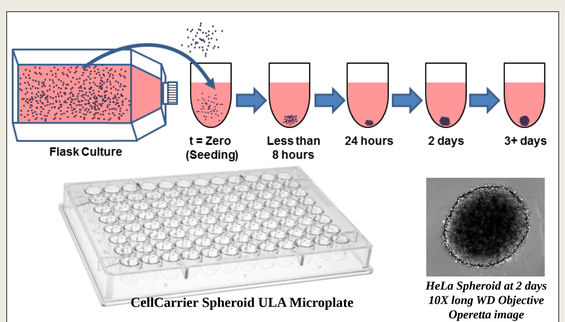
Rapid development and analysis of 3D spheroid microtissues from multiple cancer cell lines with CellCarrier™ Spheroid ULA microplates, high content imaging, and reagents from PerkinElmer

Jeanine M. Hinterneder, Richard Hellmer, Anne (Shang-pin) Kwei, and Vincent Dupriez

1 Introduction

Three-dimensional (3D) cell culture holds many potential advantages over monolayer cultures as growing cells in 3D can better simulate natural cellular interactions and mimic *in vivo* microarchitecture to provide more physiologically relevant information. Of the various options available, growing cultures as spheroid-shaped microtissues is arguably one of the best *in vitro* representations of small avascular tumors. Culturing cells in 3D allows for more complex cell-cell interactions and the formation of nutrient and oxygen gradients which tumors exhibit *in vivo*. As a result, the addition of 3D cell culture models into discovery workflows can reduce downstream costs such as secondary assay testing and *in vivo* animal testing, avoiding the high cost of stopping the progression late in the drug discovery pipeline. However, the adoption of 3D cultures in regular screening programs has been hindered by uneven culture growth, poor reproducibility, high variability, and the lack of robust methods for high-throughput analysis of 3D cultures. We present here a reliable, rapid, and straightforward method for generating 3D spheroid cultures using CellCarrier™ Spheroid ULA microplates with Ultra-low attachment coating.

2 Materials & Methods



Workflow for seeding and growing cells in 96-well CellCarrier Spheroid ULA microplates

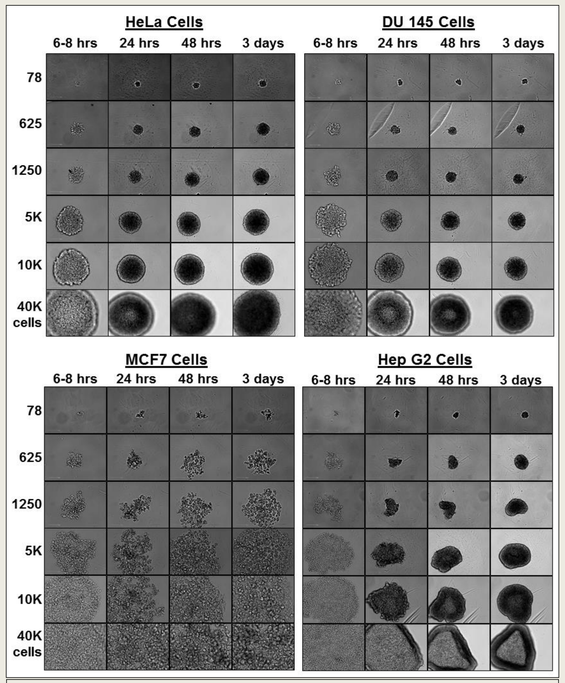
	Catalog Number	Pack Size
CellCarrier Spheroid ULA Microplates	6055330	10 pack
	6055334	Case of 40

Cell Lines Used in these Experiments

Cell Line	Cancer model	ATCC Cat. No.	Spheroid description after 2 days
HeLa	Cervical	CCL-2	Tightly Packed sphere
DU 145	Prostate	HTB-81	Tightly Packed sphere
HepG2	Liver	HB-8065	Tight, Roughly spherical
MCF-7	Breast	HTB-22	Loose clumps
HEK293	Kidney	CRL-1573	Tightly-packed sphere

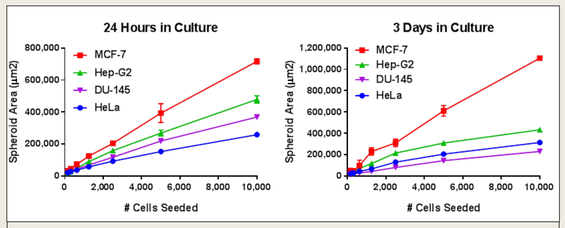
3 Imaging & Spheroid Size Analysis

Cells from 4 human cancer cell lines were raised up in flasks and seeded into CellCarrier Spheroid microplates at 12 different concentrations and grown for 3 days. Spheroid cultures were imaged daily representative images are below:

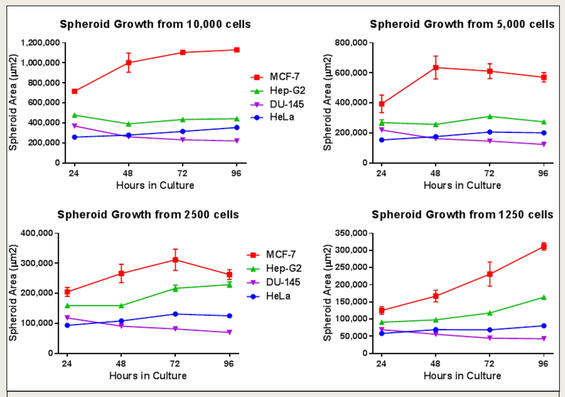


3D Spheroid cultures generated from six different seeding densities and four different cancer cell lines imaged over three days. Images were acquired daily in all microplates using the 10X long WD objective on the PerkinElmer Operetta High Content Imaging System and Brightfield optics. Spheroid culture morphologies and development profile varies among the different cell types.

HeLa cell spheroid formed from 10,000 cells seeded after 48 hours showing labeling with Cell Tracker Green dye. Imaged using the10X long WD objective on the Operetta with Brightfield and green fluorescence channels (140 µm stack, 10 µm plane distance, confocal mode (green). The maximum intensity projection images are shown here. (Scale bar = 200 µm.)



Spheroid size varies by cell type and is correlated with cell numbers seeded. The graph on the left illustrates how different cell lines produce different sized spheroids and that size increases linearly with number seeded. After 3 days in culture (right graph), spheroid size measurements show how some cell types stop proliferating in spheroid cultures.
N=3 spheres, Error = StDev.



Spheroid growth varies based on cell type and initial cell seeding density. Four graphs of spheroid area changes over 3 days demonstrate how 3D spheroids produced from four different initial seeding densities and by different cell types grow at different rates and that spheroids from some cell lines even decrease in cross-functional area (e.g, DU 145 cells).
N = 3 spheres, Error= StDev.

4 Toxicity Analysis with ATPlite™ 3D assays

New ATPlite™ 3D and ATPlite 1step 3D products, developed specifically for measuring viability and toxicity in 3D spheroid cultures, were used to analyze toxic compound effects on 3D spheroids grown in CellCarrier Spheroid microplates.

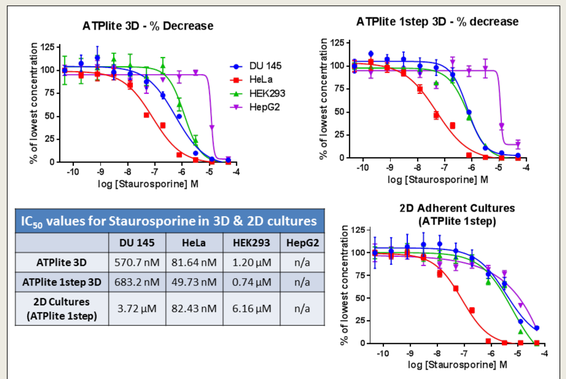
ATPlite 3D protocol

- Add 50 µL mammalian lysis solution per well
- Shake for **10 minutes**
- Add 50 µL of substrate solution
- **Pipette Mix** up and down 5 - 7 times vigorously (50 µL)
- Incubate at Room Temp for **15 minutes**
- Transfer 50 µL to 384-well HS (gray) OptiPlate
- Measure luminescence

ATPlite 1step 3D protocol

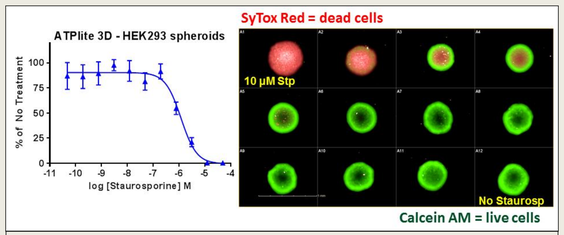
- Add 100 µL substrate solution
- Shake for **5 minutes**
- Incubate at Room Temp for **20 minutes**
- **Pipette Mix** up and down 5 - 7 times vigorously (50 µL)
- Transfer 50 µL to 384-well HS OptiPlate
- Measure luminescence (on an EnSight Multimode Plate Reader)

Assay Kit	Catalog Number	Kit Size (for 96-well plates)
ATPlite 3D Assay Kit	6066943	300 assay points
ATPlite 1step 3D Assay Kit	6066736	100 assay points
384-well HS (Gray) OptiPlates	6005310	Case of 50
	6005339	Case of 200



IC_{50} values for Staurosporine in 3D & 2D cultures	DU 145	HeLa	HEK293	HepG2
ATPlite 3D	570.7 nM	81.64 nM	1.20 µM	n/a
ATPlite 1step 3D	683.2 nM	49.73 nM	0.74 µM	n/a
2D Cultures (ATPlite 1step)	3.72 µM	82.43 nM	6.16 µM	n/a

Staurosporine toxicity profile for spheroids measured with ATPlite 3D and ATPlite 1step 3D. 4 cell types were seeded at 4,000 cells per well, grown for 3 days and treated overnight with Staurosporine and Toxicity assessed with ATPlite 3D assays. Data illustrate how different cell types in 3D spheroids differently to the same toxic compound and that toxicity can shift when cells are cultured in 3D versus 2D cultures (e.g., DU 145 cells).



Staurosporine toxicity profile for HEK293 spheroids measured with ATPlite and illustrated by Live/Dead cell stains and imaging. HEK293 cells plated at 4,000 cells per well, grown for 3 days and treated overnight with Staurosporine. A subset of the wells (spheroids) were stained with fluorescent dyes to identify live (Calcein AM; green) and dead (SyTox Red) cells; were imaged on the Operetta and then assayed with ATPlite 1step 3D (included in data in Graph A).

6 Summary & Conclusions

- **We present here a rapid, reliable and straightforward method for generating 3D spheroid cultures using ULA coated CellCarrier Spheroid microplates.**
- **We assess 3D spheroid development from 4 human cancer cell lines using fluorescent live stains and the Operetta high content imaging system to follow spheroid development over time.**
- **The effects of toxic compound on 3D spheroids from multiple cell lines were examined and compared to 2D monolayer cultures using new ATPlite™ 3D and ATPlite 1step 3D assays.**

PerkinElmer, Inc., 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602 www.perkinelmer.com