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Separation and Functional Characterization of Stem Cell-derived Exosomes from Scalable Manufacturing Processes

Introducing EVscale[™] - Extracellular Vesicles at scale

Klaus Graumann, CEO Phoenestra GmbH MSS2024, Portorož (SLO) June 7, 2024

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The Case for Extracellular Vesicles (EV)

Extracellular Vesicles (EV) are natural and universal 'cargo ships' between cells in all organisms and tissues



Li M, Fang F, Sun M, Zhang Y, Hu M, Zhang J. Extracellular vesicles as bioactive nanotherapeutics: An emerging paradigm for regenerative medicine. Theranostics. 2022 Jun 21;12(11):4879-4903. doi: 10.7150/thno.72812. PMID:35836815; PMCID: PMC9274746.

Broad Therapeutic potential in Regenerative Medicine

Conditions linked to overshooting or chronic inflammatory conditions and fibrosis, with large unmet medical needs

- Acute Lung Inflammation
- Lung Fibrosis
- Liver Fibrosis
- Heart fibrosis
- Kidney Fibrosis
- Brain and Peripheral Nerve Injury
- CNS Inflammation and Neurodegeneration

Market Potential for EV-based Therapies



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Current Main Issues for Successful Clinical Translation of EV-based Therapeutics



Торіс	Current Issues	Phoenestra's Mission
Cell Lines	 Mostly primary cells are used for EV production Donor-to-donor variability 	Key Issues
	Variable quality of cells and cell products	Batch-to-batch
Manufacturing	 Scaling difficulties Variable process performance Inconsistent quality between batches Isolation and purification technology (DSP) still underdeveloped 	variability Scalability and supply issues
Potency	 Robust potency assays or Surrogate assays for clinical efficacy are often missing 	Prohibitive Cost of Manufacturing
Analytics	 EV are complex entities Present in heterogenous mixtures Unclear composition function relationship 	(Lack of) Product understanding and definition
	 And probably, it starts already earlier: Active components driving biological function Role of vesicle corona Role of cultivation media EV/Exosome stability Confusing terminology 	4

EVscale[™] - A Holistic End-to-end Approach towards Clinical-grade Extracellular Vesicles



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The benefits of Using Telomerized MSC Lines (MSC/TERT)



Stable, telomerized MSC lines (MSC/TERT) – fully documented - GMP ready*

*Phoenestra and Evercyte have signed a commercial license agreement

Code	Tissue Source	GMP ready
ASC/TERT	Adipose tissue	Yes
BM-MSC/TERT	Bone marrow	Yes
WJ-MSC/TERT	Wharton's Jelly	Yes
P-MSC/TERT	Placenta	Yes
CP-MSC/TERT	Chorionic Plate	Yes
DP-MSC/TERT	Dental Pulp	Yes
	More in preparation	In preparation

- Homogenous starting material, continuous growth (Tiered Cell Bank concept: MCB/WCB)
- Supply of a whole product life cycle (from pre-clinical to end of commercial life)
- Virus-free cell line generation, only animal-free materials, Sartorius media in the bioreactor
- Human Telomerase is not an Oncogene, no oncogenes used for "immortalization"
- MSC/TERT display MSC-like morphology and potentials

EVscale[™] Platform Upstream/Downstream Process for Consistent and Scalable MSC/TERT-EV Supplies





EVscale[™] Cultivation of MSC/TERT and Harvest of EV from Conditioned Media (Perfusate)





- Particle concentrations in the harvest from perfusion in the range of $1.0 6.0 \times 10^{9}$ /mL
- Clinical supply quantities from a single 250 mL perfusion bioreactor within weeks (up to 43 days so far)
- Excellent overall process performance of the EV*scale*[™] perfusion setup for all currently available MSC/TERT-EV



Carriers from **bottom section** of the bed

Fine-tuning of Process Parameters and Controls Results in Significant Improvements





Consistent bioreactor performance with cell line-specific differences in the maintenance phase



Extracellular Vesicle Production in Stirred Tank Bioreactor

×10⁹

Fine-tuning of pH-control yields significant improvement of cell growth ...

... and in EV production to > 5x10e9 particles/mL (range 1-6x10e9)

Expression of MSC Biomarkers is Monitored at Seeding and at the End of Cultivation



Fluorescence Activated Cell Sorting (FACS) confirms the presence of MSC surface markers at seeding and after the end of cultivation (so far after 23 – 43 days)



Tangential Flow Filtration is a Proven Method for Purifying and Concentrating Functional EV





Step	Protein Yield [%]	Protein Recovery [%]	Particle Yield [%]	Particle Recovery [%]
Filtration	98	102	78	83
TFF	27	105	102	105

Biological Activity of TFF processed MSC/TERT-EV (EVscale[™] platform)

Extracellular Vesicles harvested over the course of perfusion runs and processed by TFF, consistently display anti-inflammatory activity in a cell-based bioassay*

P-MSC/TERT-EV P-MSC/TERT-EV BRC047 BRC053 120 120 100 100 **Dexamethasone-like activity Dexamethasone-like activity** NO secretion (%) 80 80 **Dose-response** NO secretion (%) **Consistent results** 60 60 40 40 20 20 0 0 **Dav 14** Day 21 Negative control Positive control **Dav 29** Positive Negative **Day 11 Day 18** Day 11 **Day 18** 1x 1x 1x (Dexa) control (Dexa) control 1x 1x 2x 2x

Anti-inflammatory Activity: the alteration of NO secretion of inflammation-triggered (LPS) macrophages when exposed to Dexamethasone (positive control) and respective EV preparations (TFF retentates) of different harvests throughout two independent EVscale[™] cultivations of P-MSC/TERT. The negative control is TFF buffer solution with LPS.

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Biological Activity of TFF-processed MSC/TERT-EV (EVscale[™] platform)



Comparison of anti-inflammatory (AI) activities of EV from <u>5 different GMP-ready MSC/TERT lines</u>



- Activities normalized to the same EV particle concentrations in the AI assay
- 10 µM Dexamethasone as positive control (=100% activity)

TP1-4 ... Time Points (Harvests), in chronological order

Biological Activity of TFF-processed MSC/TERT-EV (EVscale[™] platform)



Extracellular Vesicles harvested over the course of perfusion runs also consistently display antifibrotic (AF) activity in a cell-based bioassay* *In collaboration with Evercyte



TP1-4 ... Time Points (Harvests), in chronological order

Biological Activity of TFF-processed MSC/TERT-EV (EVscale[™] platform)



Comparison of anti-fibrotic activities of EV from <u>5 different GMP-ready MSC/TERT lines</u>



- Activities normalized to same EV particle concentrations in the AF assay
- PP2-treated as positive control (=100% activity)

TP1-5 ... Time Points (Harvests), in chronological order

Molecular Composition vs. Biological Functionality



Relevant Biological Functionalities - Biology
 Productive and scalable Processes - Process

Better understanding of critical quality attributes of EV preparations is key for success



Small RNAs are Abundant in EV – Comparison of miRNA Patterns Between TFF Processed Samples





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* In collaboration with TAmiRNA TAmiRNA

Top 10 P-MSC d17	Top 10 P d21	Top 10 CP d14	Top 10 CP d23	Top 10 CP d23
miR-A	miR-A	miR-A	miR-A	miR-A
miR-B	miR-B	miR-C	miR-C	miR-B
miR-C	miR-C	miR-D	miR-D	miR-D
miR-D	miR-D	miR-B	miR-B	miR-C
miR-E		miR-E	miR-E	miR-M
miR-F	miR-F			miR-F
	miR-H	miR-H	miR-H	miR-H
	miR-E	miR-M	miR-M	miR-G
	miR-G	miR-F	miR-F	miR-E
miR-K		miR-K	miR-K	

Top 10 ASC d17	Top 10 ASC d28	Top 10 ASC d36
miR-B	miR-B	miR-B
miR-D	miR-D	miR-D
miR-A	miR-A	miR-A
miR-F	miR-F	miR-F
miR-I	miR-G	miR-L
miR-G	miR-I	miR-H
miR-H	miR-L	miR-I
	miR-H	miR-G
miR-J	miR-J	miR-J
miR-L		

Top 10 BM-MSC d17	Top 10 BM-MSC d28	Top 10 BM-MSC d36
miR-D	miR-D	miR-D
miR-B	miR-B	miR-B
miR-A	miR-A	miR-A
miR-G	miR-G	miR-G
miR-H		miR-F
miR-I	miR-I	
miR-F		miR-J
miR-J	miR-F	miR-I
	miR-E	
miR-E	miR-H	miR-H

EVscale[™] - Comparison and Analysis of miRNA profiles between cell lines and harvest time points



7,0E+07	7,0E+07		7,0E+07
6,0E+07 P-MSC/TERT-EV	CP-MSC/TER	ſ-EV	6,0E+07 WJ-MSC/TERT-EV
5,0E+07 -	5,0E+07		5,0E+07
4,0E+07 -	4,0E+07		4,0E+07
3,0E+07	3,0E+07		3,0E+07 –
2,0E+07	2,0E+07		2,0E+07 -
1,0E+07	1,0E+07		1,0E+07 -
0,0E+00 DSP24-0143 DSP24-0161 DSP24-0170	0,0E+00 DSP24-0098 DSP24-0107 DSP24-0107	<mark>In du an an ais an air an an air an </mark>	0,0E+00 ■ DSP24-0072 ■ DSP24-0081
7,0E+07		7,0E+07	
6,0E+07 ASC/TERT-EV		6,0E+07 BM-MSC	/TERT-EV
5,0E+07		5,0E+07	
4,0E+07		4,0E+07	
3,0E+07		3,0E+07	
2,0E+07		2,0E+07	
1,0E+07		1,0E+07	
0,0E+00	t det att att att att att det met att det det n en ann att att att	0,0E+00	1
DSP240026 = DSP240035	■ DSP240026 ■ DSP240025 ■ DSP240044 ■ DSP240053		DSP230310 DSP240008 DSP240017

EVscale[™] - miRNA profile Analyses are Ongoing





CIMmultus QA was Selected to Separate and Characterize EV Preparations*

Exosome Harvest

TFF



* In collaboration with Sartorius BIA Separations

- Analysis of composition(s) displaying biological activities
- Options for further definition and purification of EV preparations as useful and needed for therapeutic applications



Example Anion-Exchange Chromatography: CIMmultus QA (Sartorius BIA Separations) Preparative separation is followed by UV (280/260), fluorescence (FLD) and Light Scattering (MALS, particle conc and size distribution), Chromatogram kindly provided by Darja Božič (Sartorius BIA Separations)



PATfix[®]-analysis of Preparative Chromatography Fractions



Chromatograms kindly provided by Darja Božič (Sartorius BIA Separations)



PATfix[®]-analysis of Preparative Chromatography Fractions



Graphs based on data kindly provided by Darja Božič (Sartorius BIA Separations)

Detailed Analysis of Preparative Chromatography Fractions (CIMmultus QA)







Lipid Particles (NanoFCM): >90% (MemGlow) in all fractions



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Detailed Analysis of Preparative Chromatography Fractions (CIMmultus QA)



Anti-fibrotic cell-based bioassay activity of chromatography load, flow-through and fractions



Total miRNA reads from chromatography load, flowthrough and fraction samples



L ... Load; R ... Ref Sample

Total reads per chromatography sample tested at equal EV particle counts

Detailed Analysis of Preparative Chromatography Fractions (CIMmultus QA)



miR-patterns and abundance change between chromatography fractions





EVscale[™] Platform for Product Definition and Consistent + Scalable EV Supplies Towards Clinical Translation



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The Value Proposition of EVscale[™]* - Summary

- Stable, GMP-ready MSC lines
- EVs with relevant biological functionalities and respective **potency** assays
- Innovative, **productive and scalable** manufacturing system and processes
- Excellent basis for **batch-to-batch consistency** and **leading cost of goods**
- Preparative and analytical tools for understanding EV activity better and for
- Product definition and specification



Thank you very much for your attention!





Melanie Reininger Claudia Lindner Roland Prielhofer Ingrid Hartl



Darja Božič Katja Vrabec Maja Leskovec Aleš Štrancar



Regina Grillari Giulia Corso Marieke Roefs Johanna Gamauf Alessia Brancolini



Magdalena Mecking Matthias Hackl

www.phoenestra.com

klaus.graumann@phoenestra.com