Engineered, Dynamic Materials Improve Cardiomyocyte Differentiation

Jennifer L. Young & Adam J. Engler
University of California, San Diego

Statement of Purpose: Cellular cardiomyoplasty, the process where undifferentiated stem cells are injected into an ischemic region of the cardiac muscle wall to regenerate function, is a widely used regenerative therapy that is thought to limit wall scarring and restore contractile muscle function. Until recently, however, few have appreciated the fact that scarred tissue changes extracellular matrix (ECM) elasticity, which can have a detrimental impact on therapeutic strategies. For instance, tissue scarring in ischemic cardiac muscle increases the elasticity of muscle ~5-fold from 10-15 kPa [1], and thereby limits appropriate stem cell differentiation in regenerative therapies [2]. While MSC injection can marginally improve overall cardiac function by "poking holes" into the scar and making it more compliant, these cells do not appear to differentiate into cardiomyocytes or stimulate sufficient angiogenesis in the infarct area. In fact, what results from the stem cells are small calcified lesions [3], as they have been directed by the stiff fibrotic scar to differentiate down an unintended pathway toward osteoblast-like cells, as stiff microenvironments have been shown to do [2].

Tissue-specific elasticity arises from developmental changes in the ECM [4] and suggests that traditional cultures on ECM-coated glass, gels with a static set of intrinsic parameters, and disease microenvironments may not be the most appropriate physical environment for highly contractile cardiomyocytes. Rather, such cells should be cultured in the appropriate physical conditions that mimic tissue progression, from soft, pre-cardiac mesoderm at E1 to a mature, less compliant tissue at E17 [4,5]. Limited differentiation or maintenance of a contractile phenotype even on compliant materials hints that such a dynamic property may be an important differentiation regulator [6], and to mimic this temporal ECM change in the myocardium, we have developed a "smart" material that presents temporally-sensitive elasticity to better mimic developmental matrix changes and thus improve embryonic stem cell (ESC) and progenitor cell outcomes.

Methods: Thiol-modified Hyaluronic Acid (HA) was crosslinked with polyethylene diacrylate (PEGDA) to form the gel. Degree of thiol substitution along the HA backbone was determined via NMR spectroscopy. Atomic force microscopy (AFM) was used to measure the dynamic increase in stiffness over time of both the chicken embryonic myocardium and HA hydrogel. The dynamics of HA hydrogels were tuned to closely mimic those of the developing chicken embryonic myocardium. Embryonic chicken cardiomyocytes of various developmental stages were plated onto collagen-functionalized dynamic and static materials (polyacrylamide (PA) hydrogels) and examined at the same end time point by immunofluorescence and quantitative PCR (qPCR).

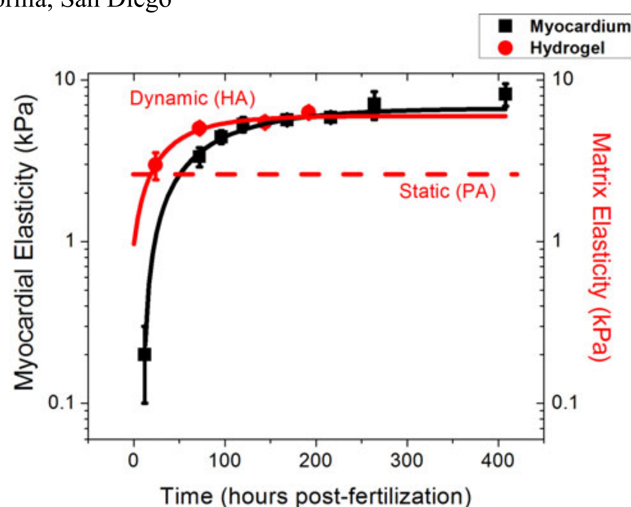

Figure 1. Plot illustrating the change in stiffness of the ventricle wall (black) as a function of time post-fertilization. Our new scaffold closely mimics this change (red, Dynamic (HA)). Static hydrogels remain at a constant stiffness throughout the time course (red, Static (PA)).

Results and Conclusions: Chicken myocardium and HA hydrogels, which both exhibited time-dependent increases in stiffness, displayed a half-max elasticity at 57.5 and 33.7 hours, respectively, while static PA gels remained constant over time (Fig. 1). Immunofluorescent staining of actin and α-actinin shows suboptimal actin cytoskeleton striation and assembly as well as altered cell morphology on cells plated on static cultures as compared with those plated on dynamic HA hydrogels. Ongoing qPCR studies to quantify changes in cardiac marker expression, e.g. troponin T, on both static and dynamic hydrogels should compliment such findings and demonstrate that there is both increased expression and improved assembly of cardiac proteins. Together, this indicates that it is not just the presentation of appropriate mechanical cues that drive differentiation, but it is the correct temporal display that appropriately regulates progenitor cell maturation. The combination of cells and developmentally appropriate matrix cues in regenerative therapies may thus significantly restore tissue function as opposed to current therapies that provide marginal improvements in patient outcome, e.g. cellular cardiomyoplasty.

References: [1] Berry, M.F. *et al.*, *Am J Physiol Heart Circ Physiol*, 2006. **290**(6): p. H2196-203. [2] Engler, A.J. *et al.*, *Cell*, 2006. **126**(4): p.677-89. [3] Breitbach, M. *et al.*, *Blood*, 2007. **110**: p. 1362. [4] E. A. Zamir *et al.*, *Ann Biomed Eng,* 2003. **31**: p. 1327. [5] Hamburger, V. and H.L. Hamilton, *J. Morphology*, 1951. **88**: p. 49-92. [6] Engler, A.J. *et al., J. Cell Sci.*, 2008. **121**(22): p. 3794-802.