OPTIMISING PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS FOR ENHANCED REGENERATIVE PHENOTYPE

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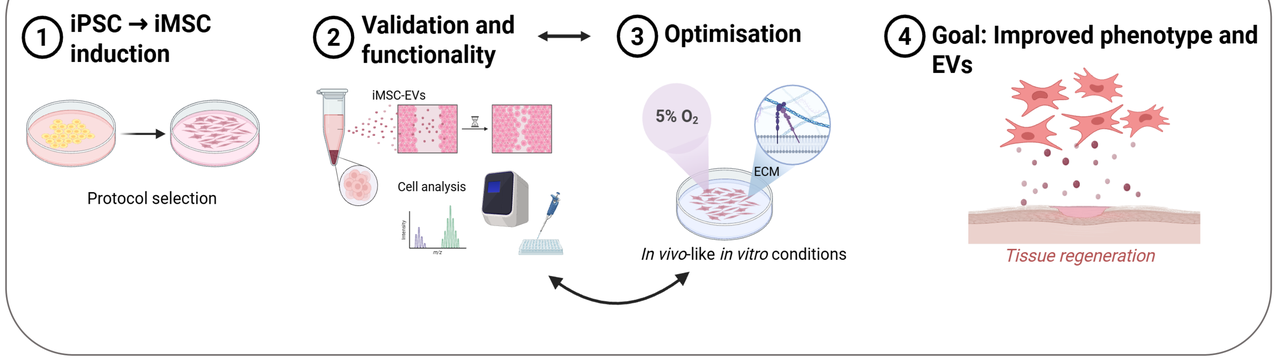
BACKGROUND

- **Mesenchymal stem cells (MSCs) and their extracellular vesicles (EVs)** hold strong potential for regenerative medicine
- Clinical use is limited by heterogeneity, senescence, and batch variation
- **iPSC-derived MSCs (iMSCs)** offer a scalable alternative, but current protocols are variable and yield inconsistent results, requiring optimisation to ensure consistent cell and EV quality.

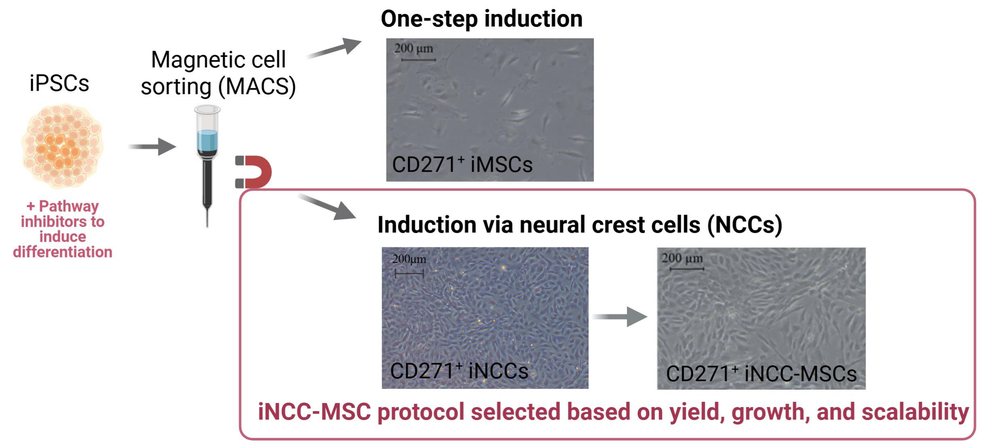
HYPOTHESIS

Mimicking *in vivo*-like conditions improves iMSC induction and functionality

PROJECT OVERVIEW



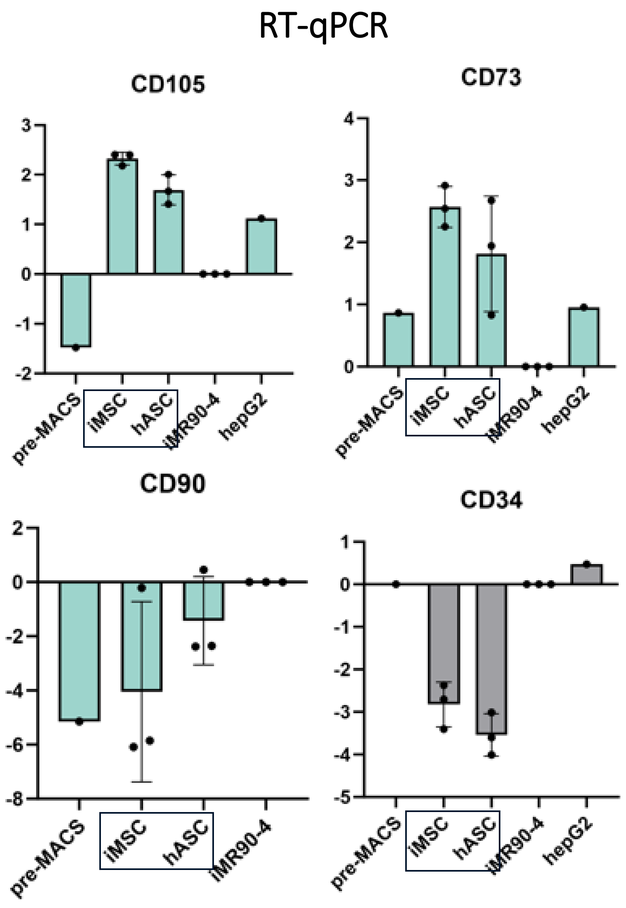
Selection and validation of iMSC induction protocol



Potential literature-based iMSC induction protocols were evaluated. A one-step inhibitor-based method[1] showed limited proliferation and low yield post-sorting. The iNCC-MSC protocol[2] was selected for its superior yield, proliferation, and scalability.

Characterisation of iNCC-MSCs

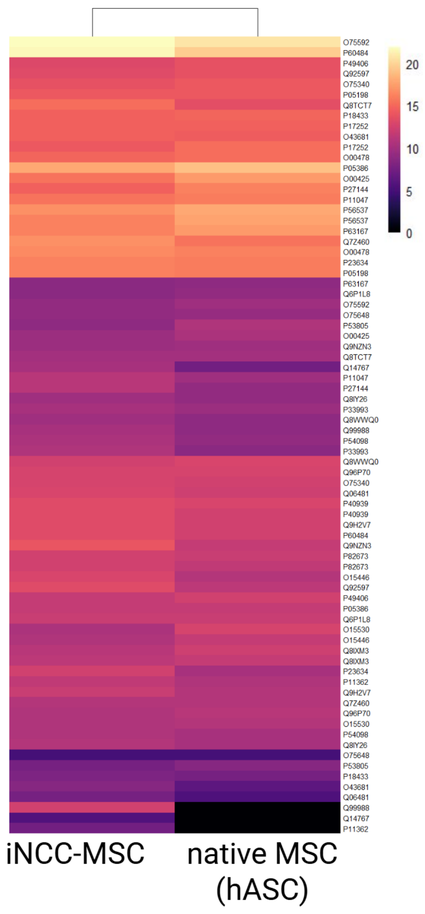
RT-qPCR



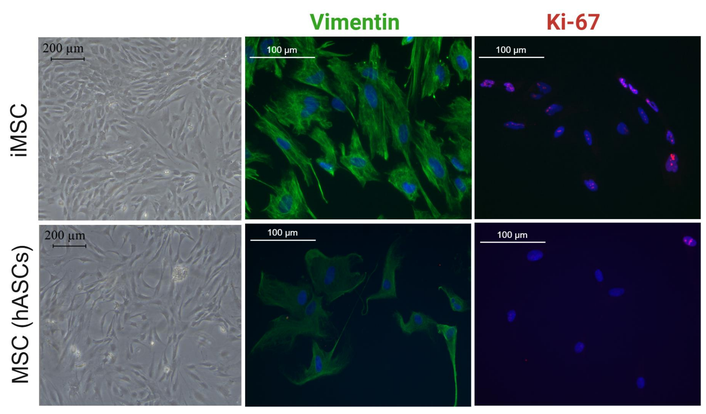
qPCR: Fold change in expression ($2^{-\Delta\Delta C_T}$) relative to iPSCs

iMSCs display MSC-like identity comparable to tissue-derived MSCs (human adipose derived stem cells, hASCs).

Proteomic heat map



Heatmap of proteins associated with MSC identity, proliferation, and regenerative pathways



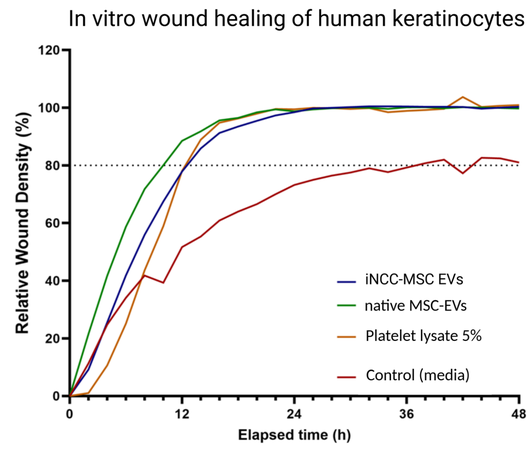
Immunocytochemistry of iMSCs revealed the presence of vimentin (green). Expression of the proliferation marker Ki-67 (red) was more prominent in iNCC-MSCs compared to hASCs.

References
1. *Leyendecker Junior, A. Stem Cells Int. (2018), 2018:1–13*
2. *Kamiya, D. et al. Npj Regen. Med. (2022) 7 (1): 47.*

Functionality of iNCC-MSC derived EVs

iMSC-EVs prior optimisation:
Comparable wound closure to hASC-EVs and platelet lysate:

Relative wound closure in human skin keratinocytes in the presence of iMSC-EVs, native MSC-EVs (hASC-EVs), platelet lysate (positive control) and untreated control (media).

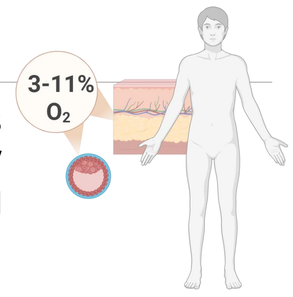


iMSC production from iPSCs mimicking MSCs *in vivo* environment

ECM optimisation

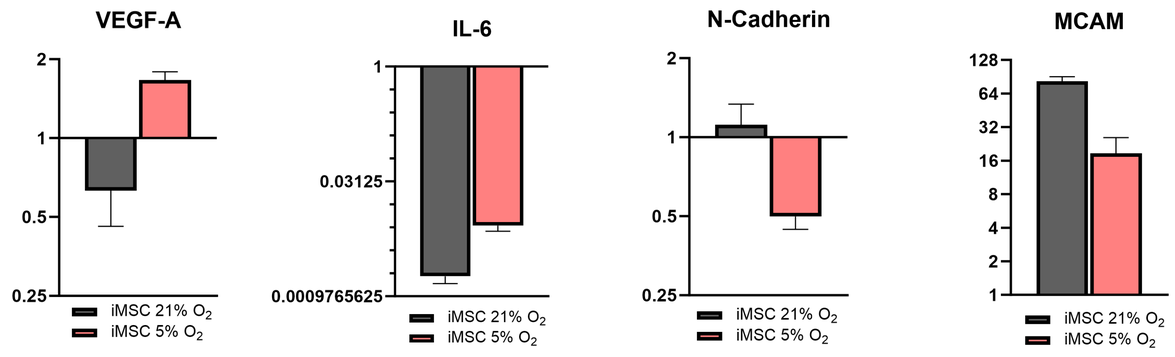
Integrin expression in tissue-derived MSCs guided the selection of protein coatings used throughout the induction. Fibronectin and laminin521 were successful compared to Matrigel, and the resulting cells maintained MSC identity as confirmed by RT-qPCR. **Fibronectin** was selected based on consistent proliferation and suitability for animal-free culture.

Physiological oxygen levels (5% O2)

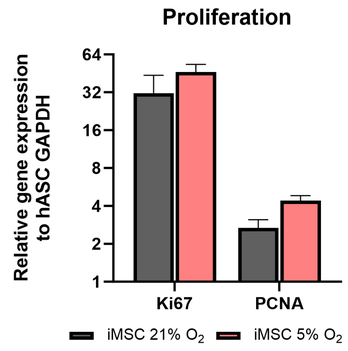


Traditional cell culture environment does not represent oxygen levels in tissues. Based on our previous findings showing enhanced MSC-EV function in hypoxic culture, iNCC-MSC **induction was performed entirely under 5% O_2** to assess effects on the resulting cells.

RT-qPCR: Functionality



RT-qPCR analysis showed stable MSC and pluripotency marker expression across conditions, comparable to hASCs. In contrast, ***Hypoxia-induced iNCC-MSCs displayed altered expression of genes associated with tissue regeneration****.*



CONCLUSIONS

Our findings highlight a promising route for generating consistent, high-yield iMSCs suitable for cell- and EV-based regenerative therapies.

Optimised culture conditions can further enhance the therapeutic potential of iMSC-derived EVs, and upcoming wound healing assays with optimised cells will help define their regenerative efficacy and translational potential.

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GENECELLNANO

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