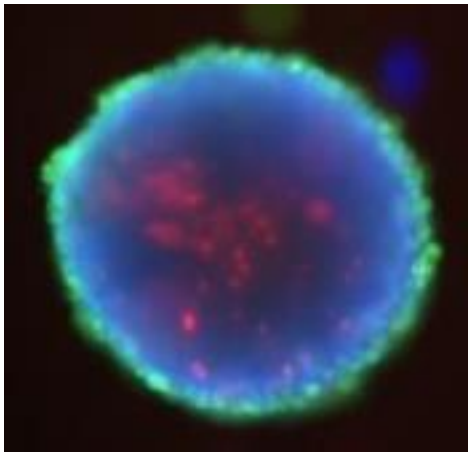
# 3D Cell Culture

## Spheroids

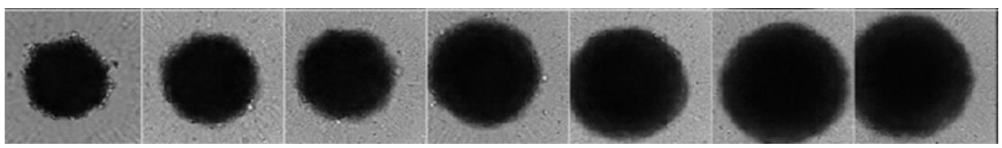
aurelia bioscience
bioassays + screening

### How it works:

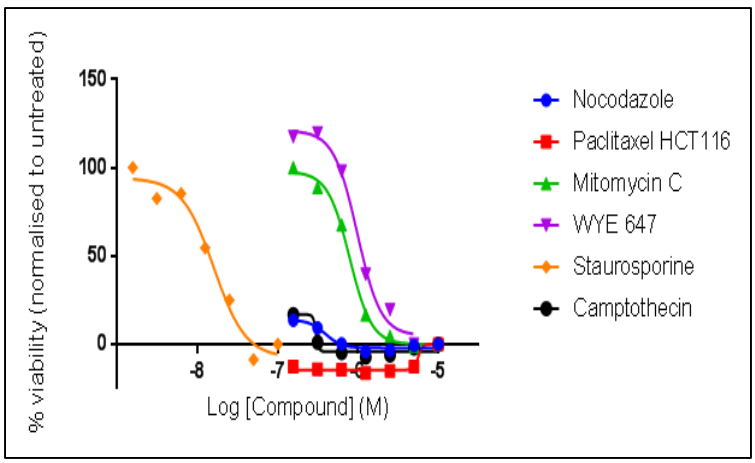
- Aurelia Bioscience uses Ultra Low Attachment (ULA) plates to generate and culture spheroids. These plates have an ultra-low attachment surface coating and a well geometry that favours the formation of a single spheroid within each well.
- Spheroids of different sizes develop gradients of oxygen, nutrients and metabolites, creating a hypoxic core and replicating cells on the outer edges.
- Drug potency and efficacy is monitored in either an imaging-based assay (on a wide-field imaging plate reader) or by using commercially available assays that track parameters such as cell viability.



U87 cells form a spheroid - stained with Hoechst (blue), Calcein AM (green) and propidium iodide (red), imaged with CellInsight™ CX5 High Content Screening Platform. The spheroid was formed from seeding 1000 cells per well and culturing in 384 well Costar ULA spheroid plates for 7 days.




Spheroids formed from U87 cells then treated with Mitomycin C. The spheroid decreases in size upon treatment and incubation with compound over 48 hours in culture




HCT116 cells were seeded at 1000 cells per well and grown for 7 days. Spheroids were then treated with increasing concentrations of compounds over a 48 hour period to examine cell toxicity. A 3-D Cell titre GLO reagent kit (Promega) was used to examine cell toxicity and data normalised to untreated spheroids.

E-mail: allenby@aureliabio.com Tel: +44 115 8370503 Web: www.aureliabio.com

Aurelia Bioscience Ltd, Nottingham, U.K.

# 3D Cell Culture

## Micro-tissues on Electrospun Scaffolds

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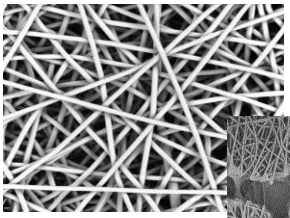
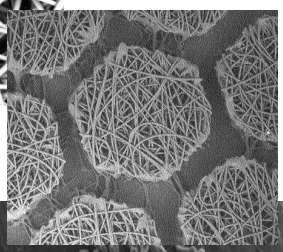
### How it works:

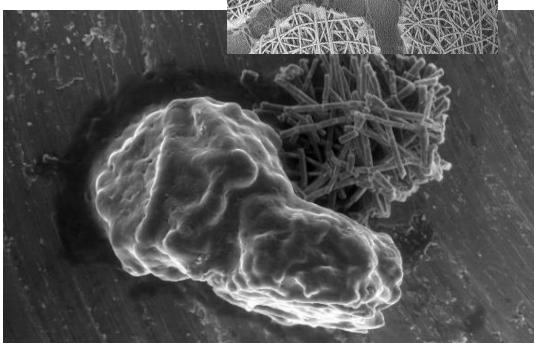
- Aurelia Bioscience has developed a 3-D micro-tissue system from electrospun material that can be used in conjunction with well plates for higher throughput screening
- Electrospun material mimics the natural extracellular matrix and provides an ideal substrate for cells to adhere to
- We have re-engineered electrospun material to form micro-scaffold islands on to which we seed, grow and differentiate cells prior to performing more conventional assays in well plates. Cells grow on, around and into the material, forming a micro-island of adherent cells that are effectively “micro-tissues in solution”
- The incorporation of iron particles into fibres during manufacture results in scaffolds that can be physically manipulated using magnetism



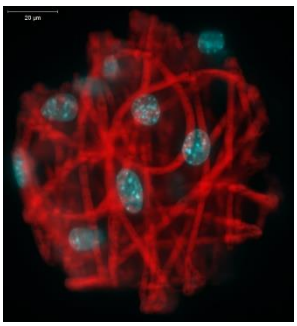
Electrospun scaffold spun from poly-L-lactide



Can be lased into specific shapes



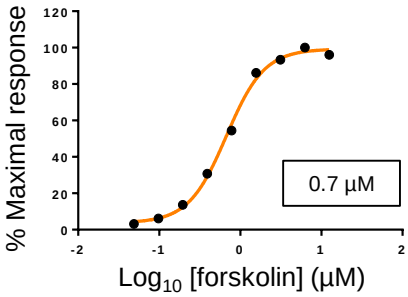
Cells populate scaffolds: Environmental SEM image of A549 cells 4 days post seeding on scaffolds. Image shows two scaffolds at 90 degrees to each other, populated with cells next to an unseeded scaffold.



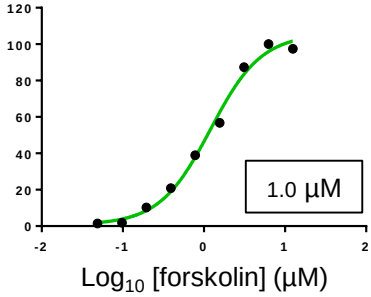
C3H/10T1/2 fibroblast cells inoculated on to scaffolds and incubated for 24hrs. Red stain – Scaffold fibre, Green – cell nucleus

- The incorporation of fluorescence dyes into fibres during manufacture means that fibres can be seen when imaging and can be used as a ‘barcode’ - two colours can be used to distinguish different cells types or populations in the same well

- Recombinant HEK293 cells stably expressing CRE-luciferase (Promega) - monitors cyclic AMP (cAMP) levels in cells. Treatment with forskolin increases cAMP levels, which acts as a transcription factor and increases synthesis of luciferase




**2D adherent**




**3D adherent**

### Advantages:

- Robust and reproducible 3-D culture environment
- Applicable to any cell type (recombinant, human primary cells and iPSC’s plus differentiation)
- Movable: from vessels-to-well and well-to-well with magnetism
- Scalable to any assay throughput
- Integrates seamlessly with all current screening and assay workflows

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