Automated, Large-Scale Targeted Differentiation of iPSCs for Cell Therapy Applications

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Introduction

Induced pluripotent stem cells (iPSCs) offer great potential as an unlimited source for regenerative therapies. However, clinical application remains challenging due to inefficient directed differentiation toward target functional cell types and labor-intensive processes that limit scalability. To overcome these challenges, scalable culture processes for neural stem cell (NSC) and hematopoietic stem cell (HSC) generation from iPSCs were established using the Quantum Flex™ Cell Expansion System (Quantum Flex, Terumo Blood and Cell Technologies).

Materials and methods

Figure 1: Culture process of NSC differentiation

- **Day −1: Coating**
  - Use small bioreactor
  - Coating reagent: Laminin 521 (BioLamina, 1 mg)
- **Day 0: Cell loading/ attachment**
  - Seed density: **1300 cells/cm²** (total 2.6×10^6 cells)
  - Medium: mTeSR Plus (STEMCELL Technologies)
- **Day 1-10: Differentiation**
  - Medium change into differentiation medium
    Differentiation medium composition:
    StemFit AK03N (Ajinomoto, without C solution)
    + 2 μM SB431542 + 2 μM DMH1
    (FUJIFILM Wako, 037-24293, 041-33881)
  - Perform medium change every other day
  - Automated metabolism monitoring by Bioprofile FLEX2 (Nova Biomedical) integrated with Quantum Flex
- **Day 10: Harvest**
  - Dissociation reagent: Accutase (STEMCELL Technologies) (60 mL, 4 min) → Collect **4.2×10^8 cells** (average)

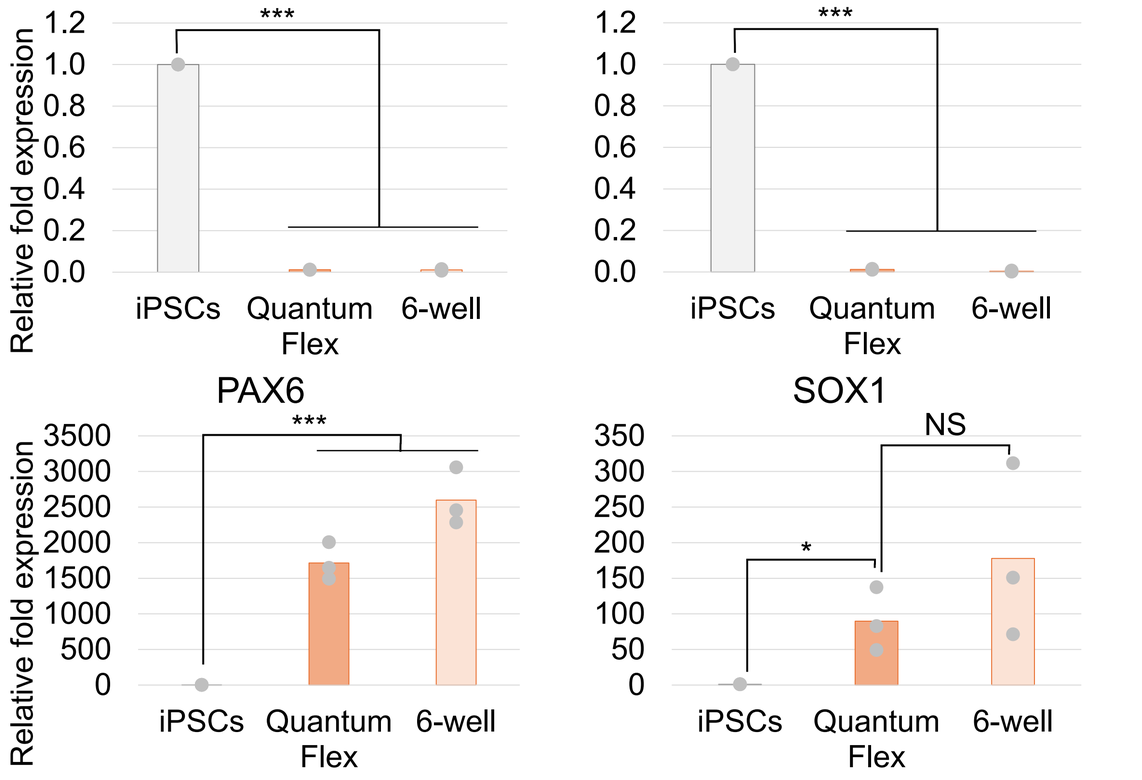
Figure 2: Culture process of HSC differentiation

- **Day −1: Coating**
  - Use small bioreactor
  - Coating reagent: Laminin 521 (BioLamina, 1 mg)
- **Day 0: Cell loading/ attachment**
  - Seed density: **500 cells/cm²** (estimated, total 2.0×10^6 cells)
  - Seeding with cell circulation inside and outside the reactor
  - Medium: mTeSR Plus (STEMCELL Technologies)
- **Day 1-3: Expansion**
- **Day 3-13: Differentiation**
  - Medium change into STEMdiff Hematopoietic Kit (STEMCELL Technologies, 05310)
  - Culture according to the official protocol
  - Automated metabolism monitoring by BioProfile FLEX2 (Nova Biomedical) integrated with Quantum Flex
- **Day 13: Harvest**
  - Collect suspension cells
    → Collect **1.8×10^7 cells** (live cells, average)

Results

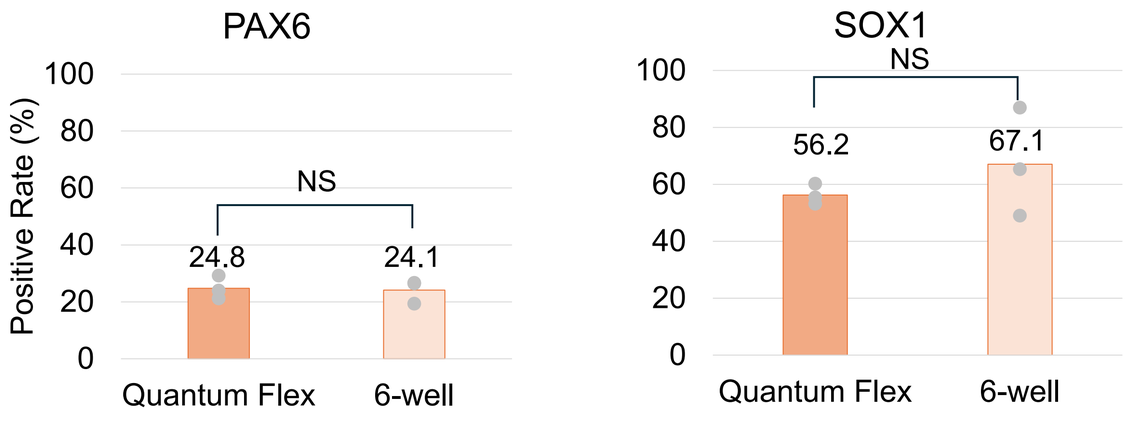
Figure 3: The quality of NSCs differentiated in Quantum Flex

(A) The results of the mRNA expression



(ΔΔCt method; n = 3, t-test; *: $P < 0.05$; **: $P < 0.005$; ***: $P < 0.0005$; NS: not significant)

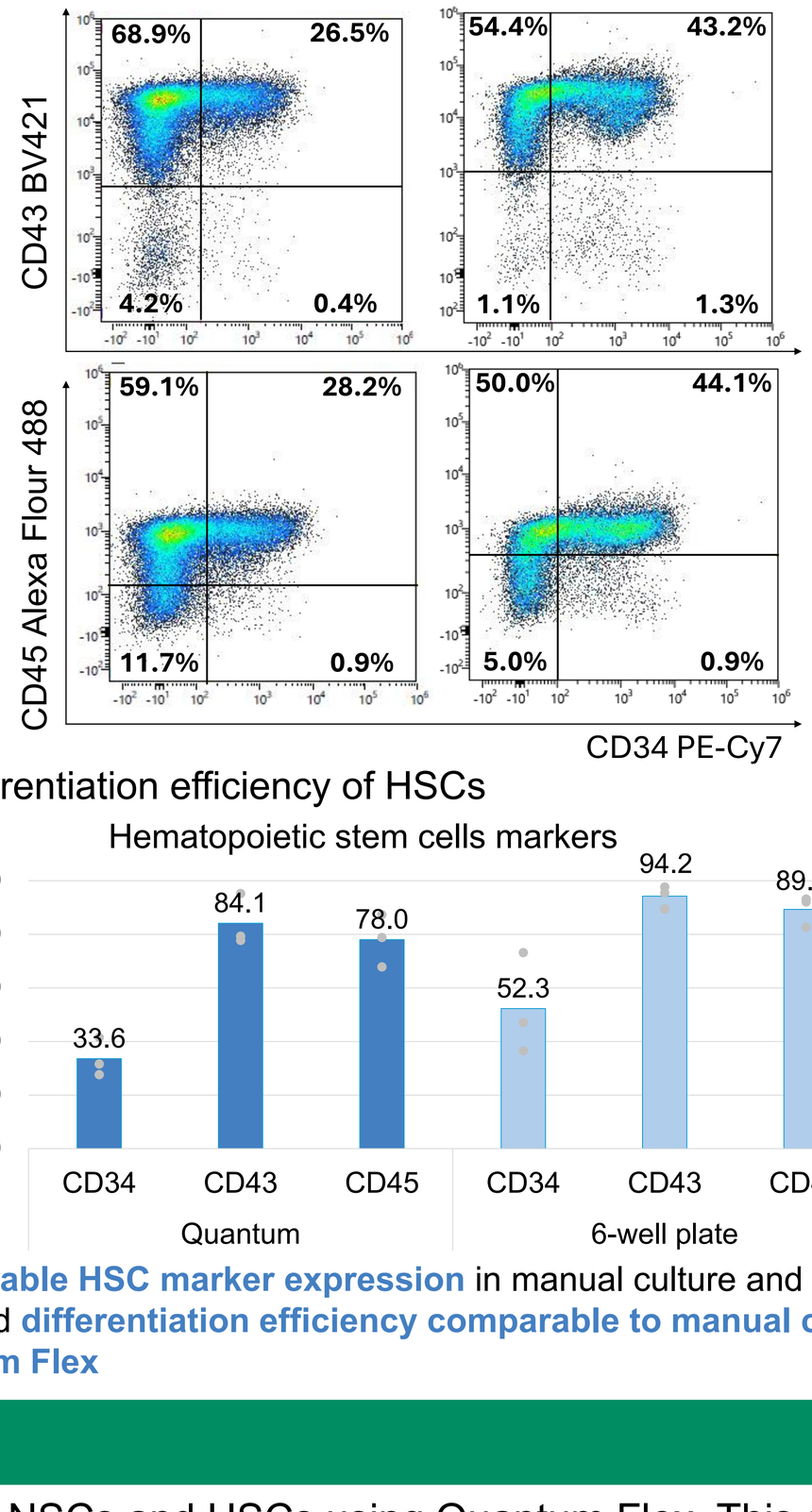
(B) Differentiation efficiency of NSCs



- ✓ Confirmed **loss of pluripotency markers and induction of NSC markers**
- ✓ Achieved **more consistent differentiation efficiency with Quantum Flex** compared with manual culture

Figure 4: The quality of HSCs differentiated in Quantum Flex

(A) Flow cytometric analysis of HSCs



(B) Differentiation efficiency of HSCs

- ✓ **Comparable HSC marker expression** in manual culture and Quantum Flex
- ✓ Achieved **differentiation efficiency comparable to manual culture with Quantum Flex**

Conclusion

We established a robust and scalable protocol for differentiating iPSCs into NSCs and HSCs using Quantum Flex. This system lays the groundwork for expanding future differentiation applications and advancing clinically relevant cell manufacturing.

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