Basement Membrane Protein Orchestrates Network Formation By Endothelial Cells in 3D: Laminin the Conductor

K Stamati[1], J V Priestley [1,2] , V Mudera[1] & U Cheema[1]

[1] Tissue Repair and Engineering Centre, Institute of Orthopaedics and Musculoskeletal Sciences, UCL Division of Surgery and Interventional Science, University College London, Stanmore Campus HA7 4LP [2] Centre for Neuroscience & Trauma, Blizard Institute of Cell and Molecular Science Barts and The London School of Medicine and Dentistry, 4 Newark Street Whitechapel London E1 2AT

INTRODUCTION: Engineering vascular networks within 3D tissue models is paramount for the survival of cells in large 3D constructs. This is critical for both *in vitro* and *in vivo* survival.
Our work has focused on network formation by endothelial cells in co-cultures with Human bone marrow stromal cells (HBMSCs) in 3D. We have examined the effect of matrix composition on endothelial cell morphology and our results emphasize the importance of basement membrane proteins for inducing network formation by endothelial cells.

METHODS: Human umbilical vein endothelial cells (HUVECs) and HBMSCs were cultured in 3D collagen type I constructs with and without the addition of the basement membrane protein laminin, to test the effect of matrix composition on endothelial cell morphology, assessed by CD31 immunostaining.

RESULTS: Differences in cell behaviour were significant, with cells showing distinct morphologies, dependent on the matrix composition they were seeded in. Cells in collagen type I only constructs aggregated in a cobblestone-like morphology, whereas endothelial cells in collagen with added laminin formed networks (figure 1). The networks showed variable characteristics, with smaller (~60μm) structures, to more multinucleate, longer (~170μm) structures.

DISCUSSION & CONCLUSIONS: Our results suggest that the interaction of endothelial cells to a basal membrane component such as laminin, is critical for network formation. Cell to matrix attachments, mediated by integrins are thought to be important regulators of cell behavior and angiogenesis in 3D [1]. Integrin expression has been manipulated extensively in experimental designs, with the addition of elements such as PMA (phorbol myristate acetate), known to increase specific expression of integrins as well as inducing collagen invasion, thereby affecting vessel-like tube formation [2,3]. Furthermore, cell-cell interactions, such as adherens junctions and tight junctions differ dependent on the extracellular matrix components surrounding the cells [1]. Notably however, endothelial cells from different sources also show variable responses to each matrix component [1].
By identifying the parameters which lead to specific types of endothelial cell aggregation, we can develop strategies to predictably control cell morphologies in 3D.

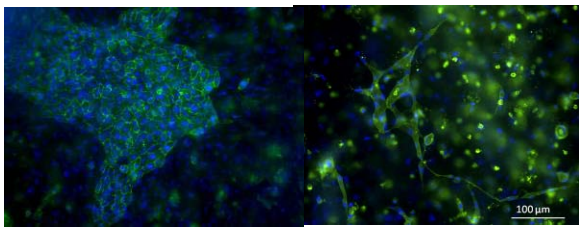

Fig 1. Cobblestone morphology in co-cultures of HUVECs and HBMSCs in 3D collagen type I (left) and network formation in 3D collagen type I with laminin (right) (CD31 staining in green, DAPI in blue).

REFERENCES: [1]Dye J.F., Lawrence L., Leach L., Firth J.A., Clark P. (2004) Distinct Patterns of Microvascular Endothelial Cell Morphology Are Determined by Extracellular Matrix Composition *Endothelium* **11**: 151-167. [2]Montesano R. and Orci L. (1987) Phorbol Esters Induce Angiogenesis in vitro from large-vessel endothelial cells *Journal of Cellular Physiology* **130**:284-291. [3]Defilippi P., van Hinsbergh V., Bertolotto A., Rossino P., Silengo L., Tarone G. (1991) Differential distribution and modulation of expression of Alpha1/Beta1 Integrin on Human Endothelial Cells *The Journal of Cell Biology* **114:**855-863

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