Morrow D, Sweeney C, Birney YA, Cummins PM, Walls D, Redmond EM, Cahill PA. Cyclic strain inhibits Notch receptor signaling in vascular smooth muscle cells in vitro. Circ Res. 2005 96:567-75

Morrow D, Scheller A, Birney YA, Sweeney C, Guha S, Cummins PM, Murphy R, Walls D, Redmond EM, Cahill PA. Notch-mediated CBF-1/RBP-J{kappa}-dependent regulation of human vascular smooth muscle cell phenotype in vitro.

Am J Physiol Cell Physiol. 2005 289:C1188-96

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SA5

Mechanotransduction and the glycocalyx

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The surface of endothelial cells (ECs) is decorated with a wide variety of membrane-bound macromolecules that constitute the glycocalyx (GCX). As the most apical structure on the EC, the GCX senses the force (shear stress) of flowing blood and transmits it via the cytoskeleton throughout the cell to sites where transduction of force to biochemical response (mechanotransduction) may occur. In this presentation the structure of the GCX and many of the experiments that demonstrate its role in mechanotransduction and vascular remodeling will be reviewed. Experiments with enzymes that degrade specific glycosaminoglycan components have been used to show that the GCX mediates the shear-induced production of nitric oxide, a central process in cardiovascular control, while the same enzyme treatments do not affect shear-induced production of prostacyclin, another hallmark of EC mechanotransduction. These experiments reinforce the concept of distributed sites of mechanotransduction in EC. The characteristic remodeling of the EC cytoskeleton and intercellular junctions in response to shear stress are dependent on the GCX as well, and the experiments that support the role of the GCX in these processes will be reviewed as well.

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SA6

Modulation of inflammatory responses of endothelial cells by changes in local shear stress

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Stable local haemodynamic microenvironments may determine the phenotype of endothelial cells (EC) in different regions of the circulation, but acute changes in flow might also modulate functional responses. We aim to understand how different levels or patterns of shear stress applied to endothelial cells regulate inflammatory responses, and in particular, leukocyte recruitment. For this purpose, we developed models in which human EC of various types (HUVEC from umbilical veins; HUAEC from umbilical arteries; HCAEC from coronary arteries) were cultured in glass capillaries coated with desired substrates. These constructs were conditioned by different levels of shear stress for different periods, or exposed to abrupt changes in shear. Conditioning could be combined with treatment with cytokines such as tumour necrosis factor-α (TNF) and interleukin-1β (IL-1), and adhesion and migration of flowing neutrophils analysed as an 'inflammatory' readout.

Initial studies showed that conditioning of HUVEC for 24h at increasing shear stress acted to powerfully suppress responses to TNF, but not IL-1, judged by neutrophil recruitment (Sheikh et al., 2003; 2005). However, in subsequent studies, responses to both cytokines were suppressed by shear conditioning for HUAEC and HCAEC. Studies in which culture medium constituents, such as basic fibroblast growth factor, were swapped, indicated that this difference between the endothelial cells arose from culture conditions rather than from an in vivo imprinted phenotype. The fact that the original 'static' cultures of each cell type showed similar abilities to support adhesion and migration of neutrophils also indicated that the phenotypes of EC were plastic and could be re-set by conditioning in vitro. Taking this further, we analysed expression of selected genes in HUVEC immediately after digestion from veins, after standard culture in vitro and then after shear conditioning. Changes were induced by initial culture, which were reversed in part at least by the return to a shear environment. Thus it seems that endothelial phenotype is highly pliable, with enviromental factors, such as shear stress and growth factors, modifying responses in an interlinked but reversible manner.

We thus investigated whether the less responsive state induced in vitro by shear stress would change when flow was ceased. This might be relevant to ischaemic conditions in vivo (for instance linked to thrombo-embolism, surgical interventions or organ transplantation), where an inflammatory response typically follows reperfusion. We found that response of EC to TNF only increased slowly over 24-48h after cessation of flow, and that if a very low level of shear stress was retained, then the response remained suppressed (Matharu et al.,2008). In all of the above, functional

changes could be linked to changes in expression of receptors such as E-selectin and the shear-sensitive transcription factor KLF-2, and in activation of NFkB. However, anomalies in the correlations between the different responses indicated that other modulatory events occurred outside of these well-described mediators.
Nevertheless, most of the changes noted were over hours and linked to modulation of gene expression. In studies of flow reduction, however, we also noted an early pro-adhesive response. In the period around 60-120min after cessation of flow, neutrophils adhered to otherwise unstimulated HUVEC, when included in medium used to ' reperfuse ' it (Matharu et al., 2008). Others have shown that an early oxidative response follows flow cessation (Manevich et al., 2001). Here, the transient neutrophil adhesion observed was attributable to oxidant-induced upregulation of expression of P-selection on the EC.
Taken together, these studies suggest that local conditioning of endothelial cells contributes to vessel- and organ-specificity in inflammatory responses, and predisposition of certain sites to development of inflammation. At the same time, acute responses of EC to disruption of flow may contribute to outcome of ischaemia and reperfusion.

Sheikh et al. (2003) *Blood* **102**, 2828-2834.

Sheikh et al. (2005) *Br J Pharmacol* **145**, 1052-1061.

Matharu et al. (2008) *J Cell Physiol* **216**, 732-741.

Manevich et al. (2001) *Am J Physiol* **280**, H2126-H2135.

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SA8

Mechanotransduction of shear stress and regulation of microvascular resistance

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Regulation of wall shear stress (WSS) in microvessels is an important local mechanism regulating microvascular resistance, thus tissue blood flow and at the same time aiming to optimize circulatory energy consumption. The pressure drop across a microvascular unit indicates the loss of circulatory energy that is primarily due to WSS. In arterioles, an increase in WSS results in a substantial, endothelium dependent dilation, whereas in venules, it elicits only a limited increase in diameter. In arterioles, only dilator mediators, such as nitric oxide (NO), prostaglandins (PGI2/PGE2) endothelium derived hyperpolarizing factor(s) EDHFs, are released, whereas in venules, in addition to these mediators, constrictor prostaglandins are also released. WSS is the function of wall shear rate (WSR) and blood viscosity (hematocrit and plasma viscosity), which parameters can be substantially different in arterioles and venules. Thus it is likely that regulation of WSS is achieved by different mechanisms in arterioles and venules. In the arterial side WSS is determined primarily by high WSR (high velocity/narrow diameter), whereas in the venular side WSS is determined primary by the hematocrit related viscosity. Accordingly, it seems that at the arteriolar side WSS is regulated primarily by substantial increases in diameter, which can be achieved, since arterioles have a substantial basal tone and because WSR is high, thus diameter changes have less impact on apparent viscosity. In the venular side however, WSR is low, thus changes in diameter can substantially affect hematocrit-induced apparent viscosity of blood. Thus in the microcirculation there is a complex interrelationship between rheological parameters, structural and functional properties of microvascular network. The nature of endothelial mediation of WSS seems to be gender specific, and it can change with age and in diseased conditions. The primary sensors of changes in WSS are likely to be the glycocalyx, as part of endothelial surface layer and platelet endothelial cell adhesion molecule (PECAM), whereas the cytoskeleton and integrins are the next serially coupled molecules of mechanotransduction. There are still several controversial issues however, such as the role of increase in [Ca2+]i and reactive oxygen species in the release of various endothelial mediators and the nature and mediation of WSS-induced responses in cerebral microvessels. The fascinating role of endothelium in the mechanotransduction of shear stress into vasomotor response is still an open field for further discoveries.

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SA9

Haemodynamic forces as *in vivo* angiogenic stimuli

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Chronic vasodilator treatment intensifies levels of shear stress in capillary beds, stimulating a specific form of angiogenesis termed longitudinal splitting, while sustained muscle overload induces the more familiar sprouting form of capillary growth (Egginton, 2001). Similar findings are observed in both rats and mice. These differently directed mechanical forces (luminal and abluminal, respectively) expand the capillary bed to a similar extent over a similar time-course, but by different growth processes characterised by unique features in structure, gene expression and protein complement (Williams et al. 2006).
Elevated capillary shear stress is transduced into an angiogenic response irrespective of the vasodilator mechanism employed, with the essential involvement of endothelial nitric oxide synthase. Surprisingly, the rate of capillary growth thus recruited

is largely mirrored by the rate of capillary regression on withdrawal of vasodilator treatment, involving reciprocal changes in VEGF and eNOS, but low levels of endothelial mitosis or apoptosis. *In vitro* responses of endothelial cells (EC) to elevated shear stress leads to differential regulation of a number of genes involved in the control of angiogenesis. Interestingly, shear appears to regulate molecules that modify VEGF effects, rather than VEGF *per se*. EC motility is increased by VEGF, although shear appears to inhibit EC migration when confluent layers are wounded in parallel to flow axis.

Capillary growth following overload is critically dependent on matrix metalloprotease activity and, as with shear-dependent growth, the presence of elevated VEGF levels. Angiogenesis does not appear to be dependent on a threshold stimulus, as seen by the differential in capillarity and EC proliferation in response to graded muscle overload. However, the response is mediated by a threshold, rather than a graded response in VEGF or Flk-1, suggesting the degree of angiogenesis is likely controlled by interactions among pro-angiogenic stimuli. There was little evidence for synergistic potentiation when applied in combination with either high flow (pharmacological dilatation) or low flow (surgical ischaemia), suggesting that feedback control limits the extent of angiogenesis in skeletal muscle.

Egginton S et al. (2001). Cardiovasc Res. 49, 634-646.

Williams JL et al. (2006). J Physiol. 570, 445-454.

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SA10

Wall shear stress distribution in the arterial system. Reconsiderations based upon *in vivo* measurements

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Wall shear stress (WSS), the drag of the flowing blood exerted on endothelial cells, is an important determinant of endothelial cell function and gene expression. WSS can be estimated from wall shear rate (WSR) and local blood viscosity, WSR being defined as the radial derivative of blood flow velocity at the wall. In large arteries in man, WSR is derived from velocity profiles, non-invasively recorded by means of ultrasound or Magnetic Resonance Imaging (MRI). In our studies we use ultrasound, because of its better spatial and especially temporal resolution than of MRI. The velocity profiles are recorded with a two-dimensional ultrasound imaging device combined with a dedicated acquisition and processing system as developed in our institute. WSS is estimated from the derived WSR and whole blood viscosity, because the thin plasma layer can be ignored relative to the size of the ultrasound sample volume (sample length 300 μm; 50% overlapping). In arterioles WSS is measured directly or estimated from WSR and plasma viscosity, WSR being derived from velocity profiles recorded with labeled blood platelets or nanometer particles as velocity tracers. Originally, the displacement and the radial position of the velocity tracers were determined by hand, a time consuming procedure. Recently a computerized two-dimensional particle tracking technique has been developed to determine radial position and displacement of the particles, automatically providing velocity profiles. In arterioles plasma viscosity can be used to calculate WSS, because the velocity tracers come as close to the wall as 0.2-0.5 μm.

The *in vivo* measurements have shown that the theoretical assumptions regarding WSS in the arterial system and its calculation are far from valid. In both arteries and arterioles, velocity profiles are flattened rather than fully developed parabolas. This implies that WSR has to be derived from recorded velocity profiles. Assuming a parabolic velocity profile will on the average underestimate derived WSR by a factor of 2-3. In humans mean WSS varies along the arterial tree and is higher in the common carotid artery (1.1-1.3 Pa; 1 Pa=10 dyn cm^{-2}) than in the brachial artery (0.4- 0.5 Pa) and the common (0.3-0.4) and superficial (0.5 Pa) femoral arteries. Only in the common carotid artery mean WSS is close to the theoretically predicted value of 1.5 Pa. The lower mean WSS in conduit arteries can be explained by the high peripheral resistance in these arteries, reducing mean volume flow and inducing reflections. Dilation of the femoral artery vascular bed results in mean WSS values in this artery not significantly different from those in the common carotid artery. This observation indicates that at rest mean WSS is largely determined locally. Although small, the difference in mean WSS between the common and the superficial femoral artery is significant, the former artery seeing reflections from both the deep and the superficial artery, while the latter one only sees reflections from its own vascular bed. Also in the carotid artery bifurcation differences in mean WSS have to be appreciated. It is of interest to note that in both the femoral and the carotid artery bifurcation the differences in mean WSS are associated with local differences in intima-media thickness (IMT): the lower mean WSS is, the larger IMT will be. Also in animals mean WSS is not constant along the arterial tree. In arterioles mean WSS varies between 2 and 10 Pa and is dependent on the site of measurement in the arteriolar network. Across species mean WSS in a particular artery decreases linearly with increasing body mass on a log-log scale, in the infra-renal aorta from on the average 8.8 Pa in mice to 7.0 Pa in rats and 0.5 Pa in humans (flow velocities being similar). A similar pattern can be found in the carotid artery, varying on the average from 7.0 Pa in mice to 4.7 Pa in rats and 1.2 Pa in man.

The observation that mean WSS is far from constant along the arterial tree indicates that Murray's cube law on flow-diameter relations cannot be applied to the whole arterial system. At the present state of the art it can be concluded that the exponent of the power law varies from 2 in large branches of the aortic arch to 2.55 in coronary arteries and 3 in arterioles. The *in vivo* findings also imply that in *in vitro* investigations no average calculated shear stress value can be taken to study gene expression by endothelial cells derived from different vascular areas or from the same artery in different species. The cells have to be studied under the shear stress conditions they are exposed to in real life.

Sensing and transduction of shear stress is likely to be in part mediated by the endothelial glycocalyx, because pretreatment of endothelial cells with hyaluronidase, leading to substantial reduction of glycocalyx dimensions, attenuates shear stress induced release of nitric oxide and shape changes of these cells. Therefore, modulation of shear stress sensing and transduc-

tion by altered glycocalyx properties, for example, in atherogenesis, should be considered.

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SA11

Endothelial phenotype plasticity in unstable flow regions of the cardiovascular system: differential microRNA expression

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Arterial endothelial phenotype heterogeneity significantly influences athero-suceptibility and athero-protection in vivo. Differential transcript profiling of endothelium in susceptible arterial regions of normal adult swine displays a balance of pro-pathological and protective transcript profiles when compared with adjacent regions that rarely, if ever, develop atherosclerosis. The endothelial phenotype in vivo and in vitro is highly sensitive to the local blood flow characteristics via mechanotransduction and transport mechanisms. Athero-susceptible locations map to regions of hemodynamic (and biomechanical) spatio-temporal complexity where transient vortices within flow separation zones promote flow reversal, oscillatory shear stresses, low flow velocities and low mean shear stresses, steep spatial shear stress gradients, and occasional turbulence (chaotic flow). Differential phenotypes are detectable in endothelium in vivo at the mRNA, protein, post-translational, and functional levels. We now demonstrate that differential microRNA expression that targets specific gene and protein expression is part of the regulation of endothelial phenotype.

Regulation of mRNA stability and translation occurs by highly conserved small non-coding microRNAs (miRNAs). Microarrays identified 3 miRNA families (let-7, miR10, miR26) as upregulated in endothelium from an atheroprotected region of thoracic aorta relative to a nearby atherosusceptible region (aortic arch). By qRT-PCR, expression levels of miR10a and 10b were 4.9 and 20.7-fold higher respectively at protected (n=8) vs susceptible (n=10) regions; in contrast miR7d and miR26b were elevated <2-fold. The copy number of miR10a was greater than that of miR10b and its preferential expression in endothelium in situ was detected by immunofluorescence. 854 putative targets of miR10a/b were organized into interactive pathways using IngenuityTM. Sequences of 138 of the most interactive genes were entered into the Sfold program that assesses target secondary structure as an important predictor of miRNA-target hybridization sensitivity. Among miR10a/b targets showing high total hybridization energy were Flt-1 (VEGFR1), Hox-D10 and VEGFA. Endothelial expressions of these genes were suppressed in protected vs susceptible regions in a reciprocal relationship with miR10a/b. Cultured endothelial cells overexpressing miR10 suppressed Flt-1 gene expression. The data show miRs to be flow responsive and suggest miR10a/b to be important regulators of endothelial gene expression in atheroprotection/susceptibility.

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SA12

Effects of shear flow on selectin expression in endothelial cells co-cultured with smooth muscle cells

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ABSTRACT

In vitro co-culture of endothelial cells (ECs) with smooth muscle cells (SMCs) induced rapid and sustained increases in EC expression of E-selectin. By using inhibitors, dominant-negative mutants, and siRNA, we found that activations of JNK and p38 are critical for the co-culture-induced E-selectin expression. Gel shifting and chromatin immunoprecipitation assays showed that SMC-co-culture increased the NF-κB-promoter binding activity in ECs. Inhibition of NF-κB activation blocked the co-culture-induced E-selectin promoter activity. Protein arrays and neutralizing antibodies showed that IL-1β and IL-6 produced by EC/SMC co-cultures contribute to the co-culture-induction of EC signaling and E-selectin expression. Pre-shearing of ECs inhibited the co-culture-induced EC signaling and E-selectin expression. These findings serve to elucidate the molecular mechanisms underlying the SMC-induction of EC E-selectin expression and the shear stress-protection against this SMC-induction.

INTRODUCTION

The aim was to elucidate the mechanisms that regulate the SMC-induced E-selectin expression in ECs and its inhibition by shear stress. This article reviews several publications in our labs [1-5].

MATERIALS AND METHODS

Cell culture. ECs were isolated from fresh human umbilical cords. SMCs were obtained from Clonetics (Palo Alto, CA).

Preshearing of ECs. ECs were seeded onto the outer side of the membrane (10-μm-thick, 0.4-μm pores, pre-coated with fibronectin) of a transwell. After incubation for 24 h, the membrane with ECs was incorporated into a flow chamber on the underside of the transwell for shear stress applications at a high (HSS, 12 dyn/cm2) or low level (LSS, 0.5 dyn/cm2) for 4 or 24 h.

Co-culture of ECs and SMCs. After EC preshearing, the inner side of the membrane was seeded with SMCs under static condition, thus forming an EC/SMC co-culture system. Controls had no cells or ECs instead of SMCs on the inner side. To study the effect of distance of EC/SMC separation, ECs seeded on the

membrane were separated by 1 mm from the SMCs plated on an outer chamber (EC/M/SMC).

RESULTS AND DISCUSSION

Pre-exposure of ECs to HSS, but not LSS, for 24 h inhibits SMC-induced E-selectin expression in ECs. EC/SMC co-culture induced an increase in E-selectin mRNA expression in ECs within 1 h. Separation of ECs from SMCs by 1 mm retarded the E-selectin expression. Pre-shearing of ECs at HSS for 24 h inhibited the co-culture-induced E-selectin expression; this was not seen with LSS. Thus, SMCs induced EC expression of E-selectin via a paracrine effect that can be inhibited by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are mediated by the JNK and p38 pathways. The phosphorylation of ERK, JNK, p38, and Akt in ECs showed transient increases after co-culture with SMCs. The co-culture-induced E-selectin expression was inhibited by inhibitors for only JNK and p38. Pre-shearing at HSS, but not LSS, for 24 h inhibited the co-culture-induced JNK and p38 phosphorylation. JNK- or p38-specific siRNA caused significant inhibition of the co-culture-induced E-selectin expression. The increase in E-selectin-Luc promoter activity in ECs by SMC-co-culture was prevented by pre-shearing at HSS, but not LSS. Thus, the SMC-induction of EC expression of E-selectin is mediated by JNK and p38 and blocked by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are dependent on NF-κB. Inhibition of NF-κB abolished the co-culture-induced E-selectin promoter activity. Co-culture with SMCs increased the NF-κB-DNA binding activity in EC nucleus, which was inhibited by HSS (but not LSS). Thus, the co-culture induced E-selectin expression is mediated by NF-κB, and this effect is inhibited by HSS.

IL-1β and IL-6 produced by EC/SMC are the major factors contributing to the SMC-induced signaling and E-selectin expression in ECs. Using a human cytokine array system, we identified IL-1β and IL-6 as the proteins released from EC/SMC at significantly higher levels than EC/EC (>4-fold). Neutralizing antibodies against IL-6 and/or IL-1β inhibited the co-culture-induced increases in E-selectin mRNA, JNK and p38 phosphorylation, and NF-κB-DNA binding activity.

IRAK and gp130 are involved in regulatory effects of SMC-co-culture and shear stress on EC E-selectin expression. The SMC-induced E-selectin expression in ECs was suppressed by siRNAs against gp130 (IL-6 receptor) and IRAK (complex with the IL-1β receptor upon its stimulation). The co-culture-induced phosphorylations of gp130 and IRAK were inhibited by pre-shearing at HSS (but not LSS) for 24 h.

These results indicate that the SMC-induction of E-selectin in ECs involves the paracrine action of IL6 and IL-1β on their receptors to activate the JNK, p38 and NF-κB, and that this effect can be inhibited by high shear stress.

1. Chiu JJ et al. (2003). Blood 101, 2667-2674.
2. Chiu JJ et al. (2005). Arterioscler Thromb Vasc Biol 25, 1-8.
3. Chen CN et al. (2006). Blood 107, 1933-1942.
4. Chen CN et al. (2006). Proc Nat Acad Sci USA 103, 2665-2670
5. Chiu JJ et al. (2007). Blood 110, 519-528.

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SA13

Shear stress, inflammation and atherosclerosis

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Atherosclerosis is the disease with the highest mortality in the western world. Despite its large socio-economical impact, the underlying mechanisms are only partially known. It has been accepted for decades that atherosclerosis is a lipid driven disease, despite the fact that risk factors related to lipid metabolism only partially explain atherogenesis. Furthermore, new therapies specially focussed upon lipid metabolism only partially reduce plaque size. Recently two concepts – inflammation and blood flow/shear stress – have undergone a renaissance and gained a lot of interest as complementary explanations for plaque formation and these concepts will be the topic of the present manuscript.

The role of inflammation became apparent from a series of mouse studies where systematically parts of the immune system were knocked down, before the induction of atherosclerosis. These studies identified inflammation as an independent mechanism attributing to plaque formation, and based upon these results and further studies atherosclerosis is considered a lipid driven inflammatory disease. The effect of blood flow in atherosclerosis is based upon the observation that plaques are not evenly distributed over the arterial system. These predilection sites are at or near side branches, i.e. where blood flow is non-uniform, or at the lesser curvature of bends, i.e. where blood velocity is relatively low. The effect of blood flow on the vessel wall is through shear stress which alters the physiology of endothelial cells. Shear stress (τ N/m2 or Pascal (Pa)) arises from the friction between two virtual layers in a fluid, and is induced by the difference in movement of the two layers (dv/dr s-1; in case of a cylindrical tube) and the "roughness" (or viscosity Pa.s) between these layers ($\tau = dv/dr * \eta$). Shear stress also arises at the interplay between blood and the endothelial layer, where it induces a shearing deformation of the endothelial cells. This shearing deformation affects the phenotype of the endothelial cells and thereby the inflammatory component and plaque progression/composition.

This paper describes the interaction between shear stress and inflammation. We will first describe recent findings on the sensing mechanism of shear stress by the endothelium. Subse-

quently, pro-inflammatory pathways modulated by shear stress in endothelial cells, followed by the effect of shear stress on plaque progression and plaque composition. At the end we will discuss new findings related to longitudinal plaque heterogeneity.

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SA14

Engineering vascular grafts

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Coronary artery and peripheral vascular disease are associated with significant morbidity and mortality. In these patients, surgical intervention including small diameter bypass grafting with autologous veins or arteries is a common treatment. However, many patients lack suitable autologous vessels, either because these vessels are diseased themselves or because of previous surgery, and in these cases, synthetic grafts are often used. Unfortunately, many of these grafts fail because of the low number of endothelial cells and the proportion of the endothelialised surface remaining after exposure to flow, which results in acute thrombosis and subsequent occlusion of the vessel.
At the University of Manchester, we are developing small calibre vascular grafts for coronary or peripheral bypass and vascular access grafts for haemodialysis. These grafts are based on electrostatically spun polyurethane and polycaprolactone with controlled porosity and biodegradability and are coated with specific vascular matrix molecules to regulate cell adhesion, migration, and growth factor bioavailability. This talk will focus on the approaches we are using to improve both the initial adhesion of endothelial cells to the graft surface and the retention of these cells to this surface following restoration of flow. Our studies have revealed significant new insights into the biology of endothelial cell attachment to surfaces coated with specific vascular matrix molecules, with important implications for the design of the next generation of vascular grafts.

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SA15

Waveform analysis and microcirculatory function

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Risk factors for cardiovascular disease mediate their effects by altering the structure and function of wall and endothelial components of arterial blood vessels. Pathological change in the microcirculation plays a pivotal role in promoting end-organ dysfunction that not only predisposes to further organ damage but also increases the risk for future macrovascular events. The microcirculation is recognised as the site where the earliest manifestations of cardiovascular disease occur that may play a pivotal role in driving the atherosclerotic process in conduit vessels(1).
Ultrasound and the Doppler effect have been long used to measure blood velocity and its temporal and spatial variation within the vascular tree in order to diagnose and monitor vascular disease. Changes in morphology of the linear flow velocity spectral envelope is not representative of any single vessel but is determined by changes in the properties and total cross-sectional area of downstream vascular networks(2). Quantitative analysis of Doppler-time velocity waveforms that reflect measures of flow pulsatility (eg resistive index, pulsatility index) can mirror changes in downstream vascular resistance and may predict future adverse clinical outcomes(3). In a series of studies in different patient groups we have shown these derived indices often provide misleading information in relation to the haemodynamic actions of drug interventions and are not sensitive in detecting early microvascular dysfunction in different vascular beds in humans(4,5). Novel algorithms that enable quantitative analysis of the Doppler velocity spectral envelope over the duration of the cardiac cycle provides more sensitive information in relation to the haemodynamic action of drugs and identification of early microvascular abnormalities in humans. Data will be presented showing the superiority of this approach in identifying early microvascular abnormalities in waveforms obtained from different arterial territories in different disease states associated with increased cardiovascular risk.
Techniques capable of detecting microvascular damage and monitoring response to therapeutic interventions, especially in vulnerable target organs of interest, may improve risk stratification and could represent a valuable surrogate for future cardiovascular outcome.

Stokes KY, Granger DN (2005). J Physiol 562, 647-653.

Lockhart CJ, Hamilton PK, Quinn CE (in press). Clin Sci 116, (00-00).

Adamson SL (1999). Eur J Obstet Gyneocol Reprod Biol 84, 119-125.

Lockhart CJ, Gamble AJ, Rea D et al. (2006). Clin Sci 111, 47-52.

Wright SA, O'Prey FM, Rea DJ et al (2006). Arterioscler Thromb Vasc Biol 26, 2281-2287.

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