Directing Stem Cell Fate in 3D through Cell Inert and Adhesive Diblock Copolymer Domains

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Statement of Purpose: Adhesions are important cell structures required to transduce a variety of chemical and mechanical signals from outside-in and vice versa, all of which regulate cell behaviors, including stem cell differentiation [1]. Though most biomaterials are coated with an adhesive ligand to promote adhesion, they often have a uniform distribution that does not match the heterogeneously adhesive extracellular matrix (ECM) *in vivo* [2]. We have previously shown that diblock copolymer (DBC) mixtures undergo interface-confined de-mixing to form nanodomains of one copolymer in another [3]. Here we demonstrate how diblock copolymer mixtures can be made into foams with nanodomains to better recapitulate native ECM adhesion regions and influence cell adhesion.

Methods: Materials: DBC foams were formed by emulsifying an oil phase consisting of styrene monomers, the crosslinking agent divinylbenze, and the diblock copolymers polyethylene oxide (PEO)-polystyrene (PS) and polyacrylic acid (PAA)-PS with an aqueous water phase containing an initiator and potassium persulfate. Charged PAA-PS should bind protein and cells while PEO-PS should not. This resulted in a high internal phase emulsion (HIPE; water phase > 74%) to form interconnected oil droplets. Characterization: To confirm and measure nano-domains, XPS and contact angle measurements were performed on foams. Chemical force spectroscopy was used to map regions of strong adhesion at 20 nm lateral surface resolution. Tapping mode AFM images and scanning electron microscope provide further structural characterization. PAA protein adsorption and cell adhesion were also tested. Cells: Human embryonic stem cell-derived mesenchymal progenitors (ESC-MP) were seeded onto and cultured in DBC foams for up to 1 week before examining cell fate by western blotting and qPCR microarrays.

Results: SEM was used to confirm the interconnected foam morphology of the material, which did not change dramatically as a function of DBC composition (Fig. 1, top). Surface roughness, as assessed by SEM (Fig. 1, middle) and AFM, also did not change as a function of DBC composition, indicating that cell behavior is likely independent of roughness and due solely to adhesion changes. XPS confirmed foam chemical composition as either pure or mixtures directly reflecting their input DBCs.

At low pH when PAA is de-protonated, foam hydrophobicity scaled with mixture chemistry as indicated by contact angle measurements, confirming at least macroscopically that mixtures were detected and functional in the foams. To assess domain formation and size relative to native ECM, adhesive forces generated by columbic interaction between the positive and negative charges of PLL and PAA were used to set a 1 nN threshold to distinguish between non-adhesive and adhesive events. Bottom panels of Fig. 1 show adhesion maps of negative (0% PAA) and positive controls (100% PAA), which should be non-flowing and uniformly adhesive due to its charge, respectively. DBC mixtures are also shown at their indicated concentrations with the PAA regions indicated by cooler colors.

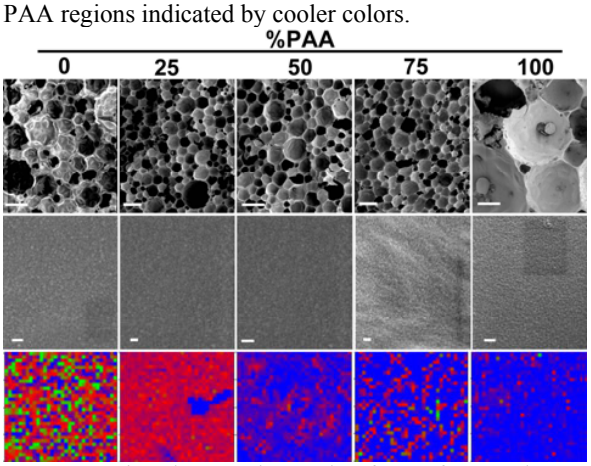

Fig. 1. Scanning electron micrographs of DBC foams at low and high magnification (top and middle, respectively) for the indicated PAA mass percent. Force spectroscopy maps of a 4 μm^2 region of the foams with high adhesion, i.e. the PEO regions, indicated by warmer colors (bottom).

Protein assembly but not adsorption on foams was affected by composition: fibronectin aggregated on scaffolds with intermediate PAA composition in discrete regions where PAA was present (e.g. 25%PAA/75%PEO) but was uniformly distributed on scaffolds with high PAA content and absent on scaffolds without PAA.

Protein distribution affected ESC-MP adhesion on the foams. For example, cells were most adherent on and expressed and assembled the most robust vinculin-containing adhesions on 25%PAA/75%PEO foams. This mixture also produced the most robust increase in lineage marker expression of adipogeness compared to undifferentiated controls, indicating that average domain size, i.e. 0.1 μm^2, and spacing, i.e. 0.5 µm, of 25%PAA/75%PEO foams may closely match native ECM [2,4].

Conclusions: Different surface mixtures can be distinguished by macro- and nano-scopic metrics and reflect previously documented ECM adhesion heterogeneity which current materials typically lack. Cell adhesion shows similar behavior and ongoing work to assess stem cell differentiation may also indicate the importance of this design criteria in future regenerative approaches.

References: [1] Engler, A.J. *et al*., *Cell*, 2006. 126(4): 677-89. [2] E.D. Hay, *Cell biology of extracellular matrix*, Plenum Press, 1991. [3] LoPresti, C. *et al*., *ACS Nano,* 2011. 5(3): 1775-84. [4] Viswanathan, P. *et al., J Am Chem Soc*, in press.