SARTORIUS

Case Study

Integrated Workflow for Reliable Generation and Selection of 3D iPSC Models

3D models such as spheroids and organoids are becoming increasingly relevant in disease research and developmental biology. However, generating these models is often complex, time-consuming, and resource intensive, particularly when building disease-relevant systems that contain specific cell populations or genetic markers. A key challenge lies in the accurate selection and manipulation of cells to successfully create a physiologically relevant system. Efficient methods for developing, monitoring, and characterizing these models are critical to support their broader use in drug discovery and toxicology. In this study, we cultured clonal iPSC spheroids from individual cells and isolated human iPSC-derived hepatic organoids from Matrigel® domes based on key morphological attributes, then selected them for further culture and analysis.

Case Profile

Objective:
Developing reliable 3D cell models, such as monoclonal spheroids and hepatic organoids, is complex and resource-intensive. This study presents an automated, standardized workflow that simplifies the generation, monitoring, and analysis of iPSC-derived 3D models. The integrated approach reduces variability, saves time, and supports the consistent development of physiologically relevant models.

Keywords:
3D cell models, Spheroids, Organoids, CellCelector Flex, Incucyte®, Monoclonal spheroids, Hepatic organoids, Disease research, Live-cell imaging

We used the CellCelector Flex to establish a simple, standardized, and robust workflow for identifying and isolating 3D cell models based on growth and morphology. Throughout the workflow, the Incucyte® Live-Cell Analysis System and iQue® High Throughput Screening (HTS) by Cytometry platform enabled real-time monitoring of growth, morphological changes, and marker expression for phenotypic characterization. This integrated approach streamlined the development of iPSC-derived monoclonal spheroids and organoids for drug discovery, disease modeling, and toxicity studies.

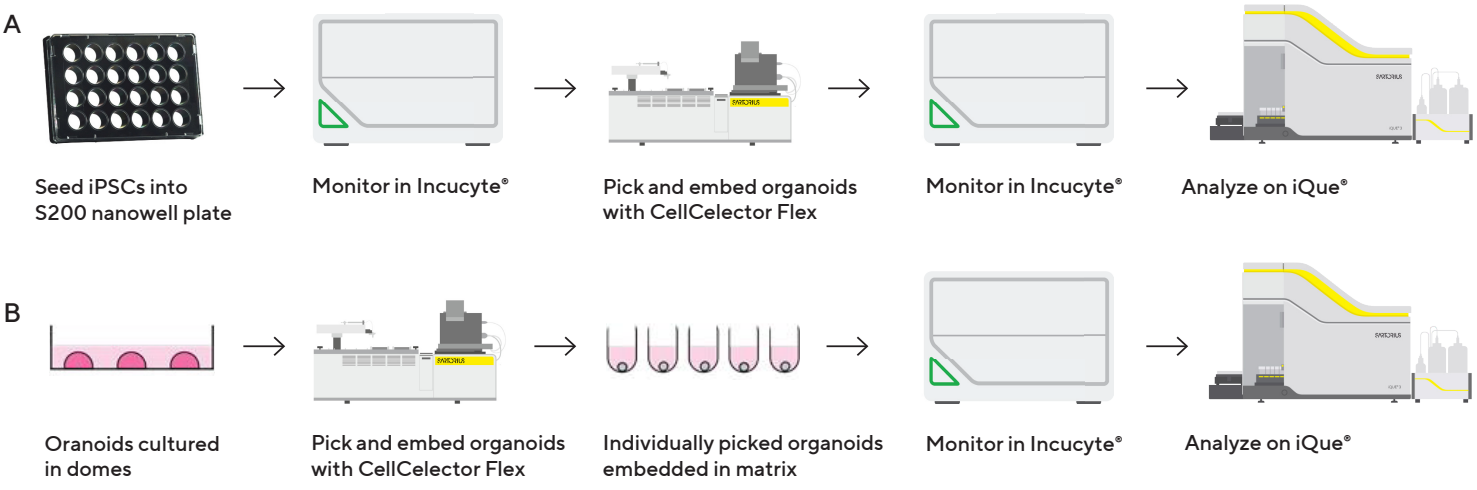


Figure 1. Schematic of 3D workflows using Sartorius systems. A) Monoclonal iPSC spheroid development. B) Organoid isolation and culture.

Challenges of Case Study

- Generation of 3D tissue models is expensive, highly complex, and manually intensive.
- Development of disease-specific models further complicates 3D model production.
- Creation of models from a single cell source is highly beneficial, but also highly complex.

Provided Solutions

- Established a simple, standardized workflow for generating 3D models using automated selection, live-cell imaging, and high-throughput screening.
- Demonstrated monoclonal spheroid generation from single iPSCs with the CellCelector Flex, enabling development of homogeneous models for disease research.
- Showcased integrated methods to monitor and analyze 3D models throughout culture and at endpoint.

Single-cell iPSC Spheroid Generation

To demonstrate the utility of this workflow, we first focused on generating monoclonal iPSC spheroids from single cells using nanowell culture and automated selection. S200 nanowell plates offer the advantage of thousands of nanowells within a single macrowell of a standard tissue culture plate. In this setup, Incucyte® Nuclight Green GFP-expressing iPSCs were seeded into a nanowell plate, ensuring single-cell seeding. After three days of culture, healthy GFP-expressing monoclonal iPSC spheroids had formed across multiple wells. The CellCelector Flex was then used to identify and pick these spheroids.

The process involved scanning and analyzing nanowells to locate GFP-positive spheroids derived from single cells (Figure 2A). Only these spheroids were targeted for picking. The CellCelector efficiently picked each spheroid from its respective nanowell and embedded it in hydrogel prior to being seeded into a medium-filled well of a 96-well ULA plate.

Plates were then transferred to the Incucyte® for a six-day post-picking period to monitor growth and morphology. Incucyte® micrographs highlighted the steady growth rate of the spheroids while maintaining GFP expression (Figure 2B). Analysis of the images captured by Incucyte® demonstrated robust spheroid growth through brightfield area measurements (Figure 2C).

Further characterization using the iQue® HTS Cytometer revealed high viability, consistent GFP expression, and retention of pluripotent markers, demonstrating the health and identity of the selected spheroids (Figure 2D).

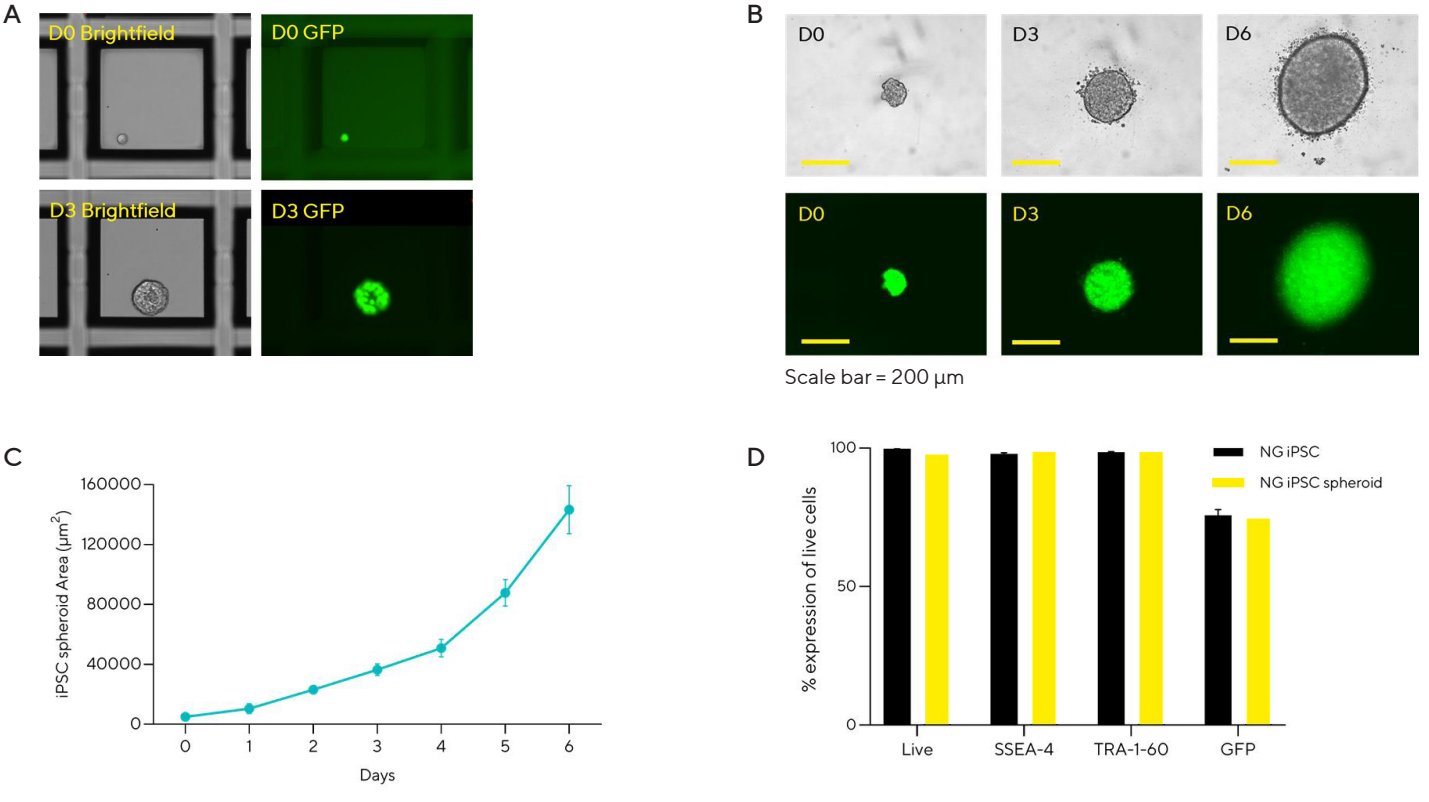


Figure 2. iPSC spheroid generation using Sartorius platforms. A) CellCelector Flex images illustrating the identification of nanowells containing a single GFP-positive cell at seed, and generation of an iPSC spheroid on Day 3. B) Incucyte® images recording the growth and fluorescence of an iPSC spheroid over 6 days post picking. C) Incucyte® analysis of iPSC spheroid area change over time, indicating growth. D) iQue® HTS Cytometry endpoint analysis of pluripotency marker expression (SSEA-4 and TRA-1-60), cellular viability, and GFP expression on Day 6.

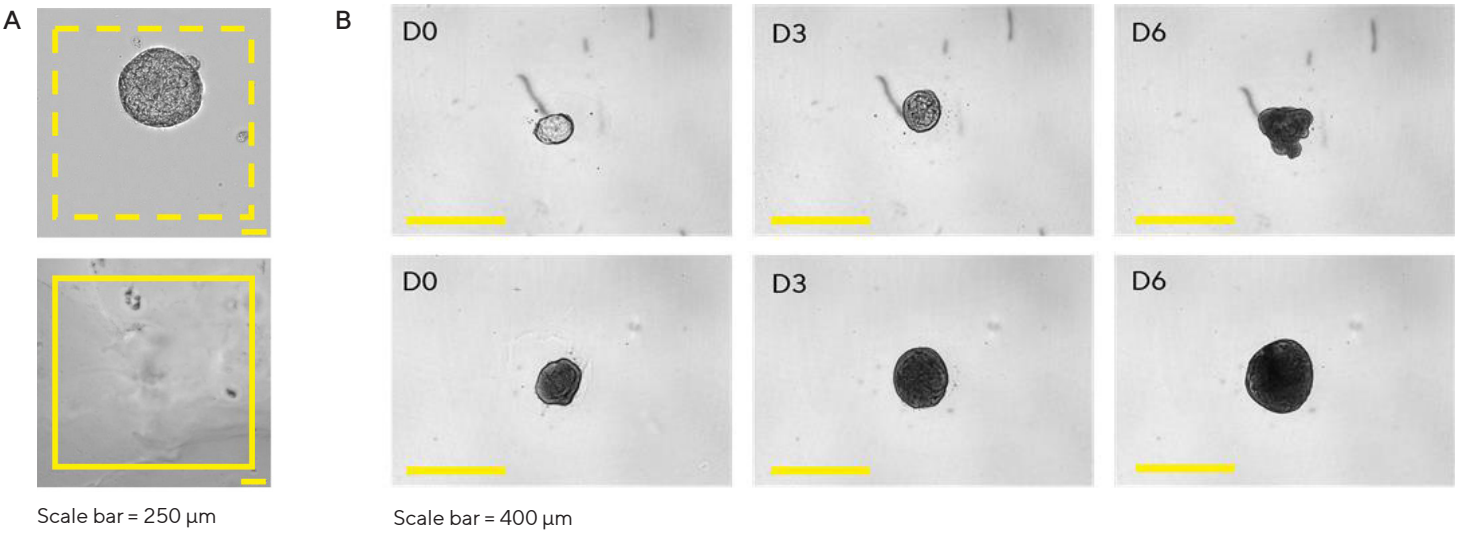
Automated Organoid Culture

We next applied the workflow to iPSC-derived hepatic organoids cultured in Matrigel® domes that were efficiently identified and picked using the CellCelector Flex. Selection criteria included size and morphology, with various sizes chosen to assess picking accuracy and success rate (Figure 3A). After picking, the organoids were embedded in a Matrigel® droplet and seeded into 96-well ULA plates.

These plates were then transferred to the Incucyte® system for continuous monitoring of growth and morphological changes over a six-day period (Figure 3B). Analysis of the Incucyte®-captured images demonstrated robust organoid growth, as measured by changes in brightfield area (Figure 3C).

Further characterization using the iQue® HTS Cytometer revealed high expression of the hepatic markers CD99 and CD184 and undetectable levels of pluripotent markers (SSEA-4 and TRA-1-60) in the iPSC-derived hepatic organoids. Additionally, there was an increase in the expression of non-pluripotency marker SSEA-1, indicating differentiation away from the pluripotent iPSC phenotype (Figure 3D).

Together, the CellCelector, Incucyte®, and iQue® HTS cytometry systems enabled an integrated, automated workflow for selecting, monitoring, and phenotypically validating iPSC-derived organoids with specific morphological and marker-defined characteristics.



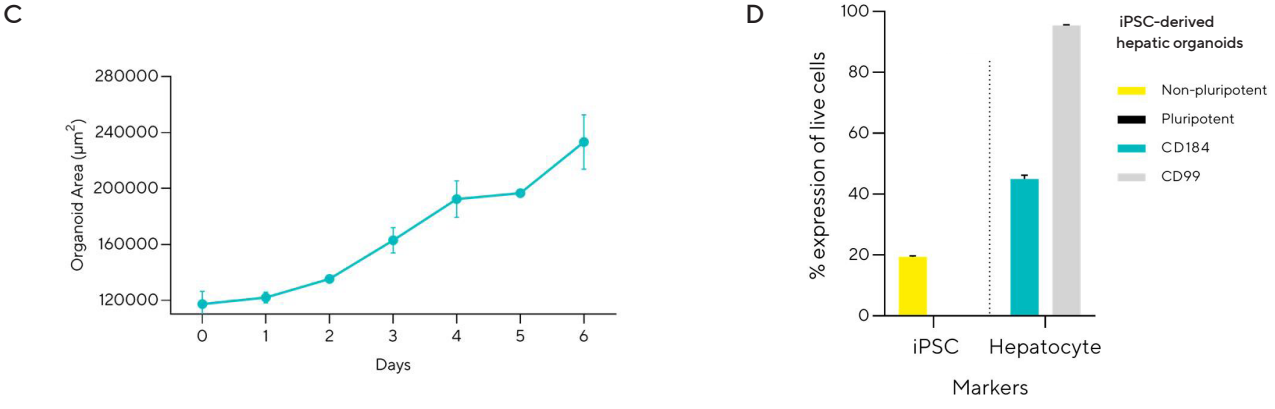


Figure 3. Organoid isolation and culture using Sartorius platforms. A) CellCelector Flex images of before and after picking of a hepatic organoid, dotted line before image, solid line after image. B) Incucyte® images of hepatic organoid growth over 6 days in culture. C) Incucyte® analysis of organoid area change over time, indicating growth. D) iQue® HTS Cytometry endpoint analysis of non-pluripotency marker (SSEA-1), pluripotency markers (SSEA-4 and TRA-1-60), and hepatic marker (CD99 and CD184) expression.

Conclusion

This study highlights a fully integrated, automated approach for developing, selecting, and characterizing iPSC-derived 3D models using the CellCelector Flex, Incucyte®, and iQue® HTS Cytometer. By combining precise selection with real-time monitoring and phenotypic validation, the workflow supports efficient generation of organoids and monoclonal spheroids, reducing complexity and accelerating research in disease modeling, drug discovery, and toxicity testing.

Download the poster here:
Automating-3D-Culture-Model-Systems-Poster | Sartorius

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Automating-the-Generation-of-Clonal-Spheroids-from-a-Single-Cell

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