PhD Open Days

GMP-compliant manufacturing of mesenchymal stromal cells-derived extracellular vesicles with immunomodulatory potential

BIOENGINEERING – CELL THERAPIES AND REGENERATIVE MEDICINE

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MOTIVATION

- **Mesenchymal stromal cells** (MSC) hold great therapeutic potential for **autoimmune diseases** due to their unique secretome
- These and other beneficial effects have been associated to **extracellular vesicles** (EV) secreted by MSC, and their use in cell-free therapies, instead of MSC, poses less regulatory challenges
- A robust, reliable, cost-effective, and **good manufacturing practices (GMP) compliant** manufacturing process for MSC and their EV is needed
- **Quality control** of these cell-derived products requires optimization and **functional assays** already established for MSC in an immunomodulatory context, such as evaluation of the **hematopoietic supportive capacity** of the MSC-EV, can be leveraged

STRATEGY

Task 1: Identification of MSC-EVs critical quality attributes (CQAs) and establishment of a characterization platform for their measurement

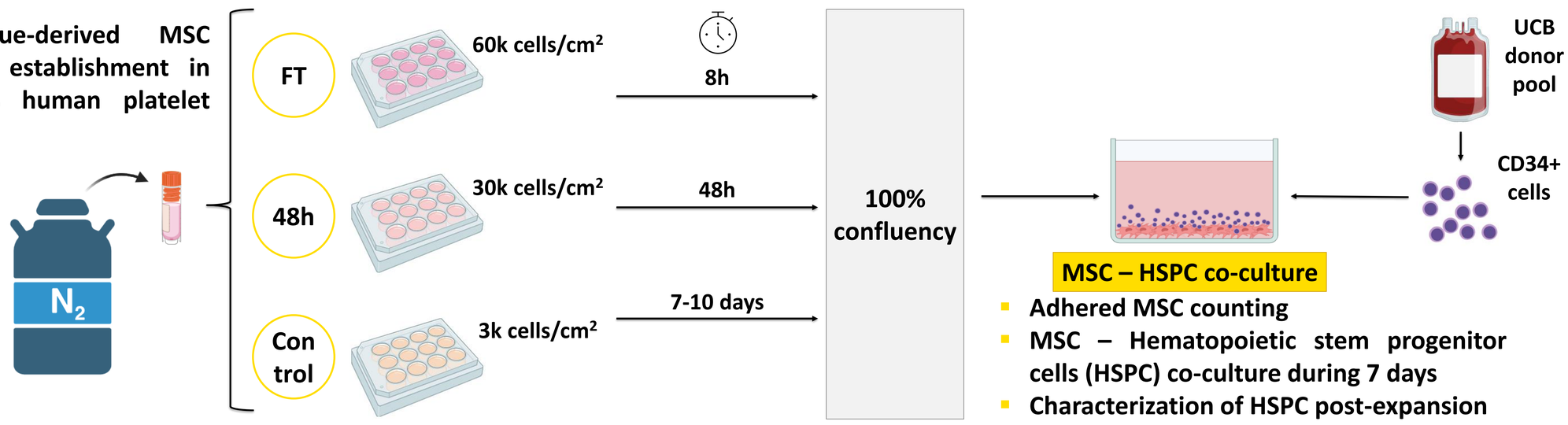
Task 2: Establishment of a GMP-compliant fully-controlled reactor system for human MSC expansion towards the maximization of EVs production

Task 3: Development and integration of a novel GMP-compliant scalable process for the isolation and purification of the MSC-EVs

Task 4: Process simulation of a manufacturing process for GMP-compliant MSC-EVs and its validation

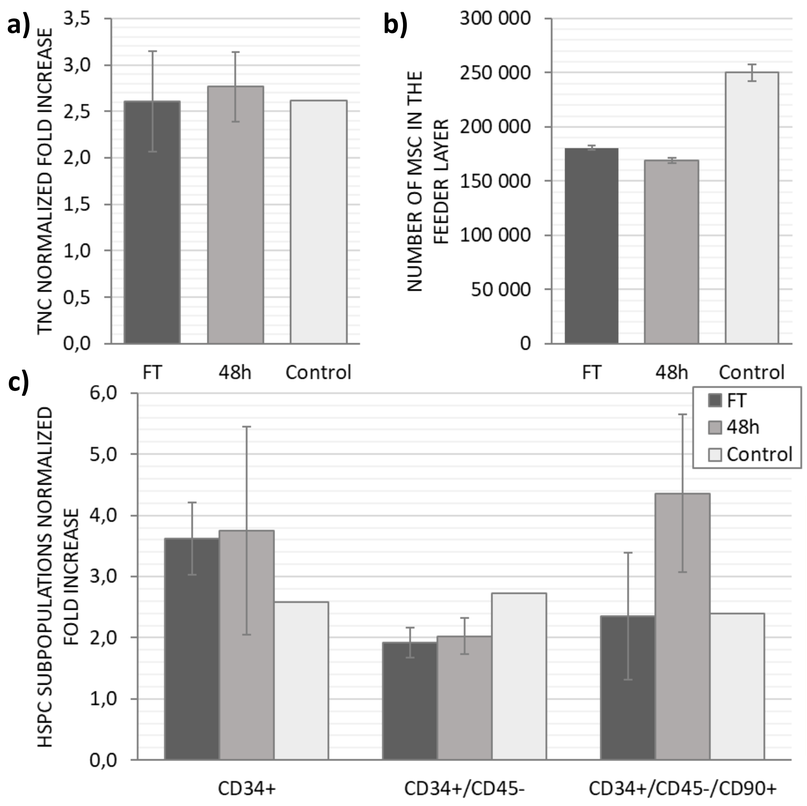
METHODS

- **Adipose tissue-derived MSC feeder layers establishment in DMEM + 5% human platelet lysate (hPL)**



- **Adhered MSC counting**
- **MSC – Hematopoietic stem progenitor cells (HSPC) co-culture during 7 days**
- **Characterization of HSPC post-expansion**

PRELIMINARY RESULTS



→ The ratio between the number of MSC in the feeder layer and the number of HSPC was approximately 3:1

→ HSPC expansion in co-culture with freshly thawed (FT) MSC and MSC revitalized in culture for 48h demonstrated comparable results to the control condition

→ Contrary to literature findings, the potency of MSC and their ability to support the expansion of HSPC remained unaffected by cryopreservation, with FT and Control results showing similarity

→ Likewise, the 48h recovery period in culture, described as sufficient for MSC to regain their characteristics, yielded no significant differences in the results

Cryopreservation and thawing of MSC **does not significantly affect** their ability to form feeder layers and support **the expansion of HSPC**

A better understanding of **MSC-HSPC interactions** during expansion will enable the **optimization of the hematopoietic support assay**, streamlining its integration into a time- and cost-effective cell-therapy manufacturing process and serving as a **potency assay for MSC**

NB: N=2 in FT and 48h conditions; N=1 in Control condition except in chart b) where N=2; error bars show SEM

FUTURE WORK

- Deepening understanding of MSC-HSPC interactions, specifically exploring the influence of MSC feeder layer fitness on the success of HSPC expansion and determining the most beneficial time frame for these interactions
- Optimization the hematopoietic support assay for MSC-EV

These **preliminary results** are of great importance as they pave the way to the **establishment of sensitive and significant potency assays for MSC**

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