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Simplifying Progress

Optimizing Human Hepatic Organoid Development: Leveraging iPSC Differentiation and Advanced Analytical Platforms with RUO Growth Factors and Cytokines

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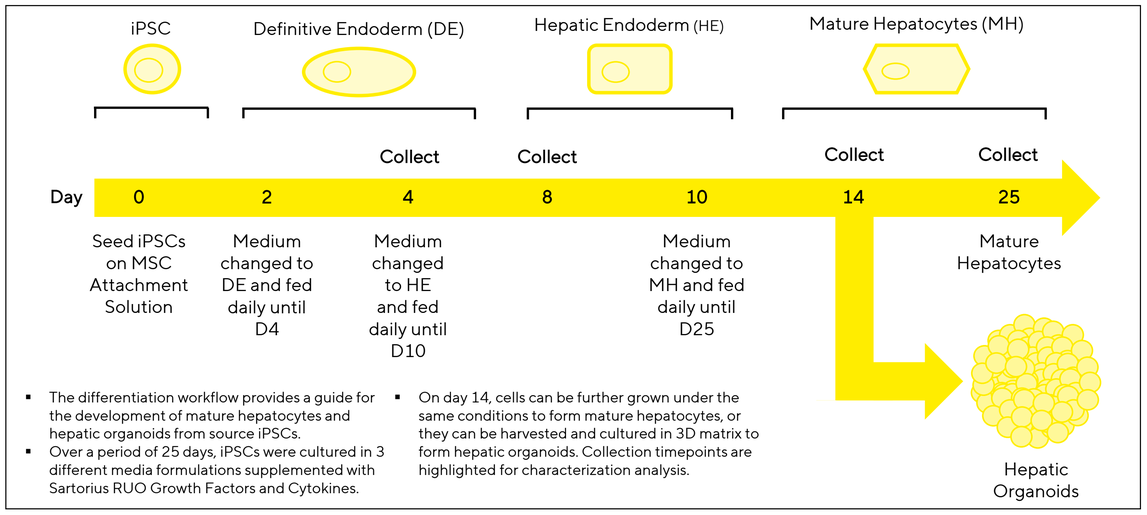
Introduction

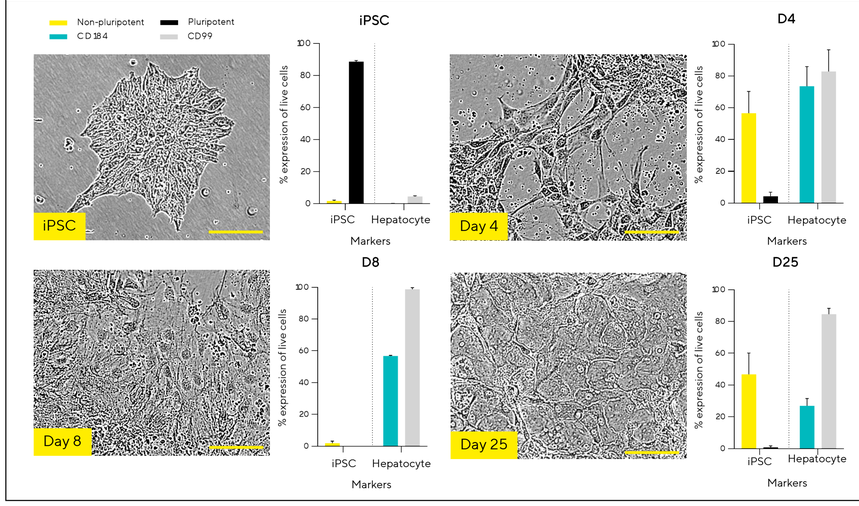
- Increasing requirement for the generation of species-specific, physiologically relevant systems for the study of disease and development has led to the expansion of 3D models, such as organoids, used in research.
- These biologically accurate tissue models can be expensive and time-consuming to produce, necessitating efficient and reliable methods for their development, monitoring and characterization.
- The utility of stem cells in laboratory and clinical research has been clearly demonstrated through an extensive number of studies exemplifying stem cells as a model for a multitude of diseases and therapeutic development.
- Differentiation of induced pluripotent stem cells (iPSCs) into somatic tissues requires specific conditions dependent on the desired tissue type.
- In this workflow, human iPSCs were differentiated into hepatocytes using three distinct media types with their own cytokine and growth factor supplementation at key stages of differentiation, providing a relatively simple method for producing mature hepatic cells.
- This enabled the production of human hepatic organoids from these source cells, demonstrating a robust method for unlimited organoid development.
- Here, we outline a simple, standardized, and robust workflow using Sartorius RUO Growth Factors and Cytokines in combination with the Incucyte® Live-Cell Analysis System and the iQue® HTS Cytometer for phenotypic characterization and monitoring of growth.
- This approach simplifies the generation of functional hepatic cells and organoids from iPSCs for drug discovery, development, and toxicity studies.

Differentiation Workflow



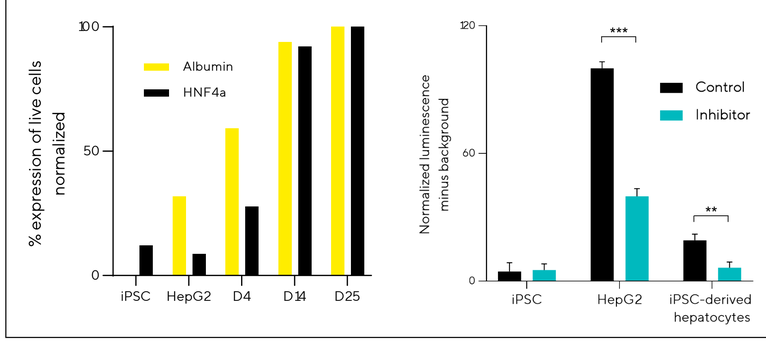
- The differentiation workflow provides a guide for the development of mature hepatocytes and hepatic organoids from source iPSCs.
- Over a period of 25 days, iPSCs were cultured in 3 different media formulations supplemented with Sartorius RUO Growth Factors and Cytokines.
- On day 14, cells can be further grown under the same conditions to form mature hepatocytes, or they can be harvested and cultured in 3D matrix to form hepatic organoids. Collection timepoints are highlighted for characterization analysis.

Monitoring iPSC to hepatocyte differentiation



- Differentiation of iPSCs into hepatocytes was monitored using the Incucyte® Live-Cell Analysis System and the iQue® HTS Cytometer.
- iPSCs treated with differentiation media begin to lose typical colony formation, becoming more spread out, eventually forming a monolayer.
- The final timepoint (Day 25) reveals a hepatocyte-like morphology, with large polygonal structure and round nuclei with prominent nucleoli.
- Confirmation of these observations through surface marker analysis, shows decreases in pluripotency markers and increases in non-pluripotency markers and hepatocyte markers CD184 and CD99 during differentiation.

Characterizing expression and functional activity of iPSC-derived hepatocytes



- Intracellular marker expression further characterized iPSC-derived hepatocytes.
- High expression of Albumin and HNF4a observed during hepatocyte differentiation.
- Expression levels greatly increased compared to iPSC and HepG2 control cell lines.
- Cytochrome P450 inhibition assay characterized functionality of iPSC-derived hepatocytes.
- Significant inhibition of CYP1A2 enzyme activity in iPSC-derived hepatocytes when treated with α-napthoflavone.

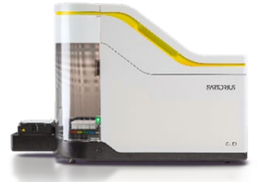
RUO Growth Factors and Cytokines, Incucyte® & iQue® Systems

Sartorius RUO Growth Factors and Cytokines

Sartorius provides a broad range of high quality RUO growth factors and cytokines which are produced using recombinant DNA technology and do not contain any animal-derived components or contaminants.

Incucyte® Live-Cell Analysis System

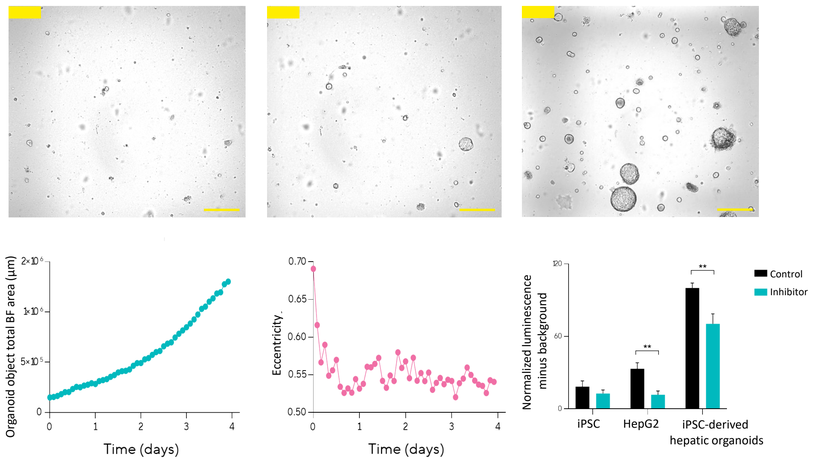
A fully automated phase contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.



iQue® HTS Cytometer

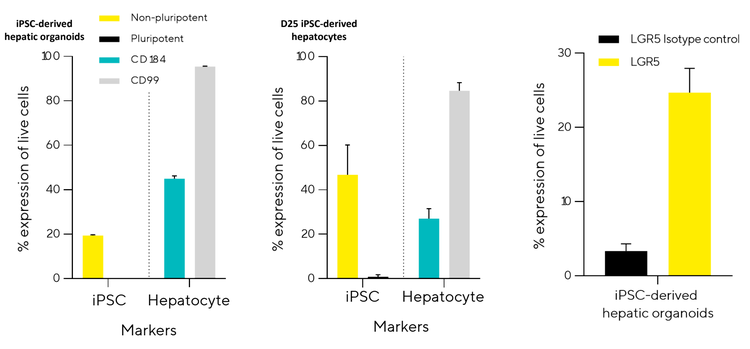
A high throughput screening cytometry platform with a patented sampling method allowing for rapid sample acquisition to deliver fast actionable results. Capable of handling 96 and 384 well plates.

Monitoring iPSC-derived hepatic organoid development



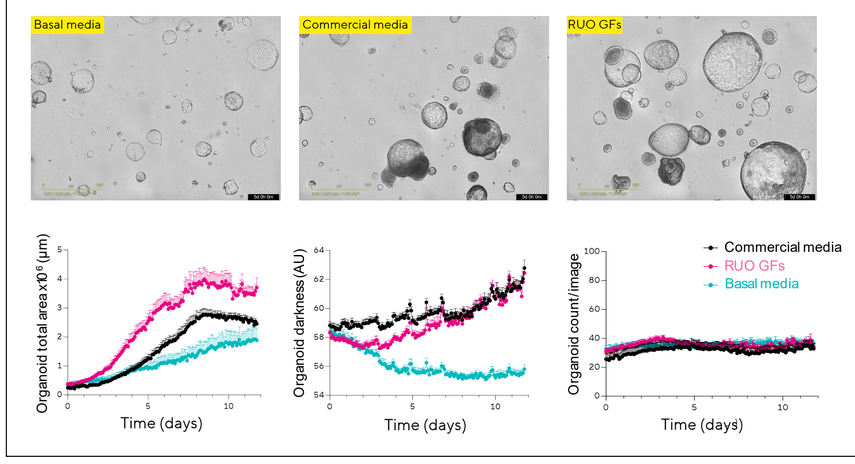
- iPSC-derived hepatocytes collected on day 14 were cultured over 7 days in 3D matrix to form hepatic organoids.
- The Incucyte® Live-Cell Analysis System monitored morphological status of the organoids.
- Rapid growth of iPSC-derived hepatic organoids, reaching up to 600 µm in diameter by day 5.
- Quantification of organoid growth, showed steady increase in organoid object area over time.
- Reduction of organoid eccentricity during early-stage growth, plateauing after day 2, indicating stable homogeneous organoid phenotype.
- Cytochrome P450 inhibition assay showed significant inhibition of CYP3A4 activity in iPSC-derived hepatic organoids treated with verapamil hydrochloride.

Characterizing iPSC-derived hepatic organoids



- Characterization of surface marker expression in iPSC-derived hepatic organoids performed using the iQue® HTS Cytometer.
- iPSC-derived hepatic organoids share similar expression profiles with iPSC-derived hepatocytes, high levels of hepatic marker CD99 and expression of CD184.
- Pluripotency marker expression undetectable in both cell types, non-pluripotency markers are expressed, indicating a differentiated phenotype.
- LGR5 expression in 25% of cells in iPSC-derived hepatic organoids, revealing a high proportion of component stem cells, indicating strong proliferative capabilities.

RUO growth factors and cytokines for organoid culture



- To evaluate Sartorius RUO Growth Factors and Cytokines in the culture of iPSC-derived hepatic organoids, different media formulations were tested.
- Hepatic organoids cultured in basal media without growth factors appeared much smaller than in the other conditions.
- Organoids cultured in media containing Sartorius RUO Growth Factors and Cytokines (RUO GFs) increased in area at a much more rapid rate compared to basal and commercially available media.
- Organoids cultured in RUO GFs displayed organoid darkness values equivalent to commercial media, but lower initial darkness, organoid counts conserved across media types.