Real-time Live-Cell Analysis of 3D Organoid growth in Matrigel® domes

Introduction

Recent advances in organoid technology have opened up new horizons for translational human disease research, disease modeling, regenerative medicine and predictive precision therapies.

Organoids are differentiated primary micro tissues formed from a variety of stem cells (SCs) that can be established within 3D extracellular matrices to mimic in-vivo architecture and genetic diversity. As self-organizing and self-renewing structures, organoids have a distinct advantage over traditional monolayer culture techniques and hold unprecedented potential for various applications. In order to effectively use these models in basic research, disease modeling and drug screening, specific and reliable in-vitro culture and analysis methods are required.

Currently, characterization and optimization of organoid cultures are limited in their ability to objectively monitor these 3D structures as they form and grow over time. The Incucyte® Organoid Analysis Software Module provides a solution to standardize and automate organoid culture workflows, simplifying culture characterization and optimization.

Assay Principle

This application note describes the use of the Incucyte® Live-Cell Analysis System and Incucyte® Organoid Analysis Software Module to study the formation and growth of organoid cultures. A proprietary Brightfield (BF) image acquisition approach enables real-time kinetic imaging of 3D organoids embedded within Matrigel® domes. Organoid size, count and morphology measurements are automatically plotted over time to provide insight on organoid differentiation and maturation characteristics.

Here we describe validation methods and data demonstrating the ability to kinetically visualize and quantify organoid formation and growth in Matrigel® domes.

Find out more: www.essenbioscience.com/en/applications/cell-monitoring-workflows/organoid-qc/
Material & Methods

Organoid Culture QC Workflow

Quick Guide

1. Harvest & resuspend organoids in matrigel

2. Pipette matrigel in plate

3. Polymerize matrigel

4. Add media

5. Monitor organoid formation & growth

Harvest and resuspend organoids or fragments in Matrigel (50 - 100 %).

Pipette Matrigel containing organoid fragments in the centre of a 24- or 48-Well plate (30 - 50 µL or 10 µL respectively).

Inoculate plate at 37°C for 10-15 mins to polymerize Matrigel, forming a dome.

Overlay polymerized dome with recommended culture media.

Place plate in Incucyte to monitor organoid formation & growth.

Optimization and characterization of organoid cultures using real-time kinetic measurements.

Defining optimal culture conditions and regimes are critical for establishing healthy cultures for use in downstream studies. Objectively defining parameters such as seeding densities, passage frequency and ensuring cultures have differentiated and display appropriate morphology is key.

Measuring proliferation to optimize growth conditions and seeding densities
To optimize organoid seeding density, mouse hepatic organoids were embedded in Matrigel® domes (100%) in 48-well plates at multiple split ratios (1:10 – 1:40 split). BF images and quantification of organoid area demonstrated that organoid growth rate and size is proportional to the number of cells seeded (Figure 3). Organoids seeded at the highest density appeared larger (> 500 μm diameter) and exhibited rapid growth reaching maximal size (73.4 × 10^4 µm^2 ± 2.3 mean ± SEM, n = 14 wells) within 120 h (Figure 3, BF images and total area time-course respectively). Conversely, at lower densities while the organoid maturation phase was extended (28.6 × 10^4 µm^2 ± 2.9 mean ± SEM, n = 14 wells at 120 h, total area), the greatest growth potential (size) was observed (Figure 3, average area time-course).

Measuring morphological features to define optimal organoid maturation phase
To define optimal organoid passaging frequency, mouse hepatic organoids were embedded in Matrigel® domes (100%) and imaged in an Incucyte® for 8 days. Hepatic organoids are typically ready for passaging when...
Tracking organoid differentiation and growth efficiency through passages

Under routine culture conditions, organoid morphology and growth capabilities are expected to remain consistent across multiple passages. To assess intestinal organoid expansion and growth efficiency across passages, a number of parameters were kinetically quantified (Figure 5). When maintained at a consistent density, intestinal organoids exhibited comparable count, area, eccentricity and darkness measurements across passages. Figure 5 exemplifies the amenability of this imaging and analysis approach to support robust and reproducible assessment of long-term organoid expansion.

Conclusions

In this application note, we demonstrate the use of the Incucyte® Live-Cell Analysis system, in combination with the Incucyte® Organoid Analysis Software Module, to facilitate kinetic assessment of organoid formation and growth. We have demonstrated:

- The ability to automatically locate and analyze 3D organoids embedded within Matrigel® domes in both 24- and 48-well plates.
- The use of integrated, real-time label-free metrics to optimize and define culture conditions and regimes.
- Optimal periods for passaging or extension of organoid cultures based on integrated morphological parameters.
- Use of this approach to assess culture quality during extended passaging.

References
