Combined Effects of Physical and Biochemical Stimuli on HUVECs *In Vitro*: A Multilayer Film Study

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Statement of Purpose:

The physical and chemical properties of biomaterials can have a profound impact on cell behavior and tissue remodeling. A given biomaterial will influence cell behavior in a cell-dependent manner. Biomaterials development therefore requires investigation of relationships between specific biomaterials and specific cell types. Polyelectrolyte multilayer films have been investigated for a variety of biomedical applications. A number of these studies have focused on effects of film properties on cell adhesion and proliferation. Here, we present results of a multilayer film study in which the combined effects of physical and biochemical stimuli on primary human umbilical vein endothelial cell (HUVEC) were determined *in vitro*.

Methods:

Multilayer Film Fabrication and Functionalization: Cells were cultured on polypeptide multilayer films composed of poly(L-lysine) (PLL) and poly(L-glutamic acid) (PLGA). In some cases, the film surface was functionalized with peptides based a collagen type I-derived motif:

CI-1: $K_{12}(GPO)_3$GFOGER$(GPO)_3$Y

CI-2: K_{12}GTPGPQGIAGQRGVVY

Functionalization was achieved by the same method used to fabricate the underlying film: layer-by-layer self-assembly.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D): Film mechanical properties were measured by quartz crystal microbalance. QCM-D data were used to calculate viscoelastic properties of films by the Voigt model. Young's modulus was calculated from the shear modulus.

Cell Adhesion and Proliferation: Responses of HUVECs to differences in film architecture were determined in short-term adhesion (2 h or 1 day) and long-term proliferation (7 days) experiments, in the absence or presence of vascular endothelial growth factor (VEGF). Fluorescence intensity was used to measure cell number with Vybrant™ and CyQuant® NF assays.

Immunocytochemistry:

Changes in cell-cell and cell-substrate adhesions were probed by staining F-actin, vinculin and cadherin with rhodamine-phalloidin, FITC-anti-vinculin and phycoerythrin-anti-human VE-cadherin, respectively.

Results:

Overall cell adhesion decreased on increasing the film thickness in serum-containing medium. Films with a positively charged surface increased cell adhesion in the presence of serum relative to films with a negatively charged surface. Film shear modulus decreased with an increase in film thickness, reflecting a decrease in film stiffness. Film functionalization enhanced cell adhesion by at least 25% and as much as 200% relative to the corresponding non-functionalized films (Figure 1).

Cell number on non-functionalized and functionalized films decreased over time and was less than 25% of the bare-plastic control after 7 days of culture, regardless of the presence of VEGF. Actin filaments were disorganized in cells culture on non-functionalized or functionalized films, and vinculin, a focal adhesion protein, was irregularly distributed on the softer films.

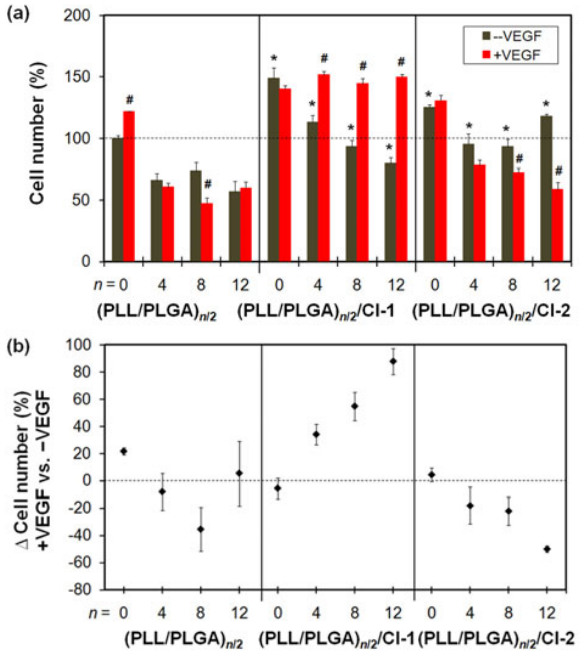

Figure 1. Short-term HUVEC adhesion on functionalized films after 2 h at 37 °C. (a) Effect of functionalization. (b) Effect of VEGF. $p < 0.05$.

Conclusions:

Mechanical properties of multilayer films appeared to have a more dominant long-term effect on cell behavior than the biochemical stimuli used in this work. The same mechanochemical stimuli tested here could be applied to many other cell types, and different stimuli could easily be tested. The general multilayer film approach is therefore a relatively straightforward means of probing cell responses to a specific and controlled environment.

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