Automated Closed-System Differentiation of iPSC-Derived Macrophages Using Prodigy and CELLA i4.0

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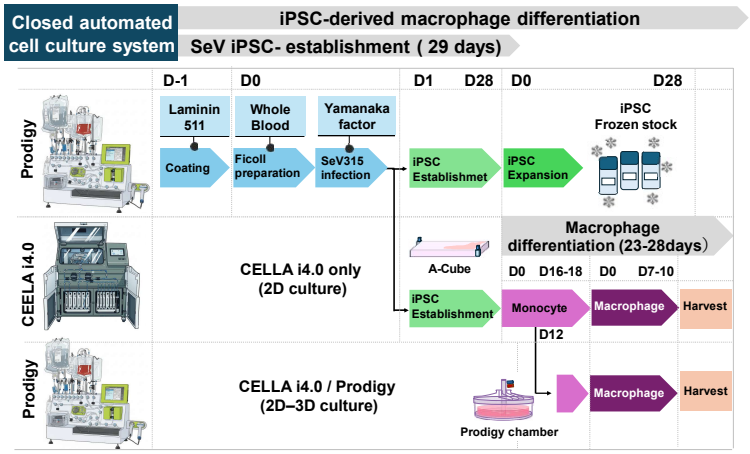
Introduction

Background : Closed-system automated culture platforms have become an important approach to improving manufacturing efficiency and reducing costs in cell production using autologous iPSCs. Our foundation has been promoting the development of a manufacturing platform based on autologous iPSCs, and methods for iPSC establishment have been reported as part of our research achievements (Shimizu et al., 2025). Efficient differentiation of macrophages from human iPSCs via a monocyte intermediate has also been reported in previously described protocols developed by Niwa and colleagues (Cui et al., 2021). However, studies applying differentiation processes from autologous iPSCs to closed-system automated culture systems remain limited.

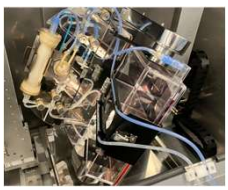
Objective : To evaluate the feasibility of implementing iPSC-derived macrophage differentiation in closed-system automated culture platforms using CliniMACS Prodigy and CELLA i4.0.

Materials & methods



iPSCs were established using the CliniMACS Prodigy system, expanded, and cryopreserved as cell stocks. In parallel, monocyte induction was performed using the established iPSCs, followed by macrophage differentiation. During the monocyte induction process, a portion of the cells was collected and transferred to the Prodigy system for macrophage differentiation.

CELLA i4.0 cultures were performed using the A-Cube® culture vessel, providing a 2D adherent culture environment, whereas CliniMACS Prodigy enables 3D suspension culture. A sequential culture strategy (2D→3D) was applied during differentiation.



A-Cube® culture vessel (CELLA i4.0)
- Surface-treated polystyrene for adherent cells
- Multilayer 2D culture surface (up to 10,000 cm²)
- Efficient expansion of adherent cells
- 10 layers ≈ 44 T225 flasks
  Single layer used in this study

Cultivation chamber (CliniMACS Prodigy)
- Polycarbonate closed culture chamber
- 3D/suspension culture with medium circulation
- Integrated automated cell processing

Result 1

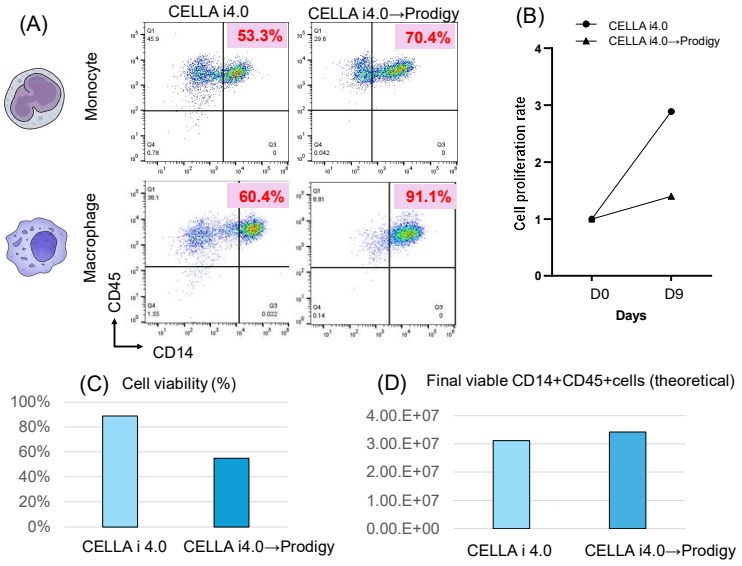


Fig1 (A) Flow cytometry analysis of monocyte and macrophage differentiation from human iPS cells using the Clini MACS Prodigy and CELLA i4.0 systems. (B) Relative cell recovery during macrophage differentiation (macrophage / input monocytes; monocytes = 1) Cell viability of macrophages obtained under each culture condition.(D) Estimated total number of $CD14^{+}CD45^{+}$ cells when all monocytes obtained under CELLA i4.0 and CELLA i4.0→Prodigy conditions were used for macrophage differentiation.

Result 2

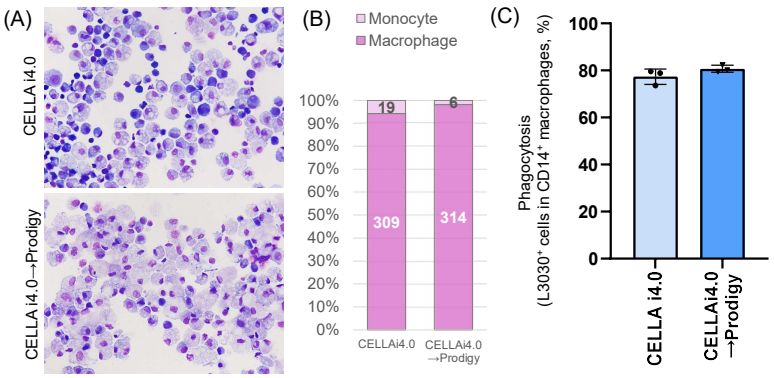


Fig. 2(A) Representative Giemsa staining images of macrophages derived from human iPS cells generated using CELLA i4.0 or the CELLA i4.0 → Prodigy sequential culture system.(B) Proportion of monocytes and macrophages determined by morphological counting based on Giemsa staining.
(C) Phagocytosis assay of iPSC-derived macrophages. The percentage of $L3030^{+}$ cells within the $CD14^{+}$ population was comparable between the CELLA i4.0 and CELLA i4.0 → Prodigy conditions.

Conclusion & Future Directions

Conclusion
- ☐ CELLA i4.0 (2D) and CliniMACS Prodigy (3D) enabled macrophage differentiation from human iPSCs.
- ☐ A 2D→3D sequential culture strategy improved macrophage induction efficiency.
- ☐ This approach supports scalable manufacturing of iPSC-derived macrophages

Future Directions
- ☐ Optimization and standardization of the automated culture process
- ☐ Development of quality control assays for iPSC-derived macrophages
- ☐ Functional characterization of iPSC-derived macrophages
- ☐ Preclinical evaluation of efficacy and safety

References & Acknowledgements

Cui D, et al. Front Cell Dev Biol. 2021;9:656867.doi: 10.3389/fcell.2021.656867
Shimizu E et al. Cytotherapy. 2025.doi: 10.1016/j.jcyt.2025.102040

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Conflict of Interest (COI) Disclosure: The authors declare no conflicts of interest related to this presentation.