Advanced technology platform for stem cell-derived exosomes manufacturing process

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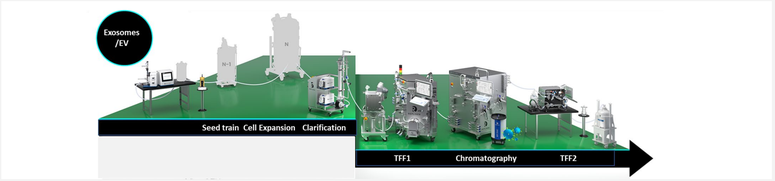
Introduction

There has been a growing demand in Extracellular Vesicle (EV) supply in recent years due to their emerging role as intercellular messengers and their therapeutic potential as targeted and natural drug delivery vehicles with high specificity and efficiency. The number of clinical trials investigating MSC-EVs as therapeutic and skincare agents has been increasing greatly over the years. The complexity and fragility of the EV products, scalability, yield, and purity of production processes are challenges to meeting demand. In this study, we used two scalable platforms to overcome those challenges.

Collaboration Objective

To deliver solutions for manufacturing of EVs using scalable and low shear technologies that enable cost-effective commercialization of these advanced therapies.

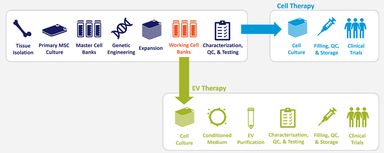
Advanced Evs Manufacturing Workflow



EV Industrial Platform

RoosterBio Upstream Platform

RoosterBio is an industry-leader in manufacturing high-quality hMSCs along with paired bioprocess medium formulations for cell growth and EV production

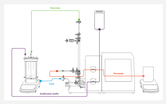


Repligen Downstream Platform

KrosFlo® TFDF® System **KrosFlo® KR2i TFF System**

Operated in clarification mode

Enabling highly efficient and scalable, harvest clarification and concentration steps



- Large pore size easily transmits large particles such as EVs
- Enclosed, single-use solution
- Scalable from 1 – 2000L
- Eliminates need for centrifugation or depth filtration
- Fast set-up
- High filtration capacity
- High flux rates (>650LMH)
- Customizable flow path complete with sensors, tubing, and connectors.
- Automated process control logic

- Compatible with hollow fiber filters and flat sheet cassettes
- Fully integrated functionality, easy to use
- Log data and control tangential flow filtration operations with KF Comm software
- Interfaces with auxiliary scales and pumps for automated process control

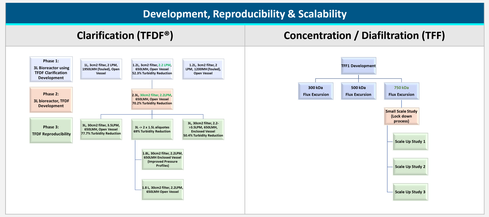
Material and Method

MSC-EVs were generated in a microcarrier-based bioreactor at 2L scale using RoosterBio xeno-free hMSCs (RoosterVial™ hMSC) coupled with RoosterBio MSC expansion media (RoosterNourish™-XF), and RoosterBio EV collection media (RoosterCollect™). Clarification was developed using tangential flow depth filtration (TFDF®) system, followed by concentration and formulation buffer exchange using a KrosFlo® KR2i tangential flow filtration (TFF) system.

For EV harvest clarification 3 cm^2 TFDF® filter with a pore size of 2-5 µm were used for development scale and 30 cm2 TFDF® filter surface area were used for 3 L.

Hollow fiber filters with MWCO of 300, 500 and 750 kDa were used in TFF development scale with 115 cm^2 surface area and HF surface area of 790 cm^2 for processing larger scale.

Study Design Workflow



EV Analytics: Critical Quality Attributes for EVs were evaluated through staining with lipid bound membrane dye, expression of EV specific tetraspanin markers (CD81, CD63 and CD9), miRNA content, DNA content and in vitro potency assay (wound assay).

Results and Discussion

TFDF®: Harvest and clarification step

Turbidity Reduction using TFDF

Run#	Phase	NTU Crude	NTU Post-TFDF
1	1b	20.3	9.8
2	1a	23.5	12.9
3	1c	27.8	11.6
4	2	19.8	5.9
5	3a	24.2	5.4
6	3b	24.2	7.5
7	3c	13.5	6.695

Key process parameters for EV clarification step, during the development phase, were identified using a 3 cm^2 TFDF® system with a pore size of 2-5 µm and a recirculation rate of 2.0-2.2 LPM resulted in a high permeate flux of 650 LMH. Identified parameters can be scaled to a 2000 L bioreactor using 0.6 m^2 TFDF® surface area with a throughput of 4000 L/m^2 and a step time of less than 2.5 hours.

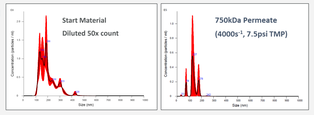
- **EV recovery yield of 86% comparable to the centrifugation control**
- **Short Process Step Time at all scales (<2hr)**
- **Sterile closed single-use solution for cell culture clarification**

TFF: Concentration and diafiltration of clarified harvest

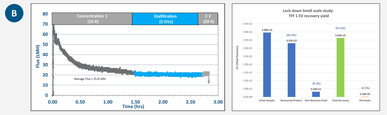
Filter Information and Process Parameters	Small Scale Study (identified process parameters)	Scale Up Experiment 1	Scale Up Experiment 2	Scale Up Experiment 3
Type of Process	UFDF (C/D/C)		UFDF (C/D/C)	
Starting Turbidity		4.8 NTU	4.1 NTU	6.7 NTU
Filter MWCO	750kDa		750kDa	
Filter Chemistry	mPES		mPES	
Filter Area	115cm2 (0.0115m2)		790cm2 (0.079m2)	
Volume Loaded	583mL	3.26L	3.6B L	2.98L
Loading Ratio	50.6 L/m2	41.2 L/m2	46.6 L/m2	37.7 L/m2
Total Particles	3.96E+12	1.79E+13	1.55E+13	2.16E+13
Loading Ratio	1.56E+15/m2	2.27E+14/m2	1.96E+14/m2	2.73E+14/m2
Shear	4000s^{-1}		4000s^{-1}	
TMP control	5psi		5psi	
Processing Steps	C1: 10 X D: 5 DVs C2: 20 X		C1: 10 X D: 5 DVs C2: 20 X	
Process Time	2.8h	2.0h	2.7h	3.2h*

TFF Small development scale (0.5 L)

For concentrating the clarified harvest post TFDF®, flux excursions and retention experiments were performed to identify scalable operating conditions and the best membrane molecular weight cutoff.

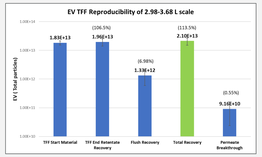


- <1% of EVs in Permeate, confirmed use of 750kDa

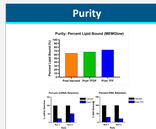
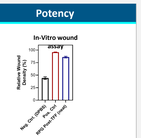


- Scalable Process conditions were identified in Small development scale TFF
- >90% process TFF step recovery yield

Scalability and reproducibility



- Great reproducibility between the three runs
- Development of Scalable Downstream Processing Platform for Therapeutic Extracellular Vesicles.

Purity	Identity	Potency
		
• ~70% of particles generated in this process stained positive with lipid membrane dye (MemGlow), indicating that those particles are EVs and they maintained their integrity throughout the process. • The developed downstream process cleared over 70% of DNA (impurity host DNA) without compromising on the generated EVs contain miRNA	• EVs generated from this process stained positive for EV specific tetraspanins markers (CD9, CD63, CD81) using western blot, further confirming the EV identity. • Sample 1: Harvested conditioned media, Sample 2: TFDF®, Sample 3: TFF	**In an in vitro wound healing assay, wounds treated with EV samples generated through TFF induced >80% wound closure (compared to 100% positive control and ~40% negative control), indicating that the EVs generated in this process maintained their potency.**

Scalability of TFDF® and TFF Downstream Steps for EV

Benchtop development scale to commercialization scale

EV Harvest Clarification using TFDF® at different scales							
Filter	Filter Area (cm^2)	Typical Batch Size (L)	Recirculation Flow Rate (L/min)	Throughput @ 20% Expansion (L/m^2)	Permeate Flux (LMH)	Permeate Flow Rate (L/min)	Process Time (h)
TFDF® 30	30	3	3	1200	650	0.0325	1.85
TFDF-450	450	45	9	1200	650	0.488	1.85
TFDF-2100	2100	210	42	1200	650	2.2750	1.76

EV Concentration and Diafiltration using TFF at different scales				
Filter Information and Process Parameters	**Small Scale Locked down process**	**Scale Up Process**	**Scalable Plan**	**Scalable Plan**
Type of Process	UFDF (C/D/C)	UFDF (C/D/C)	UFDF (C/D/C)	UFDF (C/D/C)
Filter Used	Spectrum MidiKros D02-E750-05-N	Spectrum MidiKros Sampler S02-E750-05-N	Spectrum KrosFlo Max X04-E750-05-N*	Spectrum KrosFlo Max X06-E750-05-N*
Filter MWCO (kDa)	750	750	750	750
Filter Chemistry	mPES	mPES	mPES	mPES
Filter Area (m^2)	0.0115	0.079	7.8	12.8
Volume Loaded (L)	**0.583**	**4**	**240**	**600**
Loading Ratio (L/m^2)	50.6	50.6	30.8	46.9
Process Time (hr)	2.8	2.8	1.8	2.8

Conclusion

- The identified optimal parameters yielded high EV recovery, while maintaining MSC-EV identity and potency as demonstrated by lipid membrane dye staining, positive EV markers (CD81, CD63 and CD9) and in vitro wound closure assay.
- The EV downstream clarification process step using KrosFlo® TFDF® System has been demonstrated a recovery yield of 86% comparable to the centrifugation and simplified the downstream process by eliminating secondary depth filtration Step prior to TFF1
- High recovery yield (92%) of potent EVs was achieved both at small scale and large scale
- High flux for TFDF® and the TFF enables fast process time at all scale, less than 3 hours each downstream step
- This study clearly demonstrated that integrating automated scalable single used closed system platforms, TFDF® and TFF KR2i, operated at an early development step simplified and de-risks the manufacturing process at large scale with a high recovery yield, identity, and potency of the EVs
- In this collaborative study, RoosterBio and Repligen successfully developed and advanced scalable EV bioprocessing

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