Application of the Closed Systems for Automated Production of iPSCs from Human Blood

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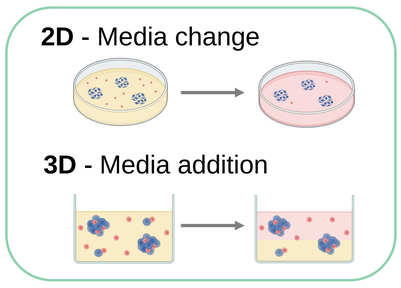
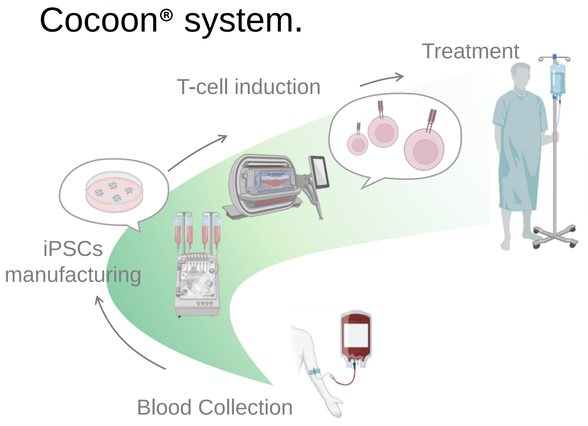
Introduction

Culturing iPSCs in a 3D system simplifies handling by eliminating the need for enzymatic dissociation during passaging, and differentiation can be initiated directly from the spheroid stage.
However, a major limitation of 3D culture is that the medium cannot be completely removed during media changes.



The closed, automated Cocoon® system integrates features of both 2D and 3D culture, providing a practical solution to this constraint. We previously established iPSCs using CD34 cell media (CD34-iPSCs) in the Cocoon® system.

Cocoon® by Lonza

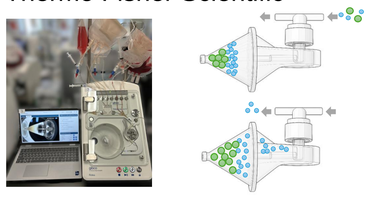


Here, we report the establishment of T cell–derived iPSCs (T-iPSCs) in this Cocoon-based workflow, which retain the original TCR and can generate rejuvenated, antigen-specific T cells with potential for personalized immunotherapy.

Materials & methods

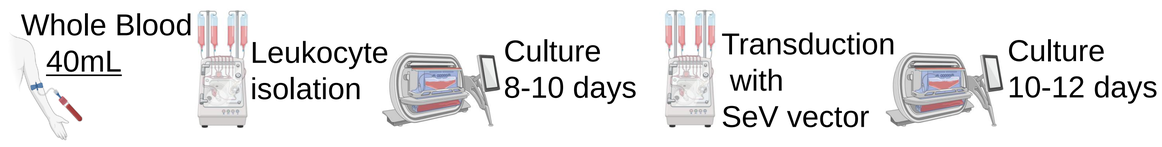
Leukocytes were isolated from 40 mL of whole blood with the Gibco™ CTS™ Rotea™ and cultured in the Cocoon ®. A total of 1.0 × 10^8 expanded T cells were then processed with the Rotea™ for transduction with a Sendai virus (SeV) vector carrying the Yamanaka factors, and subsequently cultured in the Cocoon ®.

Rotea ™
by Thermo Fisher Scientific



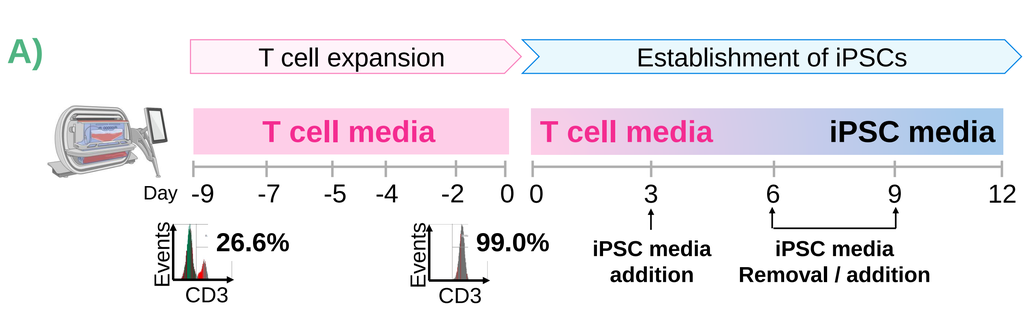
A closed, automated cell-processing platform that gently washes, concentrates, and separates cells using counterflow centrifugation.

T-iPSCs



Result ①

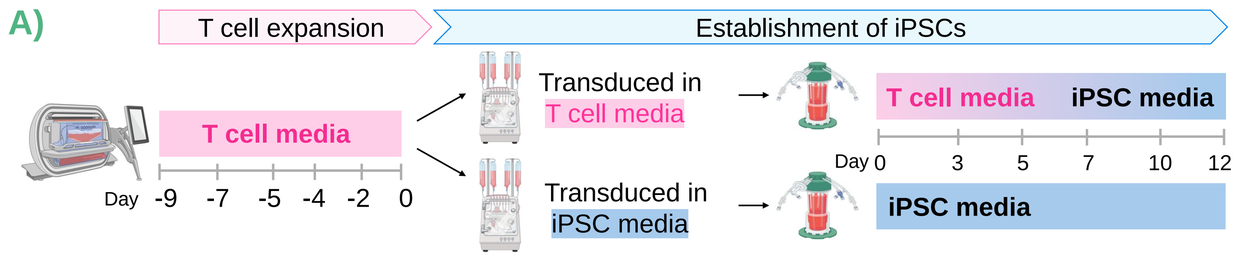
Cocoon® 1st Trial

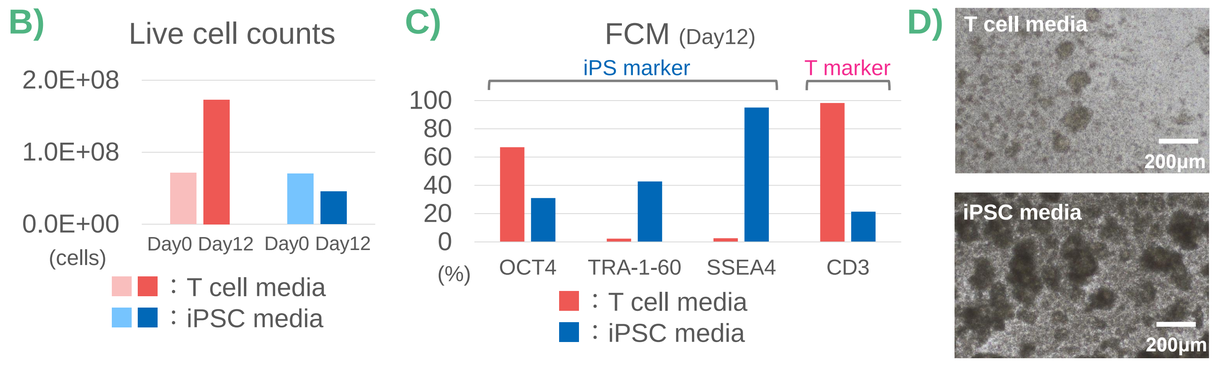


Expanded T cells were transduced with a Sendai virus vector and cultured in T cell media (Fig. A). On Day12, the number of live cells had increased 1.7-fold relative to the seeding number (Fig. B). Flow cytometry analysis showed that approximately 85% of the cells were CD3+(Fig. C).

Result ②

Switching culture media

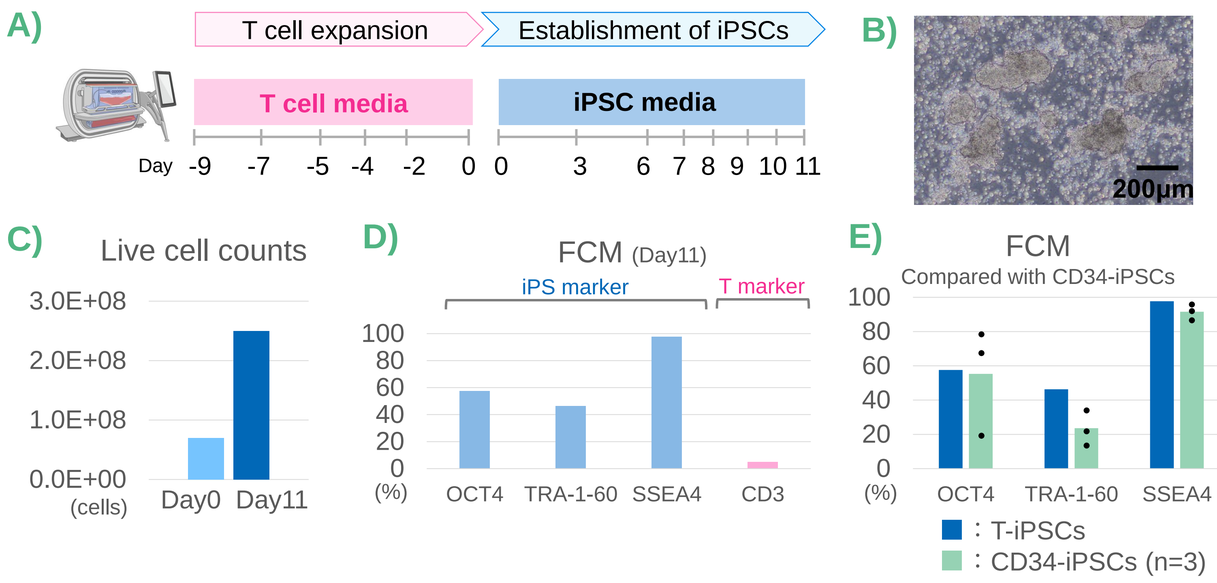




To promote iPSC dominance by suppressing CD3+ cell proliferation, we switched to iPSC media starting from the SeV transduction step (Fig. A). Under iPSC media establishment conditions, cell recovery on Day12 decreased to 0.6-fold (Fig. B), but FCM analysis showed high TRA-1-60/SSEA4 expression and low CD3 expression (Fig. C). Phase-contrast images of cells cultured under each medium condition on Day12 are shown (Fig. D). The iPSC medium condition yielded more spheroids.

Result ③

Cocoon® 2nd Trial

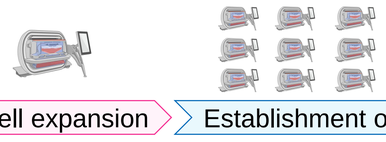


We performed the iPSC establishment in Cocoon® using iPSC media (Fig. A). Phase-contrast images of cells cultured on Day11 are shown (Fig. B). At this point, the cell number increased 3.5-fold (Fig. C). FCM analysis of iPSC marker positivity exceeded 40%, more than twice that observed in the Cocoon® 1st Trial (Fig. D). This positivity was comparable to that of CD34-iPSCs (Fig. E).

Discussion

Achievements

Successfully established T-iPSCs using the closed system
(T-iPSCs produced 58-fold more cells than CD34-iPSCs.)



Remaining challenges

Process development:
Further optimization of T-iPSC production

Characterization of T-iPSCs:
Detailed evaluation of TCR identification and differentiation potential

Acknowledgements

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