Magnetic Electrospun Micro-fibre Scaffold Assemblies: Examples of there use for 3-Dimensional Cell-Based Screening Applications

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Introduction

3-Dimensional (3-D) cell culture is being increasingly adopted in drug discovery. However there are practical issues…

- Some, but not all cell lines can be grown as spheroids – some do not grow unless given extra cellular matrices in solution
- Once formed, spheroids are impossible to manipulate and difficult to perform media changes
- Independent well-to-well culture conditions result in uneven cell growth across a plate making it very difficult to generate homogeneous spheroids

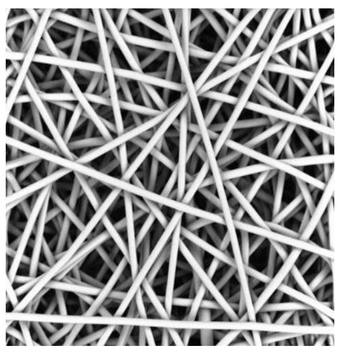
To address these issues we have developed electrospun scaffold material on to which we seed cells that grow on and into the scaffold forming a 'micro-tissue'. Importantly, scaffolds can be:

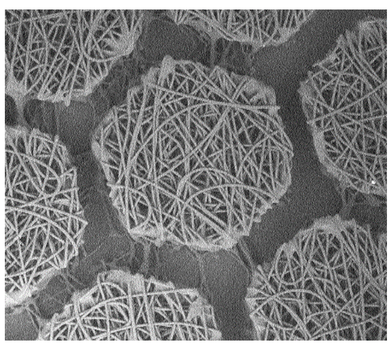
- Physically manipulated with magnetism to move micro-tissues between culture vessels and well plates without damaging cultured cells
- Manufactured to contain fluorescence dyes for imaging or used as a barcode to distinguish micro-tissue seeded with different cells but cultured in the same well
- Cells can be transfected on scaffolds
- Cells can be cryo-preserved on scaffolds
- iPSC's can be differentiated on scaffolds
- Cultures can be grown and maintained in the same vessel thereby generating micro-tissue homogeneity and separated at the point of plating
- Assay ready compounds plates can be used

Here we demonstrate the innovative utility of electrospun material, generating micro-tissues for drug discovery purposes.

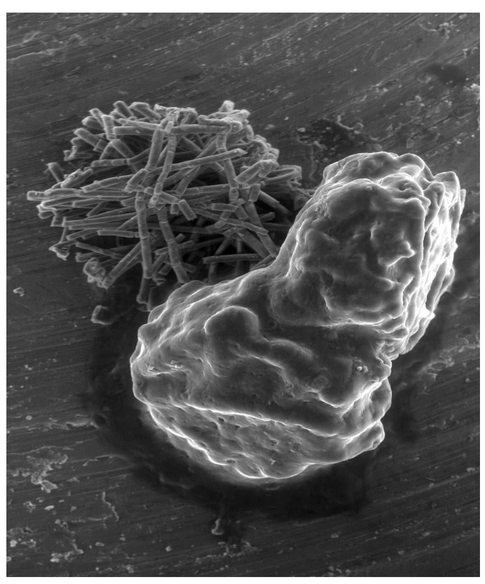
Electrospun Material - Properties

Electrospun material is made from poly-L-lactide, (FDA approved), laid down in a random pattern forming a porous structure (right). Fibres 2µm thick form pores 10-18µm, 4µm thick form pores 19-31µm





Material can be laser cut into shapes – here (left) 100µm x 100µm wide x 50µm deep hexagonal shapes. Melting of material at the edges helps maintain structure



Scanning EM image of scaffolds with and without cells. HEK-293 cells were seeded on scaffolds and culture for 96 hrs – cells colonized two scaffolds to form a micro-tissue. The third scaffold was not seeded

Inclusion of Fluorescence Dye in Scaffold Material – for Imaging and Barcoding

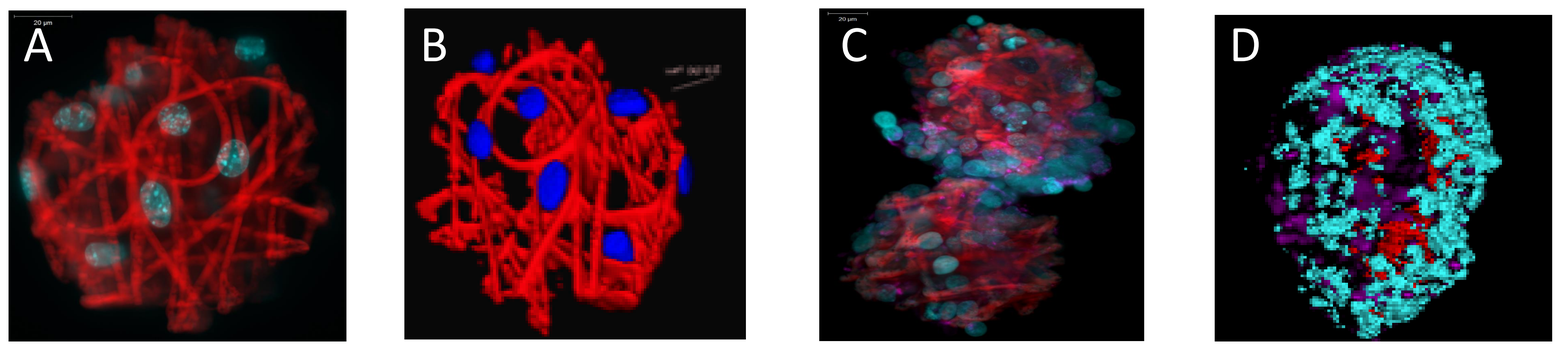


Fig. 1A. Scaffold inoculated with C3H/10T1/2 fibroblast cells, cultured for 24hrs and imaged (20x) magnification on a CellInsight CX5 imager (ThermoFisher) - Red stain = rhodamine red stained fibre, green = Hoechst stained cell nucleus. Fig. 1B. Light Sheet microscopy image of a similar treated scaffold – blue = cell nuclei. Fig. 1C. Two scaffolds inoculated with HEK-293 cells, cultured for 24hrs and stained with Hoechst (blue nuclei). Fig. 1D. Light sheet microscopy image of a similar scaffold stained with Hoechst - nuclei (blue), tubulin (purple) and scaffold material (red)

Pharmacology: (2-D -v- Scaffold) (Stable -v- Transient Expression) (Fresh -v- Cryo-preserved)

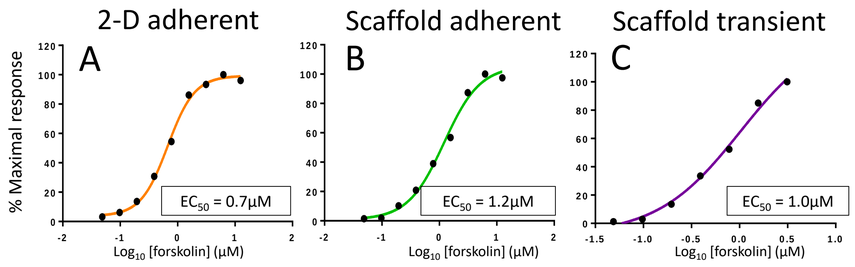


Fig. 2A & B. Pharmacological responses to forskolin are similar in 2-D and on scaffolds. HEK-293 cell line stably expressing a cyclic-AMP response element (cAMP-RA) upstream of a firefly luciferase cDNA were treated with increasing concentrations of forskolin in 2-D monolayer or on scaffolds and demonstrate similar pharmacology of the cell line on both substrates. Fig. 2C. Transient transfection on scaffolds (lipofection) with identical cAMP-RA - firefly luciferase cDNA shows similar response to forskolin.

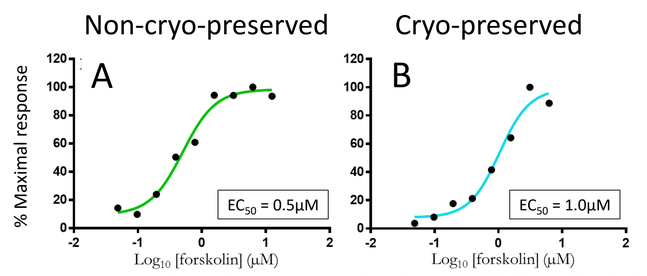
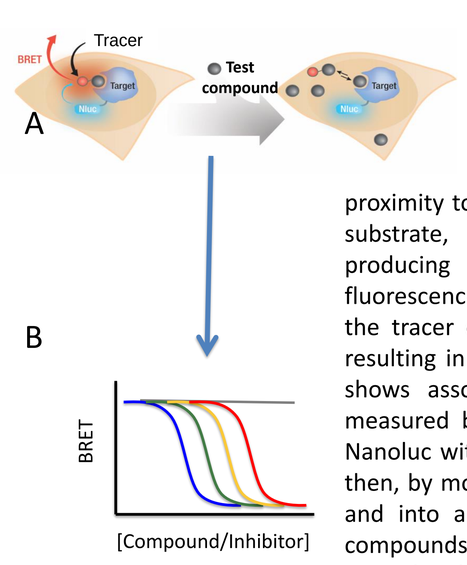
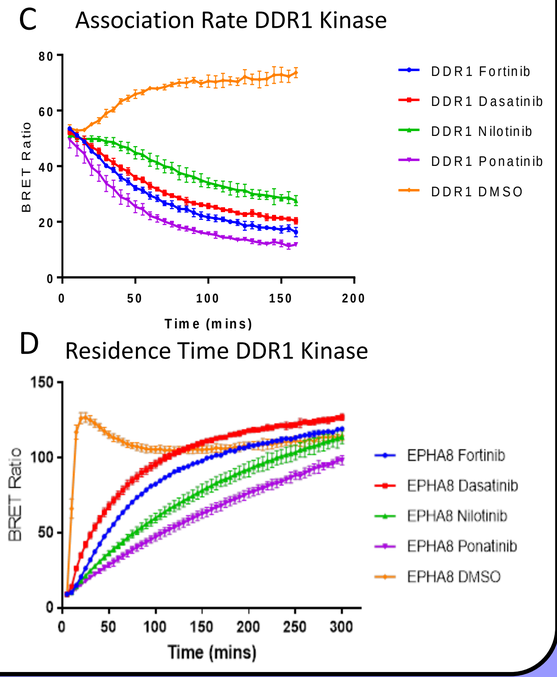


Fig. 3. Pharmacological responses of cells on scaffolds to forskolin either non cryo-preserved (A) or cryo-preserved on scaffolds (B). HEK-293 cell line stably expressing a cAMP RA - firefly luciferase cDNA were either cultured in 2-D or cryo-preserved (90% FCS: 10% DMSO) on scaffolds. Cryo-preserved cultures were incubated for 24hrs in media then treated with forskolin.

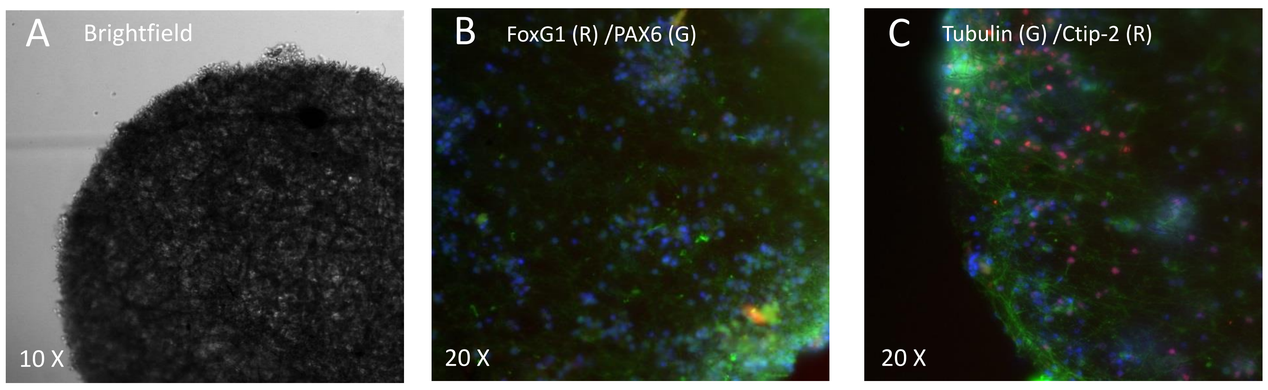
Changing the Paradigm: Moving Adherent Cells Plate to Plate for Assay



HEK-293 cells were cultured on scaffolds and transiently transfected with cDNA of a chimeric protein consisting of full length kinase (DDR-1) connected to Nanoluc™, a 19KDa luminescence enzyme, (Promega). When a Halo-tagged tracer molecule is added it binds to the kinase within the cell and the fluorescent tag comes into close proximity to the Nanoluc enzyme and, in the presence of Nanoluc substrate, Nanoluc generates photons that excite the dye producing Bio Resonance Energy Transfer (BRET) based fluorescence (Fig. 4A.). In the presence of competing compound, the tracer dissociates from the kinase and the signal decreases resulting in concentration response curves (Fig. 4B.). Fig. 4C & D. shows association and dissociation rates respectively can be measured by pre-incubating cells on scaffolds expressing DDR1-Nanoluc with either tracer or compound respectively in one plate then, by moving the scaffolds from this plate through a wash bath and into a second post-incubation plate, the cells move from compounds to tracer (or visa versa) and a kinetic read is performed immediately to detect time-dependent compound-tracer competition. This improves data quality as no cells are lost due to washing steps and the process increases throughput.



iPSC Cells Differentiated to Cortical Neurones on Scaffolds



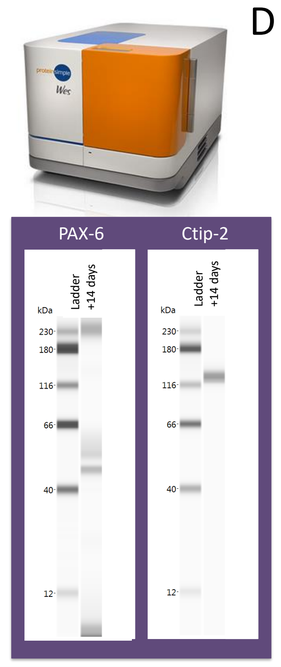


Fig. 4. Partially differentiated neuro-ectoderm cells were purchased as a cryo-preserved stock (Axol) and loaded on to 1mm scaffold discs in 384 well plates. Once adhered to scaffolds, differentiation media was replaced every 2 days for 40 days, during which time the cells differentiated into cortical neurones. Cells were fixed and imaged using the CellInsight CX5 imager in 10x brightfield (A) and 20x fluorescence (B, C). then probed with fluorescence tagged antibodies to Fox-G1 (B) (red), Pax-6 (B) (green), Tubulin (C) (green) and Ctip-2 (C) (red) used to identify mature cortical neurones. Nuclei were stained with Hoechst (blue). In other samples proteins were isolated from cells on scaffolds, run in Western Blotting (D = WES) and probed with antibodies to Pax-6 and Ctip-2. Shown in E are Western blots of protein isolated 14 day post-culture in differentiation media showing expression of mature cortical neurone proteins.

Summary

Electrospun scaffold material can be:

- Manufactured into unique shapes, sizes and sheets for different applications
- Manufactured to incorporate iron within the material, therefore it can be physically moved either vessel to well or well to well with magnetism
- Manufactured to incorporate fluorescence dyes within the material acting as a contrast in high content imaging studies or as a 'barcode' allowing different cells to be tested within the same well
- Many different cells types including iPSC's will populate the material to form a 'micro tissue' of adherent cells in solution that can be transferred into assay ready plates for compound screening
- Many different well plate formats (96/384/1536) and assay readouts can be used including fluorescence, luminescence, imaging, isolation of protein and mRNA from cells on scaffolds

[1] Aurelia Bioscience is a Contract Research Organisation. If you are interested in hearing more about our scaffold biology or other services, please come and see us at our exhibitor stand