Control of Cell-Matrix Interactions on Fibrin Bi-layers and Micro-Patterned Surfaces

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Statement of Purpose: Cells are the functional elements of tissue repair and regeneration. Successful tissue engineering hinges on the ability to induce the cells to proliferate and differentiate to appropriate phenotypes or functions. Tissue engineering scaffolds provides not only structural support, but also tensile strength and attachment sites for cells. Future progress in tissue engineering will largely depend on the ability to understand, design and manipulate the biochemical and physical interactions between cells and matrix at the molecular level. In this study, the control of cell-matrix interactions was achieved by manipulating the mechanochemical and topographic properties of the matrix, respectively [1].

Methods: Fibrin bi-layers were formed step by step on four model surfaces: hydrophobic, neutral hydrophilic, positively and negatively charged. These surfaces were achieved by self-assembled monolayers (SAMs) of alkanethiolates on gold with different terminal groups. Surface plasmon resonance sensor (SPR) was used to monitor the formation of fibrin bi-layers in real time and to measure the adsorbed amount of fibrinogen in each layer on each surface. The possible structures of the fibrin bi-layers formed on SAMs of different surface chemistries were speculated based on the adsorbed amount of fibrinogen in each layer on each surface measured from SPR with reference to a theoretical model of an adsorbed fibrinogen monolayer with "side-on" fibrinogen orientation. Bovine aortic endothelial (BAE) cells were cultured on fibrin bi-layers formed on CH_3- and OH- SAMs and characterized. Furthermore, we examined the topographic control of endothelial cell capillary-like structure (CLS) formation by culturing cells on micro-structured polydimethylsiloxane (PDMS) coated with fibronectin.

Results / Discussion: Fibrin bi-layers with different molecular arrangements were formed in a stepwise way on SAMs of different surface chemistries (Figure 1). The adsorbed amount of fibrinogen in each layer was measured by SPR. It was shown that despite the relatively low level of adsorption, fibrinogen adsorbed onto the neutral hydrophilic surface had relatively high reactivity with soluble fibrinogen when activated by thrombin. The ability of the physically adsorbed fibrinogen to form fibrin bi-layer was greatly impaired on the negatively charged surface. The overall fibrin bi-layer coverage decreases in the following order: NH_2- > CH_3- > COOH > OH. The difference in the capacity of the adsorbed fibrinogen on different SAMs to promote fibrin formation could be attributed to their orientation and conformation. Results from BAE cells cultured on fibrin bi-layers formed on CH_3- and OH- SAMs showed that fibrin bi-layers with different molecular arrangements and thus different mechanochemical properties induced by different surface chemistries could lead to different cellular responses (Figure 2). Furthermore, we described for the first time that micro-structured surfaces could induce the formation of CLS, depending on the dimension of the patterns.

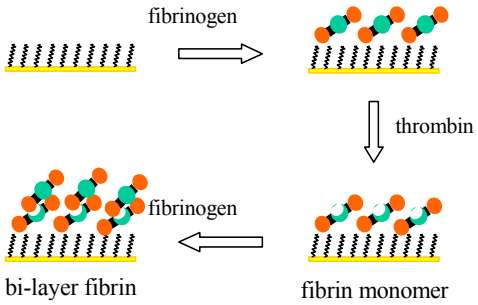

Figure 1. Model for the step-by-step assembly of a fibrin bi-layer.

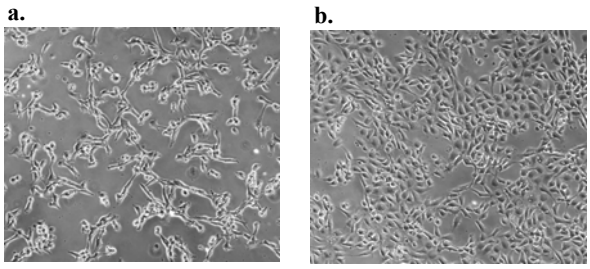

Figure 2. Phase contrast images of BAE cells cultured on CH3- (a) and OH- (b) SAMs (original magnification ´10).

Conclusions: Fibrin bi-layers were formed in a stepwise way on SAMs with different terminal groups. It was shown that the difference in the orientation/conformation of the first layer of fibrinogen induced by different surface chemistries lead to fibrin bi-layers with different structures. Fibrin bi-layers with different structures, and thus different mechanochemical properties induced the endothelial cells cultured on them to develop into distinct phenotypes. Furthermore, it was shown for the first time that micro-grooved surfaces can induce the formation of capillary-like structures by endothelial cells.

References:

1. Brash, J.; Horbett, T., ACS Symp. Ser 1987, 343.