Strain Induced Alterations in Protein Modified Surfaces and Cellular Response

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Statement of Purpose: All cells experience strain under physiological conditions. However, experiments that are performed under macroscopic strain conditions prevent a direct correlation of applied strain to cell behavior. Our focus is to measure the local stress/strain environment of the cells in a bioreactor under mechanical load and to quantify and predict biological responses at the cellular level. We begin this work by developing methods for deposition and characterization of robust protein surfaces.

Methods: Polydimethylsiloxane (PDMS) surfaces were oxidized via plasma treatment and chemically treated with either aminopropyltrimethoxy-silane[1] (APTMS) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride[2] (EDC), similarly to previous described protocols. Treated surfaces were incubated with a 4 μg/ml solution of either fibronectin or laminin overnight. Surfaces were characterized using FT-IR spectroscopy, optical interferometry, dynamic contact angle, and immunohistochemical staining. The biological response of these protein modified surfaces was characterized using smooth muscles cells (SMC) because they exhibit a distinct phenotypic response from a synthetic to a contractile state. Mechanical deformation is applied to the Bioflex culture plates (Flexcell® International, Hillsborough, NC) using an in-house system for control of biaxial stretching.

Results/Discussion: Studies investigating cellular response to mechanical deformation are performed under biaxial stretching in which elastomeric membranes are stretched by negative pressure and cells are subjected to uniform strain. Here, hydrophobic silicone membranes were coated with the extracellular matrix protein fibronectin via multiple methods. Protein modified surfaces are characterized before and after deformation to evaluate the durability of the protein coating and to determine the cellular response at the cell-biomaterial interface as a direct consequence of the surface features. FT-IR confirmed the presence of the proteins after modification. Surface roughness was measured using optical interferometry, and surface hydrophilicity was determined by dynamic contact angle. The amount of protein bound to the PDMS surface was quantified by immunohistochemical staining. A higher quantity of ECM protein, as measured immunohistochemically, was observed for surfaces initially treated with an amine-terminated silane and was retained after deformation compared to surfaces where the proteins were physically absorbed or chemically attached by EDC. The method of surface modification, and not the ECM protein, directly affected the biological response of SMCs. Surfaces that were chemically modified with fibronectin via the silane method had greater cell attachment and proliferation as compared to surfaces with physically absorbed fibronectin. RT-PCR results (not shown) and cell morphology, figure 1, confirmed that under static conditions, the SMC remained in the synthetic state, implying that all shifts in phenotype are the result of mechanical deformation applied to the membrane.

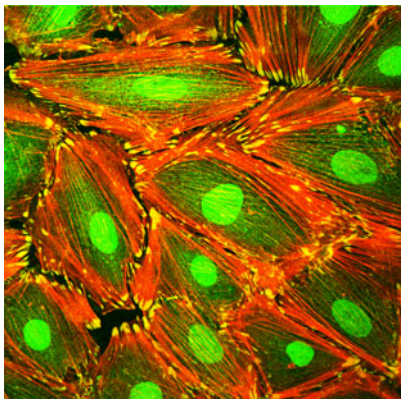

Figure 1. SMC on a fibronectin modified surface. This image highlights the cell cytoskeleton (red) and focal adhesions (yellow). Cell nucleus is shown in green. SMC shape is consistent with a synthetic phenotype.

Conclusions: Surface characterization and static culture experiments confirmed that surface features, such as roughness, and type of ECM protein does not induce a phenotypic shift in the SMC. Covalently attaching the ECM proteins to the PDMS surfaces allowed for a greater protein density after deformation and, subsequently, enables longer experimental times and better correlation to in vivo conditions. Current work is aimed at determining the ideal conditions for eliciting a phenotypic response when a mechanical stimulus is applied.

References:

[1]Altankov, G. J Biomed Mat Res. 1996;30:385-391.
[2]Volcker, H. J Mat Sci, Mat Med. 2001;12:111-119.

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