An In-vitro Model of the Glomerulus

Jack Tuffin[1], Madeline Burke[2], Simon Satchell[1], Moin Saleem[1], Adam Perriman[2] & Gavin Welsh[1]

1: Academic Renal Unit, University of Bristol **2:** School of Chemistry, University of Bristol

[1] Introduction

The Glomerulus is the filtration unit of the kidney. it permits retention of high molecular weight proteins in circulation whilst allowing passage to water, small molecules/proteins, sugars and electrolytes [1]. The selective barrier within the glomerulus is called the glomerular filtration barrier (GFB), a tri-layer structure comprised of a layer of glomerular endothelial cells (GEnCs), a layer of specialised epithelial podocytes and the glomerular basement membrane (GBM) which is situated between the two.

Fig 1: The Glomerulus. A capillary network of GEnCs (Red) can be seen to be covered by a layer of 'interdigitating' podocytes (yellow).

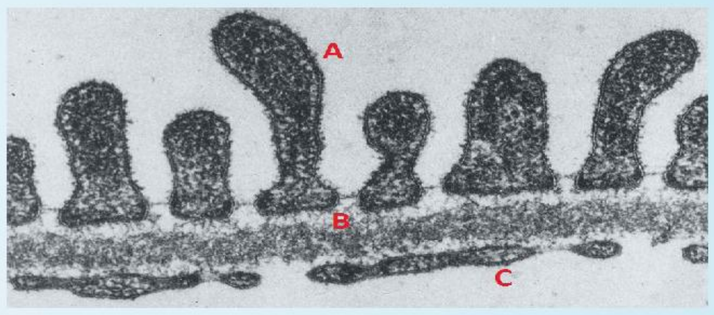


Fig 2: TEM cross-section through the GFB. (A) Podocyte layer complete with foot-processes. (B) The GBM (C) GEnC layer complete with fenestrations.

The Glomerulus is formed by (and is the site of) significant intracellular crosstalk [2]. Our lab has established cell lines of conditionally immortalised podocytes [3] and GEnCs [4] and have successfully co-cultured them using a transwell system [5]. Intracellular crosstalk is however very much a three-dimensional process and as such, an accurate in-vitro model of the glomerulus must co-culture cells on a 3D platform if possible. A 3D model of the glomerulus will also provide a more physiologically-relevant platform to model Glomerulosclerosis and will therefore prove to be a powerful tool for screening therapeutic compounds. The aims of the project are therefore:

- To establish an in-vitro 3D model of the Glomerulus through co-culturing the relevant cell types
- To induce glomerulosclerosis in this model and use it to screen disease-modifying compounds.

[2] Methods

Conditionally immortalized Podocytes and GEnCs were virally transduced with actin-GFP/actin-mCherry lentiviral plasmid pWPXL (addgene). GEnCs now stably express mCherry actin (Red) and Podocytes express GFP-actin (Green). Two different 3D platforms were used in parallel: Magnetic levitation and Tissue culture scaffold systems.

[3] Methods Continued

1: Magnetic Levitation: The first 3D tissue culture platform experimented with makes use of Magnetic Levitation technology (n3Dbio).

Fig.3:Schematic of the magnetic "bio-assembler". Cells are incubated for 24 hours with "nanoshuttle" which magnetises them. A magnet above the culture flask then allows cells to grow in 3D at the air-liquid interface.

2: Tissue culture scaffolds and gels: The second 3D culture platform uses variations of electrospun poly-glycolic acid (PGA) scaffolds coated with Fibronectin.

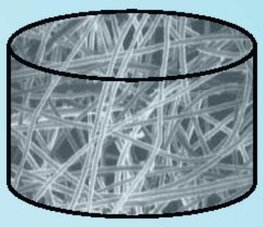


Fig:4 Schematic representation of electrospun PGA scaffold. Cells are seeded on top of scaffold and allowed to attach to fibres.

Fibrin gel was formed via polymerization of fibrinogen with thrombin. Cells were either seeded directly on top of the gel or into a scaffold filled with fibrin gel (fig.5).

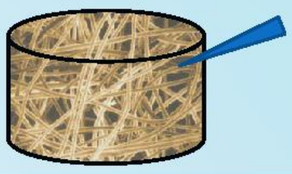


Fig:5 Fibrin gel pipetted throughout scaffold architecture. Cells are then pipetted on top and allowed to attach

[4] Results: Magnetic Levitation

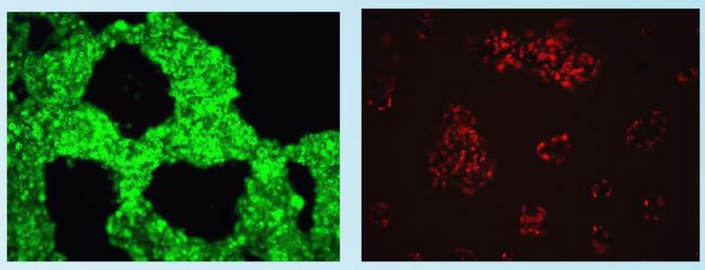


Fig.6 Widefield fluorescence images (10x) of mono cultured podocytes (Green) and GEnCs (Red). After differentiating for 5 days under magnetic levitation it is apparent that different shaped "tissues" form.

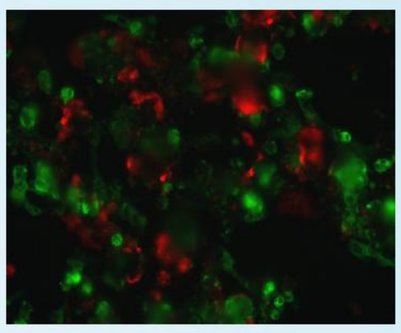
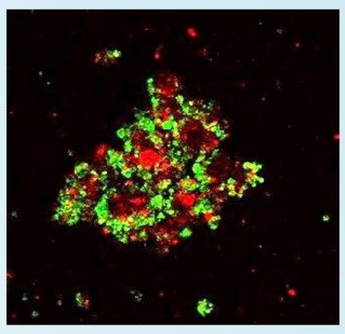


Fig 7: Widefield fluorescence image (10x) of co-cultured podocytes (Green) and GEnCs (Red). Cells brought together after 3 days of monoculture form a combined "tissue" after 2 additional days.

Fig 8: Confocal fluorescence image (10x) of co-cultured podocytes (Green) and GEnCs (Red). Cells co-cultured in undifferentiated state (72hrs) and allowed to differentiate together for 7 days.



- **Conclusion 1: Magnetic levitation** experiments show that co-cultured cells form different shaped "tissues" to each cell type grown in monoculture.

[5] Results: Scaffold Culture

An experiment was performed whereby 300,000 of each cell type was co-cultured for 72hrs on either a PGA scaffold, a fibrin gel or a PGA scaffold injected with fibrin.

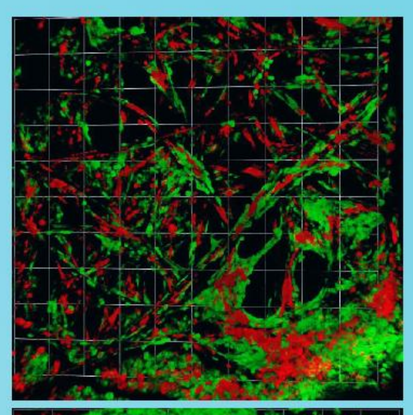


Fig.9 Confocal reconstruction (10x) of both cell types (undifferentiated) co-cultured on a PGA scaffold for 72hrs. 1 unit=145µm

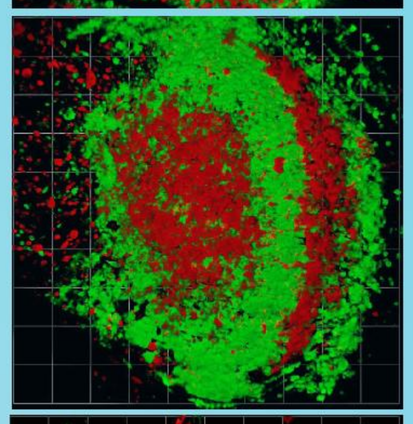


Fig.10 Confocal reconstruction (10x) of both cell types (undifferentiated) co-cultured on fibrin gel for 72hrs. 1 unit=155µm

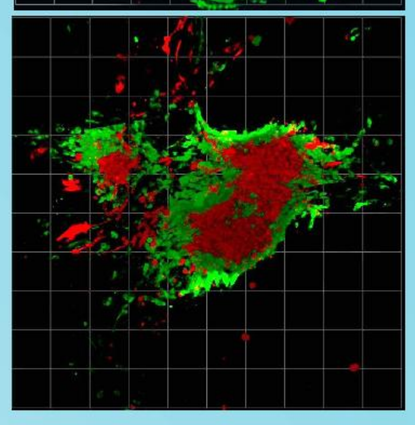


Fig.11 Confocal reconstruction (10x) of both cell types (undifferentiated) co-cultured on PGA scaffold injected with fibrin gel for 72hrs. 1 unit=135µm

- **Conclusion 2: Scaffold co-cultured cells** appear to favour a tissue shape whereby GEnCs are central, surrounded by an outer layer of podocytes.

Discussion: It seems apparent (so far) that regardless of the 3D culture platform used, co-cultured podocytes and GEnCs appear to form a similar shape, with podocytes "wrapping" around a core of GEnCs. This is exciting, as podocytes interdigitate around GEnC vasculature in-vivo (fig.1). A priority for near-future experiments will be to label for GBM proteins such as collagen IV. There are also plans to utilize time-lapse confocal microscopy to image "tissue" formation of cells co-cultured on scaffolds. If it becomes apparent that something resembling the GBM is being formed within these "tissues", experiments will shift to inducing fibrosis and ultimately examining the effects compounds may have on this.

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Figure 1: from http://www.jimstanis.com/glomerulus_9_3.html accessed 18/10/14

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Jack Tuffin

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