

High throughput production of extracellular vesicles (EVs) from immortalized Wharton's jelly mesenchymal stromal cells (iWJ-MSC)

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INTRODUCTION

Mesenchymal stromal Cells (MSC) are of particular interest in solid organ transplantation due to their **immunomodulatory and antifibrotic** functions. These functions are partially mediated by secretion products, with a special interest in extracellular vesicles (**EVs or MSC-EVs**).

MSC-EVs as therapeutic agents possess **several advantages over cellular therapies**, including low responsiveness, fewer adverse effects and easier handling.

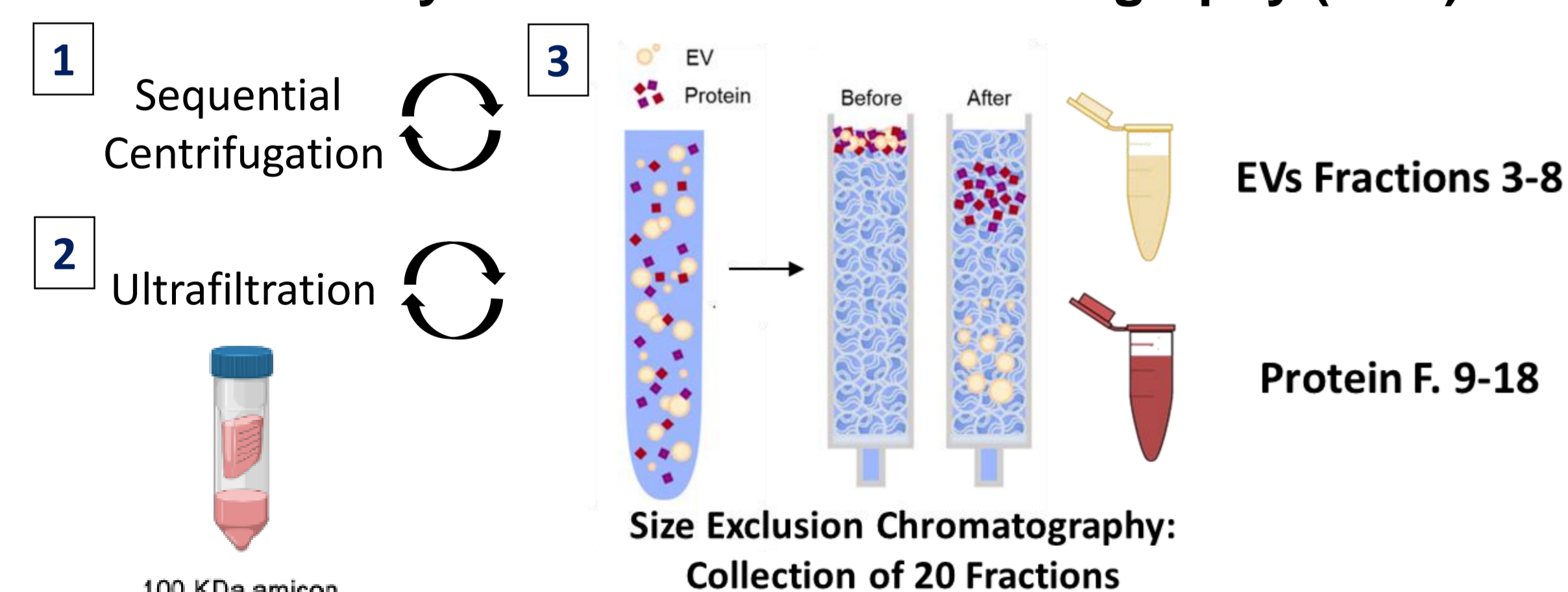
Owing to these advantages, MSC-EVs are proposed as therapeutic tools in transplantation. However, **large-scale high-throughput EV production** is still a challenge.

OBJECTIVES

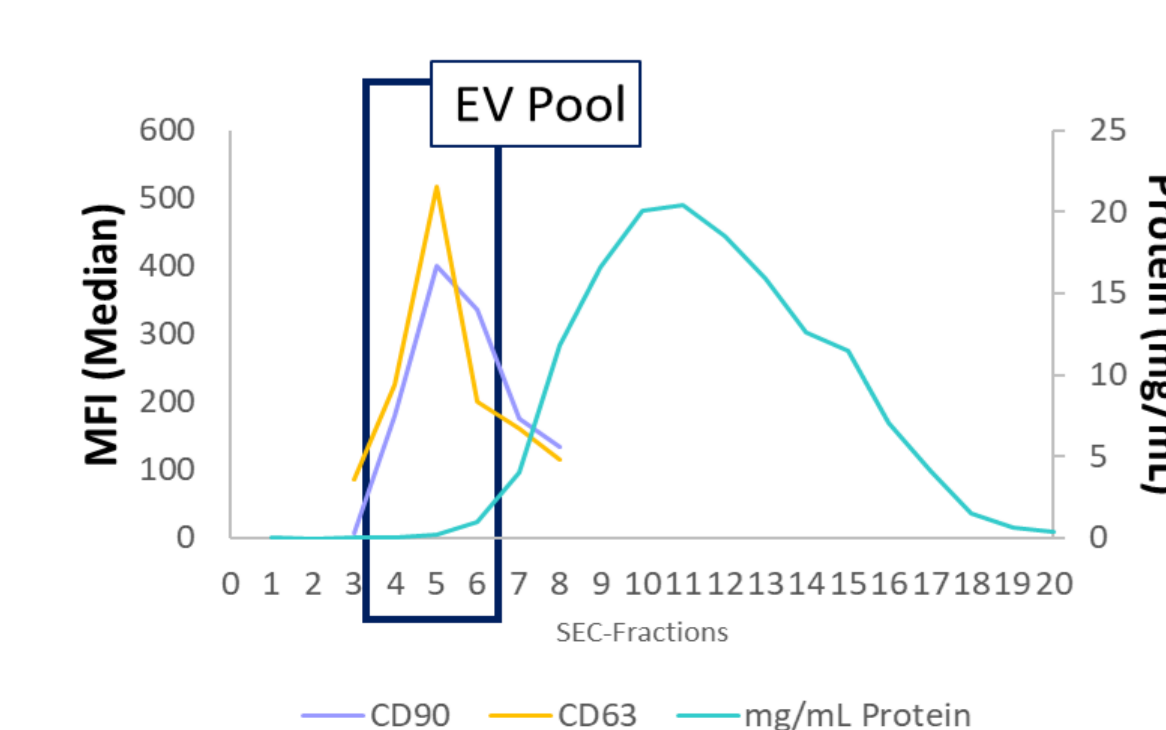
- To generate **hTERT-immortalized MSC cell lines** to produce and **isolate MSC-EVs** by **size exclusion chromatography (SEC)** with increased consistency and reproducibility.
- Extract and isolate **iMSC-EVs** from **3D culture** using **hollow-fiber bioreactor** systems for large-scale production of iMSC-EVs.
- To characterize EV obtained from **3D-cultures** and compare them to **"classical" 2D-cultured-EVs**.

METHODS

EV Isolation by Size exclusion chromatography (SEC)



EV Characterization



- EV surface markers (Flow cytometry)
- Protein Content (BCA assay)
- EV morphology (Cryo-TEM)
- Potency Assay (T cell inhibition)

RESULTS

1. iMSC 2D-culture and EV isolation

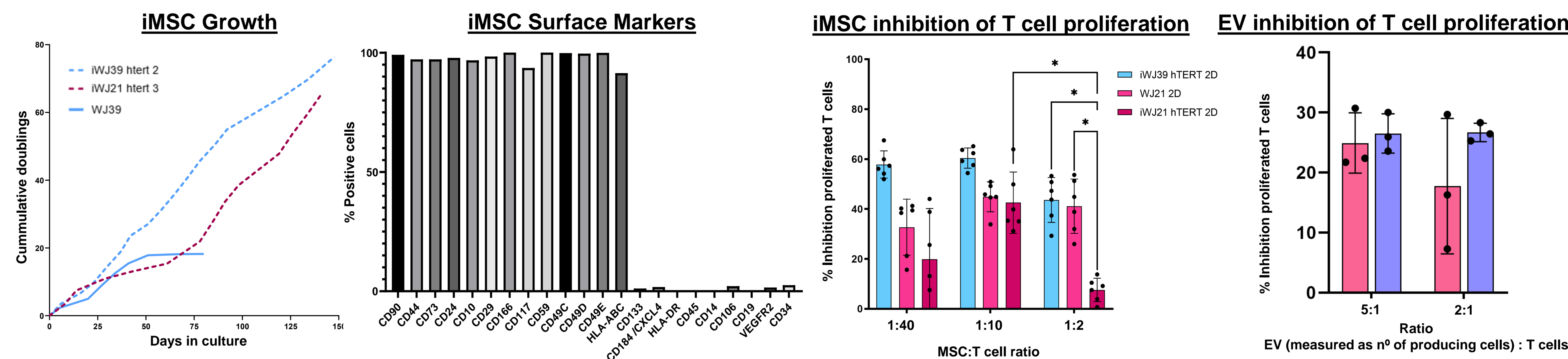


Figure 1. iMSC are comparable at phenotypic and functional levels with primary MSC, avoiding senescence mechanisms. The SEC method allows the extraction of **well-purified and defined 2D-isolated iMSC-EVs**. iMSC-EVs retain their **immunomodulatory capabilities**, with comparable T cell inhibition potency. One-way ANOVA was performed to determine statistical significance. * $p < 0.05$. One-sample T-test was performed to test statistical significance compared to the no-EVs positive control sample (not shown).

2. iMSC 3D-Culture and EV isolation

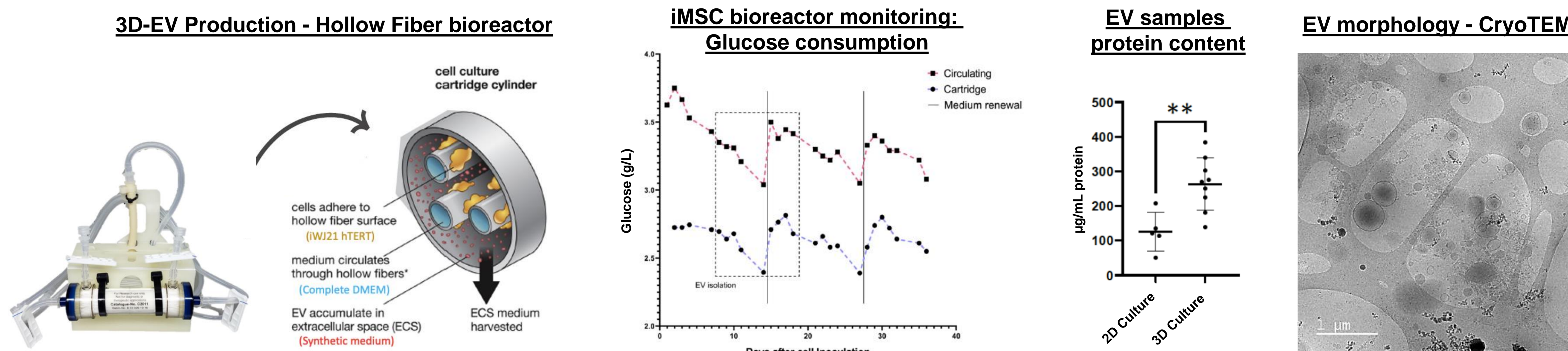


Figure 2. iWJ-MSC can be cultured in hollow fiber bioreactors using xeno-free synthetic medium. Monitoring of **glucose** in the bioreactor allows for the **monitoring of cell status**. EV can be isolated from bioreactors with **increased protein content (higher EV yield) and classical EV morphology**. Non-parametric T-test was performed to determine statistical significance. ** $p < 0.01$.

3. 3D-isolated EV characterization

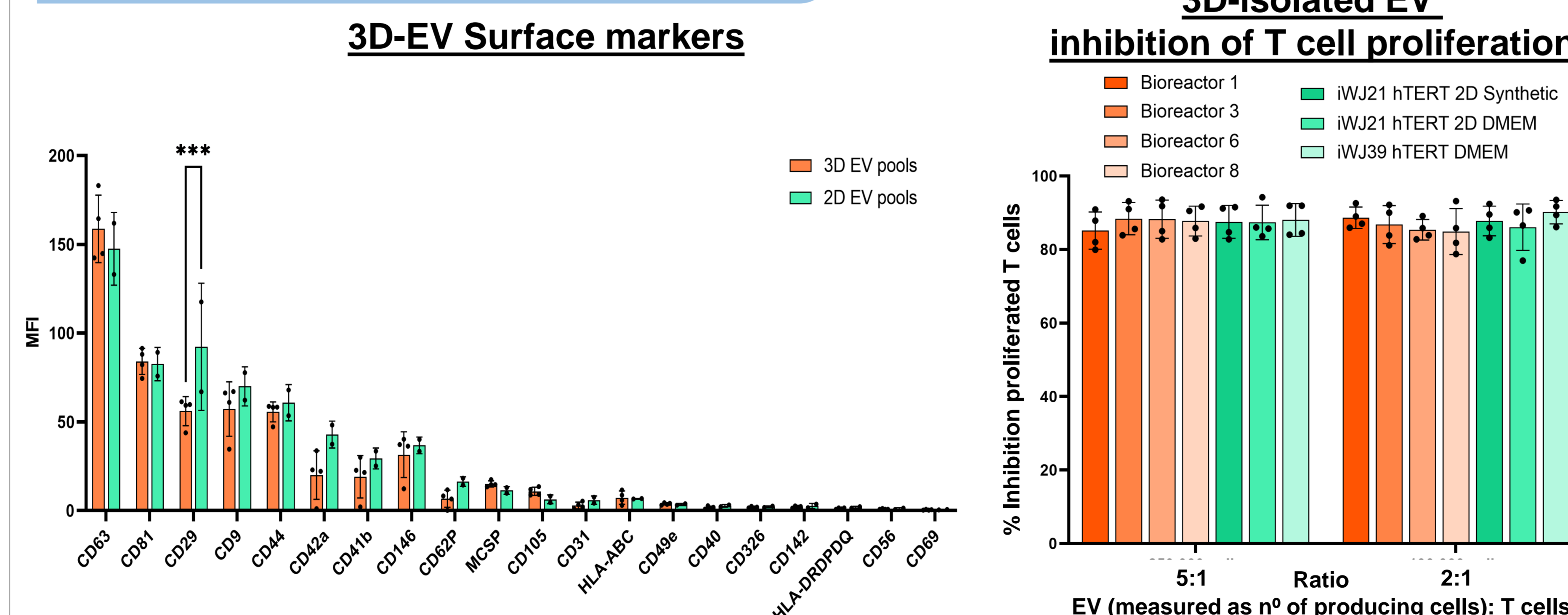


Figure 3. iMSC-EVs isolated from 3D culture maintain the classical exosome (CD9, CD63, CD81) and MSC (CD29, CD44, CD90) markers. 3D-isolated EVs also inherit the immunomodulatory capacities of iMSC, **inhibiting T cell proliferation at levels similar to those of 2D-EVs**. One-sample T-test was performed to test statistical significance compared to the no-EVs positive control sample (not shown).

CONCLUSIONS

Conclusions

- We have set up a **3D hollow-fiber bioreactor system** that allows for the obtention of **enhanced EV yields**, with **iMSC-EVs that are phenotypically and functionally comparable to 2D-EVs**.

Limitations

- iMSC exact cell number and proliferative capabilities cannot be assessed in the bioreactor. **iMSC attach tightly to fibers and cannot be recovered from the cartridge.**

ACKNOWLEDGEMENTS

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