Final Report

I am pleased to summarize below the significant progress I have made in the two years of my Regenerative Medicine Minnesota Graduate Education Award. I have focused on the structure, function and maturation of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). iPSC-CMs are derived in vitro from pluripotent stem cells by treating pluripotent cells with small molecule factors that mimic the embryonic environment of the developing heart. Since their discovery, iPSC-CMs have been investigated by researchers as a potential model of heart disease and cardiac development, as well as a potential source of cells for cell therapy, to be implanted into a diseased heart. iPSC-CMs have the benefit of being human cells, and thus expressing human, rather than animal, proteins and genes, and of being grown in vitro, eliminating the need to sacrifice animals for experiments. However, they have an immature phenotype, meaning that they are poorly suited for studying adult cardiac physiology or disease. Thus, over the past two years with funding from Regenerative Medicine Minnesota, my work has focused on two projects. First, I have utilized a novel technique to measure force development in iPSC-CMs, in order to characterize their phenotypic maturity. Second, I have used genome editing to cause increased maturation of the iPSC-CMs by overexpressing genes associated with a more mature phenotype.

In the first year, I developed a novel approach using a micro-contact printing technique to instruct myocyte morphology, and hence sarcomere assembly, as this bears critically on functional output. This approach guided the iPSC-CMs to attain an elongated rectangular shape with a 7:1 aspect ratio, identical to an adult cardiac myocyte. This is critical as it permits a single force vector along the long axis of the cell. Using this assay, I have been able to measure the amount of force produced by an iPSC-CM under controlled variables of substrate stiffness, age of differentiation, extracellular calcium concentration, as well as beta-adrenergic stimulation. My findings show that iPSC-CMs force production increases significantly with increased age of differentiation and that hydrogel stiffness bears significantly on force output. Furthermore, force development positively correlates with cell size, although not with length or width. Finally, that physiologic calcium concentrations have a significant impact on the force production and maturation of iPSC-CMs compared to those grown in low concentrations, which is the standard condition. I presented these findings at the Myofilament Conference in Madison, WI in July 2016, and they are currently written up in a manuscript that has been submitted for publication this year.

In the second year, I used genome editing to insert an inducible gene expression cassette into the AAVS1 safe harbor site in chromosome 19. The cassette contains a gene of interest, driven by a tetracycline-responsive promoter, and the tetracycline-responsive transcription factor that binds to the promoter. Our two genes of interest were SERCA2a, which is critical for calcium transient and calcium signaling in the cardiomyocyte, and cardiac troponin I (cTnI), a thin filament protein in the cardiac sarcomere, necessary for contraction and relaxation. Both genes are expressed at low levels in embryonic cardiomyocytes, but expressed at higher levels in adult myocytes. SERCA2a is notably downregulated in failing adult cardiomyocytes, and cTnI has no expression in immature cardiomyocytes, but is the sole isoform of TnI expressed in adult cardiomyocytes. We expressed exogenous SERCA2a for two weeks in iPSC-CMs and then assayed physiologic function and protein expression. We found that SERCA2a overexpression increased the adrenergic responsivity of calcium transients, specifically by increasing chronotropy and decreasing time to peak in response to isoproterenol. Furthermore, it decreased contractile time to peak at baseline and in response to isoproterenol. Through RNAseq, we found that overexpression of cTnI resulted in upregulation of genes and pathways connected to cardiac development and contractility, and downregulation of genes associated with other cell lineages.