>> Real-time analysis of 3D cell culture models using live-cell imaging



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Automated, whole-vessel imaging and analysis

In vitro models are essential for studying diseases and development. While traditional 2D cell culture models have provided valuable insights, they often fail to replicate *in vivo* complexity. This has led to increased interest in 3D models such as spheroids and organoids, which better mimic in vivo conditions.

Live-cell provides a powerful technique for studying these 3D models, enabling real-time visualization and analysis at defined time intervals.

Omni: Kinetic cell tracking

The Omni product family

- >> Assay your cells in brightfield and fluorescence
- >> Track every moment, straight from your incubator
- >> See every cell by movement of the camera
- >> Monitor and analyze your cells remotely

AI-Driven imaging software for powerful, yet simple analysis

The Omni platform software modules simplify assay setup, offer real-time cellular visualization, and enable fast analysis.







A new workflow for differentiation and characterization of iPSC-derived hepatic organoids

Introduction

The liver plays vital roles in detoxification, protein synthesis, metabolism, and hormone regulation. While it regenerates efficiently *in vivo*, expanding hepatocytes *in vitro* is difficult. Induced pluripotent stem cells (iPSCs) provide a versatile source for generating hepatic cells and organoids that mimic liver structure and function. This study presents a novel non-invasive workflow for monitoring iPSC-derived hepatic organoid development using the Omni.

Results

iPSC differentiation Distinct morphological changes occurred during differentiation: iPSCs were compact, definitive endoderm cells smaller, HPCs larger with a cobblestone appearance, and HLCs the largest with a similar arrangement.



Organoid formation HPC- and HLC-derived organoids were spherical with lumens and had similar roundness (0.85). Initially smaller, HPC-derived organoids grew 3.6-fold over 72 hours, compared to 3.0-fold for HLC-derived organoids, reflecting differences that may be related to cell maturity.



Methods

iPSCs were differentiated into hepatic progenitor cells (HPCs) and hepatocyte-like cells (HLCs) and subsequently formed into organoids. Organoid area, diameter, and roundness were determined every 4 h for 3 days.



Figure 1: Morphological changes during differentiation of iPSC towards hepatocyte-like cells. Scalebar is 200 µm and accounts for all images.

Figure 2: Change in organoid diameter and area over time for the hepatic progenitor cell derived organoids (HPC) and the hepatocyte-like cell derived organoids (HLC), including an example of organoid detection (green overlay) at 72h by the Omni.

Conclusion

This workflow demonstrates the power of live-cell imaging for real-time monitoring of iPSC differentiation and hepatic organoid formation, enhancing liver research and applications in disease modelling and therapy.

Quantifying the impact of target cell potency on CAR-T cell mediated spheroid killing

Introduction

Chimeric antigen receptor T (CAR-T) cells have transformed cancer immunotherapy. CAR-T cells efficacy largely depends on the density of target antigens like HER2, which is overexpressed in cancers such as ovarian and lung carcinomas. Traditional cytotoxicity assays, often static and reliant on 2D models, fail to mimic the complex tumour environment. This study utilizes live-cell imaging with the Omni platform to dynamically assess CAR-T cytotoxicity of cancer spheroids with varying HER2 expression.

Results

HER2 CAR-T cells exhibited a dose-dependent cytotoxic effect, with SKOV3 cells showing greater sensitivity and earlier response than A549 cells at all E:T ratios. At 72 hours, a 5:1 E:T ratio resulted in 82.4% cytolysis of SKOV3 spheroids and 40.3% cytolysis of A549 spheroids, highlighting the impact of HER2 density on CAR-T efficacy.



Methods

GFP-tagged spheroids of SKOV3 (ovarian carcinoma) and A549 (lung adenocarcinoma) cells were treated with HER2 CAR-T cells at different Effector:Target (E:T) ratios. Fluorescent intensity was used a measure of spheroid killing and to calculate the percent cytolysis.





Figure 3: Normalized green intensity results of SKOV3-GFP and A549-GFP spheroids after treatment with different ratios of CAR T-cells.

Conclusion

Higher HER2 expression in SKOV3 spheroids enhanced CAR-T cell killing compared to A549 spheroids. The Omni platform offered dynamic, real-time insights into cytotoxicity, highlighting its value in optimizing CAR-T strategies and the importance of target antigen density.

Figure 4: Brightfield (effector cells and target spheroids) and green fluorescence (target spheroids) images show the effect of different CAR T-cell ratios on SKOV3-GFP (A-H) and A549-GFP spheroids (I-P). Scalebar is 200 μm and accounts for all images.