DEVELOPMENT OF A NEW BIOMECHANICAL *EX VIVO* PERFUSION SYSTEM

STUDIES ON EFFECTS OF BIOMECHANICAL AND INFLAMMATORY STRESS ON HEMOSTATIC GENES IN HUMAN VASCULAR ENDOTHELIUM

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Abstract:

The vascular endothelium is a multifunctional interface constantly exposed to biomechanical forces such as shear and tensile stress. Biomechanical stress is involved in the pathophysiological process of the vessel wall and thus affects vascular remodeling, atherosclerosis and thrombogenesis. Many different systems have been designed to subject endothelial cells to mechanical stress. However, previous systems have had large limitations in creating physiologically relevant biomechanical stress protocols. Therefore, there is a need for more refined biological perfusion systems that as accurately as possible mimics the *in vivo* conditions. In the present work, a new biomechanical *ex vivo* perfusion system for integrative physiological and molecular biology studies of intact vessels of different sizes as well as artificial vessels was developed.

This model was constructed for advanced perfusion protocols under strictly controlled biomechanical (shear stress, tensile stress) as well as metabolic (temperature, pH, oxygen tension) conditions. The system enables monitoring and regulation of vessel lumen diameter, shear stress, mean pressure, variable pulsatile pressure and flow profiles, and diastolic reversed flow. The vessel lumen measuring technique is based on detection of the amount of flourescein over a vessel segment. A combination of flow resistances, on/off switches and capacitances creates a wide range of possible combinations of pulsatile pressures and flow profiles. The perfusion platform was extensively evaluated technically as well as biologically by perfusion of high precision made glass capillaries, human umbilical arteries as well as endothelialized artificial vessels.

Artificial vessels with a confluent human umbilical vein endothelial cell layer were exposed to different levels of shear stress or different levels of static or pulsatile pressure. Shear stress was a more powerful stimulus than static or pulsatile tensile stress. While shear stress affected mRNA expression of all six studied genes (t-PA, PAI-1, u-PA, thrombomodulin, eNOS and VCAM-1), neither gene was found to be regulated by tensile stress. Shear stress suppressed t-PA and VCAM-1 in a dose response dependent way. The expression of thrombomodulin was also reduced by shear stress. u-PA, eNOS and PAI-1 were induced by shear stress, but showed no obvious dose response effect for these genes. Further, the unexpected suppression of t-PA by shear stress was studied by using mechanistic experiments with pharmacologic inhibitors. Our data indicate that the suppressive effect of shear stress on t-PA was mediated by suppression of JNK and not by p38 MAPK and ERK1/2.

The interplay between inflammatory stress and different combination of tensile as well as shear stress was studied on six key anti- and pro-thrombotic genes in HUVEC. The endothelial cell response to TNF-α was not modulated by tensile stress. Again, shear stress was a more potent stimulus. Shear stress counteracted the cytokine-induced expression of VCAM-1, and the cytokine-suppressed expression of thrombomodulin and eNOS. Shear stress and TNF-α additively induced PAI-1, whereas shear stress blocked the cytokine effect on t-PA and u-PA.

In conclusion, these findings illustrate that biomechanical forces, particularly shear stress, have important regulatory effects on endothelial gene function. A possible pathophysiological scenario is that an unfavourable hemodynamic milieu leads to a lower threshold for the induction of genes related to endothelial dysfunction in lesion-prone areas upon negative stress, such as inflammation.

Key words: *ex vivo* perfusion system, biomechanical, endothelium, shear stress, tensile stress, pulsatile, TNF-α, JNK, hemostatic genes

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Effects of two complex hemodynamic stimulation profiles on hemostatic genes in a vessel-like environment.
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