# Synergy of Three-dimensional Soft Matrices with ECM Proteins Augments Stem Cell Differentiation towards Vascular Phenotypes

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**Statement of Purpose:** Despite significant progress in regenerating vascular tissues using mesenchymal stem cells (MSCs) there are still considerable challenges associated with the production of vascular phenotypes with high cell specificity, maturity and functional behavior. There is abundant evidence suggesting that local signals from the tissue-specific ECM micro-environments, through ligand-activated cell-matrix interaction and/or focal adhesion-induced interaction caused by matrix physical properties such as elasticity and geometry, significantly affect MSC differentiation and mature phenotype expression. It is likely that these signals together impart an instructional environment whereby MSC behavior and differentiation are directed in a highly specific manner. We previously demonstrated that 3D nanofibrous PEG gel substrates with elasticity that mimics the natural vascular stiffness can direct MSC differentiation toward vascular-like phenotypes within 24 hours (Wingate, 2012). Hereby, we expand on that approach by incorporating various extracellular matrix (ECM) proteins into our tunable 3D soft matrices. We hypothesize that the presence of specific ECM protein(s) on soft matrices augments differentiation of MSCs into mature vascular phenotype and function. To test the hypothesis, we develop a high-throughput cell microarray approach (Figure 1A) and demonstrate its utility for screening a variety of engineered microenvironments with the aim to instruct specific MSC differentiation.

**Methods:** *PEGDM/ECM Protein Electrospun Net Fabrication:* Diacrylated PEG electrospun constructs are prepared using our established spinning protocol (Wingate, 2012). To incorporate ECM proteins into the 3D PEG nanofibrous matrices, different protein solutions, including Collagen I, Collagen IV, Fibronectin, Elastin and laminin, with concentrations ranging from 5-25 pmol/well, are incubated on PEG matrices for 24 hours. *Material characterization:* To verify the absorption of proteins into 3D PEG matrices, Cy3-conjugated albumin is incubated for 24 hours and subsequently rinsed in PBS twice. The samples are then be visualized using a fluorescent microscope as well as a gene microarray scanner to verify protein absorption. The structure and mechanical properties of the PEG nets after protein absorption are evaluated using SEM, compression tests and FTIR, respectively. *Cell Culture and Immunostaining:* Human MSCs (hMSCs) from Lonza Inc. with passages 2–6 are used. Cell morphology will be obtained by staining for cell nuclei (DAPI) and cell cytoskeleton (phalloidin) after sample fixation. Immunofluorescent staining of cells with anti-αSMA, calponin, SM-22 and MHC (early, intermediate and mature SMC markers) are used to characterize vascular differentiation of MSCs.

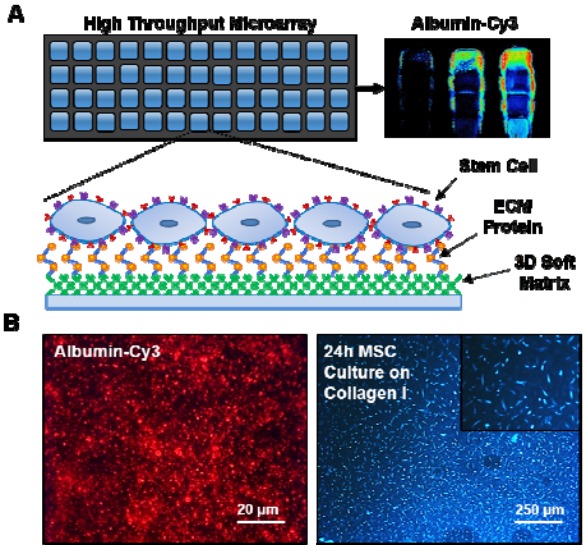


Figure 1. (A) Illustration of the microarray fixture utilized to evaluate a range of protein-matrix 3D environments and image of albumin absorption from an array scanner (left); (B) Fluorescent image of albumin absorption and MSC nuclei (blue, right) after 24hr culture on collagen-coated 3D PEG matrix.

**Results:** Protein absorption: We have evaluated protein absorption into the PEG nanofibrous nets using albumin and streptavidin with a variety of concentrations. Images from the microarray scanner verified the presence of proteins at different densities in the 3D matrix nets (Figure 1A, right). These have been further confirmed with fluorescent microscopy images (Figure 1B, left). Cell adhesion: MSCs were cultured on collagen-coated 3D PEG scaffolds for 24 hours and stained for nuclei to illustrate the cell density (Figure 1B, right). These results demonstrate substantial cell growth and spreading on the 3D PEG-protein scaffolds with significantly greater cell density and spreading as compared to PEG control scaffolds (not shown).

**Conclusions:** We have previously reported a technique to produce 3D nanofibrous PEG gels that resemble the native vascular microenvironment and soft matrices with elasticity of 12-30 kPa upregulate early SMC markers within 24 hours. Expanding on this work, we develop a high throughput method that allows for rapid screening of a diversity of 3D engineered microenvironments with tunable matrix elasticity and ECM protein combination and/or concentration by studying their influences on instructing MSC differentiation. To this end, we elucidate the synergistic role that protein ligands and substrate physical properties play on MSC differentiation.

**References:** Wingate K, Bonani W, Tan Y, Bryant S, Tan W. 2012. *Acta Biomater* 8, 1440