Abstract for thesis titled *'Probing endothelial response under physiological flow profiles using organ-on-chip platforms'*

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The endothelial responses to disturbed blood flow is a crucial factor in initiation of cardiovascular diseases. Atherosclerosis and aneurysms present contradicting vascular structures originating due to disturbances in flow, often seen in areas of vessel bifurcation. To construct organ-on-chip platform for cardiovascular disease modelling initially a hydrogel micromolding-based soft lithography technique was used to construct circular, reproducible stenosed and aneurysmal mimics, using the thermal transition of hydrogels to form a "beads-on-a-string" configuration. Later a modified simple, yet innovative glass capillary molding method was proposed to fabricate different degrees of stenosis. The transition from structurally altering the hydrogel to an altered glass tube, improved the uniformity at the stenosed junction while a droplet casting at the junction to fabricate aneurysms was used. The endothelial monolayer was grown under shear rates changing from <1 to 1000 s^{-1}, indicative of low, uniform and oscillating biophysical forces encountered at arterial walls using uniform laminar flow and periodic flows. Endothelial monolayer integrity and activation were studied under varied flow profiles first using an indirect, quantitative image analysis approach. The phosphorylation of cytoplasmic tyrosine in the structure of VE-cadherin, increases at low shear, which is a possible reason why aneurysmal areas demonstrated maximal cell-cell adhesion, even at increased shear forces alongside increased surface area at the walls, resulting in the better adhesion and growth of a uniform cellular layer. Upon endothelial culture at the area of bifurcation, the area of stenosis showed lower expression with better adhesion pre and post stenosis. This was due to reduced luminal pressure at this region, with the straighter vessels showing disturbed cellular junctions due to higher flow pressure at the lumen. Post shearing, no significant upregulation was observed upon treatment with TNF-α, inducing NF-κβ and ETS1-regulated transcription of VE-cadherin, preventing endothelial damage. Under periodic flow, the endothelial activation was characterized using the temporal and shear induced variations in expression of three key markers, ICAM-1, VCAM-1 and E-selectin. The fold change for VCAM-1 was about four times between straight and stenosed segments when the shear rate at the area of stenosis increased to 5 dyne/cm^2. The expression of VCAM-1 was however higher at the area of stenosis in straighter vessels than at vessel branches with a stenosis, while increased shear rates upregulated ICAM-1 expression on the endothelial surface at the area of stenosis at bifurcation. The altered flow profiles and mechanosensitivity of endothelial cells sufficiently discriminate between cell adhesion molecules as is seen by the low expression of E-selectin at bifurcations and no detectable changes in straighter vessels. Therefore, the variation at the area of stenosis is high in comparison to straighter parts as seen particularly in the expression of VCAM-1, ICAM-1 and reduction in VE-cadherin. Actin organization shifted from aligned stress fibers in straight segments to disorganized, faint networks under low or oscillatory shear, indicating cytoskeletal stress. This study provides an extensive mechanobiological profile of endothelial responses—at both gene and protein levels—to low, atherogenic flows, advancing understanding of early vascular disease mechanisms.

Keywords: hemodynamics, mechanobiology, microfluidics, disease model, stenosis, aneurysm