Response of Vascular Smooth Muscle Cells Under Mechanical Deformation Using Silane-Linked Laminin

Joy P. Dunkers[1] and Juan M. Taboas[2]
[1]Biomaterials Group, Polymers Division, National Institute of Standards and Technology, Gaithersburg, MD U.S.A.
[2]Cartilage Biology and Orthopedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases National Institutes of Health, Bethesda, MD U.S.A.

Introduction: For mechanotransduction studies, extracellular matrix proteins should be robustly attached to the surface to prevent cell delamination during deformation. The standard surface modification method is to incubate proteins on an oxidized, flexible surface (e.g. polydimethylsiloxane (PDMS)) without any additional surface modification. However, this results in physically adsorbed surface proteins that desorb over time even under static conditions. Also, cell response is often different on surfaces with covalently bound proteins due to changes in conformation. We have found that silane-linked proteins provide the highest coverage, uniformity, and cell proliferation over both physically adsorbed and covalently bonded proteins[1]. In this work, we evaluate the robustness of physically adsorbed and silane-linked laminin and the resulting proliferation of smooth muscle cells after equibiaxial stretching for 4 d.

Methods: Surface Modification: Bioflex cell culture plates (Flexcell Int.) were oxidized and treated with either physisorbed (plasma) or silane-linked (silane) laminin[1].

Protein Robustness via Immunochemistry: Laminin was visualized using rabbit anti-laminin IgG (Sigma-Aldrich) and Alex Fluor 488 goat anti-rabbit IgG (Invitrogen)[1]. To evaluate robustness, treated PDMS surfaces underwent continuous 5 % equibiaxial strain at 0.5 Hz for 4 d. At 2 h, 1 d, and 4 d, the strain was interrupted, samples were washed 2X with phosphate buffered saline, and five microscope images were taken on each well and normalized against of 10^{-5} mol/L fluorescein in ethanol.

Cell Proliferation: Rat aortic smooth muscle cells (SMC, A10, ATCC)[2] were seeded on the modified PDMS (45 k cells/cm^2). Some samples were preconditioned with serum containing growth medium for 24 h prior to cell seeding. After seeding, PDMS samples were strained for 1 h at 5 % peak equibiaxial strain and 0.5 Hz followed by static culture for 23 h. This cycle was repeated for 4 d, at which time cells were trypsinized and counted.

Statistics: Significant differences were assessed using analysis of variance (95 % confidence level). This is an expanded uncertainty with coverage factor k=2.

Results: Figure 1 compares the laminin retention for the plasma and silane samples under static and equibiaxial strain over 4 d. At 0 h, the samples had comparable amounts of laminin. The laminin on the static silane wells did not desorb over 4 d, while the static plasma samples decreased significantly over that time. Both the strained treatments lost laminin, with the strained plasma showing more loss as early as 2 h. Figure 2 shows SMC proliferation after 4 d of intermittent straining with laminin on both surface treatments. Although media preconditioning for 24 h did encourage significantly more SMC proliferation under static conditions (not shown), it had no effect on cell proliferation under strain. For both preconditioning treatments, the number of SMC on the silane-linked laminin was approximately double that on the plasma only laminin surface when loaded.

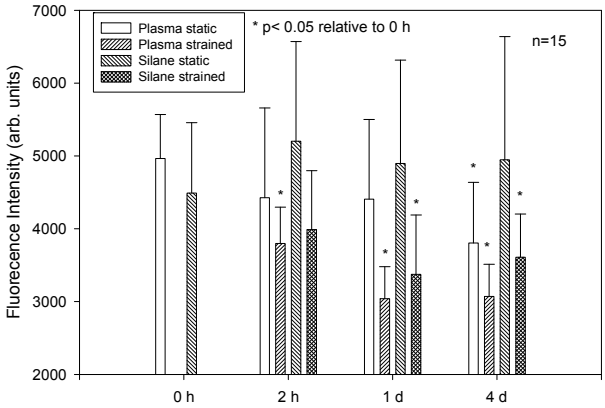

Figure 1: Comparison of Laminin Retention on PDMS Surfaces with Plasma and Silane Treatments

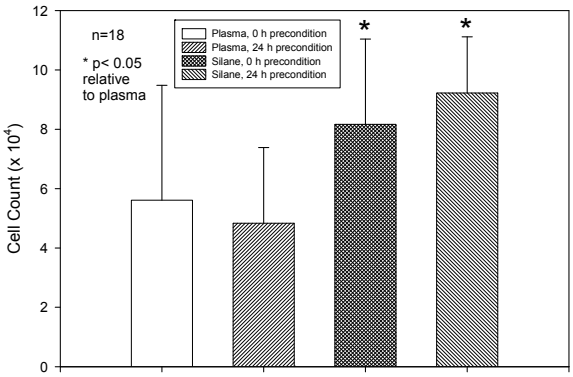

Figure 2: 4 d Proliferation on Laminin Coated Strained PDMS.

Conclusions: We have shown that more silane-linked laminin remains on the surface after strain. The silane-linked laminin also induced markedly greater proliferation and alignment (not shown) than the physisorbed laminin.

References:

1. Pakstis LM, Dunkers JP, Zheng A, Vorburger TV, Quinn TP, Cicerone MT. J. Biomed Mater. Res.-A, accepted.
2. Pauly RR, Bilato C, Cheng L, Monticone RE, Crow MT. Methods in Cell Biology 1997;52:133-154.

Acknowledgements: We thank Stefan Leigh for his statistical analysis. Official contribution of the National Institute of Standards and Technology; not subject to copyright in the United States. Certain equipment, instruments or materials are identified in this paper in order to adequately specify the experimental details. Such identification does not imply recommendation by the NIST nor does it imply the materials are necessarily the best available for the purpose.