PhD-DTP in Bioengineering Project

Directional mechanosignalling in the endothelium

Supervisor: Dr Christina Warboys (Cellular & Molecular Bioengineering Theme)

Background: Despite systemically acting risk factors it is well known that atherosclerotic lesions develop at specific sites of the arterial tree, for instance branches, bifurcations and areas of high vessel curvature whereas straight, unbranching vessels are protected. This has been attributed to local variations in haemodynamics which are sensed by endothelial cells and highlights the importance of mechanical forces on endothelial function.

Although its importance has been recognised, the patchy distribution of atherosclerosis has not been adequately explained at a molecular level. Furthermore, there is currently no agreement on what types of flow (and other mechanical factors e.g. strain) promotes endothelial cell dysfunction and atherogenesis and therefore more fundamental research is required. Recently it has emerged that flow that is multidirectional exhibits the greatest correlation with early atherosclerosis and that endothelial cells respond rapidly to changes in flow direction (Ref 1 & 2); this suggests that endothelial cells may have ‘directional sensors’. Since much of the work on endothelial mechanosignalling has been conducted under conditions of laminar compared to oscillatory shear stress (or static conditions), the analysis of endothelial responses to multidirectional flow is both timely and important.

Project Description: This project will aim to address the how endothelial cells sense and respond to multidirectional flow and furthermore how this may promote endothelial dysfunction. In particular, this project will focus on the role of β-catenin, a key regulator of endothelial function. Whilst the role of β-catenin in cancer and embryonic development is well defined, its regulation by flow and its role in endothelial dysfunction has received little attention (although there is evidence supporting an important role in mechanosignalling in osteocytes). Importantly, β-catenin is known to interact with multiple putative mechanosensors including VE-cadherin, PECAM, integrins, caveolin-1 and Frizzled receptors leading to the hypothesis that β-catenin may play a central role in endothelial mechanosignalling. Preliminary experiments reveal that β-catenin rapidly translocates to the nucleus upon exposure to flow and that after 72hrs nuclear accumulation is sustained only in ECs exposed to multidirectional flow where it may regulate the expression of genes that promote endothelial dysfunction and atherosclerosis.

This project will explore the mechanisms by which β-catenin is activated by mechanical forces using cultured human aortic endothelial cells. This will be achieved by (i) direct stimulation of candidate primary mechanosensors using magnetic twisting cytometry and/or magnetic tweezers and (ii) by acute and chronic exposure to multidirectional and uniaxial flow using the orbital shaker method (Ref 3) in combination with gene silencing techniques to knockdown primary mechanosensors. The PhD student will learn cell culture and transfection techniques, immunostaining and confocal microscopy, magnetic twisting cytometry, and western blotting and quantitative PCR to study protein and RNA expression, respectively. There may also be scope to design and develop an ex vivo organ culture model to study whole vessel responses to flow modification and exposure to multidirectional flow which would involve computational modelling of flow patterns in isolated vessels.

Supervisor details: Dr Warboys is a cell physiologist with expertise in mechanosignalling and the analysis of cellular responses to shear stress. Dr Warboys recently joined the Department as an Intermediate Research Fellow funded by the British Heart Foundation and will collaborate closely with Prof Weinberg’s group who have expertise in CFD analysis, and Dr Overby who has expertise in magnetic twisting cytometry.