Therapeutically relevant Extracellular Vesicles from stable Mesenchymal Stromal Cells

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Why EVs?

Regenerative Potential

- (Diabetic) Wound treatment
- CNS inflammation and Neurodegeneration
- Organ Injury (e.g. after heart attack)
- Osteoarthritis (OA)
- Acute Lung Inflammation
- Inflammatory bowel disease, ...

Biological Activities

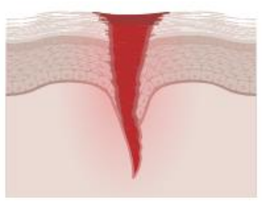
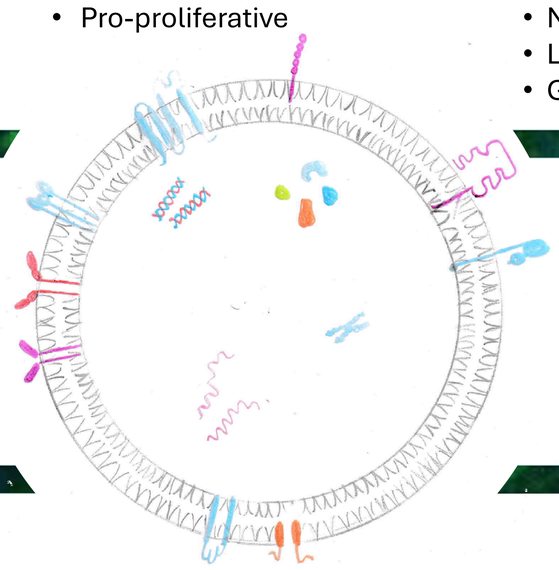
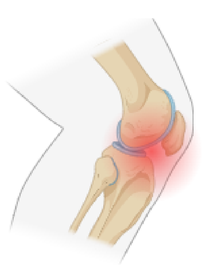
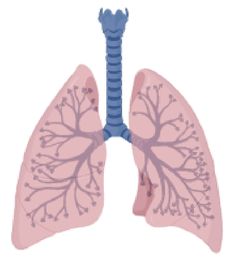
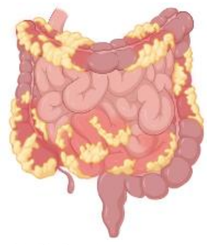
- Anti-inflammatory
- Anti-fibrotic
- Tissue-protective
- Pro-vasculogenic
- Pro-proliferative

Carriers of Active Components

- Proteins
  - Surface Markers and Ligands
  - Cytokines and Chemokines
  - Growth Factors and Enzymes
- Nucleic Acids
- Lipids
- Glycans

EVscale™
Scalable production of extracellular vesicles



Images created with BioRender.com

Why EV*scale*™?

The Problem with conventional EV technologies:

- ➢ Primary cells, including mesenchymal stromal cells (MSC), are variable and changeable
- ➢ Scaling up is difficult
- ➢ Consistent and sufficient supplies of (pre-)clinical studies are a challenge

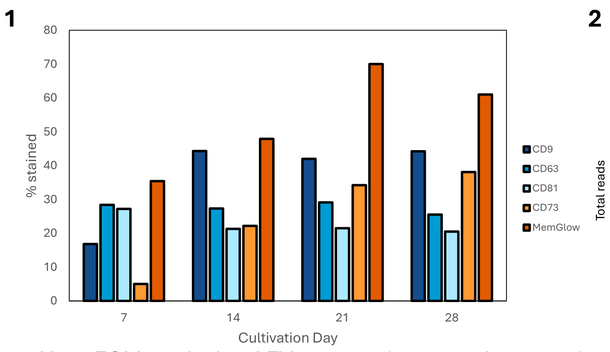
The Solution - EV*scale*™:

- ✓ Stable cell lines (telomerized MSC, MSC/TERT) from Wharton's-Jelly, bone marrow, placenta, and adipose tissue
- ✓ Cell banking (master cell bank and working cell bank) for consistent starting material
- ✓ Fully controlled perfusion bioreactor technology
- ✓ Developed and scalable downstream processing
- ✓ Consistent and sufficient supply of clinical studies and market demand become possible

Analytical Characterization of EVs

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

1



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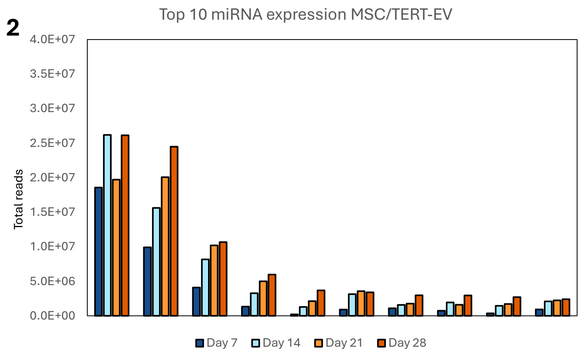


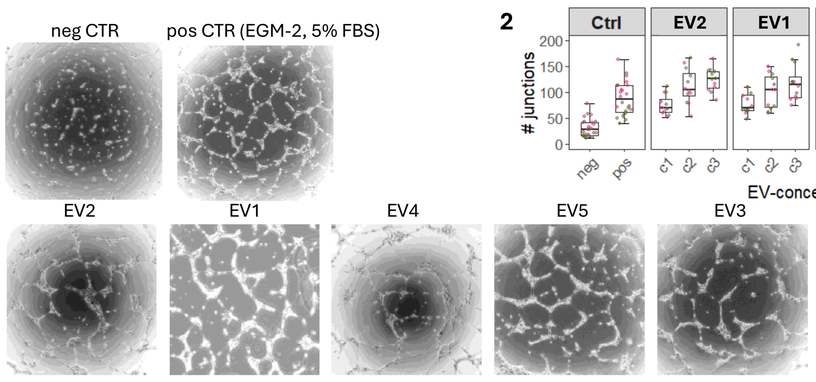
1 NanoFCM analysis of EV preparations: marker proteins CD9, CD63, CD81, the MSC EV marker CD73 and the lipid dye MemGlow data are shown over the course of a representative cultivation. **2** Micro-RNA patterns are used to assess consistency between processing runs as well as to evaluate correlations with biological functionalities. Data are shown from a representative bioreactor cultivation from different harvest time points.

With EV*scale*™ we produce biologically active EVs in large quantities and with consistent quality
Biological functions are assessed in therapeutically relevant model systems

Tangential-Flow-Filtration (TFF)-purified EV from different MSC/TERT cell lines have been tested in multiple *in-vitro* models for their activities

Blood Vessel Formation (Vasculogenesis)

1

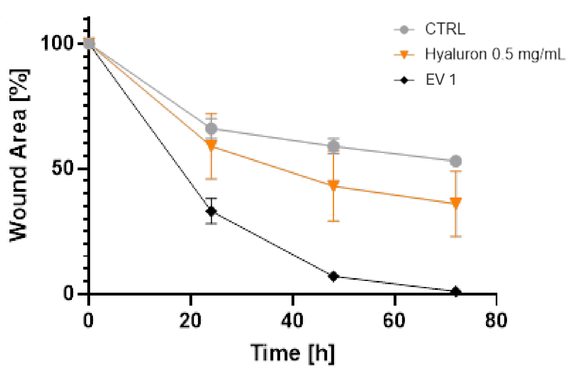


2

1-2 TFF purified retentates from different MSC/TERT cultivations were tested at 3 different concentrations in a 2D tube formation assay of human umbilical vein endothelial cells. Microscopic images were taken after 16 hours (representative images from one concentration shown in (1) and analyzed for their number of junctions between cells **(2)**. (In collaboration with Tanja Zauner, Wolfgang Holnthoner, LBI Trauma, Vienna, Austria)

Wound Healing

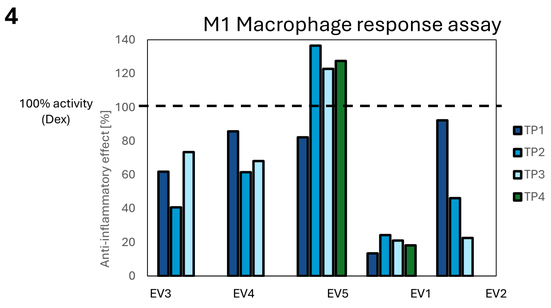
3



3 An MSC/TERT-EV preparation displays rapid scratch closure in a 2D-Scratch Assay (HaCAT cells) indicating great promise for wound healing indications. (Hyaluronic acid as positive control) (In collaboration with Ozan Fidan, Gipfel Life Sciences, Innsbruck, Austria)

Anti-Inflammation

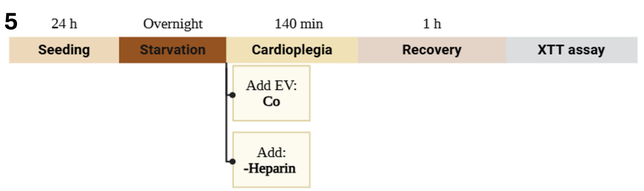
4



4 EV preparations tested for their anti-inflammatory potential (LPS triggered mouse macrophages). 2.5 µM Dexamethasone (Dex) was used as positive control (=100%). Data are compared from different experiments and have been normalized on particle concentrations and control signals. (In collaboration with Evercyte, Vienna, Austria)

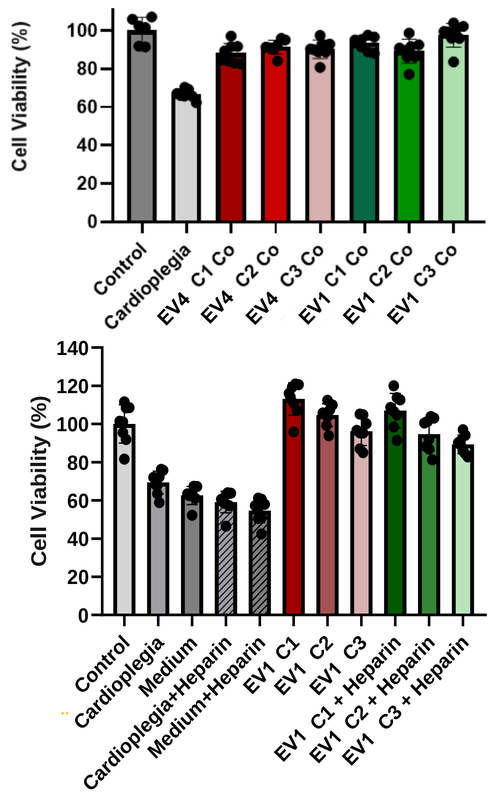
Cardiomyocyte protection

5



5 A rat cardiac myoblast cell line (H9C2) is strongly protected from a myocardial ischemia stressor by the addition of two different EV preparations. The effect was observed at all 3 tested concentrations. In one EV preparation, it is shown that heparin addition did not interfere with the protective effect of EVs on cell viability (In collaboration with Ana Goncalves, Attila Kiss, Bruno Podesser, MUW, Vienna, Austria).

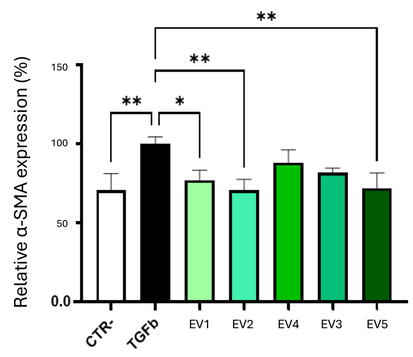
Additional information: Cardioplegia is a technique used during cardiac surgery to intentionally and temporarily stop the heart (cardiac arrest) to allow surgeons to perform procedures in a still and bloodless field. However, the reperfusion of the heart afterwards induces stress on the tissue (Reperfusion Injury).



Anti-Fibrosis

3D-Liver organoid

6



6 EV preparations from different cultivation MSC/TERT lines were tested for their ability to reduce α-SMA (α-smooth muscle actin) mRNA expression after TGF-β trigger in a 3D liver co-culture (HepG2 and LX-2 cells) model. (In collaboration with Stefania Bruno, UNITO, Torino, Italy)

Conclusion: EV*scale*™ yields EV preparations with combinations of biological activities and generates therapeutic product candidates for regenerative medicine indications with unmet clinical need

References:
Harrell CR et al.: Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. Cells 2019, 8, 1605; doi:10.3390/cells8121605
Kumar MA et al.: Extracellular vesicles as tools and targets in therapy for diseases. Signal Transduction and Targeted Therapy (2024) 9:27 ; https://doi.org/10.1038/s41392-024-01735-1

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