PhD Open Days

An Integrated Approach to the Production of Extracellular Vesicles from Human Induced Pluripotent Stem Cells in Stirred-Tank Bioreactors

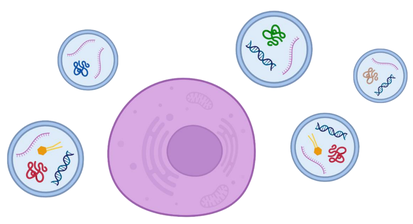
PhD Program in Bioengineering

William Salvador (william.salvador@tecnico.ulisboa.pt)

Relevance

Extracellular vesicles (EVs) are lipidic nanostructures secreted by cells that carry complex biological cargo composed of proteins, lipids, RNA and DNA. Depending on their cargo, **EVs** can:

- Stimulate cell proliferation
- Prevent cell death
- Promote vascularization



Coupled with the fact that **EVs** are not directly targeted by the immune system and can easily cross biological barriers, they have increasingly come to be seen as promising therapeutic agents for **regenerative medicine**. For example, **EVs** can be used to treat acute myocardial infarction and retinal degeneration.

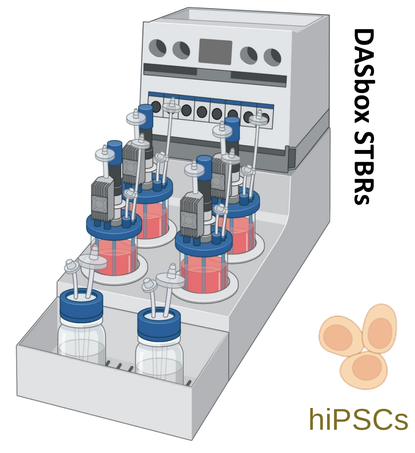
EVs from **human induced pluripotent stem cells (hiPSCs)** have been observed to have higher regenerative potential than committed cell types.

Problem

Treating a single patient can require up to 100 billion **EVs**, an amount difficult to acquire with traditional production methods. **Stirred-tank bioreactors (STBRs)** are ideal platforms for scaling up the culture of **hiPSCs** and **EV** production because they:

- Integrate monitoring and automation
- Allow for high cell densities
- Are highly scalable

Once **EVs** are secreted by cells they need to be recovered and purified. Among the several strategies available to achieve this, **tangential flow filtration (TFF)** is highly scalable and offers an acceptable tradeoff between yield and purity.

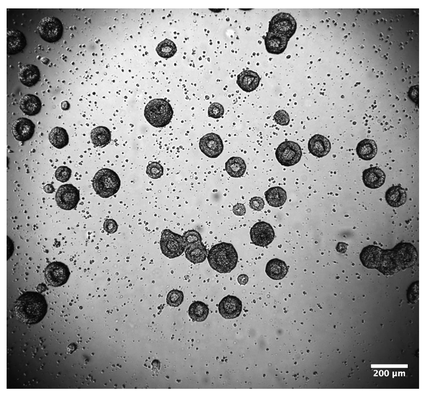


The **objective** of this work is to establish an **hiPSC-EV** production platform that integrates an **STBR** and a **TFF** system for **EV in-situ recovery**.

Methodology

hiPSC Expansion

- Free-floating aggregates in Eppendorf's DASbox Mini Bioreactor System
- Optimization of agitation rate, medium exchange strategy and culture media (**mTeSR1**, **E8** and two formulations from **Defined Bioscience**: **HiDef-B8** and **A8**)
- Assessment of hiPSC pluripotency (flow cytometry and immunostaining)

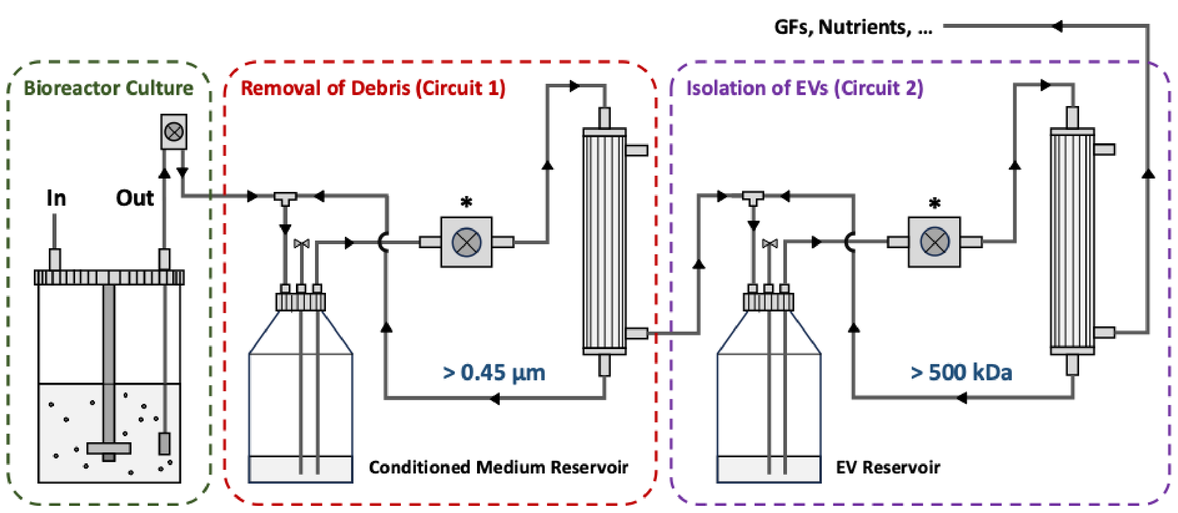


hiPSC free-floating aggregates

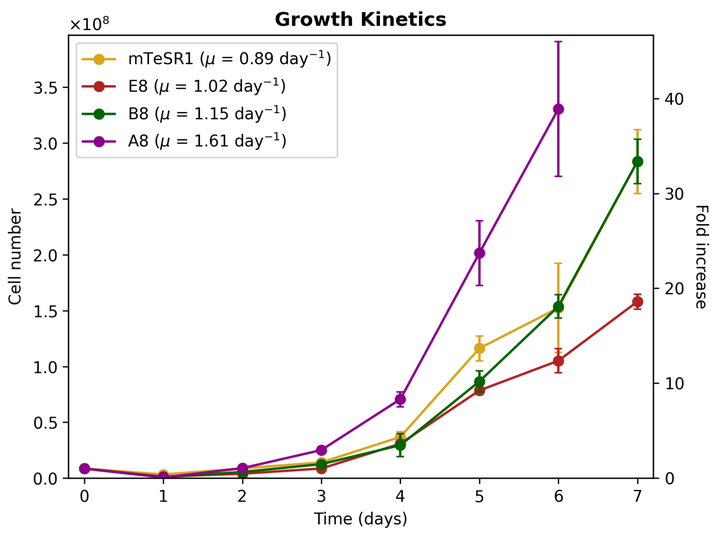
Two-Step Hollow Fiber TFF System

1st Pore size of 0.45 µm
Removes cell debris

2nd Pore size of 500 kDa
Retains and concentrates EVs
Removes small biomolecules



Results and Conclusions



Fold Expansion

- **mTeSR1**/**B8** – 33×, 7 days
- **E8** – 18×, 7 days
- **A8** – 39×, 6 days

Pluripotency Maintenance

- **mTeSR1**/**A8** – Expression of all markers higher than 95%
- **E8**/**B8** – Notably decreased expression of TRA-1-60

A8 presented improved cell survival (38%) and faster growth rate, aside from being the second cheapest medium after **B8**

Medium	Particles/mL	Total Particles
E8	$(0.82 \pm 0.13) \times 10^{10}$	$(0.68 \pm 0.11) \times 10^{12}$
A8	$(7.98 \pm 0.44) \times 10^{10}$	$(6.38 \pm 0.35) \times 10^{12}$

Particles in Medium (measured by nanoparticle tracking analysis)

- **A8** – 9.4× more particles than **E8**
- **mTeSR1** – masking effect due to large number of particles in medium

A8 presents itself as the best culture medium for optimized **hiPSC-EV** production. In the future, further analysis of particles to guarantee they are EVs and characterize their contents will be performed.

Acknowledgments: The authors acknowledge the funding received from FCT – Fundação para a Ciência e a Tecnologia, I.P. granted to William Salvador (2024.05042.BD), the projects CardioWheel (PTDC/EQU-EQU/29653/2017) and SMART (PTDC/EQU-EQU/3853/2020), as well as to iBB – Institute for Bioengineering and Biosciences (UIDB/04565/2020 and UIDP/04565/2020) and to the Associate Laboratory i4HB – Institute for Health and Bioeconomy (LA/P/0140/2020). The authors further acknowledge the funding received from GFI – The Good Food Institute granted to the project VitaminSea (22-CM-PT-DG-1-317). The authors also express their sincere gratitude for the support of Phoenestra GmbH and Defined Bioscience.

TÉCNICO LISBOA

Carlos Rodrigues, Klaus Graumann, Joaquim Cabral

PhD Program in Bioengineering

phdopendays.tecnico.ulisboa.pt