Optimizing Human Pluripotent Cell Culture Differentiation, and Reprogramming Using Callisto, an Automated Cell Culture System

T. Guo1, S. Boutet1, J. Gibson2, M. Watson1, N. Devaraju1, J. Davila3, C. Gifford4,5,6, G. Harris1, Y. Lu1, G. Sun1, M. Unger1, D. Srivastava4,5,6, M. Wernig3, C. Nelson2, N. Li1

1 Fluidigm Corporation, 2500 Sand Hill Court, South San Francisco, CA, USA
2 Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA
3 Department of Stem Cell Biology and Cancer Medicine, University of California, San Francisco, CA, USA
4 Department of Pediatrics and Developmental Biology, University of California, San Francisco, CA, USA
5 Department of Pediatrics and Developmental Biology, University of California, San Francisco, CA, USA
6 Department of Pediatrics and Developmental Biology, University of California, San Francisco, CA, USA

Introduction

In recent years, advances in the stem cell field have opened up many opportunities for disease modeling and cell-based therapies. However, finding the optimal condition for cell expansion, differentiation, and reprogramming remains laborious and costly. To address these challenges, we have developed an automated cell culture system for cell manipulation with environmental control. Using this system, we have developed a protocol for reprogramming human somatic cells into pluripotent cells by viral infection. Analysis of pluripotency gene expression demonstrates efficient reprogramming of human fibroblasts on an integrated fluidic circuit (IFC), similar to the results on a standard well plate. Reprogrammed cells can be exported live from individual chambers and replated onto standard plates for expansion. Furthermore, on-IFC differentiation assays can be used to confirm pluripotency of the reprogrammed cells, and to optimize conditions for differentiation. Multiple commercially available differentiation kits have been tested. In summary, the automated microfluidic platform employs precise control of the microenvironment of cells, facilitates studies of multifactorial combinations, and enables development of robust, reproducible, and chemically defined cell culture and manipulation.

Results

Figure 1. Major system components

The major components of the Callisto system include: (A) an IFC (integrated fluidic circuit) to provide fluid paths and cell culture microchambers for cell seeding and treatment; (B) an instrument to provide thermal, pneumatic, and environmental gas, humidity control of the IFC to enable long-term cell culture and dosing; (C) software to design, monitor, and record experiments; and (D) a reagent kit to support cell loading, live harvest, and lyse and harvest.

Figure 2. Efficient transduction of human fibroblast by Sendai virus induces early pluripotent marker expression

One week after virus infection, small colonies started to appear on the IFC. Live staining with TRA-1-81 (green) and SSEA4 (red) on day 10 confirmed the emerging colonies were positive for pluripotent cell markers (A). Fixed staining with OCT4 (green), SOX2 (red), and NANOS (magenta) at the same time also confirmed that high numbers of cells on IFC were converted to pluripotent stem cells (B). Reprogrammed cells can be exported from individual chambers through live harvest and replated in tissue culture plates for expansion. We showed that live cells harvested at different time points during reprogramming can be expanded on feeder layer or under feeder-free conditions (C).

Figure 3. Gene expression analysis of reprogramming process

To monitor gene expression dynamically, we lysed cells from selected Callisto culture chambers and performed qRT-PCR. Pluripotent genes OCT4 and SOX2 show strong expression 1 day after virus infection indicating efficient induction. The mRNA levels of multiple pluripotent genes stabilized from day 3 to day 8 (A). Principle component analysis (PCA) shows reprogrammed cells clusters away from the starting cell types (BJ and HFF (human foreskin fibroblasts)) (B).

Figure 4. Colonies formed on IFC and expanded in well plates

Figure 5. Retrovirus-mediated reprogramming of multiple cell types into hiPSCs

Human BJ fibroblasts, endothelial cells (HUVEC), or smooth muscle myocytes (HSMM) were infected on Callisto with different dose of a polystratice virus mediating the expression of OCT4, SOX2, KLF4 and c-MYC. Seven days post infection, cells were live-stained respectively with antibody against SSEA4 (A). Partially reprogrammed cells can also be live-harvested four days post infection and expanded and matured in standard 6-well plates (B). Analysis of pluripotency gene expression demonstrates an efficient reprogramming of BJ’s on IFC similar to the one in the standard well plate (C).

Figure 6. Differentiation of hiPSC toward definitive endoderm

Many stem cell applications involve differentiating cells into one of the three germ layers. The Callisto system allows easy adaptation and optimization for differentiation protocols. Here we show that using a commercial differentiation kit (Thermo Fisher Scientific) hiPSCs can be robustly differentiated into definitive endoderm on Callisto as indicated by BRACHYURY (T) (red) and SOX17 (green) staining.

Figure 7. Differentiation of hiPSC toward cardiomyocytes

The Callisto system allows long-term cell culture and three-day hands-free operation, suitable for long differentiation protocols. By adapting a Wnt antagonist-based protocol2, we were able to differentiate hiPSCs into cardiac progenitors expressing GATA4 (green) and HAND1 (red) at day 6 (A). A calcium-sensing GFP-reporter hiPSC line3 clearly demonstrated the generation of contracting cardiomyocytes on IFC (B). Tropinin (green) and NKX2.5 (red) staining confirmed cardiomyocyte identity (C). Strong green fluorescence associated with each contraction can be monitored. Fluorescence intensity pseudocolor is shown in (D).

Figure 8. Differentiation of hiPSCs into neurons using NGN2 lentivirus

We also demonstrated that differentiation of hiPSC to neurons using virus expressing nR2 and a virus expressing eGFP, NGN2, and purumycin resistance gene as a fusion protein linked by P2A and T2A sequences and driven by a TetO promoter4. hiPSC cells were infected with different doses of lentivirus stocks and the expression of NGN2 was induced for 24 hours, selected with puromycin for an additional three days, and cultured in neuronal medium to allow for maturation of the induced neurons for three days. At day 8, the cells were fixed and stained on IFC using MAP2 and βIII-tubulin antibodies with DAPI as nuclear staining.

Conclusion

- The Callisto system allows long-term cell maintenance, precise cell treatment, and flexible and dynamic analysis using methods such as live staining, fixed staining, lyse and live harvest.
- We have developed a streamlined workflow for efficient reprogramming of different human somatic cell types into pluripotent stem cells. The process is robust and highly efficient.
- The flexibility of the Callisto system also allows easy setup for stem cell differentiation. It serves as an automated platform for validating and optimizing commercial reagent kits as well as developing new protocols.

References