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Endothelial Cell Culture Model (ECCM) for Evaluation of Aortic Endothelial Cells Exposed to Normal and Disturbed Flow

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Atherosclerotic lesions occur non-randomly at vascular niches in bends and bifurcations where fluid flow can be characterized as 'disturbed' (low shear stress oscillatory flow) [1]. Endothelial cells (ECs) at these locations experience significantly lower average shear stress without change in the levels of pressure or strain which affects the local balance in mechanical stresses. Endothelial cells cultured under conditions of disturbed flow *in vivo* are associated with polygonal and randomly oriented phenotype, high EC turnover, increased accumulation of LDL, increased oxidative stress, increased DNA synthesis and higher expression of proinflammatory adhesion molecules [2-3]. Common *in vitro* models of atherosclerosis focus primarily on shear stress without accounting for pressure and strain loading [4]. However, ECs *in vivo* rely on a well balanced stress sensing machinery and experience pulsatile pressure and cyclic strain in addition to shear stress to maintain phenotype and function. To determine if alteration in the balance of mechanical stresses rather than changes in shear stress alone more accurately replicates the *in vivo* atherogenic EC phenotype, new systems capable of concomitant stimulation with realistic physiologic and pathophysiologic pressure, flow, strain and shear stress waveforms are necessary.

To accomplish *in vivo*-like mechanical loading, we designed and fabricated an Endothelial Cell Culture Model (ECCM) based on existing mock flow loops. Cells within this system are cultured on a thin (~ 500 μm) planar membrane within a rectangular flow channel and subject to constant fluid flow. Pulsatility is introduced using a programmable pneumatically controlled collapsible chamber. Using this system, pressure, stretch and shear stress profiles (Fig. 1) associated with normal and disturbed flow patterns typically seen in atherosclerosis susceptible regions like the proximal internal carotid were replicated. Human aortic endothelial cells (HAECs) were cultured within the ECCM under normal and disturbed flow conditions and evaluated using confocal microscopy. Cell size, shape, orientation along with staining for F-actin and β-Catenin clearly indicate that cells cultured under disturbed flow exhibit a randomly oriented polygonal phenotype with expression of low levels of F-actin and intermittent β-Catenin expression indicating generation of a proatherogenic phenotype within the ECCM under conditions of disturbed flow (Fig. 2).

Reference

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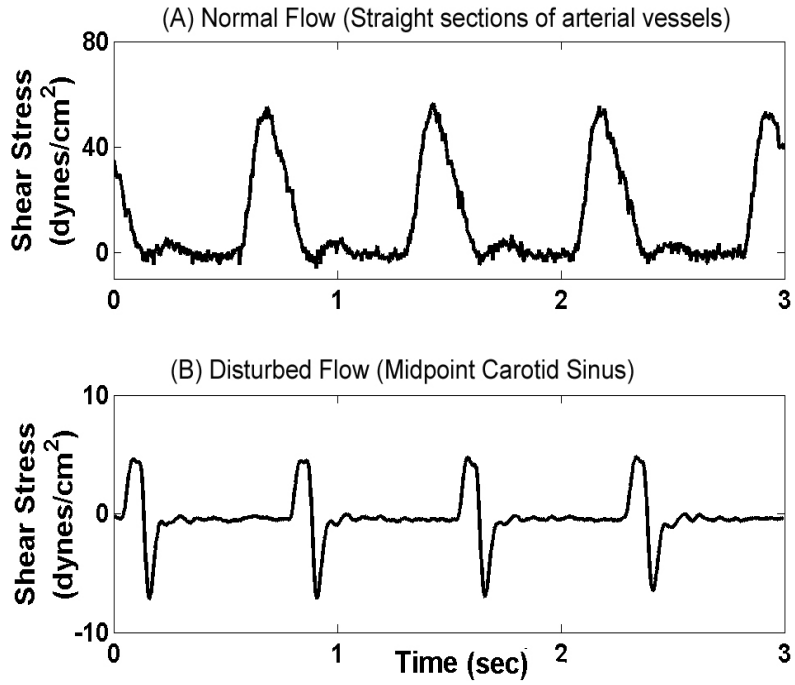

Figure 1. Comparison of shear stress profiles used to culture HAECs under **(A)** Normal and **(B)** Disturbed flow conditions. The shear stress waveform closely mimics the shear stress waveform seen in the proximal internal carotid. Pulsatile pressure was maintained at 120/80 mm Hg and cyclic strain of 6-11% was also accomplished.

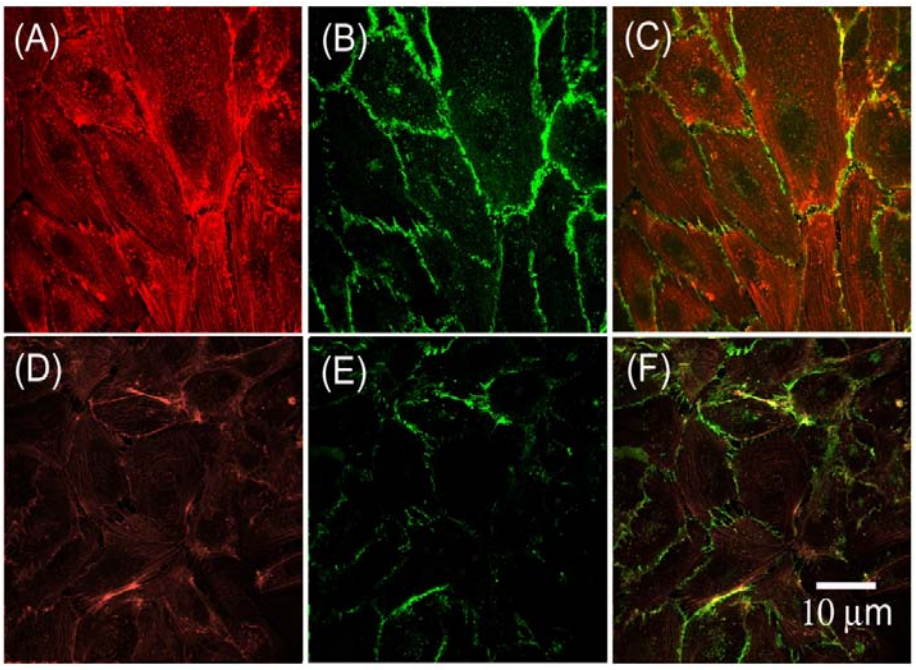

Figure 2. HAECs cultured under conditions of normal flow **(Top)** and disturbed flow **(Bottom)**. staining with F-actin **(A, D)**, β-catenin **(B, E)** and combined images **(C, F)**.

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