EXTRACELLULAR VESICLES AS CELL-FREE TOOLS FOR THERAPIES

Dr. Yarúa Jaimes, Group for cell activation and deactivation

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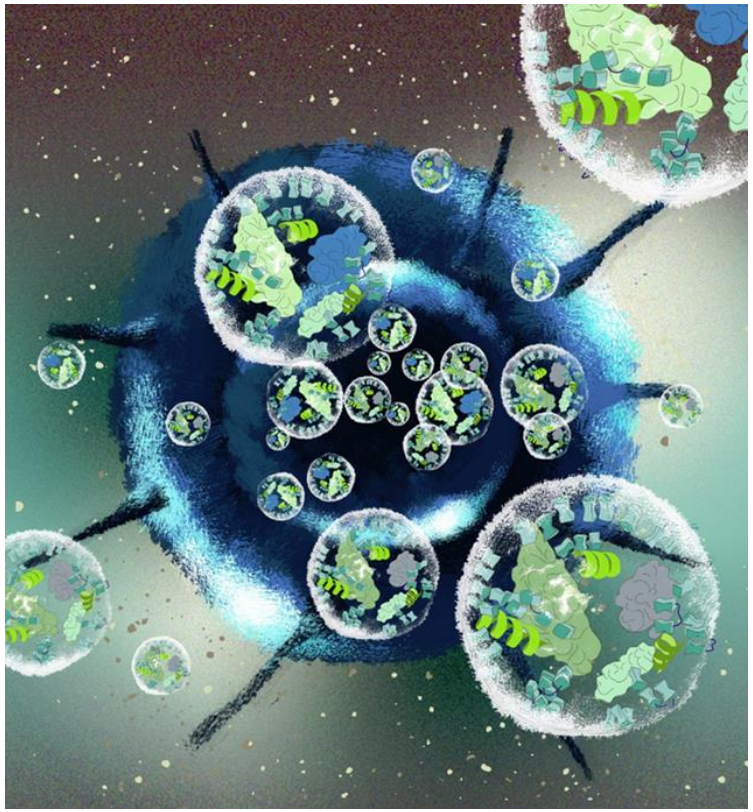
Extracellular vesicles (EV)

- Potent carriers in intercellular communication
- Influence cell function by transferrin nucleic acids, lipids or proteins from their cell of origin.
- Strong biological function in diseases
- Potential tools for new therapeutic approaches and diagnostics.



At **Fraunhofer IZI** we use EVs to design new targeted therapies for a wide variety of diseases

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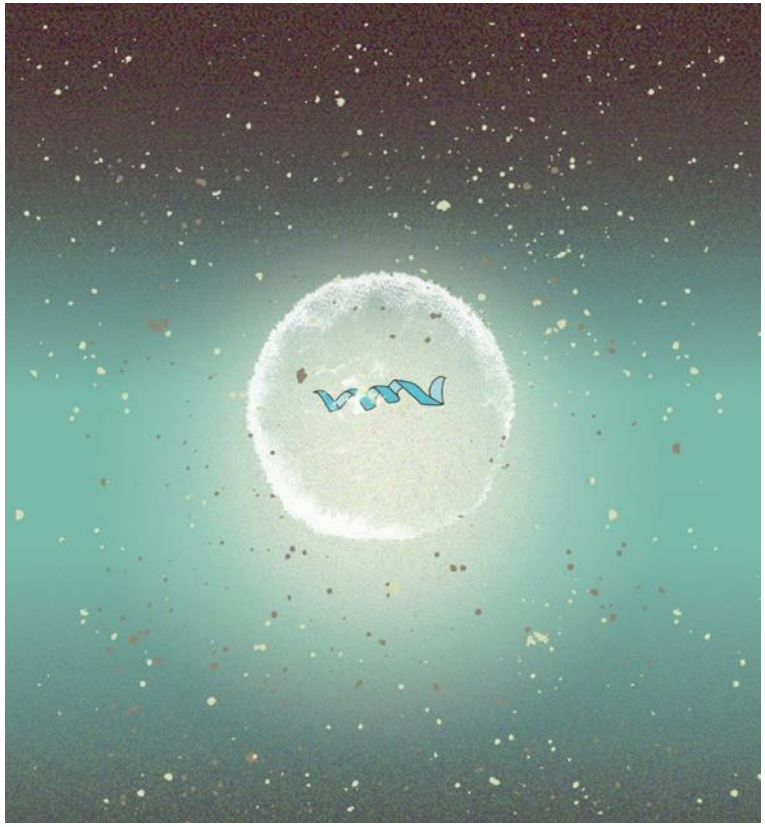
Mesenchymal stroma cells-EV (MSC-EV)

Advantages as therapeutic tools:

- Remarkable immune modulators
- Well tolerated after transplantation or transfusion
- Less storage problems than for MSCs
- Small size allows crossing biological barriers
- Low toxicity and tumorigenicity
- Provide high stability to the transported molecules
- Safety
- Increased off-the-shelf availability
- Prolonged half-life in circulation (> 6 days), higher than liposomes



Enhanced EVs for therapies development

Our expertise:

- Production and testing of MSC-EVs for therapeutic purposes
- Customization of MSC-EVs by MSC genetic modification
- Biological testing of modified MSC-EVs
- Functional testing of MSC-EVs in disease-relevant immunological assays

At **Fraunhofer IZI** we are specialized at customizing and testing MSC-EVs

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MSC-EV production and testing

- We routinely produce, isolate, evaluate and store EVs from human adipose derived stem cells (ADSCs).
- ADSCs are characterized for their pluripotent capacity and immunomodulatory effect.
- ADSCs can be modified for the introduction of interest molecules that will be naturally packaged in the ADSC-EVs.
- Modified or unmodified ADSC-EVs are isolated from cell culture supernatant by gradient ultracentrifugation.
- Modified ADSCs and ADSC-EVs are molecularly and functionally characterized (proteins, microRNAs, effect on cell-model of interest).
- ADSC-EVs are particularly attractive to be used as therapeutic agents for the treatment and prevention of inflammatory diseases and new developments are rapidly rising.

Proprietary pipeline at Fraunhofer IZI

- We offer our expertise at EVs-*in vitro* testing for optimization and standardization of industry research interests
- We offer our own therapeutic products for further uses
  - ExoTherAl: Extracellular Vesicles as therapy for Alzheimer

  - EVRA: Extracellular Vesicles for Rheumatoid Arthritis

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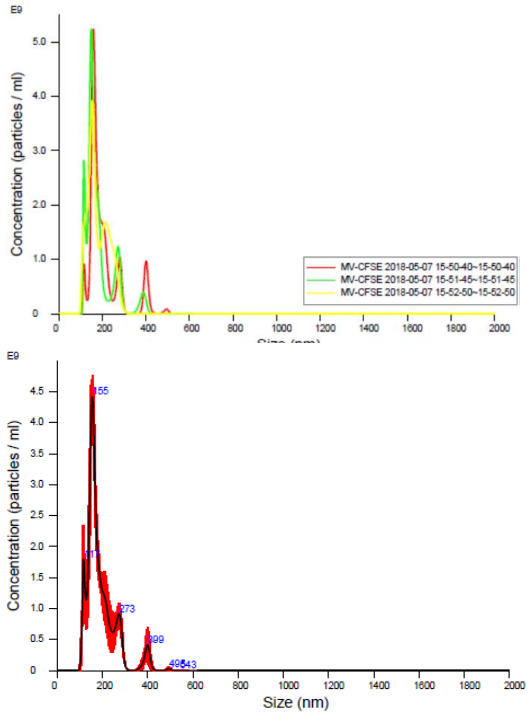
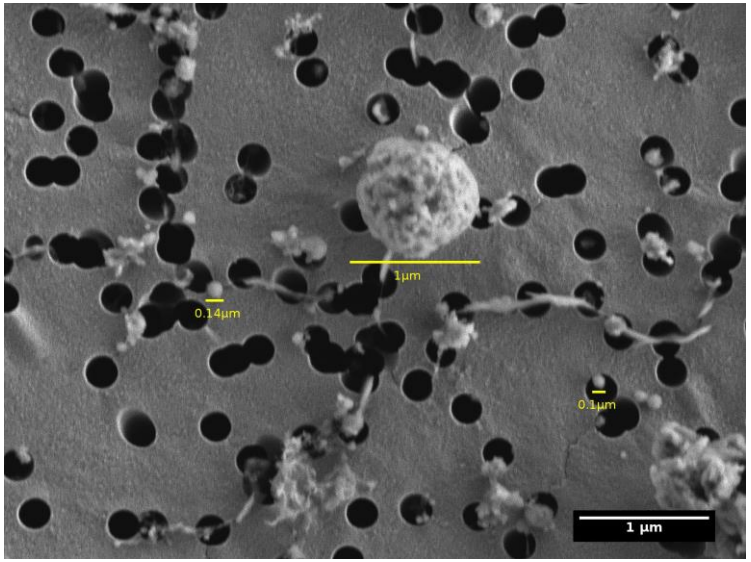
Human derived MSC-EVs size distribution and morphology





Our human MSC-EVs size range comprises from 50 to 1000nm

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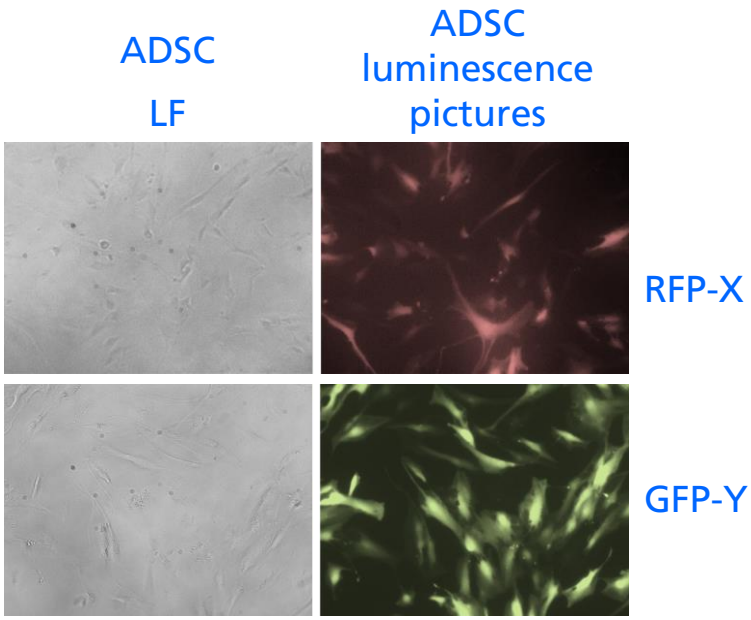
Overexpression of molecules X and Y in ADSC

- We have developed a stable and efficient method for transduction of ADSCs.
- The pictures show the reporter expression after introduction of vectors for overexpression of a cytoplasmic molecule (X) and a membrane molecule (Y).



- ADSC: Adipose derived stroma cells
- LF: Light-field
- GFP: Green fluorescent protein
- RFP: Red fluorescent protein
- X & Y: undisclosed therapeutic molecules

We stably introduce vectors for overexpression of therapeutic molecules (miRNA, enzymes, cytokines, membrane molecules) in ADSCs

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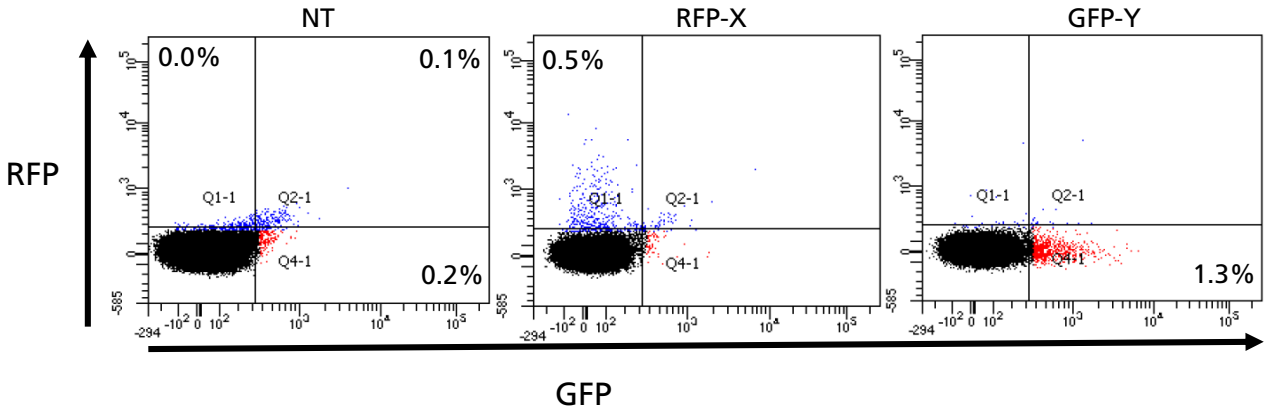
Introduction of molecules X and Y in MSC-EVs

- NT: Non-transduced
- GFP: Green fluorescent protein
- RFP: Red fluorescent protein
- X & Y: undisclosed therapeutic molecules



- Molecules introduced to the cells are packaged in their EVs.
- Reporter molecules are detectable by flow cytometry in MSC-EVs.
- The GFP molecule, cross-linked to a membrane molecule, is detected with higher intensity than the RFP reporter, which is cross-linked to a cytoplasmic molecule. Possible hint to Y-surface expression on EVs.

Molecules introduced to ADSCs are packaged in their EVs

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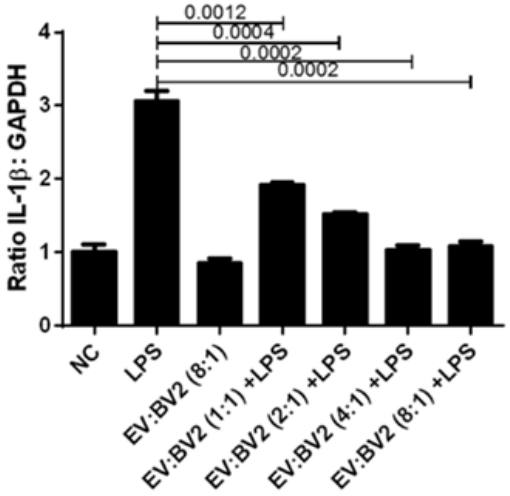
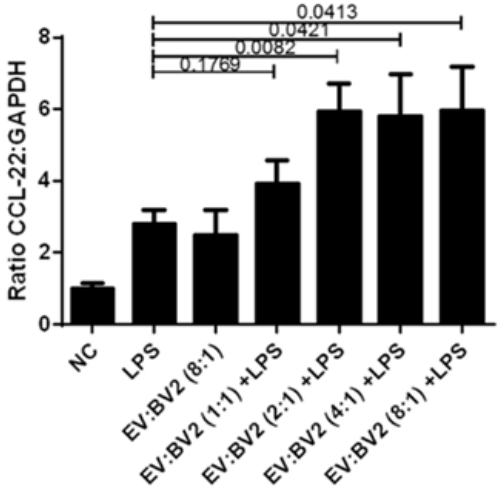
In vitro Proof-Of-Concept

Pro-inflammatory gene transcription



Anti-inflammatory gene transcription



- Effect of MSC-EVs as modulators of Microglia activation.
- MSC-EVs prevent up-regulation of pro-inflammatory genes (IL-1β, TNF-α, IL-6, iNOS, PTGS2) by microglia cells after LPS stimulation.
- MSC-EVs induce up-regulation of anti-inflammatory gene as CCL-22 by microglia cells.

- NC: Non-stimulated cells
- LPS: Lipopolysaccharides
- EV: Extracellular vesicles
- BV2: mouse microglia cell line
- (8:1): Ratio EV: BV2 cells.

MSC-EVs modulate microglia activation by LPS

Jaimes et al. Stem Cells. 2017 Mar;35(3):812-823

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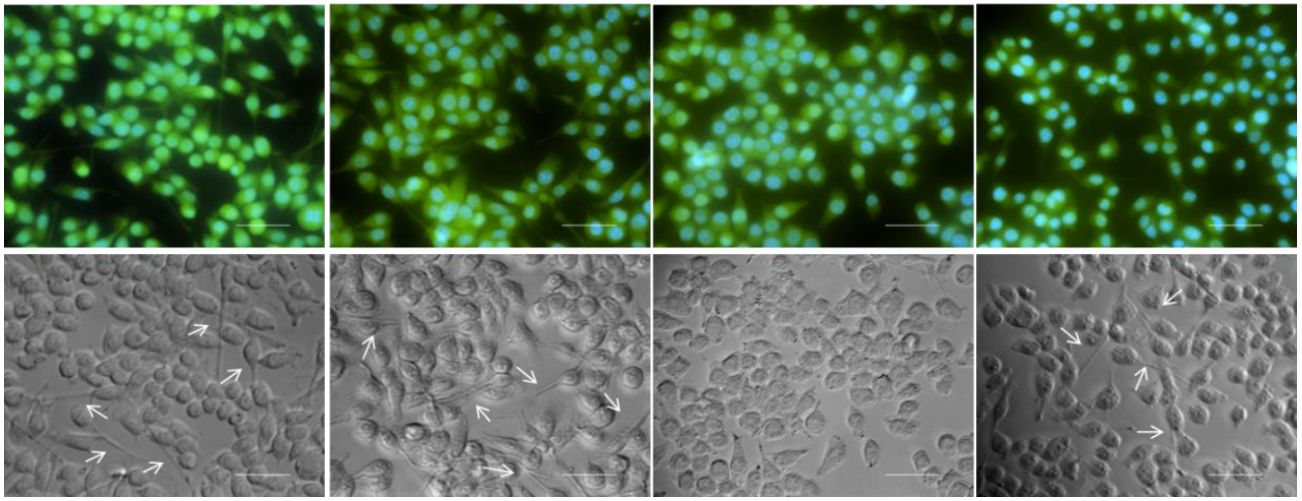
Immunosuppressive effect of MSC-EVs on Microglia cells

Morphology of microglia cells after stimulation with LPS and in presence of MSC-EVs



LPS	-	-	+	+
MSC-EV	-	+	-	+

Activation of microglia cells is prevented by MSC-EVs

Jaimes et al. Stem Cells. 2017 Mar;35(3):812-823

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Summary

- We offer standardized methods for production, isolation, modification and analysis of EVs.
- We are the only group worldwide with the technology to introduce molecules in EVs without further altering their content.
- We are looking for industry partners interested in testing new EV technologies.
- We are offering our expertize to support industry partners in their EV-products development.
- We have developed modified vesicles with introduced undisclosed molecules interesting for Alzheimer and Rheumatoid Arthritis therapies.