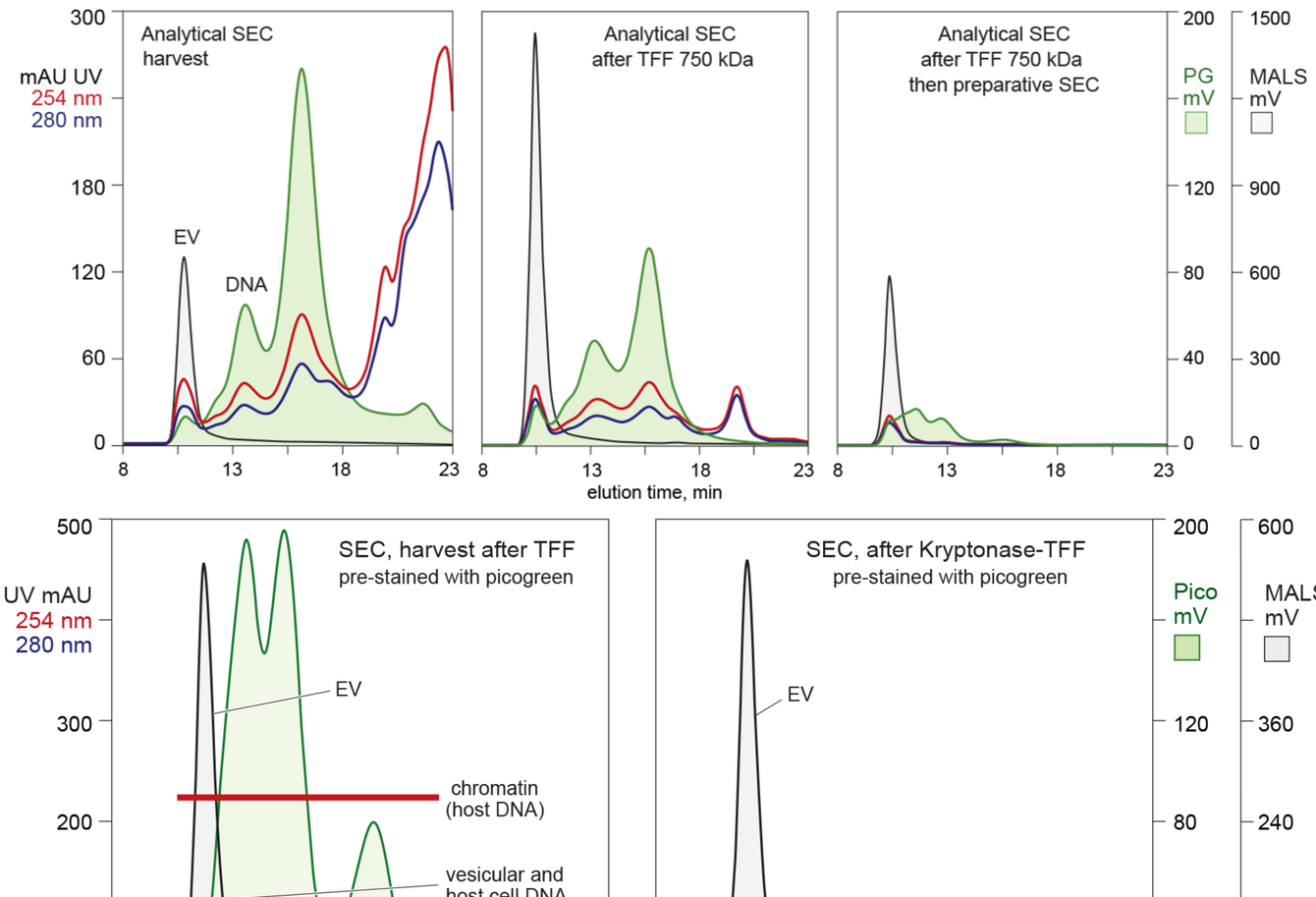

Figure 1. Distribution of dsDNA (green) during fractionation by size exclusion chromatography (SEC). Samples were stained with fluorescent reagent Picogreen and monitored with fluorescence detector. MALS was used to monitor EVs and UV detector was used to monitor UV at 254 and 280 nm.

TFF removes the low-MW DNA fragments, while retaining high-MW DNA and protein aggregates (chromatin). Observing by UV profile, contaminating DNA seems to be removed by preparative SEC, but staining with Picogreen reveals a DNA population persists in the final product.

Figure 2: Removal of chromatin contaminants by Kryptonase approach. Analytical SEC-Picogreen illustrates sample pre-treated by TFF, before and after Kryptonase treatment. Low-MW host cell proteins and DNA are removed by TFF through 750 kDa filter. Staining with Picogreen reveals large-MW dsDNA-containing contaminants persist in the TFF retentate (left). Kryptonase is added *in-situ*, it digests nucleic acids and releases the aggregates formed with histones and other proteins. The by-products are then removed in the second TFF step.

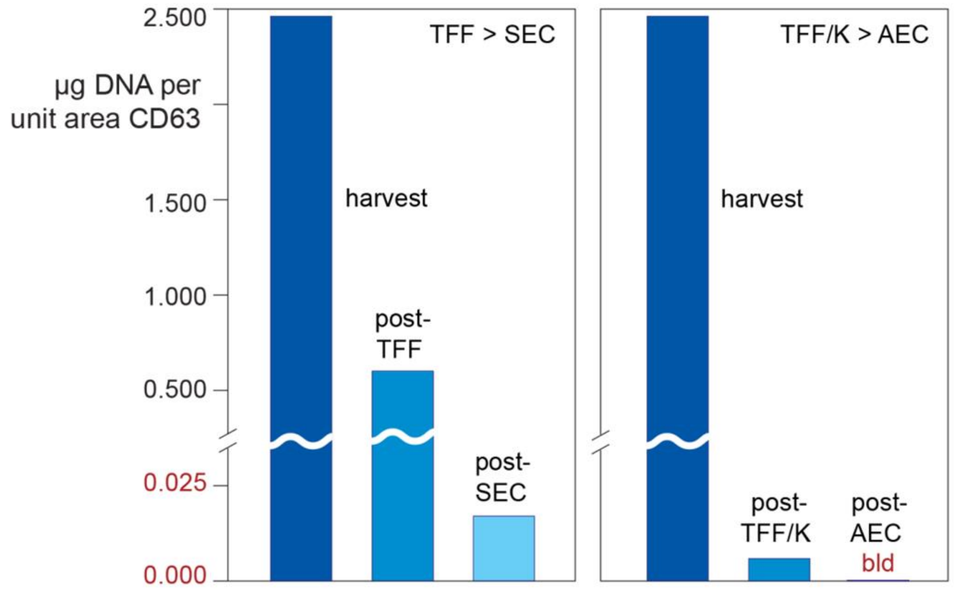

Figure 3: Relative concentration of dsDNA during purification process. DNA concentration was measured with Picogreen. Relative exosome concentration was measured using immunological chromatographic approach using CD63 exosome marker (SEC-IF). DNA concentration was expressed per unit area of CD63.
TFF>SEC (left): both TFF and SEC retain some dsDNA contamination, DNA persists to the final sample (post-SEC).
TFF/K>SEC (right): The majority of dsDNA is removed by TFF/Kryptonase step, the remaining population of DNA is finally removed by AEC.

CONCLUSIONS

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