Impact of Technology Selection on iPSC 3D Cell Culture Expansion

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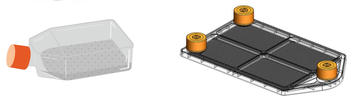
Introduction

Human pluripotent stem cells (hPSCs) are widely used in life sciences research for drug discovery and disease modeling, as well as for regenerative medicine and clinical therapeutic development. While various applications may require different quantities of hPSCs, all methods of expansion are optimized for maintenance of pluripotency and critical cellular attributes, utilizing either two-dimensional (2D) or three-dimensional (3D) culture systems. In this study, we set out to understand the impact of technology selection on induced pluripotent stem cell (iPSC) aggregate formation and expansion. Utilizing either Corning® Elplasia® technology, static 3D culture in vessels with Ultra-Low Attachment (ULA) surface coating, or dynamic 3D culture in shaker flask technology, iPSC aggregates were characterized for yield, fold-expansion, viability, and pluripotency over 3 days. Aggregate counts, morphologies, and size distributions were rapidly quantified using Flow Imaging Microscopy (FIM) with FlowCam® by Yokogawa Fluid Imaging Technologies. FlowCam's highly efficient assessment of 3D cell culture provides high-throughput data in real-time via high-resolution imaging.

3D Cell Culture Technologies

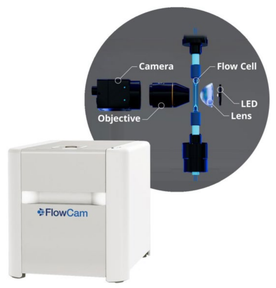
Corning 75 cm² U-Shaped Flask and CellSTACK® 1-Chamber with ULA Surface

Corning 125 mL Erlenmeyer Flask



Corning Elplasia 12K Flask and 48K Vessel

3D Cell Culture Imaging and Analysis



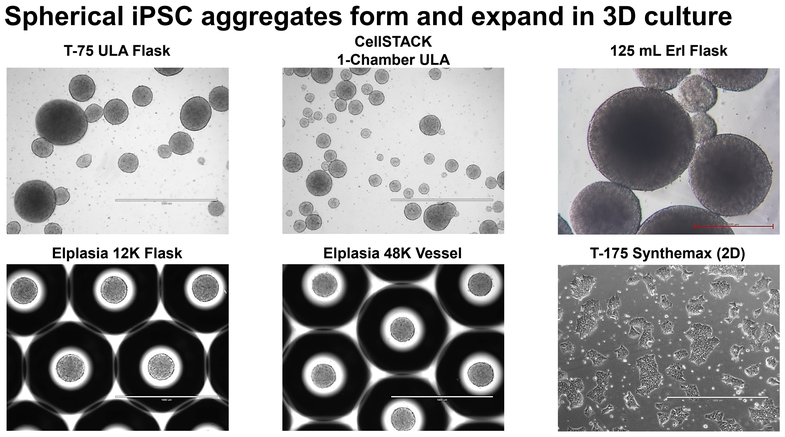
Yokogawa Fluid Imaging Technologies FlowCam 8000

Materials and Methods

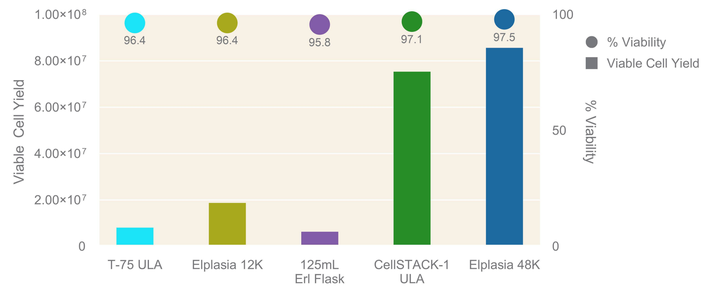
- Normal human iPSC (iXCells Biotechnologies) were expanded in 2D using Corning Synthemax® II-SC Substrate coated Corning CellBIND® cell culture flasks, harvested into single cells and seeded into various Corning vessels for 3D aggregate culture. mTeSR™ Plus Medium (STEMCELL Technologies) was used for all 2D and 3D cultures with 10 µM Y-27632 (Sigma) added during seeding and removed after 24 hr.
  - **Corning Elplasia 3D culture:** Corning Elplasia 12K flask or Corning Elplasia 48K vessel prototype seeded at 500 cells per microcavity ($6x10^6$ and $24x10^6$ iPSCs, respectively).
  - **Static ULA 3D culture:** Corning 75 cm² U-shaped flask with ULA surface coating seeded at $6x10^6$ cells. Corning CellSTACK 1-chamber with ULA surface seeded at $24x10^6$ cells.
  - **Dynamic 3D culture:** Corning 125 mL Erlenmeyer flask seeded at $3x10^6$ cells in 20 mL total volume and placed on an orbital shaker (1.9 cm orbital diameter) set to 70 rpm.
  - **2D control:** Corning Synthemax coated CellBIND 175 cm² flask seeded at $10x10^3$ cells per cm².
  - iPSCs were cultured for 72 hrs. in a 37°C, 5% CO_2 humidified incubator. Images of aggregates were obtained with an inverted microscope at 24 hr. and 72 hr. timepoints using 2X and 4X objective in brightfield mode. Representative images at 2X magnification were analyzed manually for aggregate size and shape using ImageJ measuring program.
- iPSC aggregates were collected at 72 hr. and processed for FIM analysis, confirmation of pluripotency, and ability to return to 2D culture conditions:
  - Real-time image and data analysis with FlowCam: 1 mL iPSC aggregate samples in Corning Phosphate Buffered Saline were run in triplicate using FlowCam 8000 (Yokogawa Fluid Imaging Technologies) configured with 4X magnification lens, FOV 600 flow cell, a sample flow rate of 1 mL/min. and ~90 sec. run time.
  - Remaining iPSC aggregates were collected and dissociated into single cells:
    - Viable cell density was determined via NucleoCounter® NC-200™ (ChemoMetec).
    - Pluripotency marker expression was assessed via flow cytometry (MACSQuant® analyzer, Miltenyi Biotec) using BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit (BD Biosciences).
    - Corning CellBIND 6-well plates coated with Corning Synthemax were utilized for return IPSC to 2D culture seeded at 10e3 cells/cm² and maintained until cultures showed mature morphology (highly nucleated with compact colonies) and reached >70% confluency.

Results

Spherical iPSC aggregates form and expand in 3D culture



Representative images of 72 hr. iPSC aggregates in Corning 3D cell culture technologies, or 2D culture control flask, obtained using 4X objective in brightfield mode. Rounded iPSC spheroid morphology observed in all 3D culture conditions, with Corning Elplasia technology generating uniform sized and shaped cultures throughout, while others show various aggregate sizes.



	T-75 ULA Flask	Elplasia 12K Flask	125 mL Erl Flask	CellSTACK 1-Chamaber ULA	Elplasia 48K Vessel
Doubling Time (hr.)	148	43	62	43	38
Fold Increase	1.4	3.1	2.1	3.1	3.8

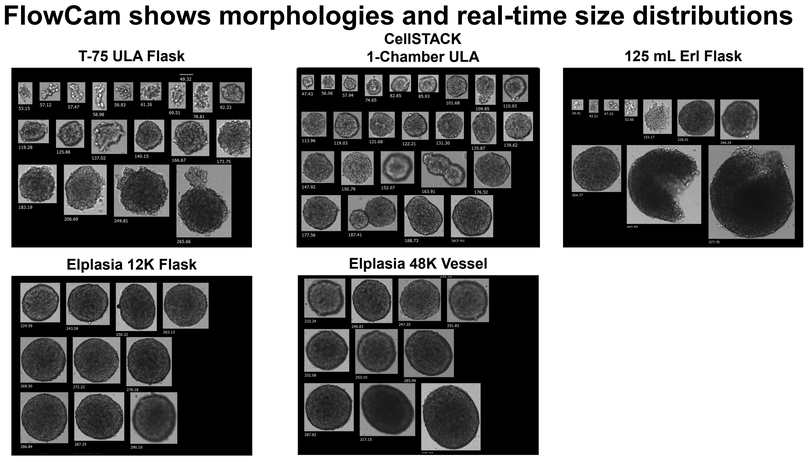
Cell expansion over 72 hr. determined via viable cell counts of dissociated 3D iPSC aggregates. While all technologies resulted in iPSC expansion, Corning Elplasia technology demonstrated greater than 3-fold expansion over 72 hr. and linear scalability from 12K to 48K vessel capacity.

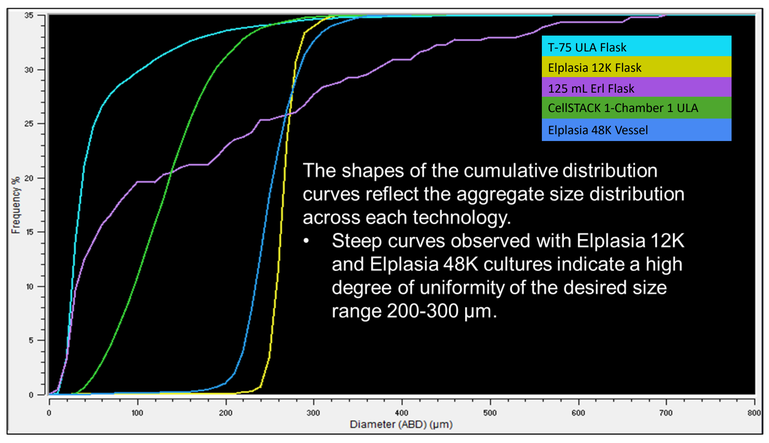
iPSC pluripotency is maintained after 3D culture expansion

Markers	T-175 Synthemax 2D Control	T-75 ULA Flask	Elplasia 12K Flask	125 mL Erl Flask	CellSTACK 1-Chamaber ULA	Elplasia 48K Vessel
SSEA-4	95.2%	99.9%	99.6%	99.9%	95.0%	95.6%
TRA-1-60	99.3%	98.4%	98.4%	99.9%	92.0%	94.3%
SOX2	99.3%	97.8%	97.5%	99.4%	99.1%	99.0%
NANOG	91.8%	91.1%	93.8%	93.6%	95.9%	93.6%
OCT3/4	94.1%	94.0%	96.5%	99.0%	99.6%	99.1%

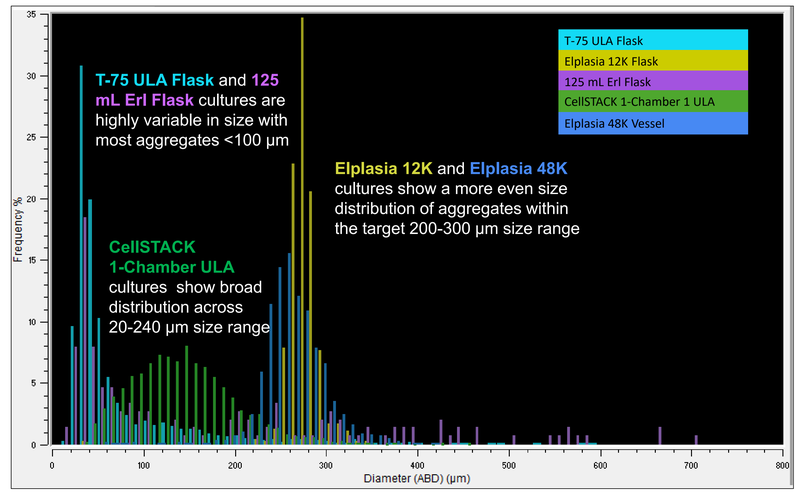
Flow cytometry analysis indicates that all 3D iPSC cultures maintain pluripotency with expression >90% after 72 hr. regardless of cell culture technology selection. Successful return of iPSC to 2D culture confirmed (data not shown).

Rapid, high-resolution iPSC aggregate image capture with FlowCam shows morphologies and real-time size distributions

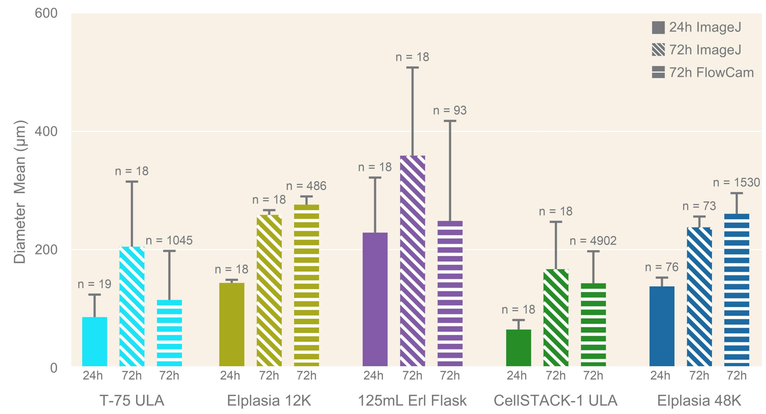




FlowCam analysis of aggregate diameter and particle counts highlights range of size distribution of iPSC aggregates



High-throughput FlowCam analysis of iPSC aggregate growth confirms in-culture image analysis in 90 seconds



High-throughput FlowCam analysis of collected iPSC aggregates at 72 hr. compared to and ImageJ processing of representative images of iPSC in culture obtained at 24 hr. and 72 hr. timepoints show increase in iPSC aggregate diameter over time in all 3D culture conditions. Rapid high-resolution imaging using FlowCam at 72 hr. collection confirms average aggregate diameter with ability to process many more particles and perform high-throughput data analysis in real-time compared to hours required for manual acquisition of images and ImageJ analysis. Corning Elplasia technology results in the narrowest distribution range of iPSC aggregate diameters using both methods with scalable increase between Elplasia 12K and Elplasia 48K vessels. Static ULA culture in Corning 75 cm² U-shaped flask and Corning CellSTACK-1 chamber, and dynamic culture in 125 mL Erlenmeyer flask show greater variability and range of aggregate diameter.

Conclusions

- Corning provides various technologies for generating 3D iPSC cultures under both static and dynamic culture conditions, all resulting in cell expansion and healthy spheroid formation with variability in aggregate size uniformity and fold-expansion.
- Yokogawa Fluid Imaging Technologies FlowCam 8000 provides rapid processing of 3D iPSC cultures with real-time analysis.
- Coupling Corning technology selection with FlowCam provides an efficient platform for optimized iPSC expansion with an accurate measurement system to ensure critical cell culture attributes are maintained for iPSC 3D cell culture.