



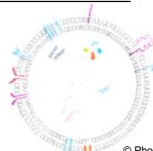
Extracellular Vesicles from stable Mesenchymal Stem/Stromal Cells (MSC) for therapeutic applications

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The benefits of MSC-EVs as therapeutic agents

They possess **Regenerative Potential** for **multi-faceted diseases** due to their **combinations of biological activities**:

- Anti-inflammatory
- Anti-fibrotic
- Vasculogenic
- Tissue-protective
- Pro-proliferative



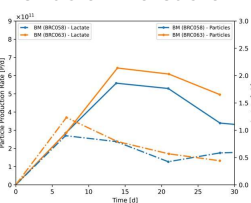
- Less safety concerns
- Less variability of quality (with EVscale™)
- Scalable manufacturing (with EVscale™)



Stable Cell Lines

MSC from different tissues (WJ / BM / P / CP / ASC)
Virus-free telomerization
GMP-ready

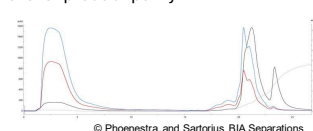
Perfusion Bioreactor Process



- Serum- and hPL-free process
- Fully controlled, reproducible
- Continuous harvest over 20+ days
- Productive at low footprint
- Economy of scale

Downstream Processing

Combinations of modular unit operations (Depth/Membrane Filtration, Tangential Flow Filtration (TFF) and preparative chromatography to achieve the required level of product purity



EVscale™ delivers EV preps with **consistent quality** in **scalable quantities**

- ✓ Stable cell lines (telomerized MSC, MSC/TERT) from WJ, BM, Placenta (2x), and adipose tissue (GMP-ready)
- ✓ Cell banking for consistent starting material (MCB/WCB)
- ✓ Standard unit operations and equipment for easy tech transfer and scale-up
- ✓ EV with excellent biological properties
- ✓ Consistent, sufficient and cost-effective supplies of clinical studies and market demands become possible with EVscale™

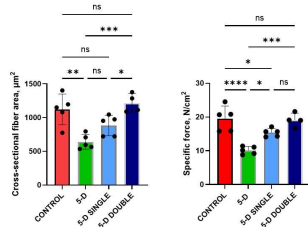
EVscale™ EV products show excellent *in vitro* and *in vivo* activities

Assay	EV1	EV2	EV3	EV4	EV5
Anti-Fibrosis 2D	***	**	**	**	-
Anti-Fibrosis 3D	**	***	+	+	***
Anti-Inflammation	+/+	n.s.	+	+	***
Proliferation - Nerve	***	+	n.s.	***	n.s.
Wound Healing 2D	***	n.s.	n.s.	n.s.	n.s.
Diabetic Mouse Wound Healing	***	n.s.	n.s.	n.s.	n.s.
Vasculogenesis 2D	***	***	***	***	***
Vasculogenesis 3D	***	***	***	***	+
Cardioprotection	***	n.s.	n.s.	***	n.s.

Overview of *in vitro* and *in vivo* model data. EV1-5 represent EV preparations from MSC/TERT cell lines with different tissue background. They show similarities with some distinct differences. Details available under NDA.

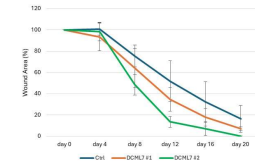
Ventilation-induced Muscle Loss

A double dose of EV1 fully preserves diaphragm muscle diameter and strength in a ventilated rat model (courtesy Prof. Lars Larsson, Univ. Uppsala, SE and Leksum LLC, Boston, MA USA)



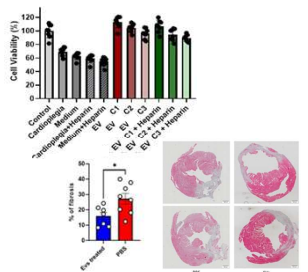
Wound Treatment

EV1-hydrogel combination products accelerate wound closure in a full thickness wound diabetic mouse (DBM) model. Using an innovative hydrogel enhances efficacy over a standard-of-care hydrogel (in collaboration with Gipfel Life Sciences (Innsbruck, AT) and LBI Trauma (Vienna, AT))



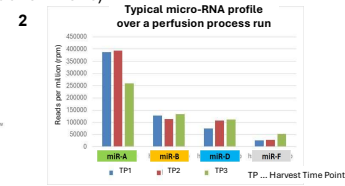
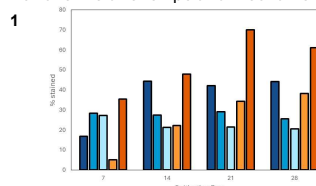
Cardio-protection

EV1 and EV4 protect heart cells from apoptosis in an *in vitro* cardioplegia model. The viabilities of rat myoblast and cardiomyocytes as well as HUVECs are maintained at around 100% while the standard cardioplegia solution (St. Thomas) yields around 60% cell viability in a head-to-head comparison. EV1 improve functional parameters and reduce scarring/fibrosis in an *in vivo* Myocard Infarction (MI) model (both Prof. Kiss/Podesser, Med. Univ. Vienna (MUW), Vienna, AT)



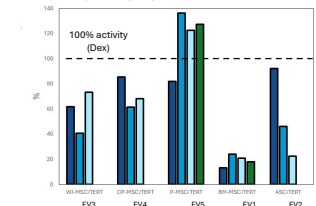
Analytical Characterization of EVs

EV preparations are characterized by orthogonal methods to assess differences in EV composition and to correlate these with biological activities. (defining composition-function relationships and mechanisms-of-action MoAs)

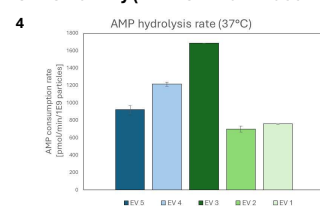


1 Nanoflow cytometry analysis (NanoFCM) of EV preparations: marker proteins CD9, CD63, CD81, the MSC EV marker CD73 and the lipid dye MemGlow data from representative processing samples from bioreactor harvest timepoints. 2 Micro-RNA patterns are used to assess consistency between processing runs as well as to evaluate correlations with biological functionalities

Anti-Inflammation

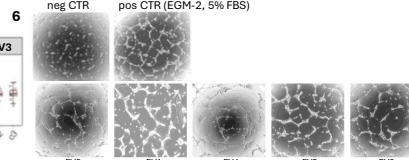
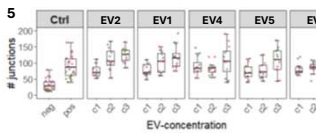


CD73 Activity (NLRP3/Inflammasome)



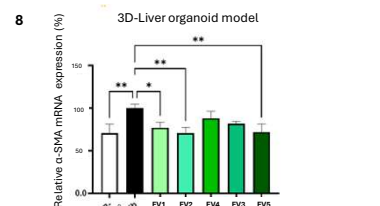
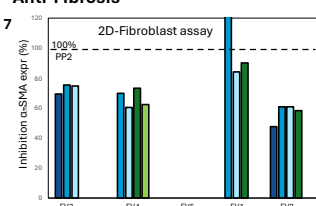
3 Anti-inflammatory potential (LPS triggered mouse macrophages). 2.5 μM Dexamethasone (Dex) as positive control (=100%). Data are compiled from different experiments and have been normalized. 4 Comparison of different EV preparations based on their specific CD73 activity (AMP hydrolysis) which is annotated to NLRP3/Inflammasome inhibition.

Blood Vessel Formation



2D tube formation assay of human umbilical vein endothelial cells with EV1-5 (6) and analyzed for their number of junctions between cells (5)

Anti-Fibrosis



7 Reduction of α-SMA (α-smooth muscle actin) expression upon TGF-β trigger in a 2D fibroblast assay. 2 μM Src-kinase inhibitor PP2 as positive control. Data are compiled from different experiments and have been normalized. 8 Abundance of α-SMA mRNA in a 3D liver organoid co-culture (HepG2 and LX-2 cells) model.