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Simplifying Progress

Research Use Only Growth Factors and Cytokines for Organoid Generation and Culture

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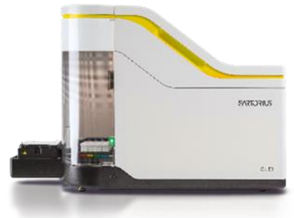
Introduction

- Cell lines and 2D culture systems are conventionally used in drug discovery due to their ease of use and availability. However, it is well established that these monolayer cultures do not adequately reflect the *in vivo* situation and can lead to drug failure in later clinical trials due to inaccurate predictions of efficacy.
- Advanced culture systems including spheroids and organoids provide a more representative model of organ function, development, and disease due to their 3D architecture. There is a shift towards using these for pharmacological testing, cancer research, modelling of tissues and neurodevelopmental biology.
- There is also a shift towards using patient-derived cells to develop therapies that will be compatible with specific genotypes and pathologies.
- For example, human induced pluripotent stem cells (iPSC) can be directed to differentiate into cell types of interest by controlling growth conditions including adding certain growth factors at precise time points.
- These cell types can be used to create organoids from tissues that are hard to obtain, opening up the potential for new therapeutic areas.
- Growth factors and cytokines are a fundamental component of organoid culture medium as they maintain a self-renewing stem cell population within the structure to allow for organoid expansion, whilst preventing differentiation.
- Here we demonstrate the growth and monitoring of organoids of both adult stem cell and iPSC origin using the Incucyte® Live-Cell Analysis System, iQue® Advanced Flow Cytometry Platform and Sartorius Research Use Only (RUO) Growth Factors and Cytokines.

Incucyte® & iQue® Systems

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® Advanced Flow Cytometer

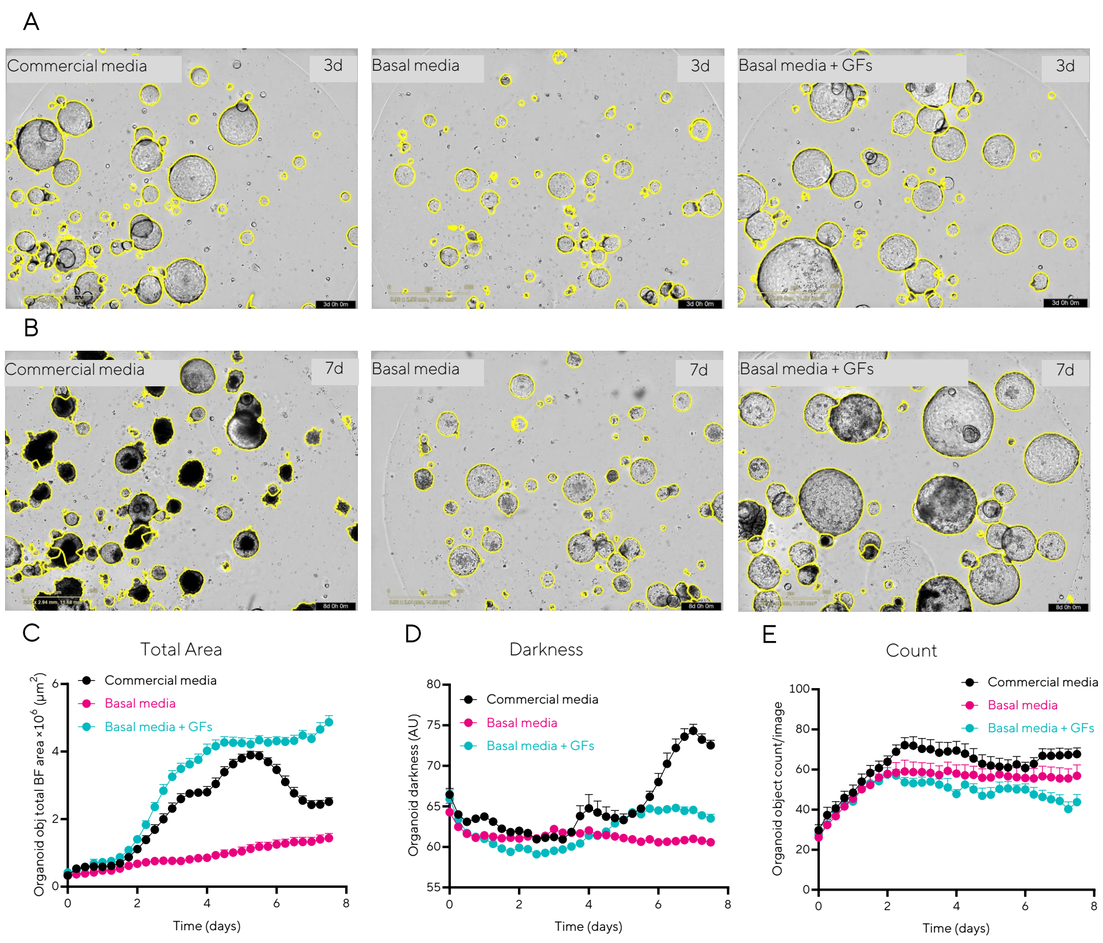
An advanced flow cytometry platform with a patented sampling method allowing for rapid sample acquisition to deliver fast actionable results. Capable of handling 96 and 384 well plates.

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.

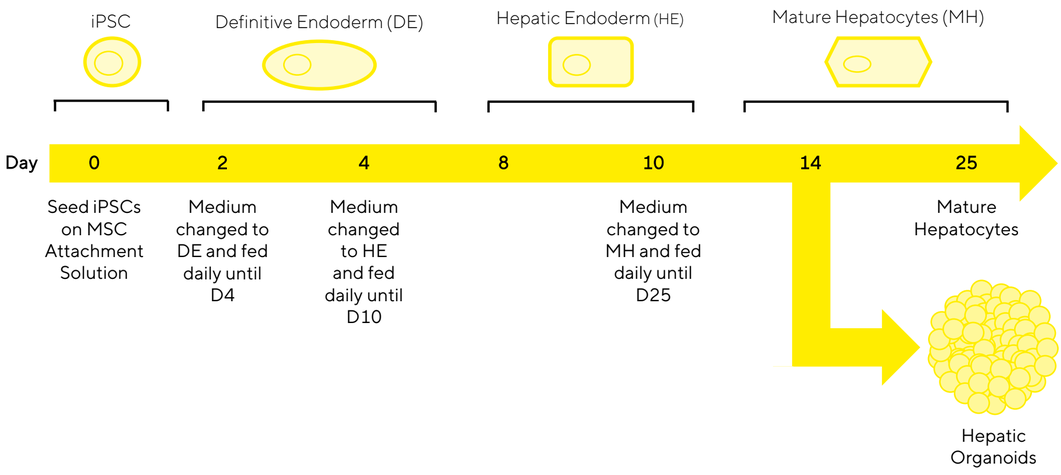
Improved growth of organoids using growth factors

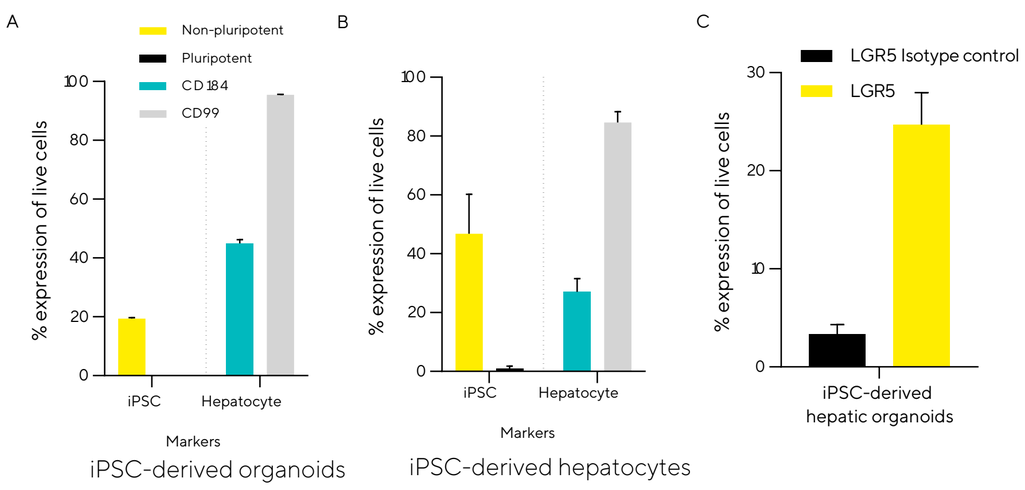
- Mouse hepatic organoids were cultured in either a commercially available media, basal media without growth factors, or basal media containing Sartorius RUO Growth Factors EGF (50 ng/ml), FGF10 (100 ng/ml), HGF (50 ng/ml), and R-spondin1 (1 µg/ml) .
- Organoids were automatically located and changes in size (area) were tracked using the Incucyte® Organoid Analysis Software Module. Images show brightfield images including the organoid segmentation mask (yellow outlines).
- Organoid growth metrics total area, darkness, and organoid count were automatically quantified.
- Organoids cultured with Sartorius RUO Growth Factors demonstrate improved growth and prolonged health when compared to the controls without growth factors or in commercially available medium.



Generation of hepatic organoids from iPSCs

- Human iPSCs were directed to differentiate into hepatocytes using media formulations containing Sartorius RUO Growth Factors and Cytokines. They were transferred into Matrigel™ for the formation of hepatic organoids.
- Phenotypic analysis of surface marker expression using the iQue® showed a decrease in iPSC pluripotency markers (SSEA-4, TRA-1-60) and an increase in hepatocyte makers (CD184, CD99).
- Expression of hepatic stem cell marker LGR5 was detected in the differentiated organoids, indicating the presence of a stem cell population within the organoids and allowing propagation and expansion for downstream applications.





Growth of human hepatic organoids

- Following derivation from iPSCs, human hepatic organoids were cultured in either a commercially available media, basal media without growth factors, or basal media containing Sartorius RUO Growth Factors EGF (50 ng/ml), FGF10 (100 ng/ml), HGF (25 ng/ml), and R-spondin1 (1 µg/ml).
- Hepatic organoids cultured in basal media without growth factors appeared much smaller than in the other conditions, illustrating the importance of growth factors for organoid growth.
- Organoids cultured in media containing Sartorius-RUO Growth Factors and Cytokines increased in area at a much more rapid rate compared to basal media and commercially available media, reaching a maximum diameter of around 1.2 mm at 7 days, which was maintained for the remainder of the culture period.

Investigation of individual growth factor effects

- R-spondin1 is often provided to organoids within conditioned medium, which contains an unknown concentration of R-spondin1. Using recombinant growth factors provides a more controlled and defined environment for organoid culture.
- To optimize concentrations of R-spondin1, mouse hepatic organoids were treated with a concentration range of R-spondin1 and monitored using the Incucyte® Live-Cell Analysis System.
- Organoids demonstrated concentration-dependent growth profiles in response to R-spondin1.
- Including R-spondin1 in the media delivered increased growth compared to commercially available media.