TISSUE ENGINEERED MODEL OF THE ARTERIAL INTIMA SUBJECTED TO WALL SHEAR STRESS

Dalit Shav, Ruth Gotlieb, Zaretsky Uri, David Elad, Shmuel Einav

Department of Biomedical Engineering, Faculty of Engineering, Tel-Aviv University, Israel

Introduction

The response of the vascular wall to the stimuli of hemodynamic forces is dependent on the interactions between vascular endothelial cells (EC) and smooth muscle cells (SMC). Understanding the effect of these interactions is critical for the prevention of vascular disease development and progression. Although wall shear stress (WSS) mechanotransduction in EC has been extensively studied and reviewed, the role of SMC and its interaction with the endothelium in this process is still not clear [Shi and Tarbell, 2011]. In order to investigate the interdependency between EC and SMC, several co-culture models were introduced [Truskey, 2010], revealing the presence of a mechano-signal transduction coupling between the EC and the SMC [Hsiai, 2008]. The present study aims to investigate the effect of flow on the cellular complexes that form the EC-EC and EC-SMC connections in such models.

Methods

In order to study the interactions of the two cell types in different *in vitro* models of the vascular wall, three models of the arterial lining were exposed to WSS: (i) EC mono-layer; (ii) EC cultured directly on a mature SMC layer (i.e., "1-side model"); and (iii) EC and SMC cultured on opposite sides of a membrane (i.e., "2-side model"). For these models we used human umbilical vein EC (HUVEC) and human umbilical artery SMC cultured on a polytetrafluoro-ethylene (PTFE) membrane. We studied the attachment of EC to the matrix through the expression of focal adhesion kinase (FAK), EC-EC connection through the expression of VE-Cadherin and the EC-SMC connections through the expression of myoendothelial (MEJ) junction proteins Connexin (Cx) 37 using immuno-fluorescence staining and flow cytometry.

Results

Results show that the amount of MEJ protein Cx 37 was highest in the 1-side model, where EC is cultured directly over the SMC. On the other hand, the FAK expression was lowest in the 1-side model, which was supported by the staining of FAK. VE-Cadherin staining was thicker in the 1-side and 2-side models compared to the EC monolayer. Following the exposure to WSS the continuous line of the VE-Cadherin seemed disintegrated, with a limited effect in the 1 side model (Figure 1).

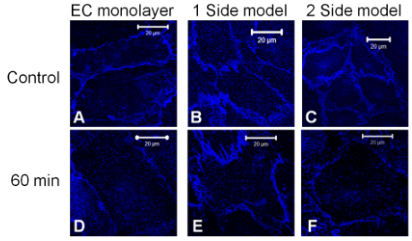

Figure 1: VE-Cadherin staining following the exposure of 12 dyne/cm^2 to 0 (control) and 60 min. The VE-Cadherin marks the border of the cells, with wider band in the 1-side and 2-side models (B and C). Following the exposure to WSS the VE-cadherin stain seemed fenestrated (D-F). In the 1-side model, this fenestration is not as significant as in the other models (E).

Discussion

The results indicated quantitative changes of the MEJ protein Cx 37 in the different models, suggesting that the EC-SMC connections were reduced when introducing a membrane between EC and SMC. In addition different focal adhesion complexes were formed, when EC were cultured directly on SMC. The changes in the EC-EC which can be noticed through the width of the VE-cadherin staining, suggest a leaky phenotype in the presence of SMC. These results indicated that the connections between the cells constructing the vascular wall as well as to their matrix were important in the response to WSS and should be considered when studying the role of blood flow on the vascular wall.

References

Hsiai TK, Am J Physiol Cell Physiol, 294: C659-61, 2008.

Shi ZD and JM Tarbell, Ann Biomed Eng, 39: 1608-19, 2011.

Truskey GA, Int J High Throughput Screen, 1: 171-181, 2010.