Automation for iPSC Processing in Cell Therapy Development: A Closed System Approach.

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Abstract

Induced pluripotent stem cells (iPSCs) hold great promise in the field of regenerative medicine and biomedical research. iPSCs provide a flexible platform for diverse clinical developments that have incredible potential to address scalability issues and help improve patient outcomes. In the work described here, we cultured and expanded iPSC up to 3 billion cells in a 10-layer Nunc™ Cell Factory ™ System (CF) to mimic large scale adherent cell culturing for iPSC master bank preparation. Manual processing and harvesting in 10-layer cell factory system can be labor intensive and prone to contamination. Utilizing Gibco CTS™ Rotea™ counterflow centrifugation system can help minimize human intervention at multiple stages, including removal of media, washing of cells, addition of cell detachment media, collection and concentration of iPSCs, buffer exchange to resuspend into cryoprotectant reagent and delivery into final collection bags. Using this closed system, we can process and harvest large number of iPSCs in a single batch to create a master bank for downstream cell therapy development. The iPSCs prepared using this method retain pluripotency characteristics with good viability and expansion capability.

In summary, using the Gibco™ Rotea™ CTS™ Counterflow Centrifugation System reduces manual touch points thereby helping minimize the risk of contamination in large scale iPSC expansion and master banking. The closed automated processing of cells described here will be useful for incorporation into cGMP-compliant clean room manufacturing environment.

Introduction

iPSCs can be differentiated into various cell types, such as neurons, heart cells, or pancreatic cells, that can be transplanted into patients to replace damaged or diseased tissues. iPSC based derived cell therapy approach has shown promise in preclinical and early clinical trials for conditions like spinal cord injury, heart disease, and diabetes. Allogeneic cell therapy for cancer treatment is a developing field, and in very recent years, iPSCs have drawn unique interest for the development of clinical manufacturing of CAR-NK and CAR-T cells. iPSC culture and differentiation involves multiple steps with several manual touch points. To circumvent the drawbacks of human handling necessary during cell and gene therapy workflows, automation needs to be improved and there are currently limited closed automated solutions available. Utilization of closed automated instrument systems like Rotea can minimize the number of manual steps at multiple stages of iPSC processing thereby helping reduce processing time and human error related quality risks.

Cell therapy manufacturing: automated iPSC processing.

Challenge

- Maintain pluripotency and viability of sensitive iPSCs during processing.
- Reduce the risk of contamination during processing
  - Expansion
  - Harvest
  - Scale up to 3x10^9 iPSCs
- Labor intensive workflow

The answer: CTS Rotea System

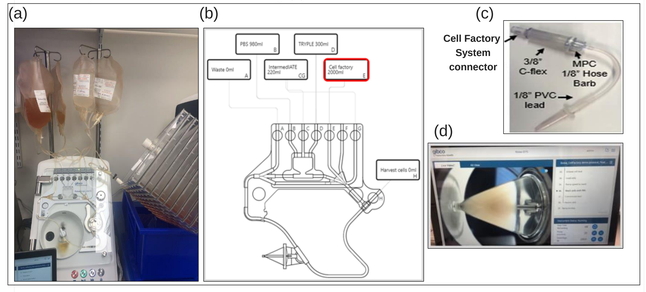
- Closed, automated cell processing system.
- Cell harvesting via direct-weld of the CTS Rotea system to a large-scale cell culturing system.
- Efficient, automated wash and cell concentration up to 3 billion iPSCs per system loop.
- Maintenance of iPSC cell viability and pluripotency during cell processing.

Table 1 Cell culture method: 2 million iPSCs from frozen vials were expanded up to 3 billion in Nunc™ Cell Factory™ system (CF system).

Week 1			Week 2	Week 3	
Thaw frozen vials containing 2 Million iPSCs	**Culture and stabilize iPSC growth**	**Culture 3 Million cells in T75 flask**	**20-30 Million cells directly passed into 2 stacks cell factory system**	**200 Million cells passed into 10 stacks cell factory system.**	**Use CTS Rotea system for iPSC harvest and wash/concentrate**
Post thaw allow 1 week for iPSCs to recover for better growth	3 days of expansion 6 well plate	3 days of expansion up to 20-30 Million cells in one T75 flask. Check for cell confluency. Should be >70% before next passage	3-4 days of expansion up to 200 Million cells in two stacks CF system. Check for cell confluency. Should be >70% before next passage	3 days of expansion up to 3 billion cells	iPSC characterization for pluripotency. Use intracellular and extracellular pluripotency markers with/without CTS Rotea system process.

Reference for Pluripotent Stem Cell Scale Out: https://www.ncbi.nlm.nih.gov/books/NBK571710/

Figure 1. Direct, closed-system harvesting of iPSCs. The 10-layer cell factory system was connected to the single-use kit with custom tubing assembly (a). Single use kit diagram after the bags have been connected in line with the respective tubing (b). Customized tubing system to connect cell factory system and CTS Rotea system single use kit (c). iPSCs collected in CTS Rotea system cone before harvesting (d).



Results:

Figure 2. iPSC Pluripotency Characterization. iPSCs were cultured in 10-layer cell factory (CF) system. After reaching 70% confluency, iPSCs were processed and harvested by using CTS Rotea system. Expression of pluripotency markers (CD30 and TRA1-60) were determined on the same day pre and post processing with CTS Rotea system. The upper panel represents the iPSCs with no CTS Rotea system processing and the lower panel represents the iPSCs with CTS Rotea system process. Viable cells gating (a), expression of CD30 pluripotency marker (b) and the expression of TRA1-60 pluripotency marker (c).

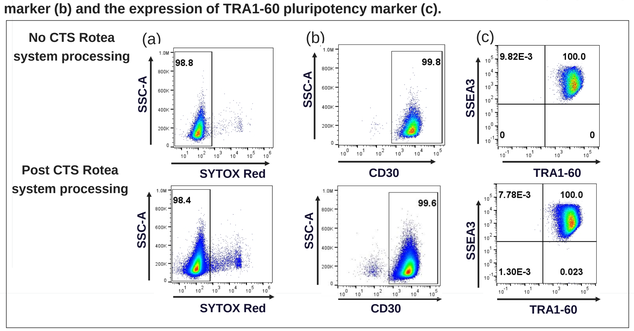


Figure 3. Additional iPSC pluripotency characterization post iPSC expansion in CF system and CTS Rotea system processing. The iPSCs were fixed and permeabilized for cytoplasmic protein (OCT4) evaluation. Flow analysis data shows the selection of iPSCs by forward versus side scattered gating (a). Pluripotency was assessed using SSEA4 (b) and OCT4 expression (c). The upper panel and lower panel represent iPSCs processed without or with CTS Rotea system respectively .

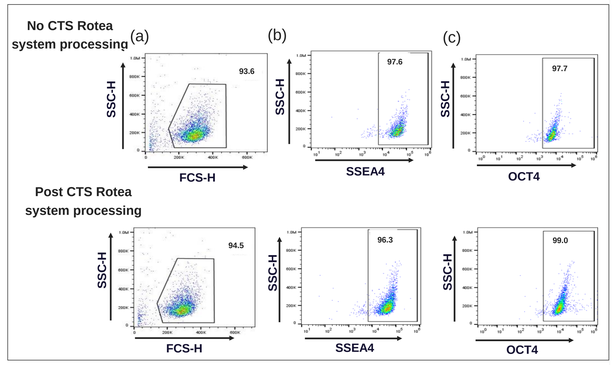


Figure 4. CTS Rotea system processed iPSCs maintained colony forming characteristics similar to manual processed iPSCs. CTS Rotea system processed and manual processed iPSCs were cultured for 4 days for iPSC colony formation. Resulting iPSC colonies were imaged by microscope. Manual processed iPSC colony image (a) and CTS Rotea system processed iPSC colony image (b).

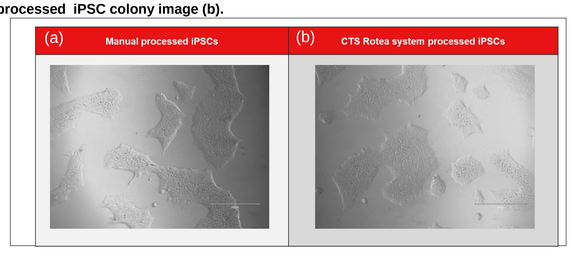


Figure 3. iPSC pluripotency characterization by immunocytochemistry. The CTS Rotea system processed and manually processed cells were cultured and expanded for three weeks. IPSCs were then processed for immunocytochemistry staining. The processed cells were imaged using fluorescent microscopy for protein expression. Green and red fluorescence represent SSEA4 and OCT4 pluripotent protein expression respectively. Manual processed iPSC colony images (a) and CTS Rotea system processed iPSC colony images (b).

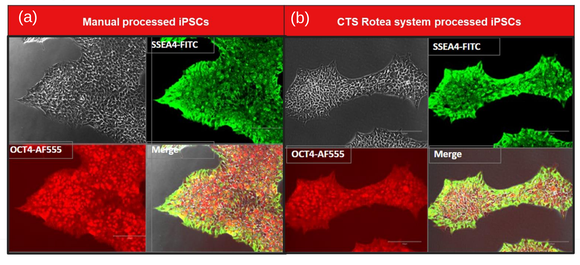
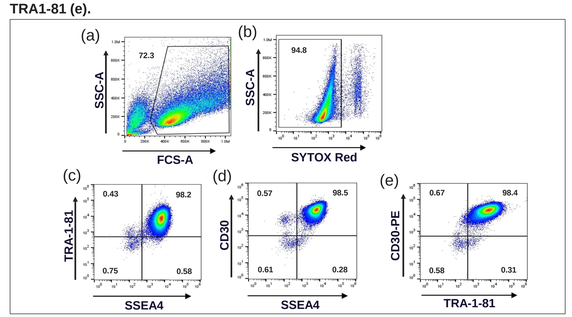


Figure 5. iPSC pluripotency characterization post three-weeks of cell expansion. CTS Rotea system processed and manually processed iPSCs were cultured for 4 weeks. iPSCs were evaluated for pluripotency marker analysis by flow cytometry. Cell gating by forward and side scatter (a). Viable iPSC selection by SYTOX red staining (b). The double positive gating of pluripotent markers TRA1-81 versus SSEA4 (c), CD30 versus SSEA4 (d) and CD30 versus TRA1-81 (e).



Conclusions

iPSC processing using the CTS Rotea System

- Completely closed system harvest can help reduce the risk of contamination.
- Direct-weld of the CTS Rotea system to the Cell Factory System™ stacks.
  - Efficient, automated wash and concentration (15-fold) of more than 3x10^9 iPSCs in 48 min.
  - Maintenance of pluripotency and viability of sensitive iPSCs during processing.

Robust, scalable expansion of iPSCs

- Methods described here enable Pluripotent Stem Cell Culture scale out: https://www.ncbi.nlm.nih.gov/books/NBK571710/
- Cell Factory™ System provides consistent high-quality iPSC cell culture performance (2-stack and 10-stack).

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