Pluripotent Stem Cell Expansion In QUANTUM FLEX Cell Expansion system

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Abstract

Aim

Use Minimal Feed to achieve iPS cell expansion with less media, demonstrate scale up, and automate metabolite monitoring.

Method

iPSCs were expanded in Quantum Flex Cell Expansion System [Terumo BCT] integrated with Bioprofile®FLEX2 [Nova Biomedical] for automated monitoring.

Conclusion

iPSCs showed effective expansion and linear scaling in both small and standard bioreactors of Quantum Flex integrated with an auto-sensing device, FLEX 2.

Material & Method

Quantum Flex Cell Expansion System
Automated/Closed cell expansion platform

Two sizes of bioreactors
Small (Sm, **0.1x**)/Standard (St, **1x**)

1 week — Thawing/reviving cells

1 week — Acclimation to mTeSR

Day -1 Coating Laminin521
Sm: 1 mg, St: 5 mg

Day 0 Load/Attach
Seed density: **2600 cells/cm²**
Sm: 5.2E+06 cells, St: 5.5E+07 cells
Attachment: Overnight

Day 1-7 Expansion

Quantum Flex

FLEX2 (Nova Biomedical)

Minimal Feed protocol
Focuses on minimizing feed flow
↔**Sufficient Feed protocol**
Focuses on maintain pH stability
Metabolism measurment by FLEX2
Auto-sampling: every 12 hours

Day 7 Harvest
Accutase Sm: 60 mL, St: 180 mL

Study 1: iPSCs expansion in small bioreactor
To optimize growth and media consumption, the method of medium supply was changed from **Minimal Feed (MF)** to **Sufficient Feed (SF)**, and the impact on collection number and cell quality was assessed.

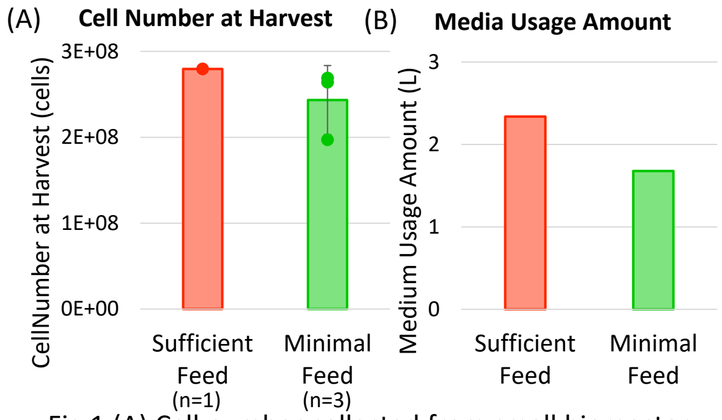
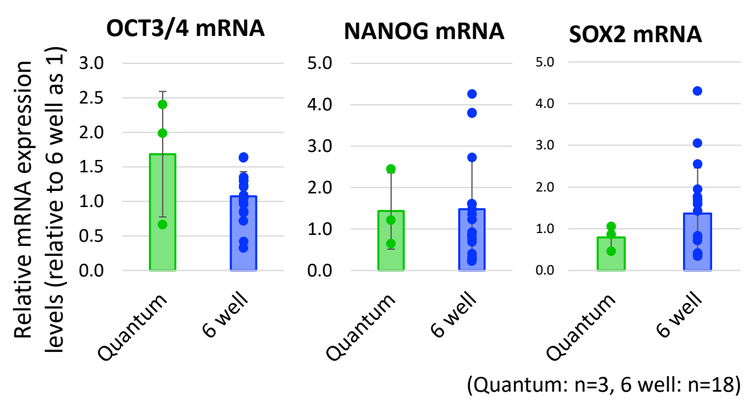


Fig.1 (A) Cell number collected from small bioreactor. (Averarage viability: SF 76.6%, MF 95.2%)
(B) Media usage amount of each feed strategy. Minimal Feed reduces media usage by 28.2%.

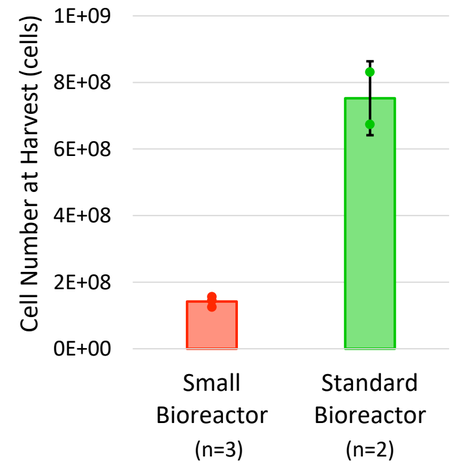


(Quantum: n=3, 6 well: n=18)

Fig.2 The expression level of pluripotent markers. iPSCs cultured with MF showed equivalent levels of pluripotency markers compared to the control and no chromosomal abnormalities were detected (KaryoStat data, not shown).

The Minimal Feed protocol achieves a culture that maintains pluripotency while reducing medium consumption.

Study 2: iPSCs expansion in standard bioreactor
To demonstrate linear scale up across Quantum bioreactor sizes, it was compared with the small and standard bioreactors integrated with **automated monitoring system.**



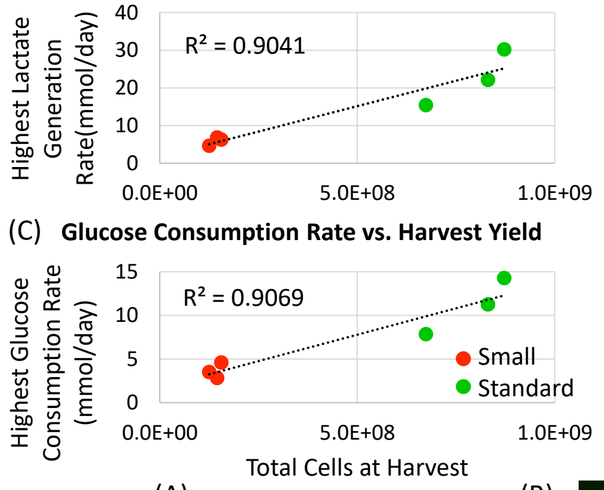


Fig.3 (A) Cell number collected from small bioreactor. (Average viability: Sm 97.8%, St 94.1%) (B,C) Correlation of cell nember at harvest with (B)grucose consumption rate(GCR) and (C)lactate generation rate (LGR) on harvest day. GCR and LGR showed a strong correlation with the number of harvested cells and showed similar metabolism in both small and standard reactors.

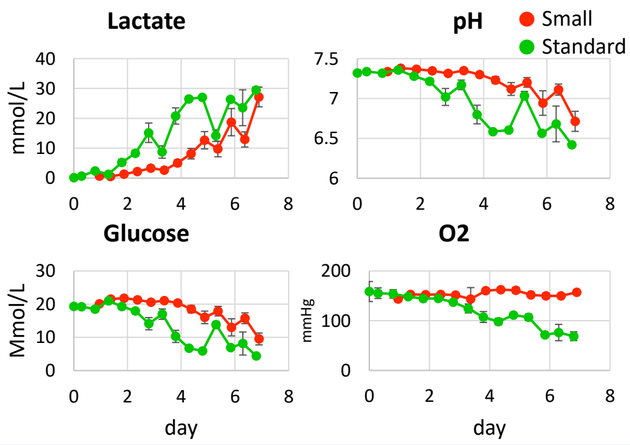
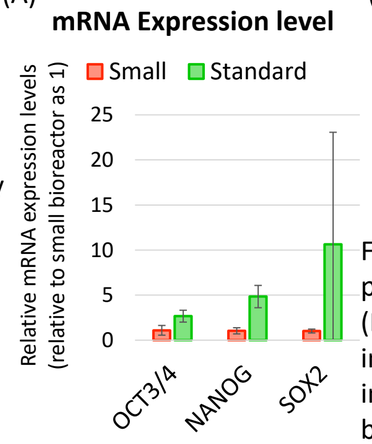


Fig.4 Media monitoring data taken by FLEX2. Automatic sampling was performed every 12 hours, measuring metabolites, pH, gases, and ions. The graphs show representative parameters.



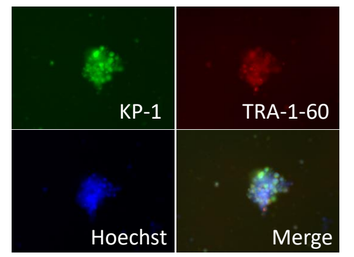


Fig.5(A) The expression level of pluripotent markers (Sm: n=3, St: n=2) (B) Fluorescence immunostaining images of KP-1, TRA-1-60 expression in single cells collected from standard bioreactor.

The process developed in the Small Bioreactor was successfully scaled up to Standard bioreactor. Integration with FLEX2 enabled automated metabolite monitoring throughout the culture.

CiRA Foundation

Established to bridge technologies such as iPS cell production and quality evaluation to the industry. CiRAF is promoting two projects, **iPS Stock Project** (allogeneic) and **my iPS Project** (autologous).