

### **Engineering 3D Vascularized-Cardiac Constructs through Electromechanical Stimuli**

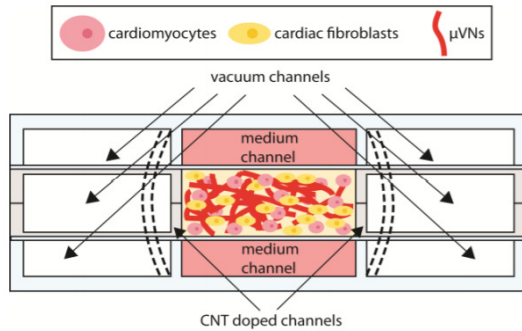
**Background:** Despite significant studies in recent years, progress in the field of engineering functional tissue constructs has been hampered by limitations in traditional culture systems to reconstitute physiological factors in the cellular microenvironment in a spatiotemporal manner. This is particularly relevant for cardiac tissue constructs given the multiple levels of coordinated physical cues (mechanical contractions, electrical activity, hydrostatic pressure) that drive heart function. In fact, numerous studies have validated the underlying role of mechanical stimulus and electrical inputs, in the native cardiac extracellular matrix, to elicit the contractile properties of cardiomyocytes (cardiac muscle cells). [1] Therefore, the coupled application of mechanical and electrical stimuli is imperative to fully reproduce the physiological microenvironment leading to the establishment of cardiac functions in the engineered construct. While the integration of multiple physical cues in the engineered system has become a major obstacle in the field, adequate tissue vascularization remains as the most pervasive challenge to date. [2]

Owing to the high cell population and continuous metabolic activity in the cardiac environment, substantial amounts of oxygen are required by cardiac cells to sustain heart function. Hence, a key objective to engineer fully-functional cardiac tissue is to reconstitute a vascular system within the construct thus providing a continuous pathway for oxygen, nutrient and waste transport. Numerous studies have undertaken this prevailing challenge by implementing microfabrication-casting methods in natural or synthetic matrixes [3] to generate a predefined vascular network lined with endothelial cells. Although this approach provides a high degree of morphological control over the formation of the vascular bed, this method typically generates vascular systems with large-scale geometry when compared to the actual microvascular networks ( $\mu$ VNs) in the native tissue. As an alternative, researchers have adopted co-culture techniques of endothelial cells with fibroblasts or mesenchymal stem cells which allows paracrine signaling between the different cell types to induce the self-organization of endothelial cells into perfusable  $\mu$ VNs (vasculogenesis) thus achieving capillary-sized vessels that better resemble natural vascular patterns. [3]

Ultimately, efforts to address challenges in cardiac tissue engineering have been focused exclusively on either inducing contractile activity or integrating a vascular system in the engineered construct. In addition, most of these studies are carried out in macroscale bioreactors that require large volumes of cell and expensive reagents and have limited imaging capabilities during experimentation. In order to improve our current ability to engineer functional cardiac constructs, I propose a microfluidic co-culture platform that will provide precise spatiotemporal control over electromechanical inputs in the extracellular environment to establish stable contractile function of cardiomyocytes with simultaneous functional  $\mu$ VNs within the construct by inducing vasculogenesis with cardiac fibroblasts and endothelial cells.

**Research Plan:** The device design for this research consists of a multi-channel, layered microfluidic chip (**Fig. 1**) with a 3D central gel region for the injection fibrin gel containing human umbilical vein endothelial cells (HUVEC) co-cultured with primary human cardiac fibroblasts (HCF) and human cardiac myocytes (HCM). The upper and lower medium channels flank the central gel region through a porous membrane thus allowing a continuous exchange of nutrients and waste during cell culturing. To induce uniaxial tensile strain, a series of vacuum channels are implemented at each side of the gel region connected to a computer-controlled vacuum generator to induce mechanical strain under controlled conditions. [4] When a negative pressure is applied to these channels the thin lateral walls in the middle chamber will deflect outwards thus inducing uniaxial strain to the tissue construct. The outer-middle vacuum channels in the device are casted with a pre-polymer mix containing carbon nanotubes (CNT) which provides an electrical path

within the device for electrical stimulus. [5] After 24 hours of initial cell seeding, perfusable  $\mu$ VNs will be generated within the construct due to the paracrine signaling between the HUVEC and HCF. [6] Subsequently, the tissue construct will be stimulated with a simultaneous electrical pulse of 5V at 1 Hz (characteristic of the native myocardium [7]) and cyclic strain with magnitudes of 2.5%, 5% or 10% at 1 HZ.



**Figure 1.** Device Schematic Cross-section

After electromechanical stimulus, the contractile properties will be evaluated by exposing the tissue to only electrical stimulus at different magnitudes and measuring, via high-resolution imaging, the contractile motion of the HCM accordingly to the electrical input. In order to analyze vessel morphology, the construct will be imaged via traditional immunostaining to quantify lumen diameter, branch number and length, and total vascularized area. In addition, perfusability of the networks will be evaluated by establishing a pressure

gradient across the medium channels and perfusing the vessels with fluorescent microbeads. Finally, differences in cardiac-specific markers will be quantified to assess the phenotypic alteration in cardiac cells due to either the magnitude, frequency or coupling of physical stimuli. Comparisons will be drawn between the known physiological characteristics of cardiac tissue in *in vivo* systems and the engineered construct from each experimental condition to optimize the magnitude and frequency of the physical factors and achieve physiologically-functional cardiac constructs. I will perform this research in the [REDACTED] Lab at [REDACTED] under the supervision of Prof. [REDACTED] (letter writer), a leading expert in cellular and tissue biomechanics with extensive published work in vascular formation and mechanics. Thus, I will consolidate the resources and expertise at the lab with my strong research background to exceed the objective of this project.

**Intellectual Merit:** Success in this novel platform will translate to advances in tissue engineering techniques to induce adequate physiological properties of the tissue construct leading to the generation of vascularized, thick tissue sections and ultimately whole organoids. Furthermore, the results of this work will provide fundamental insight about the mechanisms underlying cardiac function and vascular formation under superposition of exogenous electrical and mechanical stimuli in the cellular microenvironment.

**Broader Impacts:** On average, 287,000 Americans die each year from heart failure. The results from this research will directly translate to reduce this frightening statistic by developing a technique for functional cardiac constructs amenable for treatments against cardiac diseases. In addition to the direct scientific implications my proposed study will have, I will also focus my efforts on broader social contributions. I plan to do so by imparting part of my research skills on a one-to-one basis as a graduate student mentor to minority students participating in the [REDACTED] Summer Research Undergraduate Program. I will also facilitate a broader understanding of my research by submitting my findings to the local press such as the [REDACTED] so that the general public can learn how their tax dollars are being invested into science and technology.

**References:** [REDACTED]

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