Innovative Spheroid Analysis Software workflow for drug response studies on single 3D models combining spheroONE™ and Incucyte®.

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Introduction

Complex three-dimensional (3D) in vitro cell models, such as spheroids, tumoroids, and organoids, have become essential in fundamental physiology research, drug discovery, and personalized medicine. Ensuring reproducible drug toxicity and efficiency testing with 3D cell models requires automated systems capable of monitoring spheroid response over time. Spheroids generated using conventional approaches exhibit size and functional discrepancies, which can impact their drug responses, thus the assay reproducibility.
In this study, an innovative workflow is introduced to demonstrate the pharmacological utility of standardized and isolated 3D cell models. The workflow combines spheroONE, for isolating and sorting spheroids, with Incucyte® Live-Cell Analysis System for continuous drug response monitoring. Here, the response of GFP-expressing Human Embryonic Kidney (HEK) spheroids to a cytotoxic compound treatment, camptothecin, was used as model to demonstrate the suitability of the spheroONE + Incucyte workflow for drug toxicity assays.
The results demonstrated the synergy between spheroONE and Incucyte® Live-Cell Analysis System advanced technologies, offering researchers a potent tool to gain robustness and throughput for drug testing and expedite scientific discoveries.

Workflow description

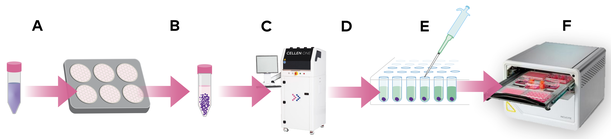

Fig 1. Workflow overview

- HEK293 cells were seeded into a spheroid forming microcavity plate (Fig. 1A).
- Three days after cell seeding, spheroids were harvested (Fig. 1B) and loaded into spheroONE (Fig. 1C).
- spheroONE was used to sort and isolate one, and only one, HEK spheroid into each individual well of a 96-well ULA plate (size gate 190 - 260 µm) (Fig. 1D).
- After isolation, the ULA-plate was placed into Incucyte® Live-Cell Analysis System for automated t=0 imaging of the 96 isolated spheroids.
- Different concentrations (0.1 to 1000 nM) of camptothecin were then added to different wells (Fig. 1E).
- The spheroid response to camptothecin was monitored using Incucyte® Live-Cell Analysis System every 6 hours for 7 days (Fig. 1F).

To learn more about spheroONE, scan this QR code:

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Technology

spheroONE features:

- Up to 100% single cellular aggregate isolation accuracy.
- For cell aggregates ranging from 100 to 600 µm diameters.
- Gentle dispensing technology maintaining integrity and viability.
- Improved cellular debris removal compared to manual isolation.
- Compatible with standard multi-well plates (e.g., 96 and 384) and any custom labwares.
- Visual inspection of samples and recorded images of isolated aggregates.

Incucyte® Live-Cell Analysis System features:

- Simple, flexible sample prep, reduced troubleshooting with lab-tested protocols.
- Walk-away, user-friendly, and guided experimental setup.
- Compatible with over 600 vessels.
- Acquire and View Images Over Time, up to five fluorescence channels combined with HD phase. Vessel View for quick visual survey of results.
- Analyze in Real Time. Efficient and reproducible image analysis.
- Powerful visualization of images and kinetic measurements, including IC50.
- Integrated solutions to automatically monitor and quantify tumour spheroid growth and death.

Manual pipetting vs. spheroONE isolation

spheroONE isolation delivers highly homogenous population with no debris which then facilitates automatic and accurate object detection and reproducible assays using Incucyte® Live-Cell Analysis System (Fig. 2).

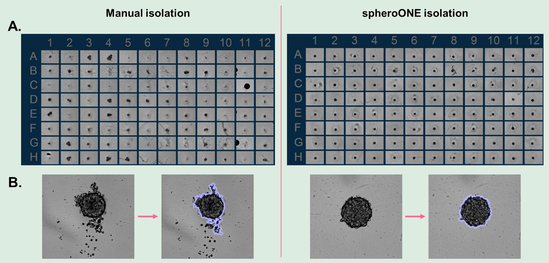

Fig 2. Manual pipetting- (left) vs. spheroONE- (right) isolated spheroids. (A) Full plate image and (B) Brightfield Average Object Area detection using Incucyte® Live-Cell Analysis System.

Results

Spheroids drug response was measured using size (Brightfield Object Average Area), as well as fluorescence intensity (Largest Brightfield Green Mean Intensity).
Thanks to Incucyte® Live-Cell Imaging and Analysis, isolated HEK spheroids were tracked and analyzed live over 7 days (Fig. 3).

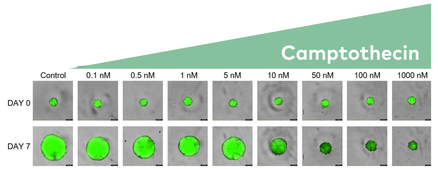

Fig 3. HEK-GFP spheroids growth dynamics during camptothecin treatment tracked with Incucyte® Live-Cell Imaging and Analysis software. Images captured at 10X magnification. Each data point represents mean ±SEM, n = 7.

At the highest camptothecin concentration (1000 nM), spheroid growth was completely inhibited after 1.5 days (Fig. 4A). At intermediate concentrations (50 to 100 nM), growth stopped by day 4. In contrast, spheroids exposed to lower concentrations (0.1 to 10 nM) grew throughout the experiment, although their growth rates inversely correlated with increasing camptothecin concentrations (Fig. 4B). The median effective concentration (EC_{50}), based on either spheroid growth or GFP expression at day 7, was 9.06 nM (Fig. 4C) and 13.6 nM (Fig. 4D), respectively.

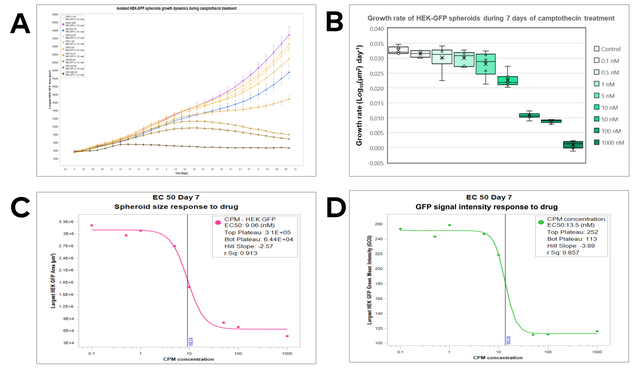

Fig 4. HEK-GFP spheroids dynamics analyses using Incucyte®. (A) The graphs display the Largest Brightfield Object Area (µm²) over the course of 7 days with 6-hour intervals showing specific treatment-dependent kinetic profile of spheroid growth and shrinkage. (B) Growth rate over 7 days of treatment for each camptothecin concentrations. (C,D) Response to different camptothecin concentrations and EC_{50} at day 7 using spheroid detected areas (C) and mean fluorescence intensities (D).

Conclusion

This innovative workflow represents a significant advancement in drug testing assays, whether for generic drug discovery or personalized medicine, by shifting experimental strategy from quantity to quality.
The use of spheroONE increases data consistency by (i) selecting and isolating uniformly sized and viable spheroids and (ii) limiting debris and dead cell background signals. As a result, subsequent drug treatment and data analyses could be undertaken on fewer but highly homogenous spheroids.
The use of Incucyte® Live-Cell Analysis System allowed low-disturbance incubation and imaging to continuously assess spheroid size dynamics in response to drug treatment. Additionally, thanks to the Incucyte fluorescent imaging capacities, it can be combined with a range of proprietary non-cytotoxic viability dyes which are particularly useful when working with non-proliferating cellular aggregates such as certain patient-derived tumoroids.
When combined, these two technologies leverage each other's strengths, allowing live and continuous monitoring of highly reproducible assays using fewer homogeneous 3D cell models. Together, these technologies overcome reproducibility issues which have long limited the implementation of spheroid assays as models of choice in drug discovery and personalized medicine applications.