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

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Introduction

Induced pluripotent stem (iPS) cells offer great potential as an unlimited source for regenerative therapies. However, their clinical use remains constrained by two key challenges: achieving efficient differentiation into target cell types and scaling production to clinically relevant quantities. Here, we present a large-scale protocol for generating neural stem cells (NSCs) from iPS cells using the Quantum Flex™ Cell Expansion System (Quantum Flex, Terumo BCT).

Materials and Methods

Figure 1: Culture process

Day −1: Coating
- Coating reagent: Laminin 521 (Biolamina, 1 mg)

Day 0: Load/attach cells
- Seed density: 1300 cells/cm^2 (total 2.6 × 10^6 cells)
- Medium: mTeSR Plus (STEMCELL Technologies)

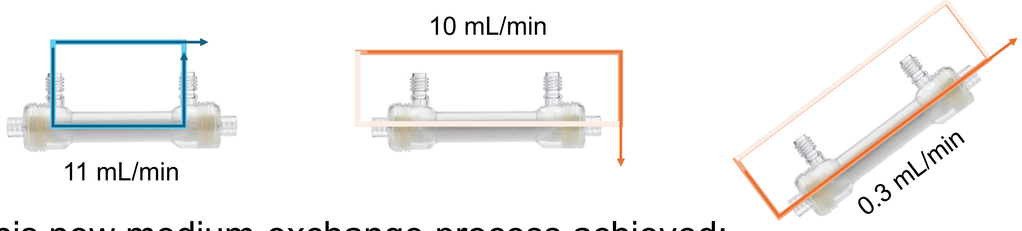
Day 1: Start differentiation
- Medium change into differentiation medium
  Differentiation media composition:
  StemFit AK03N (Ajinomoto, without C solution)
  + 2 μM SB431542 + 2 μM DMH1 (FUJIFILM Wako, 037-24293, 041-33881)

Day 1-10: Differentiation
- Perform medium change every other day using new protocol (Figure 2)
- Automated metabolism monitoring by Bioprofile FLEX2 (FLEX2, Nova Biomedical) integrated with Quantum Flex
- Dissociation reagent: Accutase (STEMCELL Technologies) (60 mL, 4 min)

Day 10: Harvest

Figure 2: Medium replacement protocol

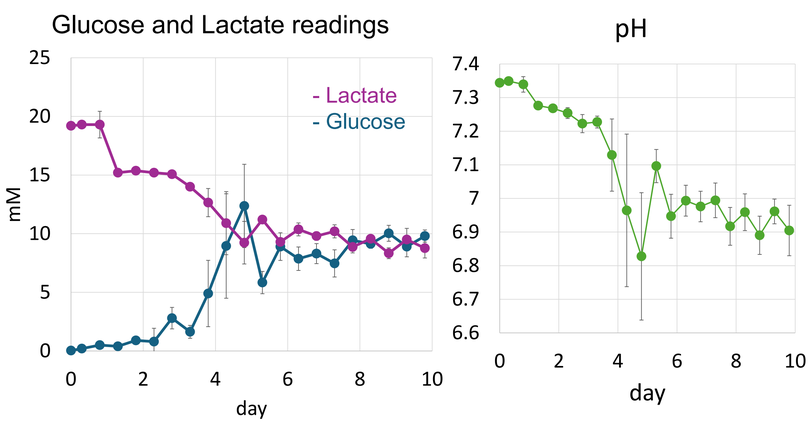
Step 1: Extracapillary circuit → Step 2: Intracapillary circuit (reactor exterior) → Step 3: Intracapillary circuit (reactor interior)



This new medium-exchange process achieved:
- ✓ **Minimal cell loss** during medium exchange (< 1.5%)
- ✓ **High medium-exchange efficiency** (> 90%)
- ✓ **Rapid medium exchange** (3.5 h)

Results

Figure 3: Monitoring by FLEX2



- ✓ Integration with FLEX2 enabled high-frequency auto monitoring that **simplified culture condition management**

Figure 4: mRNA expression

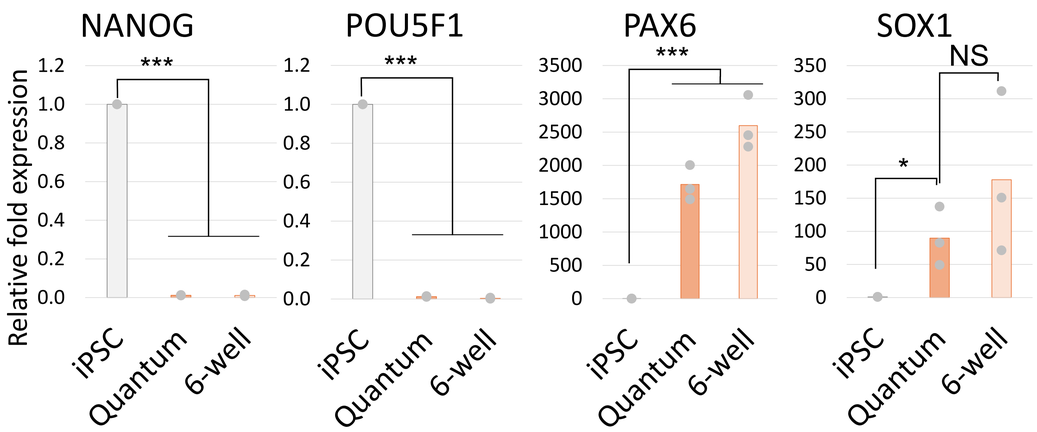
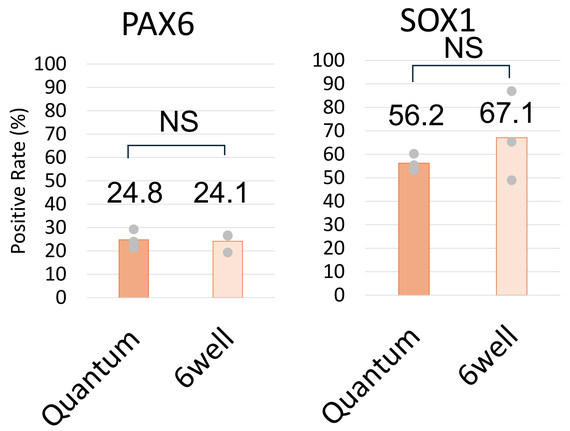


Figure 5: Flow cytometry



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

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

Conclusion

We established a robust and scalable protocol for differentiating iPS cells into neural stem cells using the Quantum Flex system. This platform lays the groundwork for expanding future differentiation applications and advancing clinically relevant cell manufacturing.

TERUMO BLOOD AND CELL TECHNOLOGIES

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