Molecular Engineering of Smart Protein Scaffolds for Cardiac Stem Cell Differentiation

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"Smart scaffolds" with tunable ligand density and mechanical stiffness have been engineered to mimic the cardiac extracellular matrix (ECM) and enhance the differentiation of human embryonic stem cell derived cardiomyocytes (hESC-CMs). The protein scaffolds are synthesized in *E. coli* bacterial hosts, enabling molecular-level control over the material design. The engineered proteins contain RGD-binding sequences interspersed with an elastin-like sequence to promote cell adhesion and mechanical integrity, respectively. The resulting scaffolds are optically transparent and easily micro-molded in a sheet or 3D construct. Crosslinking is achieved with a water-soluble, bi-functional N-hydroxysuccinimide (NHS) ester molecule, which has excellent cell biocompatibility. By changing the degree of crosslinking, we can create scaffolds ranging in elastic moduli from 0.1 – 1.0 MPa. These protein scaffolds demonstrate several key features of the ECM, including elasticity and cellular adhesion. Unlike current natural and synthetic materials, the ligand density and elasticity can be engineered independently.

Beating clusters of H9 hESC-CMs were plated on protein scaffolds (~1 x 10^8 ligands/cell area) and monitored for 10 days in culture. As a negative-control, the RGD sequence was scrambled (RDG) to create scaffolds with identical hydrophilicity, molecular weight, and mechanical properties. Viability of the hESC-CMs was maintained on both protein scaffolds for the length of the experiment. Phase contrast images show hESC-CMs cultured on the RGD peptide scaffold attach and are well-spread in comparison to the RDG scramble scaffolds, as well as contract rhythmically *(http://microsystems.stanford.edu/~jblundo)*. The rate and amplitude of contraction of the hESC-CM clusters was captured using video microscopy and analyzed using edge detection software. Cell samples were fixed in 4% paraformalin and stained for DAPI nuclear marker. Immunofluorescence staining for smooth muscle actin and the cardiac specific marker, troponin, was used to analyze levels of cardiac expression in the culture. The mechanical properties of the scaffolds were measured by nanoindentation with atomic force microscopy (AFM). AFM probe data of protein scaffolds with identical RGD-ligand density and varying crosslinker density showed increasing crosslinker density yields a stiffer material.

In conclusion, our results demonstrate molecular engineered RGD-elastin protein scaffolds are a viable matrix for hESC-CMs. Continued study on the effect of scaffold stiffness and contractility of hESC-CMs is planned.