52. Role of Rhythmic Breathing Motions on In Vitro Pulmonary Vascular Remodeling

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Introduction

In addition to mechanical forces caused by blood flow, lung vascular network undergoes additional forces in the form of cyclic stretch, generated by respiration. These forces act on the microvascular wall and play an important role in the regulation of vascular structure. Vascular cells can sense variations in mechanical forces and transduce the mechanical signal into a biological response (mechanotransduction). For instance, an over-extension of the lung may lead to large deformations that cause cells to trigger an inflammatory response. However, the precise mechanisms of vascular cell mechanotransduction remain a mystery, particularly, in the lung because of the complexity of local distension patterns in the lung parenchyma. Current in vitro microvasculature models investigating cellular response induced by changes in the mechanical environment are limited to the study of the effect of the shear stress generated by the blood flow. The existing models that incorporate mechanical stretch are usually limited to 2D cell culture platforms, which lack the dimensionality seen by cells comprising cyclically expanding organs, such as the lung. Advanced 3D in vitro microvasculature models better mimic the in vivo conditions and offer a potential alternative to traditional animal experiments. These platforms are seen as promising tools to address underlying mechanisms of pathophysiological mechanotransduction. In this research we developed a dynamic microvasculature platform to investigate the effect of 3D cyclic stretch from the rhythmic breathing on human microvascular remodelling.

Materials and methods

Experiments were carried out using a microfluidic platform made of PDMS. The platform was a multi-layered system that consists of a thin polymeric membrane and a fibrin gel layer with an incorporated microvasculature cavity (Fig. 1). A perfusable microvasculature was reproduced in the fibrin gel layer by seeding human endothelial cells in a tube formed by a needle that was removed after gelation of the fibrinogen. After formation of an endothelial barrier, the microvasculature was subjected to a 3D cyclic strain. After immunostaining, confocal images of the microvasculature were analysed to examine the microvascular structure and morphology. To measure the vascular permeability, 70 kDa RITC was loaded in the microvessel. The vascular permeability was calculated based on the fluorescent intensities across vascular walls over time.

Results

Physiological cyclic stretch appears to improve the integrity of the microvasculature, by assisting the formation of tighter vessel walls, with smaller gaps sizes (Fig. 2). Results of the experiments exposed that cell membranes, cell attachment sites, and cytoskeletal network serve as primary mechanosensors because of mediating signal transduction by activating transmembrane receptors of cadherin (cell-to-cell) and integrin (cell-to-substrate) families. The model revealed strong differences in the permeability, morphology and structure of microvessels in either static or dynamic conditions.

Conclusion

This in vitro 3D dynamic model of human lung microvasculature represents an exciting approach because it permits to study the effect of physiological and pathological cyclic stretch on isolated human microvessels. Dynamic lung microvasculature provides the possibility to study the mechanotransduction mechanism and the vascular remodeling in greater detail than in vivo studies. This model may help development of novel therapies for the treatment of pathologic conditions such as pulmonary hypertension and ventilator-induced lung injury in the future.

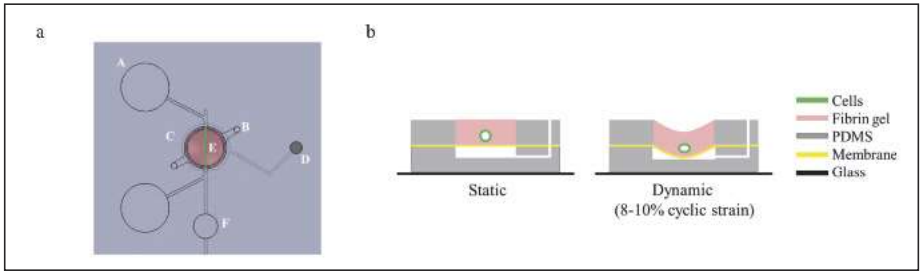

Figure 1: a) Layout of the dynamic microvasculature chip. A: Reservoirs. B: Inlet ports for injection of fibrin gel. C: Well containing gel scaffold and channel for cell culture. D: Connection port to breather. E: Microvasculature. F: Plug forsealing channel. b) Side view of the chip with and without cyclic stretch.

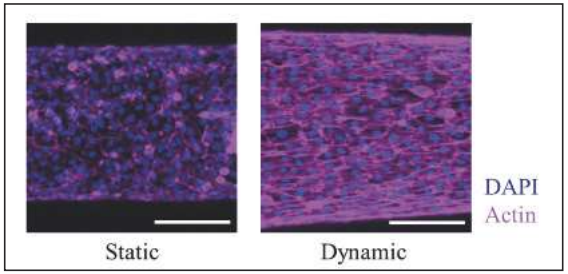

Figure 2: Z-projections of half of the microvasculatures in static and dynamic conditions. 24 hours of physiological cyclic stretch significantly increases theintegrity and tightness of the endothelial barrier. Scale bar: 100μm.