Cell based assays have widely been used in drug discovery, however, it is becoming increasingly clear that the traditional 2D model of cell culture is not truly representative of how cells grow and respond in vivo. To address this disparity, 3D cell culture technology has made vast improvements in recent years and is fast emerging as a new approach to drug discovery allowing for a greater understanding of drug efficacy and toxicity before drugs move into the clinical trial phase.

Aurelia has implemented 3D cell culture techniques which can easily be adapted into an HTS format. Ultra-low attachment (ULA) plates allow spheroids to form naturally. This technology relies upon a cells innate ability to grow and interact in a 3D format to form a spheroid. This provides a better representation of in vivo cell physiology which is lost in the tradition monolayer format. Using this technology we were able to monitor drug potency and efficacy in either an imaging-based assay (on the CellInsight™ CSX High Content Screening [HCS] Platform) or by using commercially available assays such as the CellTiter Glo® 3D viability assay (Promega).

Here we test two spheroid cultures derived from either a HCT116 (human colorectal carcinoma) or U87 (human glioblastoma) cell line. Each cell line produces a compact spheroid. We have used a number of readouts to generate a pharmacological profile of each spheroid type.

HCT116 and U87 cells were cultured in DMEM media supplemented with 10% FBS in TC-treated flasks at 37°C, 5% CO2. For spheroid formation, each cell line was seeded at 400 cells/well (40µl/well) in cell culture medium in 384-well ultra low attachment (ULA) microplates (Corning). The plates were incubated at 37°C, 5% CO2 for a period of 4 days to allow the cells to cluster and form a spheroid. Compounds were added on day 4 at a 1:2 dilution (40µl/well) and incubated with cells for a further 4 days before measurements were taken.

The effect of each compound was determined in three different assays. Viability was measured using the CellTiter Glo® 3D Viability Assay (Promega). An equal volume of reagent was added to the culture medium. Plates were mixed for 5 minutes on an orbital shaker to ensure good cell lysis. The plates were allowed to equilibrate at room temperature for 20-25 minutes after which time luminescence was determined using the Enspire® Plate Reader (PerkinElmer). The luminescent signal is directly proportional to the level of ATP within the cells and hence gives a measurement of metabolite activity and cell viability.

Over time, the size of the spheroid was monitored using brightfield microscopy on the CellInsight™ CSX High Content Screening (HCS) Platform (Thermo Scientific). An algorithm was used to define the area of the spheroid using a 10× objective.

In addition to monitoring spheroid size, we also stained the spheroids with DRAQ7™, a far-red DNA stain that will only stain the nuclei of dead or permeabilised cells. Spheroids were stained with DRAQ7™ at a 1:2 dilution to give a final concentration of 1.5µM.

Summary and Conclusion
- All compounds tested affected cell viability in a dose dependent manner (CellTiter Glo® 3D Viability Assay). While there was some variation in potency, not all compounds were able to restore viability to untreated levels within the concentration range tested.
- Further investigation with a DRAQ7™ dead cell stain revealed that those compounds which showed reduced viability appeared to have a necrotic core. This effect was also dose dependent.
- Growth inhibition was determined by calculating spheroid area. We were able to monitor growth inhibition in a dose- and time- dependent manner in both spheroid types tested. Only nocodazole and paclitaxel increased spheroid size. They appeared to cause shedding of peripheral cells and a loss of the tight spheroid morphology.
- Taken together, we were able to build a pharmacological profile for each compound tested.

The Use of High Content Imaging and Viability Readouts for Screening with 3D Spheroids

Alison Gordon1 & Gary Allenby1
1 Aurelia Bioscience Ltd., Biocity, Pennyfoot Street, Nottingham, NG1 1GF

Methodology

Spheroids were allowed to form in ULA plates for 4 days, this was an optimum time for cell compaction prior to drug treatment. To assess cellular viability, spheroids were cultured and then tested with an increasing concentration of compound. Each cell line showed a dose-dependent decrease in viability with an increasing concentration of compound.

Spheroid size was quantified on the CellInsight™ CSX High Content Screening (HCS) Platform using an overlay on the brightfield imaging channel.

Prior to treatment, spheroid sizes showed an initial decrease due to cell compaction. After this time, in the absence of compound, spheroids grew in a linear manner up to day 8 of the experiment. Compounds induced a dose-dependent inhibition of growth as characterized by a reduction in the spheroid size. This was consistently seen in the U87 cell line. In comparison, compounds paclitaxel and nocodazole induced a looser cytoskeletal organization in HCT116 spheroids at higher concentrations.

Spheroid structure is characterized by a mixed population of cells whereby a hypocic core is surrounded by a viable and replicating population of cells on the periphery. In the absence of compound, DRAQ7™ staining increased in untreated spheroids over time.

Spheroids were treated with compounds early in their growth phase prior to the formation of distinct necrotic core. Compounds induced a gradual increase in DRAQ7™ staining over time in a dose-dependent manner. Treatment with nocodazole or paclitaxel significantly increased DRAQ7™ staining, this may be due to the loose nature of the spheroids in the presence of compound.

Table 1: Estimated IC50 values generated from the spheroid size at day 8 derived from spheroids comprising of U87 or HCT116 cells.

Table 2: Estimated IC50 values derived from the spheroid size at day 8 derived from spheroids comprising of U87 or HCT116 cells.

Table 3: Estimated IC50 values derived from DRAQ7™ intensity in spheroids - comprising of U87 or HCT116 cells.