

ENGINEERING CARDIAC TISSUES WITH THREE-DIMENSIONAL BIOPRINTING FOR
BIOMEDICAL APPLICATIONS

MATTHEW ALONZO

Doctoral Program in Biomedical Engineering

APPROVED:

Binata Joddar, Ph.D., Chair

Felicia S. Manciu, Ph.D.

Munmun Chattopadhyay, Ph.D.

Sylvia L. Natividad-Diaz, Ph.D.

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

Copyright ©

by

Matthew Alonzo

2021

Dedication

This one's for me.

-M.A.

PREVIEW

PREVIEW

ENGINEERING CARDIAC TISSUES WITH THREE-DIMENSIONAL BIOPRINTING FOR
BIOMEDICAL APPLICATIONS

by

MATTHEW ALONZO, M.S.

DISSERTATION

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Biomedical Engineering

THE UNIVERSITY OF TEXAS AT EL PASO

May 2021

Acknowledgements

I would like to acknowledge the mentorship and guidance of my advisor Dr. Binata Joddar, the faculty and staff in the Department of Metallurgical, Materials, and Biomedical Engineering, as well as those at the Border Biomedical Research Center at The University of Texas at El Paso (UTEP). I would further like to acknowledge the help of Dr. Chattopadhyay's lab at Texas Tech University Health Sciences Center El Paso (TTUHSC), Dr. Ito's lab at RIKEN in Japan, Dr. Sugg's lab at The University of Texas at Austin, and Dr. Willerth's lab at University of Victoria (UVIC), for their assistance and expertise. I also thank my peers and lab mates in the Inspired Materials and Stem-Cell-Based Tissue Engineering Laboratory (IMSTEL) at UTEP. Additionally, I appreciate my committee members for their service, mentorship, and input in making me a better scientist/engineer. In terms of academic funding, I would like to acknowledge the Gates Millennium Scholarship Program, National Science Foundation Partnership for Research and Education in Materials, Patrick E. and Eloise B. Fellowship, NSF grants #1828268, # 1927628, #1854008, NIH SC1 grant #1SC1HL154511-01, and NIH 1SC2HL134642-01 for supporting me throughout my doctoral studies. I am humbly grateful for you all. Thank you for molding me into a better researcher and engineer throughout the last couple of years and making this dissertation possible.



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 heterogeneous 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.

Table of Contents

Dedication.....	iii
Acknowledgements	v
Abstract.....	vi
Table of Contents	viii
List of Tables	xiii
List of Figures.....	xiv
List of Illustrations	xvi
Chapter 1: Introduction and Declaration of Specific Aims	1
1.1. Biomedical Engineering: An Interdisciplinary Field	1
1.2. Heart Disease: A Global Health Problem.....	2
1.3. Tissue Engineering: A Potential Solution	7
1.4. Hypotheses and Specific Aims	8
1.4.1. Specific Aims 1A & 1B.....	8
1.4.2. Specific Aim 2	9
1.4.3. Specific Aim 3	9
Chapter 2: Methodology	10
2.1. Three-Dimensional Bioprinting.....	10
2.1.1. Types of Bioprinting.....	10
2.1.2. Hydrogels/Bioinks	12
2.1.2.1. Fibrin	13
2.1.2.2. Gelatin	14
2.1.2.3. Alginate	15
2.1.3.4. Matrigel	16
2.2. Mammalian Cell Culture	16
2.2.1. Two- and Three-Dimensional Cell Culture	17
2.2.2. AC16 Human Cardiomyocyte Cell Line	18
2.2.3. Human Fibroblasts.....	19
2.2.4. Human Dermal Microvascular Endothelial Cells.....	19

2.3. Material Characterization of Hydrogels	19
2.3.1. Scanning Electron Microscopy	19
2.3.2. Fourier Transform Infrared Spectroscopy	21
2.3.3. Rheology	21
2.4. Probing Cultured Cells and Tissues	23
2.4.1 Cell Viability (Live/Dead) Assay	23
2.4.1 PKH26, PKH67, and CellTrace Violet.....	23
2.4.2 Tissue Staining & Immunohistochemistry	24
2.5. Microscopy	24
3.5.1 Brightfield Microscopy	24
3.5.2 Confocal Microscopy	25
3.5.3 Fluorescent Microscopy	25
Chapter 3: The 3D Bioprinted Cardiac Patch	27
3.1. Abstract.....	27
3.2. Introduction	28
3.3. Materials and Methods	34
3.3.1. Bioink Composition.....	34
3.3.2. Biofabrication	36
3.3.3. Biomaterial Characterization.....	39
3.3.4. Cellular Studies	39
3.3.4.1. iPSC & AC16 Cell Characterization, Cellular Viability	39
3.3.4.2. Cardiomyocyte Morphology Orientation, and Specificity	40
3.3.4.3. Coupling of CM with FB.....	40
3.3.5. Statistical Analysis	42
3.4. Results and Discussion	42
3.4.1. Design and Structure	42
3.4.2. Material Characterization of Bioprinted Scaffold, Cell Viability	43
3.4.3. AC16 CM Morphology, Specificity, and Orientation within Bioink	43
3.4.4. CM-CF Orientation and Coupling	45
3.5. Conclusion	48
Chapter 4: The Cryo-Induced Myocardial Infarct Rat Model	50
4.1. Abstract.....	50

4.2. Introduction	51
4.3. Materials and Methods	54
4.3.1. Rat Model	54
4.3.2. Chemicals	55
4.3.3. Preoperative Procedures	56
4.3.4. Cryo-Induced Myocardial Injury	57
4.3.5. Tissue Processing	58
4.3.6. Quantification of Muscle Density	59
4.3.7. Quantification of Fiber Length	60
4.3.8. Quantification of Fiber Curvature	60
4.4. Results and Discussion	60
4.4.1. Macroscopic Characteristics of Cryo-MI	60
4.4.2. Muscle Density	62
4.4.3. Fiber Length	64
4.4.4. Fiber Curvature	65
4.4.5. Contraction Band Necrosis	66
4.5. Conclusion	67
Chapter 5: Engineered Alginate-Gelatin Bioprinted Scaffold with Three Heterogenous Cardiac Cell Types	69
5.1. Abstract	69
5.2. Introduction	69
5.3. Materials & Methods	71
5.3.1. Biofabrication	71
5.3.1.1. Preparation of Bioink	71
5.3.1.2. Three-Dimensional Bioprinting	72
5.3.2. Material Characterization of Alginate-Gelatin Hydrogel Scaffold	75
5.3.2.1. Stability of Structures	75
5.3.2.2. Swelling Analysis	76
5.3.2.3. Scanning Electron Microscopy	76
5.3.2.4. Rheology	77
5.3.2.5. Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance	78
5.3.3. Cellular Studies	78

5.3.4. Statistical Analysis	79
5.4. Results and Discussion	79
5.4.1. Biofabrication	79
5.4.2. Structure Stability, Transparency, and Average Pore Size	81
5.4.3. Swelling of Three-Dimensional Construct	83
5.4.4. Rheological Assessment	85
5.4.4.1. Bioink Characteristics	85
5.4.4.2. Temporal Mechanical Properties of 3D-printed Scaffold	87
5.4.5. Chemical Composition	89
5.4.6. Cellular Studies	91
Chapter 6: Effect of Varying Scaffold Mechanical Properties on Cardiomyocytes: A Cardiac Fibrosis Model and Guide for Bioink Selection	97
6.1. Abstract	97
6.2. Introduction	98
6.3. Materials and Methods	100
6.3.1. Materials	100
6.3.2. Optimization of Hydrogels	101
6.3.3. Gel Formulation	103
6.3.4. Rheology	103
6.3.5. Scanning Electron Microscopy	104
6.3.6. Swelling Analysis	104
6.3.7. Three-Dimensional Cell Culture in Bulk Hydrogels	105
6.3.8. Cell Viability	106
6.3.9. Statistical Analysis	106
6.4. Results and Discussion	106
6.4.1. Embryonic, Physiologic, and Fibrotic Alginate Gels	106
6.4.2. Average Pore Size and Hydration Behavior of Three Gels	109
6.4.3. Cellular Reaction to Mechanical Environment	110
6.4.4. Confirmation of Network Formation	112
6.4.5. Cell Viability	115
6.5. Conclusion	116

Chapter 7: Conclusion and Closing Remarks	118
References	120
Appendix A: Figure Permissions.....	136
Chapter 1	136
Chapter 2	137
Chapter 3	139
Chapter 4	148
Chapter 5	149
Chapter 6	150
Appendix B: IRB Forms for Animal Protocol	152
Vita	171

List of Tables

Table 1.1: Various concentrations of biomedical engineering with some principles used in this dissertation in boldface.	1
Table 2.1: Comparison between inkjet-based, extrusion-based and laser-assisted printing techniques. This table was obtained from [34].	11
Table 3.1. Components and their respective concentrations in 1 mL of bioink are listed.	35
Table 3.2. Bioprinting parameters for the construction of the cardiac patch are listed.	37
Table 3.3. Crosslinking components and their respective durations are listed.	39
Table 5.1: Printing parameters for the scaffold developed in this study.	73
Table 6.1: Concentrations of alginate, calcium carbonate, and GDL are shown for embryonic, physiologic, and fibrotic gels	103

PREVIEW

List of Figures

Figure 3.1: The design of the engineered cardiac patch for this study was inspired by the geometry of native human cardiac tissue (A) and was constructed using CAD modeling (B) before being 3D bioprinted (C). This figure was adopted with permissions from [47, 64].	43
Figure 3.2: Immunoassayed AC16 CM against Myocardin, cardiac Troponin-T, and DAPI markers reveal elongated morphology characteristic of cardiomyocytes. This figure was taken with permission from [47].	44
Figure 3.3: A comparison between cardiomyocytes in the developed bioink that are 3D printed (A) versus manually cast in bulk hydrogel (B) reveal a sense of preferred organization in bioprinted constructs after 24 hours of culture. This figure was adopted with permission from [47].	45
Figure 3.4: Coupling of cardiomyocytes and fibroblasts are confirmed within fibrin-gel-fu scaffolds after 24 h culture in two-dimensional wells (A), and in 3D-bioprinted constructs (B, D). Heterogenous cells align themselves to a higher degree in bioprinted samples (C). This figure was reproduced with permission from [47].	47
Figure 3.5: Connexin-43 expression is seen to a higher degree in bioprinted constructs as compared to controls of cells mixed in bulk. This figure was reproduced with permission from [47]	48
Figure 4.1: Inspection after cryo-infarction reveals a zone of reddish-brown tissue (A) which is roughly the same dimensions as the cross-section of the 3 mm diameter stainless steel probe (B). This figure was reproduced with permission from [66].	61
Figure 4.2 Representative H&E sectioned tissues for sham (A) and cryo-infarcted (B) samples. Reduced muscle density (C), fiber length (D), and nonlinear fibers (E) are seen in cryo-injured specimens as compared to controls. This figure was reproduced with permission from [66].	63
Figure 4.3: Microscopic images (40x) over a larger area of sham (A) and cryo-infarcted (B) tissue shows decreased muscle density on lesioned rats. This figure was reproduced with permission from [66]	64
Figure 4.4: Contraction band necrosis is observed in tissue sections of cryo-infarcted rats, but not in control samples. Figures were taken from [66, 94]	67
Figure 5.1: Bioprinted ring structures directly after the crosslinking procedure is shown to be transparent and rigid enough to be picked up with forceps. Structures are to fit in 24-well plate inserts.	80
Figure 5.2: Integrity of the scaffold over 3 weeks of culture show minimal signs of degradation. Furthermore, the scaffold remains transparent throughout the 21-day period, making it accessible to imaging. The average pore size within the scaffold increases as the structure swells.	82
Figure 5.3: Alginate-gelatin scaffolds were bioprinted and subjected to swelling for 21 days. (A) shows the scaffold with no swelling on the left column and at equilibrium swelling on the right. The degree of hydration for the scaffolds was monitored for 21 days (B).	84
Figure 5.4: Shear-thinning properties of the composite bioink are graphically represented. Viscosity of the hydrogel is seen to decrease with increasing shear rate, thus making the material composition a good bioink candidate for extrusion bioprinting. Axes on the graph are in logarithmic scale.	87
Figure 5.5: Rheological characterization of alginate-gelatin bioink and scaffold shows an unstable bioink composition (left) that gets stabilized into a rigid structure when crosslinked	

(right). The solid-behavior of these bioprinted scaffolds decreases as the scaffolds swell with media.	89
Figure 5.6: FTIR spectra of crosslinked alginate-gelatin spectra after 3 and 21 days of swelling. A difference between the two spectra reveals minor degradation of the alginate crosslinks and dissolving of the gelatin from the structure.	91
Figure 5.7. Confocal fluorescent microscopic images of the three heterocellular cell types on the 3 rd day of culture. Cells, specifically CM and FB begin coupling.....	93
Figure 5.8. Confocal fluorescent microscopic images of the three heterocellular cell types on the 10 th day of culture First signs of all three cell types coupling occurs at this timepoint.....	94
Figure 5.9. Confocal fluorescent microscopic images of the three heterocellular cell types on the 21 st day of culture The total number of couplings where all three cell-types are involved is significantly higher at this time.	95
Figure 5.10: Number of couplings per unit area of the three cell types are seen to increase over time with a significant difference at day 3 timepoint (blue). Coupling of all three cell types together is also seen to increase over time with a significant difference at day 21 (maroon).....	96
Figure 6.1: Three concentrations of MVG alginate (4, 7, 10 % w/v) were each cross-linked with three different concentrations of calcium carbonate (10, 30, 50 mg/mL) and fitted with a quadratic equation in order to find optimum polymer and crosslinking concentrations that produce hydrogels with varying degrees of stiffness. This figure was taken with permission from [37]	102
Figure 6.2: The average storage, loss, and elastic moduli of three distinct bulk alginate gels are graphically depicted (A). Crosslinking density of gels increases by increasing either monomer or crosslinking agent concentration (B). This figure was taken with permission from [117].	108
Figure 6.3: Material characterization of three alginate gels shows an interconnected, porous structure (A-C). Average pore size decreases with increasing stiffness (D). Swelling decreases as stiffness increases (E), This figure was taken with permission from [37].	110
Figure 6.4: PKH26-stained cardiomyocytes in all three cell types showed signs of proliferation from 4 days to 7 days in culture. Cells in softer 3D bulk gels showed a longer morphology and a higher degree of networking as compared to the other progressively stiffer 3D bulk gels. This figure was taken with permission from [37].....	111
Figure 6.5: Brightfield microscopic images at 7 days of culture show CM cellular networking in embryonic, physiologic, and fibrotic 3D bulk gel types. Networking decreases with increasing stiffness. This figure was taken with permission from [37]	113
Figure 6.6: Florescent microscopic image of PKH26-stained AC16 CM show clustered, rounded cells in alginate gels without the inclusion of Matrigel. Without RGD adhesion moieties, these cells cannot elongate. This figure was reproduced with permission from [37].....	114
Figure 6.7: Live/Dead assay after 5 days of culture reveals a decrease in cardiomyocyte viability as the stiff-like character of the gel increased. Live cells are stained green by calcein AM, while dead cells are stained with ethidium homodimer-1 which fluoresces red. This figure was taken with permission from [37]	116

List of Illustrations

Illustration 1.1: Many pathologies are associated with heart disease, with coronary artery disease being the most prevalent. This illustration was obtained from [7].	2
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].	3
Illustration 1.3: The three stages of myocardial repair after injury. This figure was reproduced with permission from [15].	5
Illustration 2.1: Various methods of bioprinting and their method of function. Extrusion bioprinting will be used This figure was taken with permission from [33].	12
Illustration 2.2: The chemical composition of gelatin is a linear backbone of amino acids. The arginine-glycine-aspartic acid sequence in gelatin allows for cell adhesion.	14
Illustration 2.3: When alginate becomes crosslinked with divalent cations, such as Ca ²⁺ , two guluronic acid moieties create an "egg-box" shaped link. This figure was taken with permission from [37].	15
Illustration 2.4: Commonly used equipment for cell culture experiments included use of a biosafety cabinet, centrifuge, incubator, inverted microscope, and water bath.	17
Illustration 2.5: A Hitachi	21
Illustration 2.6: Anton Paar MCR 92 rheometer with a 25 mm parallel plate geometry was used for rheometric analysis of hydrogel samples in this work.	22
Illustration 3.1: Graphical abstract of a 3D printed cardiac patch presented in <i>Chapter 3</i> of this dissertation. This figure was reproduced with permission from [47].	28
Illustration 3.2: Current treatments for treating ischemic heart disease by reperfusing the myocardium include an angioplasty procedure (A), a stent implant in the arterial wall (B), and a CABG surgery (C). This figure was made using figures from [53-55].	30
Illustration 3.3: The presence of a healed fibrotic scar in the thick myocardium after an MI is highlighted by the arrow in a transverse human heart section. This figure was obtained from [56].	31
Illustration 3.4: Cardiac tissue in this study was printed with the Biobot 1/Allevi 2 bioprinter.	36
Illustration 3.5: Visible light generated with an Intelli-Ray 600 was used in the first crosslinking step of the bioprinted scaffold.	38
Illustration 3.6: A schematic of the chemical crosslinking mechanism of gel-fu with visible-light. This illustration was taken with permission from [62].	39
Illustration 4.1: Graphical abstract of the cryo-induced myocardial infarction murine model developed in <i>Chapter 4</i> of this dissertation. This figure was reproduced with permission from [66].	51
Illustration 4.2: LAD ligation of a mouse heart using a suture. This image was taken from [76].	53
Illustration 4.3: Surgical set-up to induce myocardial infarction in a rat model using a cryoprobe. This figure was reproduced with permission from [66].	57
Illustration 4.4: A fresh, naturally-occurring myocardial infarct from a human is shown. Discoloration highlighted by the arrows show necrotic tissue. This picture was taken from [88].	62
Illustration 4.5: Wavy fibers at the sight of an infarct. This illustration was taken from [92]	66

Illustration 5.1: Cellink’s Bio X extrusion-based bioprinter was used for biofabrication of circular constructs in this study.	74
Illustration 5.2: Leica microscope was used for low magnification analysis of scaffold degradation in this study.....	75

PREVIEW

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, arrhythmias, and malfunctions of the valves, as depicted in *Illustration 1.1* [6].

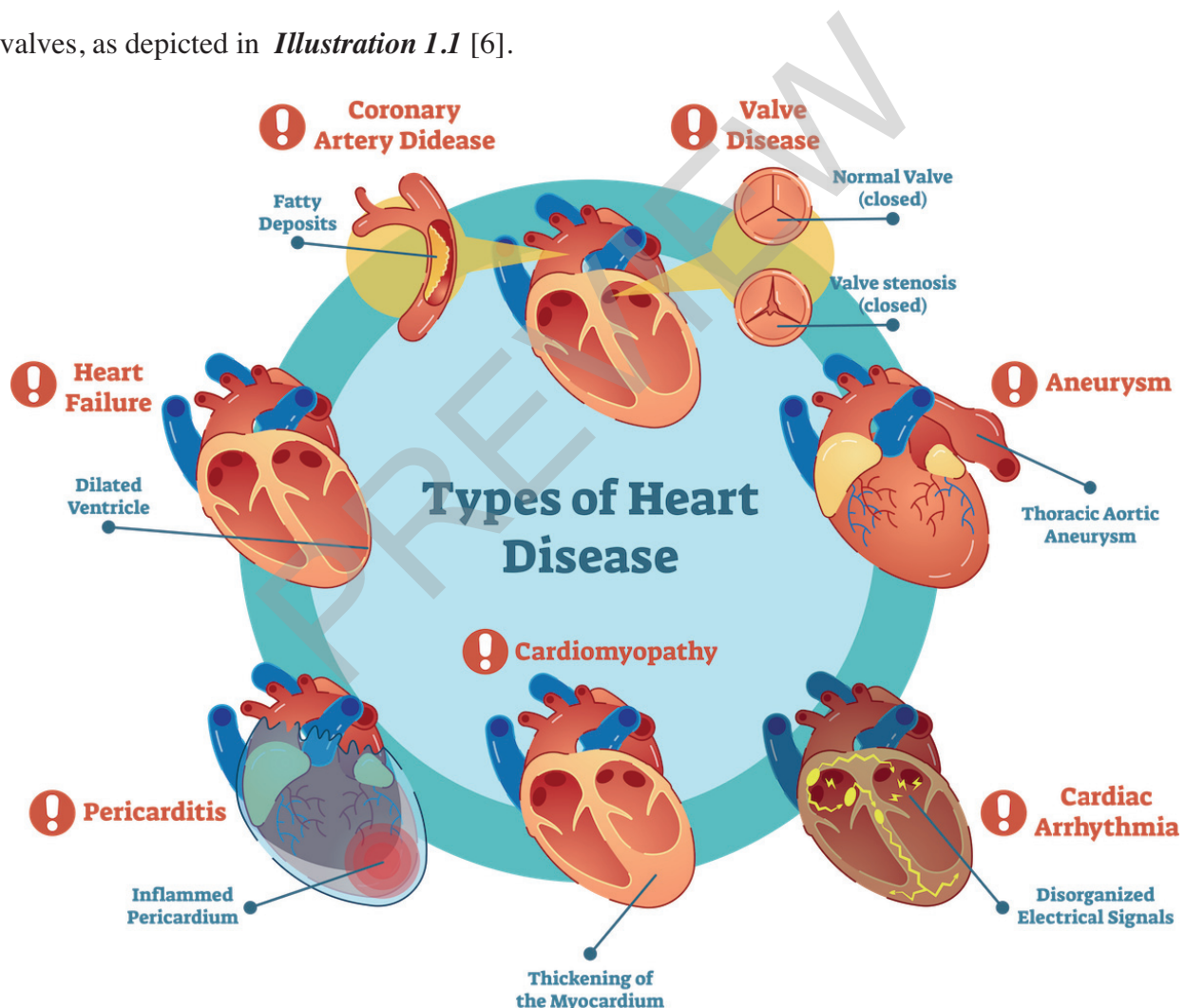


Illustration 1.1: Many pathologies are associated with heart disease, with coronary artery disease being the most prevalent. This illustration was obtained from [7].

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.

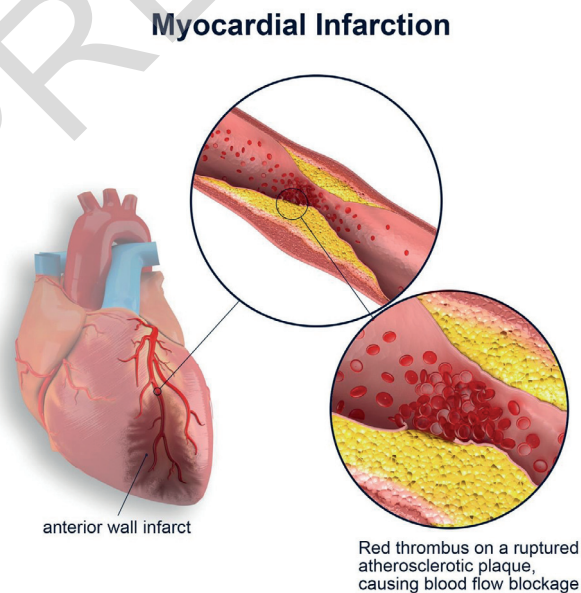


Illustration 1.2: Myocardial infarction is characterized by occlusion of the heart's arteries by plaque or a blood clot that causes death of the myocardium. This illustration was obtained from [12].

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 in *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.

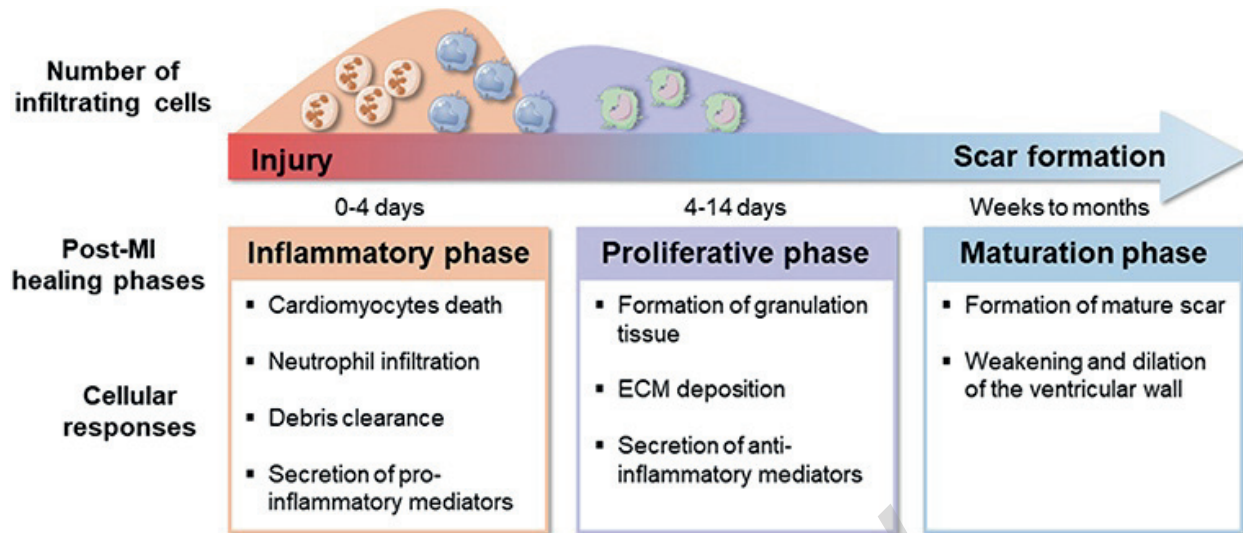


Illustration 1.3: The three stages of myocardial repair after injury. This figure was reproduced with permission from [15].

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.