Scalable Production of Modified HEK293T Extracellular Vesicles Using Adherent Packed Bed Bioreactor Cell Culture System

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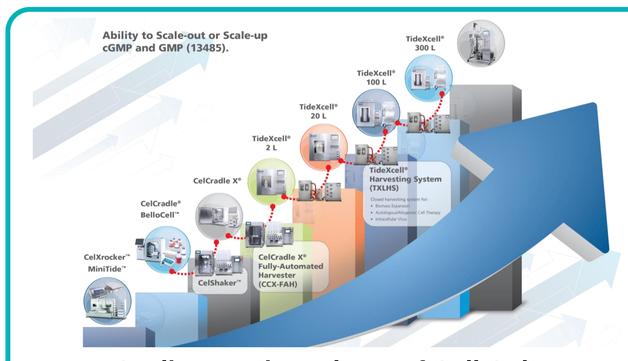
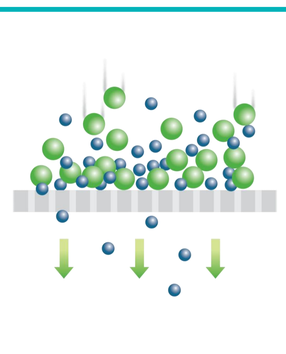
Summary

Our study highlights the efficacy of our proprietary adherent packed bed bioreactor for a scalable production of extracellular vesicles (EVs). Leveraging on the innovative 'Tide Motion' principle, which alternately exposes the cells to air and nutrients to maintain the cell culture system. In a commercial endeavor, we engineered HEK293T cells to express a specific protein on the surface. The cells were seeded in our smallest bioreactor, the CelXrocker®, as a proof-of-concept evaluation. We successfully demonstrated the feasibility of cultivating the cells in our bioreactor and the CelXrocker® may minimize the cost and time for early-stage process development. Subsequently, the cell culture were scale up in the CelCradle® and to support the high-density cell growth, a perfusion system was attached to the bioreactor (CelCradle X®). Our data shows that the bioreactor can support the high-density cell growth of the modified HEK293T cells, and this significantly increased the EVs production yield. A correlation between the cell culture parameters and the quality of EVs can be established. We carried out downstream processing to purify the EVs and analytical characterizations were carried out to confirm the identity of the EVs.

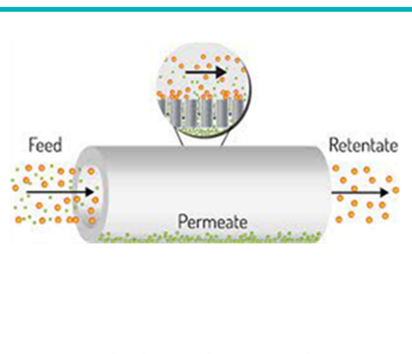
General Process Development Steps



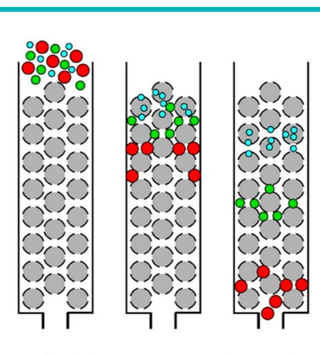
Scaling Up the Volume of Cell Culture Collection of Conditioned Medium



Clarification of Conditioned Medium



Isolation of EVs using Tangential Flow Filtration



Polishing Step using Size Exclusion Chromatography

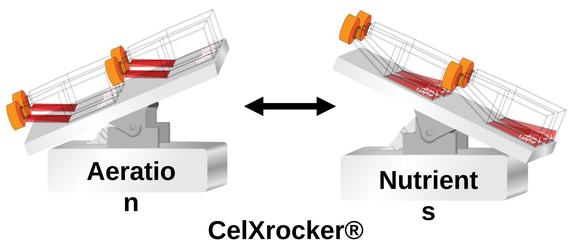
Further Steps:
e.g., Formulation with API
Fill and finish

Upstream Process Development

The Heart of the Bioreactor

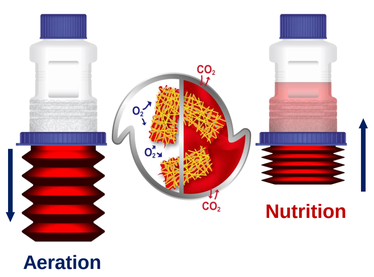
BioNOC II® Macrocarriers

Small Scale Feasibility Study using CelXrocker®

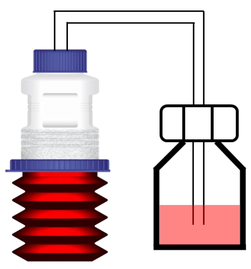


CelXrocker®

Scale up of EV Production with CelCradle®



CelCradle®



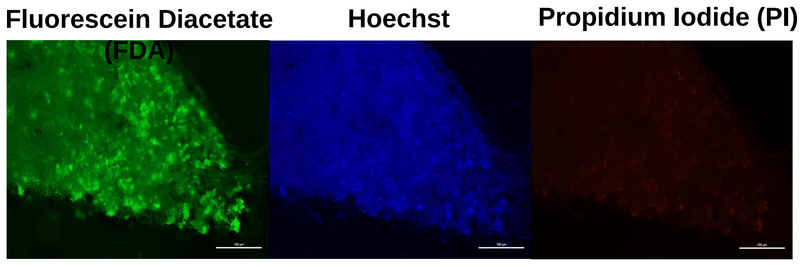
CelCradle X®

- **Consist of 100 mL packed bed volume of BioNOC II®**
- **Volume of cell culture medium : 500 mL**
- **Volume of cell culture medium in perfusion system: 700 mL**

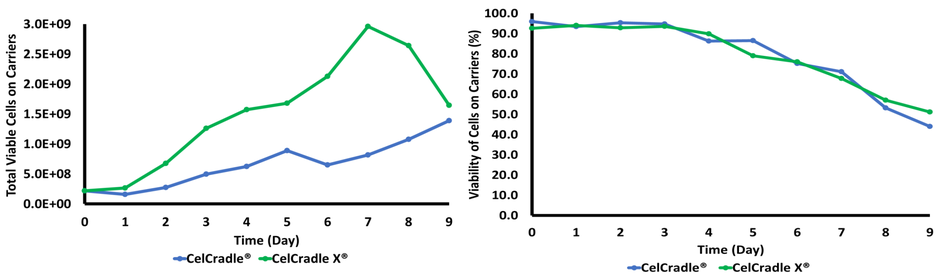
Optimization of Cell Seeding Density

Seeding Density (cells/carrier)	1 x 10^5	2 x 10^5	3 x 10^5	6 x 10^5
Number of carriers	50			
Total number of cells seeded	0.5 x 10^7	1 x 10^7	1.5 x 10^7	3 x 10^7
Volume of medium (mL)	30			
Attachment efficiency (%)	99.2	97.3	87.6	83.1
Actual number of cells on carrier	0.49 x 10^7	0.97 x 10^7	1.30 x 10^7	2.40 x 10^7

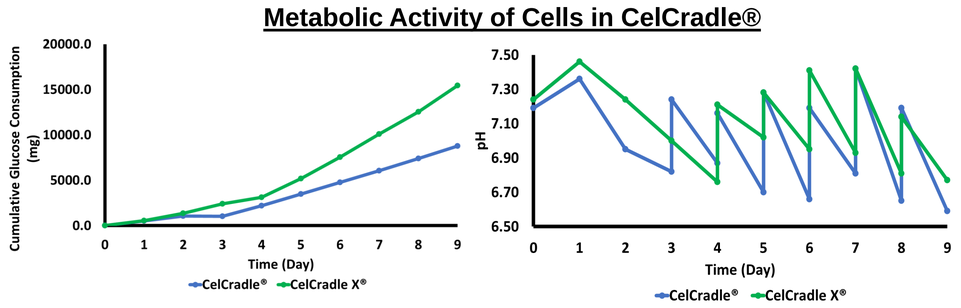
Fluorescent Imaging of BioNOC II®



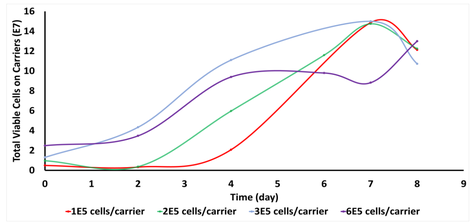
Cell Growth and Viability in CelCradle®

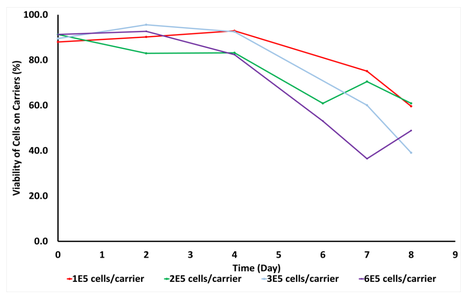


Metabolic Activity of Cells in CelCradle®

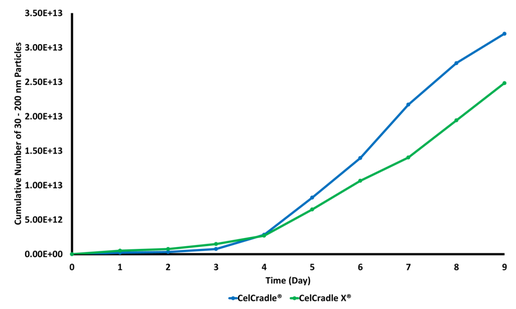


Cell Growth and Viability in CelXrocker®

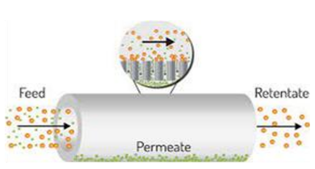




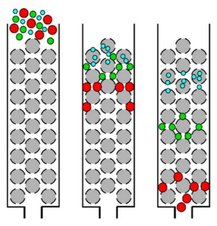
Yield of EV Particles in Cell Culture Medium



Downstream Process Development and Analytical Characterization

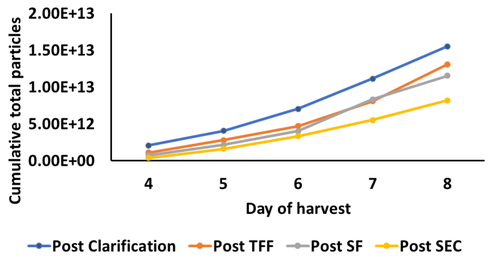


Tangential Flow Filtration (TFF)

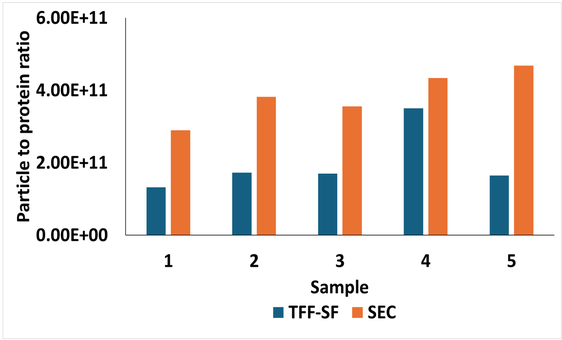


Polishing Step using Size Exclusion Chromatography

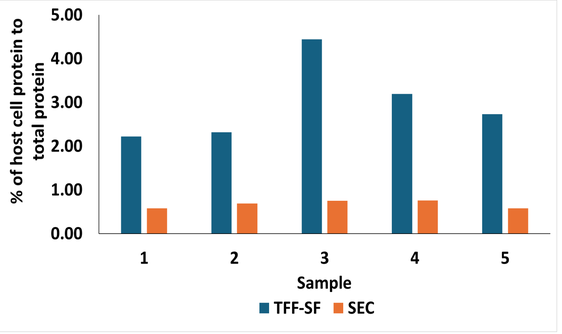
Yield Recovery of EVs



Particle-to-Protein Ratio After SEC

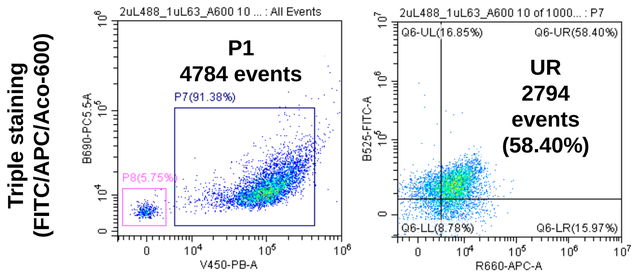


Percentage of Host Cell Protein After SEC



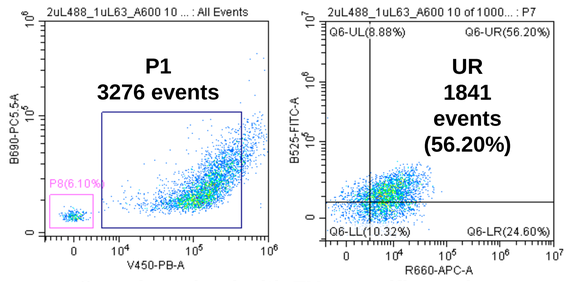
Characterization of EVs using Flow Cytometry

Batch 1



- ***Fluorescent staining of lipid bilayer on EVs (Acoerela Aco600)***

Batch 2



- ***Sample stained with EVs specific markers***

ABOUT ESCO ASTER: CDMO organization focuses on cGMP compliant end to end manufacturing process. We focus on offering vaccine-, bio-, cell- and gene-therapy development manufacturing services using primarily its proprietary Adherent Tide Motion Platform supplemented by single-use suspension, downstream bioprocessing, and custom bioengineering equipment for client specific therapeutics

References:
- ***Shekari et al., J of ExtracellularBio.2023;2:e115***

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