A 3D Culture Model for Screening of Cancer Therapeutics

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Summary & Impact

- Growing evidence suggests the need for physiological relevant 3D models that mimic the *in vivo* micro environment (e.g. cell polarity, cell interactions, physiological extracellular matrix).
- Here we describe a 96-well kinetic tumor 3D assay for spheroid growth and shrinkage utilizing Ultra Low Attachment (ULA) microtiter plates (Corning®) in conjunction with the IncuCyte ZOOM.
- CellPlayer™ ULA 3D spheroid assay provides a technically simple, economical, and fully kinetic approach to creating 3D spheroids, equivalent to executing a 2D assay.
- The images and time-lapse movies provide important information and validation regarding spheroid morphology and the effects of test samples.
- IncuCyte Zoom metrics spheroid size (area, confluence) and fluorescence intensity are informative, and can be readily gathered to monitor spheroid growth and shrinkage over time . Tumor shrinkage is an important translational paradigm for drug treatment of existing solid tumors.
- These assays should prove useful for medium throughput, quantitative screening of test samples for effects on the growth and shrinkage of micro-tissues.
- The 3D spheroid assay can be applied as a more physiologically relevant *in vitro* co-culture model to study the tumor micro-environments.
- Cell proliferation can be directly compared in 2D and 3D.

Cell Player ULA Spheroid Assay Workflow

Cell Seeding

Harvest and seed cells into 96-well ULA plate (Corning 7007)

- 100 µl per well, 1 – 5K cells per well
- Centrifuge plate (1000 RPM, 5 minutes at ambient temperature)

Spheroid Formation

Place ULA plate within IncuCyte ZOOM (4x or 10x)

- Image Phase & Fluorescence
- Scan every 6 hr (72-96 hr)

Sample Addition

Prepare treatments at 2X and add to plate containing spheroids

- Aliqout 100µL 2x treatments to wells containing spheroids and media
- Return plate to IncuCyte ZOOM

Spheroid Growth & Shrinkage

Monitor and quantify spheroid parameters over time

- Size (mm², confluence) & fluorescence intensity metrics
- Real time, automated analysis

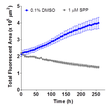
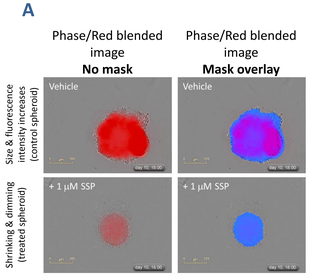


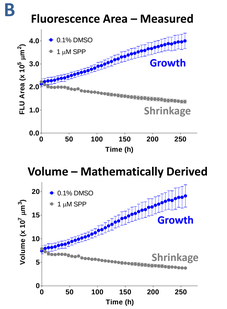
Image Processing & Quantification

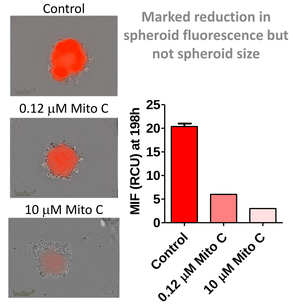
A. Image Processing
- Phase Contrast & Fluorescent images are acquired.
- Due to technical difficulties associated with masking 3D images in ULA plates, fluorescently labelled cells are used for analysis/masking.

B. Quantification
- IncuCyte ZOOM can automatically quantify spheroid size (fluorescence area and confluence). Volume can be mathematically derived.

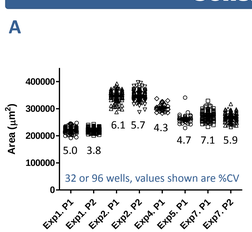
C. Time-dependent reduction in spheroid fluorescence intensity (brightness) is used for agents that cause little or no change in spheroid size, however cause a marked reduction in spheroid fluorescence.

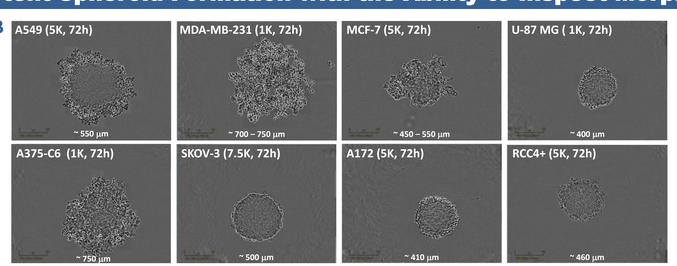






Consistent Spheroid Formation with the Ability to Inspect Morphology

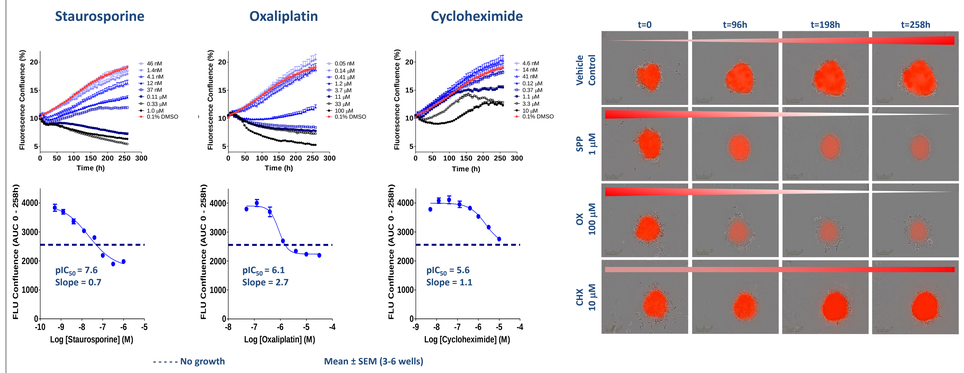




A. A549 NucLight Red cells seeded at 2.5K cells/well and fluorescent size was analyzed at 72 hours.
- Spheroid size at 72h is highly homogenous within and across plates

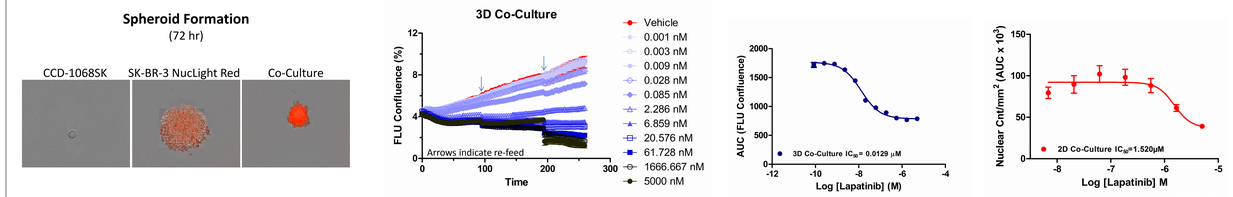
B. Shown are HD phase images of spheroids formed from a range of tumor cell lines at 72 hours.
- Images validate quantification (verify size and fluorescent intensity)
- Single and centrally located spheroids can be inspected for different morphologies (tight spheroids, compact and loose aggregates)

Quantitative Pharmacology of 3D Spheroids



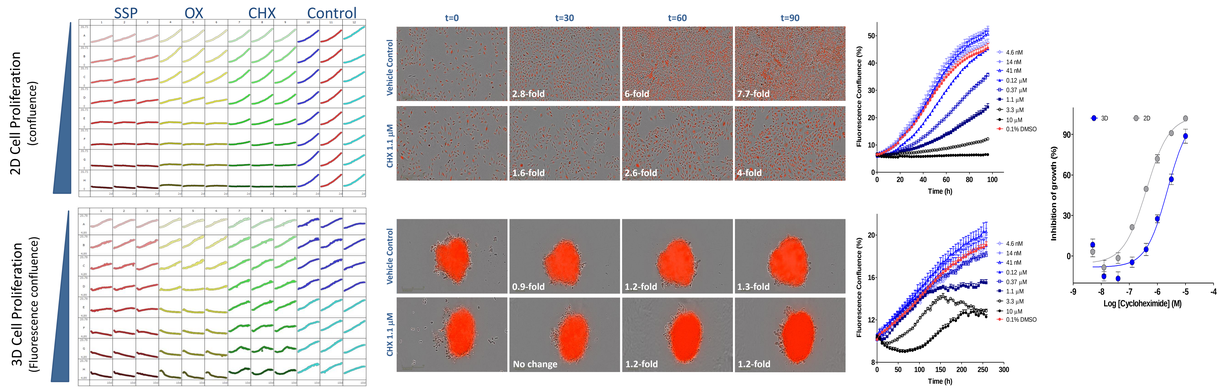
A549 NucLight Red Cells were seeded in a round bottom ULA plate (5K cells/well) and allowed to form spheroids over 3 days. Cells were treated with decreasing concentrations of either Staurosporine (SSP), Oxaliplatin (OX) or Cycloheximide (CHX). Kinetic response was plotted as fluorescence confluence and EC50 values were calculated using Area Under the Curve (AUC). The dotted line on the concentration response curves represents no growth of the spheroid. Note: known cytotoxic compounds, SSP and OX, cause the spheroid to shrink to a size smaller than its starting size while this is not observed with the cytostatic compound CHX. Quantification was validated by inspection and visualization of images.

Effects of Lapatinib in a 3D Co-Culture Approach



In order to determine the effect of Lapatinib in a 3D co-culture model, SK-BR-3 (breast adenocarcinoma) and CCD-1068SK (mammary derived stromal cells) were first seeded in mono-culture (10k/well) or co-culture (10k/cell line/well) to determine their ability to form spheroids. Images show that CCD-1068SK cells are required for SK-BR-3 to form a compact spheroid (SK-BR-3 cells form loose aggregates in the presence of 5% Matrigel, image not shown). Co-cultures were then seeded and allowed to form spheroids prior to treatment with 3-fold decreasing concentrations of Lapatinib, a tyrosine kinase inhibitor. Fluorescence confluence metrics were used to determine spheroid size, and kinetic data was used to determine Area Under the Curve (AUC). The IC50 value using the 3D co-culture method (0.013 µM), is similar to the previously published IC50 value of 0.037±0.031 µM (Konecny, Cancer Res 2006). Interestingly, in a 2D co-culture model, CCD-1068SK fibroblasts have a rescue effect on SK-BR-3 cells from Lapatinib (IC50 = 1.52 µM). Although the 3D co-culture model IC50 did not shift from previously published mono-culture data, images suggest that fibroblasts play an important role in tumor formation in the more physiologically relevant 3D *in vitro* culture model.

Differential Effect of Cycloheximide in 2D vs 3D Biology



A549 NucLight Red Cells were seeded in both a standard flat bottom 96-well plate (2.5K cells/well) and a round bottom ULA plate (5K cells/well). Cells were treated with decreasing concentrations of either Staurosporine (SSP), Oxaliplatin (OX) or Cycloheximide (CHX). Kinetic response was plotted as fluorescence confluence and the EC50 value of Cycloheximide was calculated using Area Under the Curve. Looking at the proliferation time-courses we can see the dramatic differences in the temporal profile of CHX in 2D vs 3D. Assay metrics are verified by visual inspection of the cells, showing striking differences between 2D and 3D proliferation (noted as fold increase from t=0). At the 90h time point, we see selective inhibition of 2D proliferation (~ 2-fold decrease in cell confluence compared to control) with little or no effect on the proliferation of the spheroid. EC50 data shows a that CHX is almost 10-fold more potent in 2D inhibition of growth.