ENGINEERING CARDIAC TISSUES WITH THREE-DIMENSIONAL BIOPRINTING FOR BIOMEDICAL APPLICATIONS

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Dedication

This one's for me.

-M.A.

ENGINEERING CARDIAC TISSUES WITH THREE-DIMENSIONAL BIOPRINTING FOR BIOMEDICAL APPLICATIONS

by

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DISSERTATION

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Abstract

The role of a biomedical engineer is to solve unmet health-related needs that face society. For the past couple of decades, heart disease (HD) has remained the leading cause of mortality and morbidity worldwide. The disease is characterized by various pathologies that affect the heart. Indeed, malfunction of one of the body's most vital organs is bound to manifest poor health and even death in patients. Despite advances in medicine and technology, heart disease continues to be prevalent and diminishes the quality of life for many around the world. Certainly, there exists a need for developing new tools to better understand and treat HD. Tissue engineering cardiac tissues in-vitro offers an alternative, useful platform to study diseases and navigate towards generating biologically relevant artificial tissues for clinical translation.

Tissue engineering combines biomaterial scaffolds, cells, and construction methods to grow viable tissues in-vitro and in-vivo. Advent of three-dimensional bioprinting has allowed for creation of living tissues that mimic the geometries and physiologies of native tissues. In accordance to the principles of this field, this work aims to exploit the benefits afforded by extrusion-based 3D-bioprinting to engineer viable cardiac tissues in-vitro for biomedical applications. Studies include development of a furfuryl-gelatin-fibrinogen bioink that was crosslinked enzymatically by the conversion of fibrinogen into fibrin and with photo-oxidation with visible light. Cardiomyogenesis was observed in bioprinted constructs, characterized by the coupling of human cardiomyocytes and fibroblasts. Furthermore, cells within bioprinted constructs showed characteristic cardiac biomarkers and preferred cellular organization in the direction of bioprinted filaments. In another investigation, a murine animal model was adopted to develop a method of cryo-inducing myocardial infarction in-vivo. A reproducible zone of necrotic myocardium that resembled many of the histopathological characteristics of the naturally-

occurring MI was produced using a cryo-probe. Developing protocols for generating controlled infarcts on demand provide a beneficial in-vivo model for future implantation of 3D-bioprinted cardiac grafts. Development of 3D-bioprinted constructs was further explored through the development of a scaffold able to culture endothelial cells, a third major cell type found in the heart. The scaffold was tailored to house all three heterogenous cardiac cell types for future study of microgravity-induced cardiac atrophy during spaceflight. Finally, modulation of cardiomyocyte physiology was probed in response to variation in hydrogel mechanical composition. A hydrogel system was adopted to mimic various developmental stages of the heart. Mechanical properties of gels were optimized to mimic stiffness of embryonic, physiologic, and fibrotic cardiac tissue. CMs cultured in softer gels showed enhanced cellular networking and alignment within hydrogel scaffolds as compared to those cultured to stiffer ones. Results from this work give insight into biomaterial selection for three-dimensional culture and their effect on the construction of cardiac tissues in-vitro.

Generating viable cardiac tissues on-demand with three-dimensional bioprinting has the potential to greatly benefit society and provide an extra tool to understand and battle heart disease. Bioprinting creates architecturally similar tissues to those of the native heart, as well as provide physiologically responsive platforms for disease modeling and pharmaceutical testing. They further hold potential to be used for as grafts to replace necrotic or fibrotic myocardium. Work from this dissertation sets a foundation for further development of 3D-bioprinted cardiac tissues in-vitro.

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Chapter 1: Introduction and Declaration of Specific Aims

1.1. Biomedical Engineering: An Interdisciplinary Field

Biomedical engineering is an interdisciplinary field that uses engineering principles to research and develop medical technology to help solve some of the biggest healthcare problems facing society [1]. One of the main goals of a biomedical engineer is to understand, modify, or control biological systems as well as design and manufacture products that can monitor physiological functions and assist in the diagnosis and treatments of patients [1]. The field has progressed from being focused on developing medical instruments in the mid 1900s to a breadth of other activities [1]. A list of some of these activities are laid out in ***Table 1.1*** below with topics explored in this dissertation highlighted in boldface. Besides the technology developed, one of the greater benefits of biomedical engineering is the identification of impediments and unmet needs of our present healthcare system [1]. Thus, biomedical engineers provide the tools and techniques that make our healthcare system more effective, efficient, and improve the quality of life for patients [1]. With the role of a biomedical engineer in mind, this dissertation aims to understand and tackle a huge burden that currently faces today's global society: heart disease.

Table 1.1: Various concentrations of biomedical engineering with some principles used in this dissertation in boldface.

Genres of Biomedical Engineering			
Biomechanics	**Biomaterials**	**Tissue Engineering**	Drug Design and Delivery
Regenerative Medicine and Cell Therapies	**Personalized Medicine**, Genomics, and Proteomics	Prosthetics	Telemedicine
Biosignals and Biosensors	**Physiologic and Disease Modeling**	Medical Robotics	Biomimetics
Clinical Engineering	Medical and Bioinformatics	Biotechnology	Medical Imaging

1.2. Heart Disease: A Global Health Problem

According to the United States' Centers for Disease Control and Prevention [2], and the World Health Organization [3], heart disease has remained the leading cause of death in the global population for the last couple of decades [4, 5]. Cancer and stroke trail behind in second, on the American and international stages, respectively [2, 3]. Heart disease, a type of cardiovascular disease, is the term given to myriad disorders that affect the heart and its blood vessels [5, 6]. Examples of heart disease include coronary artery disease, arrythmias, and malfunctions of the valves, as depicted in ***Illustration 1.1*** [6].

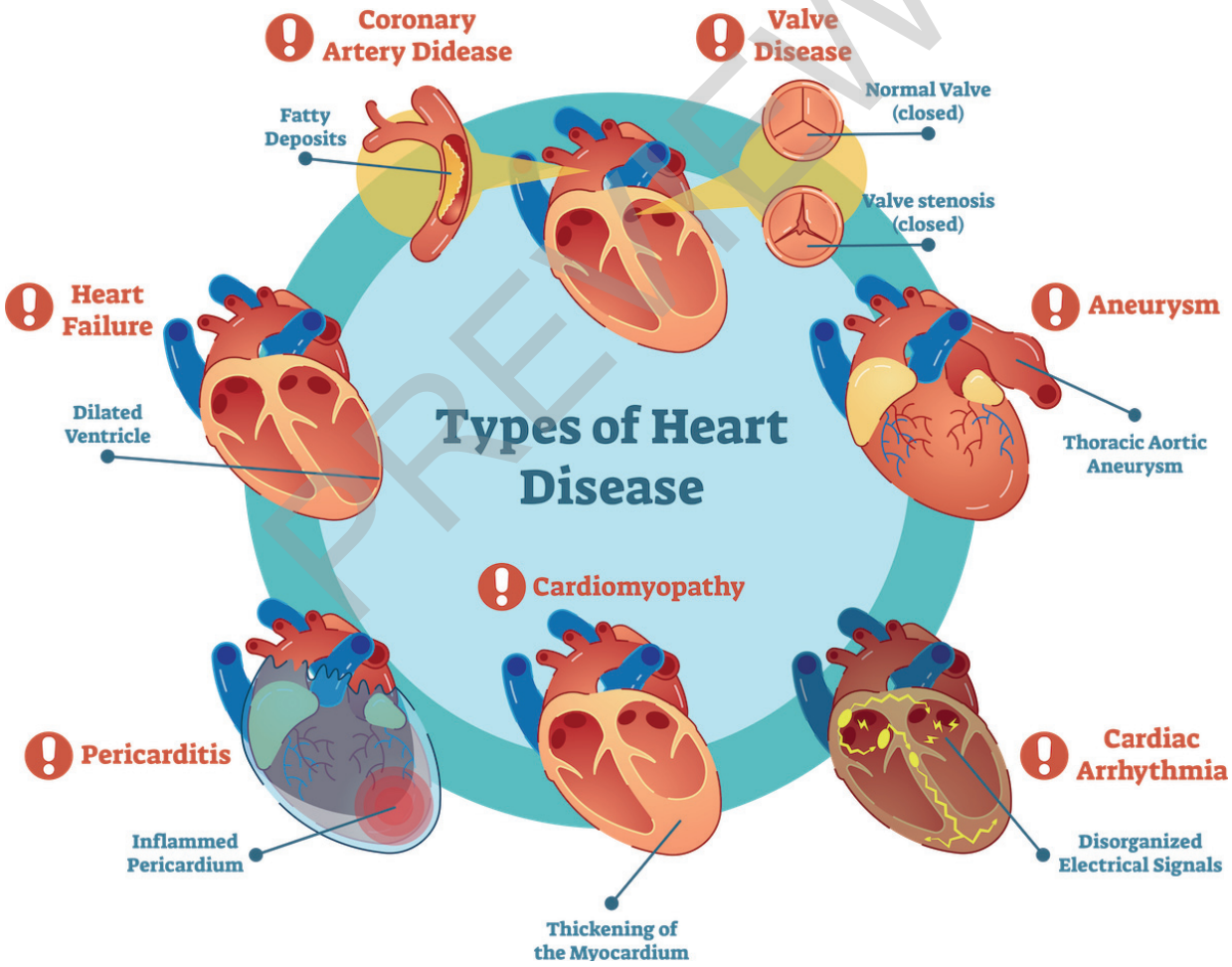

Illustration 1.1: Many pathologies are associated with heart disease, with coronary artery disease being the most prevalent. This illustration was obtained from [7].

Indeed, malfunction to one of the most vital organ systems in our body is bound to manifest poor health, even death, in patients. Ischemic heart disease is the largest contributor to the global death toll from all forms of heart disease [8]. It accounts for about 1.72% of the world population [8]. As its name suggests, ischemic heart disease is characterized by occlusion of the blood vessels that supply the organ with oxygen-rich blood. The occlusion is most commonly caused by the accumulation of fat deposits or a thrombus within an artery, usually one of the coronary arteries of the heart [9]. As circulation ceases, cardiac cells in the thick myocardial wall begin to experience hypoxia and begin to asphyxiate[10]. This event where the muscle of the heart is suffocated and dies due to the blockage of vessels is known as a myocardial infarction (MI), more commonly referred to as a heart attack [10]. This phenomenon is represented in ***Illustration 1.2***. Unfortunately for cardiac myocytes, the main cell-type of the myocardium, they do not possess the capacity to regenerate unlike other tissues in our body [11]. Yet, the heart still has a mechanism in place to try to heal itself and attempt to restore homeostasis.

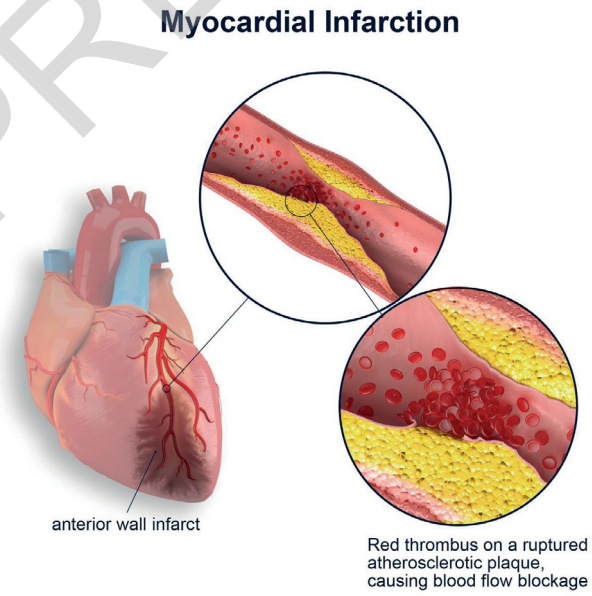

Illustration 1.2: Myocardial infarction is characterized by occlusion of the heart's arteries by plaque or a blood clot that causes death of the myocardium. This illustration was obtained from [12].

Cardiac repair after acute myocardial infarction begins with inflammation to the myocardium [13]. During this phase inflammatory cells are recruited and infiltrate the damaged heart wall [13]. Enzymes, such as matrix metalloproteases, begin digesting the extracellular matrix (ECM) to free the cellular debris caused by hypoxia-induced apoptosis [13]. Macrophages then begin to remove the cellular debris loosened from the digested ECM [13]. This process usually lasts for a couple of days, after which inflammation wanes and neovascularization and the reparative phase begin [13]. During this stage that lasts for about two weeks, new vasculature begins to form, and fibroblasts begin depositing a thick collagenous scar to replace the damaged myocardium [13]. Weeks after acute MI, a complete scar has formed on the myocardial wall [13]. Stages of cardiac repair are summarized inn ***Illustration 1.3***. Due to the poor contractile properties of the scar, the heart's pumping efficiency is impeded as it has to overcompensate to carry the dead weight contributed by the robust collagen deposit [14]. Over time, the vitality of organ dwindles and the organ's ability to function, and leads to heart failure [14]. Heart failure is when the heart cannot pump enough blood to meet the body's demands [14]. Recalling from ***Illustration 1.1***, heart failure is another form of heart disease. Therefore, if the onset of acute myocardial infarction does not kill you, you are still at further risk of developing heart failure and dying from that. Heart disease is consequentially a serious biomedical area of interest where much more can be done to reduce the damage caused by this disease.

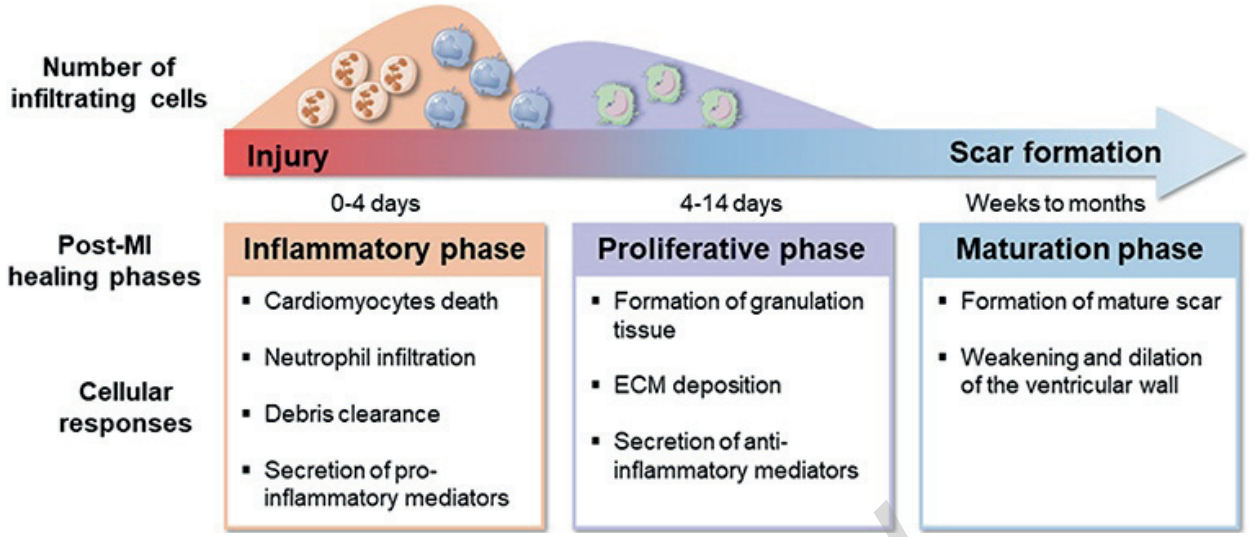

Illustration 1.3: The three stages of myocardial repair after injury. This figure was reproduced with permission from [15].

Current treatments to mitigate the effects of myocardial infarction range from lifestyle changes to invasive surgeries [16]. If the heart attack is mild, patients are advised to exercise once recovered in order to strengthen their heart [16]. Additionally, removing any stress, drugs, alcohol, and a cleaning up one's diet can lower one's risk of inducing an MI [16]. Furthermore, physicians also analyze the cause of the infarction by locating the occluded cardiac blood vessel [16]. If the vessel can be cleared by a catheter, the artery regains perfusion ability [16]. Sometimes, a mesh-like metal stent is inserted to hold the target vasculature dilated for blood flow to occur [16]. If the artery is unable to be cleared for reperfusion, a bypass surgery is performed in which vasculature from another part of the body, usually the great saphenous vein, is harvested and connected in such a way that reroutes blood flow from the site of blockage to the other end of the blocked artery [16]. This, as its name states, bypasses the occlusion to deliver blood flow to myocardial tissue. A visual summary of these treatments can be referenced in ***Illustration 3.2***. While a stent and bypass surgery certainly reperfuse the myocardium, they don't address whatever damage has already been done to the cardiac muscle.

Patients who develop a weakened heart due to the added stress on the heart from the collagen scar (i.e. develop heart failure), will require outside reinforcement in pumping to reduce its workload. For these people, a ventricular assistive device (VAD) is implanted [16]. While VADs unburden the heart from overworking itself, they produce some burdens of their own. They may cause blood clots, require open heart surgery, sometimes have device malfunctions, and may cause heart failure anyway [17]. Thus, patients may be prescribed blood thinning medications which could cause complications if the patient were to bleed [17]. One of the last options for most patients progressing toward heart failure is a heart transplant [16]. There are more than 7,300 people in the United States alone that are currently on a list for a heart transplant, with only 2,000 transplants actually performed per year [18]. Clearly the availability of vital organs cannot keep up with the rate of people who need one [18]. Furthermore, even if patient is lucky enough to obtain a vacant heart, they still run the risk of having their immune system reject the organ [19]. Physicians therefore prescribe immunosuppressive drugs to reduce this possibility; however, there is added risk of the patient succumbing to another illness [19]. These immunosuppressive drugs are prescribed for life, and therefore the immune system would be too weak to fight off any foreign agents into the host's body [19]. These treatments are currently our limit in combating heart disease as it relates to ischemic heart disease and heart failure. Certainly, there is room for improvement in combating the disease responsible for claiming the most human lives. Tissue engineering offers an alternative, potentially better solution.