Elastin-Based Biomaterials for Embryonic Stem Cell Derived Cardiomyocyte Applications

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Statement of Purpose: Cardiovascular disease is the single biggest cause of death in the United States. Therapy is complicated by the fact that cardiovascular muscle does not, of its own, regenerate. The use of stem cell-based implantation therapy has emerged as a promising new alternative(*1*), and using biomaterials to aid cell implantation may substantially increase the therapeutic efficacy by providing cell-biomaterial interactions that control cell behavior. Using recombinant protein technology, we have synthesized a family of protein-engineered biomimetic materials that can be used to independently tune multiple critical properties of the biomaterial that will guide cell adhesion and contractility (3). A modular biomterial design strategy enables independent tuning of the initial mechanical properties, the density of cell adhesion ligands, and the rate of proteolytic remodeling of the biomaterial. Human embryonic stem cell-derived cardiomyocytes (hESC-CM) cultured on our protein-engineered biomaterials have demonstrated excellent viability, proliferation, and morphology comparable to positive controls. Furthermore, hESC-CM retain their functional differentiated phenotype based on positive immunostaining and spontaneous, regular, coordinated contractions over multiple weeks. Our promising preliminary data demonstrates the use of protein-based biomaterials as a viable option for cell-compatible, biodegradable therapeutic scaffolds.

Methods: Recombinant DNA technology has been used to manufacture all protein biomaterials. The protein sequences are designed with a modular design strategy. Structural integrity is ensured by the inclusion of a peptide sequence that mimics elastin, a fibrous protein found in connective tissue that is known to provide elasticity. In addition, peptide sequences that mimic the cell adhesion domains of natural extracellular matrix proteins (specifically, the RGD domain from fibronectin) have been included. The elastin sequence has been modified with lysine residues so as to facilitate crosslinking using a chemical crosslinker. It is possible to modify the stiffness of the material by simply tuning the crosslinker:biomaterial stoichiometry. The adhesivity of the biomaterial may be modified independently of the elastic modulus by varying the number of RGD cell adhesion ligands presented to the cell. It is hypothesized that the relative adhesivity of the material will have an impact on hESC differentiation into cardiomyocytes and cardiomyocyte contractility. A mixture of beating and non-beating H7 hESC embryoid bodies (formed without feeder lines) was seeded onto substrates with varying concentration of RGD ligands. In a separate experiment, H9 hESC were cultured using feeder lines (*2*), differentiated into cardiomyocytes, and cultured on biomaterial substrates containing different levels of RGD.

Results: Preliminary data suggests that increasing biomaterial RGD content may induce contractility in a larger number of hESC colonies, Figure 1.

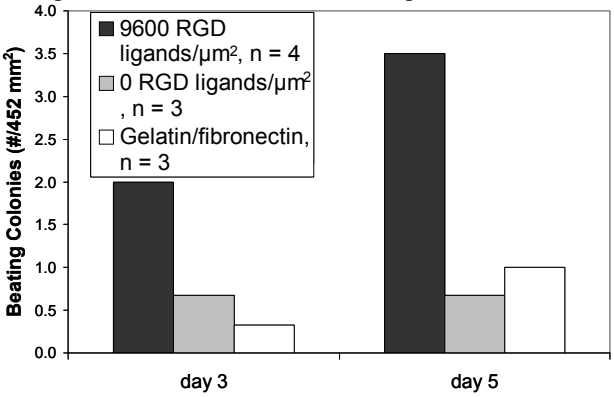

Figure 1: Number of contractile H7 hESC embryoid body colonies per 452 mm^2 of various culture subtrates.

The number of H7 hESC colonies that spontaneously initiated regular contractions was highest on substrates containing 9600 RGD ligands/μm^2. In contrast, the negative control protein-engineered substrate (no RGD ligand) performed similar to the commonly used gelatin/fibronectin substrate. Additionally, contractile H9 hESC-CMs demonstrated continued contractility over a period of three weeks on all protein-engineered substrates, with a general increase in beats per second observed for cells cultured on substrates with increased RGD concentration. Taken together, these data suggest that biomaterial adhesivity may impact hESC-CM contractility, perhaps by influencing cellular morphology.

Conclusions: The above preliminary data demonstrate that biomaterial properties may impact hESC differentiation along a cardiomyocyte lineage. hESC-CMs have been demonstrated to survive and proliferate on our protein-based biomaterials and demonstrate contractility and morphology similar to gelatin controls. In addition, preliminary data suggest that RGD concentration may promote hESC-CM differentiation and contractility. These biomaterials may be molded into patches for future *in vivo* hESC-CM experiments and for eventual tissue engineering applications.

References:

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