Exosomes: Scalable Production, Purification, and Characterization Methods using 293F Cells

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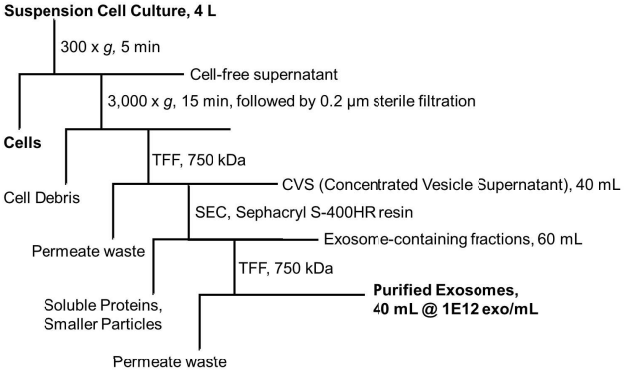
Abstract

Capricor is developing the StealthX™ platform, an engineered exosome delivery system, for vaccinology and therapeutics. To support the StealthX™ program, we have developed a scalable exosome isolation method, using a combination of tangential flow filtration and size exclusion chromatography, which is capable of processing liters of cell culture supernatant. The isolated exosomes are highly concentrated at relevant concentrations (trillions of exosomes per mL) to support R&D activities including, but not limited to, in vitro assays and preclinical studies. This method has been used to isolate exosomes from multiple cell lines, and the isolated exosomes have been characterized by a variety of assays developed to analyze exosome size, concentration, surface marker expression, protein content, lipid content, RNA content, DNA content, and expression of engineered content. With the success of this method, Capricor is scaling up the process further to a GMP scale exosome isolation method capable of supporting future clinical trials and the potential commercialization of StealthX™ exosomes while maintaining the critical characteristics of exosomes.

Methods



Exosome Characterization Methods:

- Size Distribution, Concentration by Particle Metrix ZetaView NTA
- Structural characterization by TEM
- Total protein content by BCA
- Exosome Identity by CD81 capture bead flow cytometry assay
- Total lipid content by fluorescence-based assay
- Absence of cellular DNA/RNA by Agilent Bioanalyzer
- Absence of cellular/vesicle contamination markers by JESS Western Blot
- Confirmation of presence of surface engineered protein by flow cytometry
- Quantitation of engineered exosome protein content by ELISA

Conclusions

- Scalable exosome isolation process produces exosomes at yields suitable for supporting R&D activities, proof-of-concept work, and IND-enabling studies. A GMP-scale process is currently in progress for use in clinical trials.
- Purified exosomes demonstrate a size (100-140 nm), uniformity (polydispersity index of < 0.2), and surface marker expression (CD81 and CD9) of typical exosomes.
- Purified exosomes do not contain contaminating cellular or vesicular markers (GM130, Calnexin, HSP60, CytC). No or low DNA/RNA was detected by bioanalyzer.
- The protein content of engineered exosomes is detectable by flow cytometry bead-based assays, JESS Western Blot, TEM, and ELISA.

Disclosure

StealthX™ is proprietary of Capricor Therapeutics, Inc (NASDAQ: CAPR)

Results

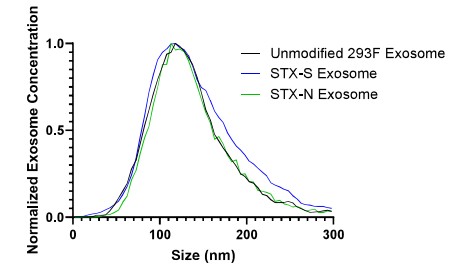


Figure 1. Size distribution by ZetaView NTA. Exosomes from unmodified 293F exosomes show similar size distribution to StealthX™ exosomes engineered to express Spike or Nucleocapsid protein.

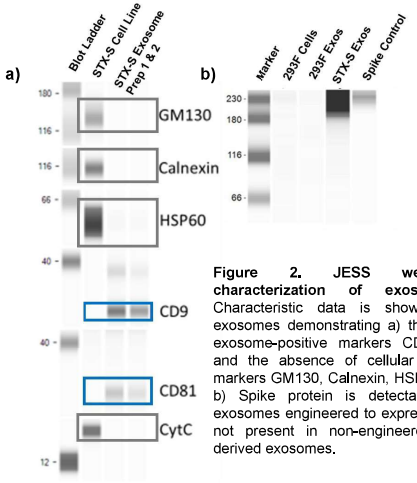


Figure 2. JESS western blot characterization of exosome preps. Characteristic data is shown for STX-S exosomes demonstrating a) the presence of exosome-positive markers CD81 and CD9 and the absence of cellular contamination markers GM130, Calnexin, HSP60, and CytC. b) Spike protein is detectable in STX-S exosomes engineered to express Spike but is not present in non-engineered 293F cell-derived exosomes.

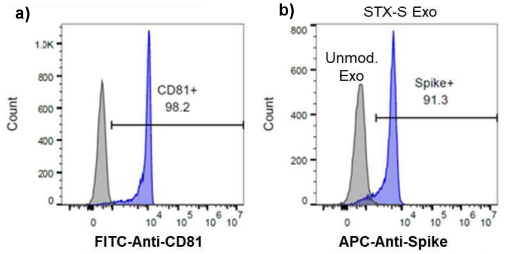


Figure 3. Flow cytometry bead-based assay showing a) CD81 was detected on STX-S exosomes engineered to express Spike (blue) compared with no signal from isotype control antibody (grey). and b) Spike was detected on STX-S exosomes engineered to express Spike (blue) but not on 293F parental exosomes (grey).

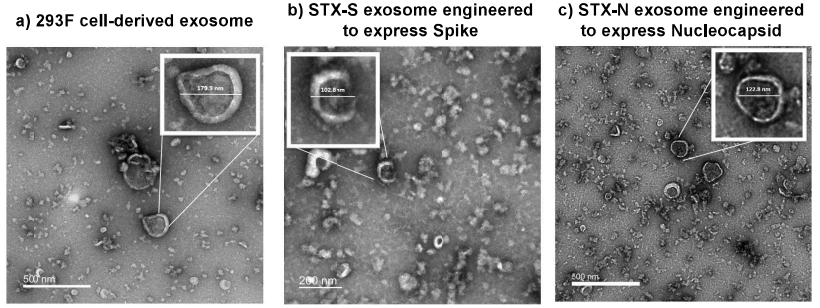


Figure 4. TEM characterization of exosomes isolated using Capricor's isolation process. Isolated exosomes demonstrate the characteristic lipid bilayer. A representative exosome is shown for a) 293F cell-derived exosome (179.9 nm), b) STX-S exosomes engineered to express Spike (102.8 nm), and c) STX-N exosomes engineered to express Nucleocapsid (122.8 nm)

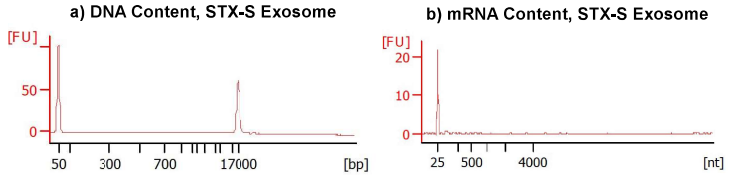


Figure 5. Agilent Bioanalyzer Electropherograms demonstrating that no a) cellular DNA or b) cellular mRNA are detected in exosome preparations. Peaks present are from internal control markers.

Table 1. Summary of biophysical and biochemical characterization of exosome preps.

Biochemical and Biophysical Characterization	Unmodified 293F Exosomes	STX-S Exosomes engineered to express Spike protein	STX-N Exosomes engineered to express Nucleocapsid protein
Exosome Yield per mL starting material	2.92E9 ± 1.23E9	4.07E9 ± 2.79E9	4.18E9 ± 3.34E9
Average Diameter	142 nm	126 nm	141 nm
Polydispersity Index	0.169	0.128	0.147
Protein Concentration (normalized to 1E12 exosomes)	0.30 ± 0.04 mg/mL	0.42 ± 0.09 mg/mL	0.43 ± 0.08 mg/mL
Lipid Concentration (normalized to 1E12 exosomes)	0.74 ± 0.02 mg/mL	1.28 ± 0.22 mg/mL	1.09 ± 0.05 mg/mL
Exosome Identity by CD81	POSITIVE	POSITIVE	POSITIVE
Presence of cellular DNA	NEGATIVE	NEGATIVE	NEGATIVE
Presence of cellular RNA	NEGATIVE	NEGATIVE	NEGATIVE
Concentration of engineered protein (normalized to 1E12 exosomes)	n/a	253.77 ± 140.80 ng/mL	40.59 ± 15.48 ng/mL