UNIVERSITY OF CALIFORNIA, SAN DIEGO

Effects of Materials on Electrophysiology when Interfaced with Whole Heart and Cardiomyocytes

A Thesis submitted in partial satisfaction of the requirements for

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in

Bioengineering

by

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Signature Page

The Thesis of Priya Sundaramurthy is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2010

DEDICATION

I would like to dedicate my thesis to my mom, dad, and Keerthi for their unconditional love and unfaltering support. - Priya

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ABSTRACT OF THE THESIS

Effects of Biomaterials on Electrophysiology when Interfaced with Whole Heart and Cardiomyocytes

by

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Master of Science in Bioengineering
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Myocardial infarction (MI) is occlusion of the heart coronary artery. The resulting breakdown of extracellular matrix and adverse remodeling of the myocardium lead to heart failure. Cell and material transplantation therapies have been investigated to help in regeneration and improve heart function. Some cell types like skeletal myoblasts show increased risk for arrhythmias compared to saline injections, but arrhythmia inducibility of materials has not been studied. To assess the safety of injectable materials, programmed electrical stimulation (PES) was used with a rat MI model. This involves burst and extra-stimulation of the heart with rapid pulses alone or preceded by pulses of lower frequency. We found similar arrhythmia inducibility in myocardial matrix, Polyethylene Glycol, and PBS injections. Arrhythmogenicity of materials being similar to that of PBS indicates that use of materials is safe. The

materials were injected largely into the infarct region (already a conduction block), which may be a reason for the low occurrence of arrhythmias. Since materials can be injected with cells, a study of electrophysiological effects of the biomaterial on cardiomyocytes (CM) is necessary, and was done with optical mapping (OM) of monolayers. OM uses a voltage sensitive dye that, when the monolayer is stimulated, fluoresces and is recorded to measure action potential duration (APD) and conduction velocity (CV). This showed significantly lower CV and APD at 20% repolarization in cells cultured on myocardial matrix versus collagen. Reduced APD indicates increased maturity of the CM, while lower CV suggests reduced connexin gap junctions and current channels.

1. INTRODUCTION

1. INTRODUCTION

1.1 Myocardial Infarction and Heart Failure

Myocardial infarction (MI) is the occlusion of the coronary artery in the heart. Once the heart has been injured by the lack of blood flow, heart failure (HF) ensues. HF is the leading cause of death in the United States and the western world [1], and occurs when the heart is unable to pump enough blood to meet the oxygen demands of the myocardium and other organ tissues. Nearly 5.8 million people in the US have HF, and about 670,000 new cases of heart failure are reported each year in the US. Furthermore, two-thirds of those who suffered from heart attack will develop HF and one fifth of those with HF will die within one year of diagnosis [2].

Unlike a few species in the world, the human heart lacks the ability to regenerate its injured tissue. Inflammatory response occurs, only leading to more damage in the extracellular matrix due to the fact that substances secreted by immune cells such as macrophages, monocytes, and neutrophils break down the extracellular matrix. This damage in turns leads to myocytes being unable to interact closely with the surrounding extracellular matrix, which causes them to slip away and widen the infarct region. Initially, after the pump capacity decreases, the heart tries to compensate via the activated adrenergic nervous system, cytokine system, and so on. However, though the compensatory mechanisms seem to be able to restore normal heart function for a few

weeks, they lead to increased tissue damage and the heart is not able to keep up its efforts in compensation [3].

As with any body response to injury, collagen deposition occurs following the inflammatory phase. Because 75% of the healthy myocardium is made of non-myocytes and 95% of non-myocytes are collagen-producing fibroblasts, different varieties of collagen are deposited in the infarct region [4], [5], [6], [7]. Most of this collagen is not resilient and confer resistance to stretch and deformation in the heart wall [8]. This forms the scar tissue, which has different mechanical properties from that of the healthy myocardium, creating adverse stress-strain relationships in the injured wall [9], [10], [11], [12], [13]. Eventually, due to negative remodeling in the heart wall, thinning of the infarct zone and significant decrease in heart function occurs (with ejection fraction reduced to less than 35% of normal) [14], [15], [16], [17].

The first few weeks after MI are critical as the heart undergoes a variety of responses from different aspects of the normal healing process. Within the first 24 hours, edema occurs, followed by necrosis and apoptosis, acute inflammation, and extracellular matrix degradation. By two weeks, healing of the infarct zone is underway and collagen deposition has begun though it is still at a minimal amount. This time period is therefore critical to carry forth any therapy to slow heart failure. In the late stage (up to 6 weeks post-MI), significant increase in scar formation is observed and migration of cells into the infarct zone is limited. Past this stage (in the order of months to years), scarring of the infarct zone is very significant and fibrosis spreads into viable

regions of heart. Negative remodeling in the affected ventricle continues and worsens with time until ultimate death [18].

1.2 Current Therapies

Heart transplantation is the only successful treatment to MI and HF available currently. It is, however, limited by the availability of donor hearts. Few other therapies are accepted as treatment for myocardial infarction (before the onset of heart failure) and are utilized based on the severity and elapsed time since the attack. Generally, if the patient is brought to medical attention within a few hours of the onset of the symptoms or if there is a wait for surgical intervention, they are given the fibrinolytic therapy. Here, the patient is injected with substances that dissolve blood clots or relax the muscle cells of the arteries. This method is not used in patients with increased risk of bleeding [19]. For surgeries, revascularization of the blocked coronary artery through percutaneous intervention (such as coronary angioplasty and stenting) is the most commonly practiced therapy today [19]. Stenting, however, can result in restenosis and introduce other risks. Timing is also critical as the mortality level increases by the minute from the time of the attack. Alternatively, bypass grafting with a vein is used as a last resort in patients where percutaneous intervention failed [19]. These therapies do not target treating the necrotic region of the heart. Instead, it focuses on preventing further injury to the remaining viable myocardium.

In the case of treating heart failure, several pharmacological agents are used to attenuate adverse stresses during remodeling [20], [21]. Other therapeutics are being

developed to target angiogenesis, limit non-myocyte proliferation, curb extracellular matrix degradation, and so forth [22]. Medical devices such as restraint devices (meshes) were also designed to physically limit dilation of the ventricle. Pacemakers and ventricular assist devices (VAD) are also used to improve contraction and assist the pumping of blood respectively [23].

1.3 Cell and Material Transplantations

For several years now, cell transplantation has been explored as a potential treatment for heart failure. Skeletal myoblasts are used for their myocyte phenotype and their ability to tolerate ischemia. These cells are also able to regenerate and proliferate following an injury and thus were deemed to be a strong path to pursue for cardiac regeneration. Moreover, after cell transplantation, they are able to be patterned along the lines of cardiac stresses [24], [25], [26]. However, they cannot be differentiated to have a cardiac phenotype [27], [28]. Also, their ability to electrically couple with the host cardiomyocytes is unclear and may depend on time [29]. Recent studies have shown their tendency to provoke arrhythmias in transplanted animals and patients with Holter monitoring [30], and in animals with programmed electrical stimulation. Though spontaneous occurrence is few, induced sustained arrhythmias is significantly more than that in sham injected hearts. The mechanism for arrhythmia induction possibly lies in the cell's ability to form electrical interfaces. As a result it was recommended to be used for transplantation only in patients implanted with a cardiac defibrillator.

Mesenchymal stem cells are able to functionally couple with cardiomyocytes and show fewer tendencies for arrhythmias. However, they are not able to differentiate into CMs [31]. Autologous adult stem cells from bone marrow are more promising in terms of arrhythmogenicity. Other cells types (hematopoietic stem cells) are known to promote angiogenesis. Patients treated with cell transplantation so far are those enrolled in clinical trials. Some cell types injected in the heart have shown to slightly improve heart function. However, retention and survival of injected cells is still low.

Biomaterials have been explored more recently, injected into the heart wall with and without cells. The ability to tailor the mechanical strength and properties like viscosity is a great advantage with materials, as the mechanical support provided to the heart is critical in determining the progression of negative remodeling. To date, several injectable materials have been used to study their effects on LV remodeling and heart function post-MI. Injectable biomaterials for myocardial tissue engineering, including fibrin [32], [33], collagen [34], [35], matrigel [36], [37], myocardial matrix [1], and many others, have thus been investigated as potential treatments for MI. Material transplantation, or *in situ* tissue engineering, has shown improvements in LV function and cell retention when cells were injected using the material as a scaffold. Injectable materials like the myocardial matrix have also shown improvement towards neovascularization and improvement in the ability of cells to differentiate (refer to section 1.4 on myocardial matrix) [1], [38]. Few materials have gone into clinical trials for injection into patients. Though functional studies have been done with injected

biomaterials with and without cells, the arrhythmia inducibility of either has not been tested so far.

The advantage of using injectable acellular scaffolds versus materials delivered with cells is that cell-based therapies can cause complications such as immunogenicity [39] that may be avoided. It is also difficult to find a reliable cell source. Also, there may be problematic differences in the interaction between the native and injected cell types. This includes intrinsic differences in automaticity (where the cells act like independent pacemakers) [40], [41], alterations in action potential durations [42], and lack of proper electrical coupling between the cardiomyocytes and injected cell type [41], [43], [44]. Other issues eliminated by using acellular scaffolds include cell culture time and cell source [39]. Overall, injected biomaterials have the potential to attenuate the effects of HF by thickening the LV wall, reducing wall stress, and thus mitigating the negative LV remodeling process.

Skeletal myoblast transplantations have shown arrhythmia inducibility in animals and patients. To test such adversities with materials, this study uses injected materials without cells. The purpose of this research is to study the effects of materials on the electrical conduction of the heart when injected into the infarct zone of the left ventricle. Programmed electrical stimulation allows us to study the effects of injected materials on electrical propagation in heart wall through the inducibility of arrhythmias.

For the case where materials are injected along with cells, it is critical to learn about how the electrical properties of the injected cells are affected when they interact

with the material surrounding. Thus, electrical properties, such as conduction velocity and AP duration, of cardiomyocytes cultured on plates coated with different materials are studied. Moreover, this study will provide insights on the electrical maturation of the cells when they interact with the different environmental compositions. This aspect is studied by optically mapping a monolayer of cardiomyocytes grown on different extracellular components.

1.4 Myocardial Matrix

The myocardial matrix is a solubilized form of a decellularized myocardium, and is thus a complex composition of the different components of the extracellular matrix. Developed in the Christman lab, this material is showing a lot of promise *in vitro* and *in vivo*, shows preservation of cardiac function, and is able to be delivered to the myocardium via catheter. It is therefore the material of interest in this study. Singelyn et al. [1] showed through an *in vitro* study that the myocardial matrix significantly increases vascular cell migration into the material. This indicates that when injected into the myocardium, the myocardial matrix has the potential to allow for vascular formation. Furthermore, in an *in vivo* study, Singelyn et al. showed a significant increase in arteriole density after injection of myocardial matrix into a healthy rat heart.

DeQuach et al. showed the effects on maturation of cells when cultured on myocardial matrix, compared to those cultured on gelatin [38]. The maturation of cells is much improved when cultured on the myocardial matrix. This and the results from

Singelyn et al. strongly indicate that the material has promise for clinical translation as an acellular and cellular therapy.

More recently, Singelyn et al. [45] showed with Magnetic Resonance Imaging that cardiac function is preserved at 6 weeks post-MI (4 weeks after injection with the myocardial matrix) in a rat ischemia-reperfusion model. Many other materials have also shown preserved heart function in small animal models through direct epicardial injection, but the myocardial matrix is able to be delivered through percutaneous transendocardial injection using a catheter in large animal models, showing its potential to be translated to the clinic for a minimally invasive procedure. While some other materials have also shown catheter deliverability in large animal models, the myocardial matrix is the only one that mimics the chemical and structural characteristics of native extracellular matrix in the myocardium.

1.5 Arrhythmia Causes and Pathophysiology

There are several causes and types of arrhythmias. These abnormal heart rhythms can be a symptom of heart disease, or even stress, alcohol, and medicines. Arrhythmias are created when the pacemaker of the heart develops an abnormal rhythm or if an ectopic pacemaker (pacing from abnormal locations) starts sending pulses along with the normal pacemaker. Arrhythmias can be distinguished into different types by the location of their initiation. Atrial arrhythmias originate from or around the sinus node. The sinus node could fire signals at a very rapid pace, causing the heart to beat really fast as in a sinus tachycardia. An atrial flutter causes the atria to contract rapidly,

causing the heart to beat unusually fast, whereas a fibrillation occurs when the atria is made to contract in a very uncontrolled way. Such a scenario leads to not enough blood filling in the atria and therefore not enough blood pumped by the ventricles, which receives signals in an irregular way. Ventricular arrhythmias originate in the ventricles, and are caused when the ventricle produces electrical signals of its own, sometimes overriding those sent by the sinus node. This again causes the heart to beat very fast as in ventricular tachycardia (VT) [46]. The mechanism by which arrhythmias are induced during PES is by trying to create VT by pacing rapidly in the ventricles. The pacing can cause ventricular reentry, which is facilitated by the use of extra-stimuli or burst pacing.

Reentrant arrhythmias are caused if the stimuli from the pacemaker find lower-resistance electrical conduction pathways or surpass normal pathways due to an abnormally high resistance in a blocked region. In such a case the action potential duration could differ between the fast and slow pathways, inducing reentry. After a myocardial infarction, the injured tissue starts to lose cardiomyocytes, leading to breaks in the electrical conduction pathway. The signal is only able to travel around this region in order to get past it (or in a unidirectional block, the signal is able to travel in one direction but is blocked in the opposite direction) [47]. This slowed conduction can be dangerous when the signal arrives at a spot after a signal that took a faster path had already depolarized the cells in that area. If the slower signal reaches this spot at a time where the depolarized signal starts to repolarize and is in a relative refractory period, the spot of concern can be depolarized again before it is able to complete its natural

refractory period. This creates an irregular beat. In severe cases this reentrant arrhythmia cycles in a very irregular manner and can cause the heart to not contract properly. In the case of injected materials, reentrant arrhythmias may occur due to unidirectional blockage in conduction where the material is injected, forcing the conduction pathway to slow down around the injected region (Figure 1). Thus, there is relatively faster conduction through the pathway not interrupted by the blockage, leading to a difference in tissue excitability. The same mechanism of arrhythmogenesis can be used for injections of cells for HF treatment. There are additional causes of arrhythmias in this case, however, including the formation of new electrical coupled junctions between native cardiomyocytes and transplanted cells. These junctions do not behave the same way compared to those between two native cardiomyocytes. This, in its unique way, can lead to different conduction pathways, ultimately resulting in reentrant arrhythmias again.

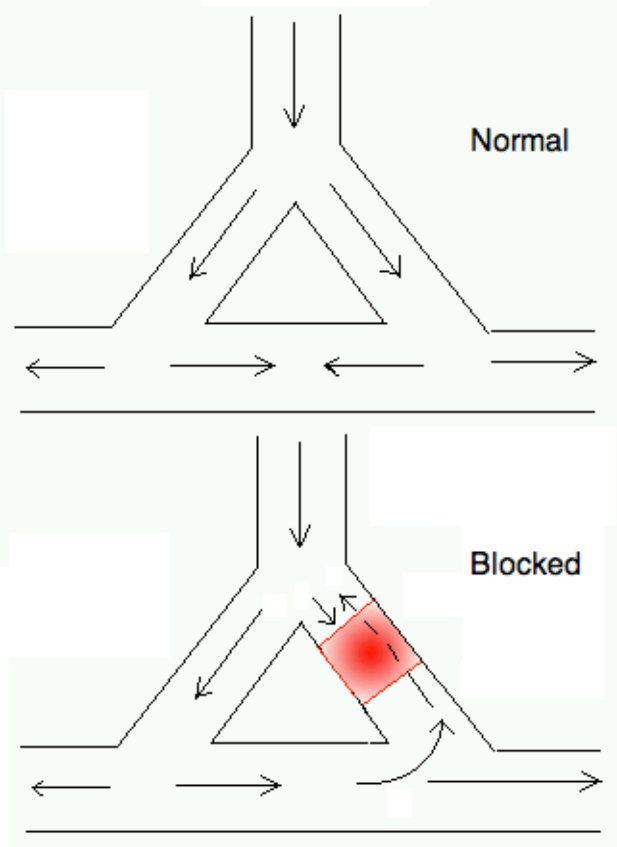


Figure 1: Schematic of process involved in reentrant arrhythmias [47].

Extra-Stimuli-pacing induction used in the PES protocol acts by shortening the refractoriness of the ventricles by applying consecutive premature stimuli, thus creating a reentry circuit [48], [49]. The penetration into a reentrant circuit is partly dependent on the duration of the fixed rate pacing [50] and the rate of the extra-stimulus that is applied immediately after [49], [51], [52] (or rate of burst pacing without a fixed rate pacing preceding it). Moreover, extra-stimuli pacing can induce arrhythmias by slowing conduction of electrical signal in a region of the myocardium. This heterogeneous distribution of conduction velocity throughout the heart can also allow for development

of reentry circuits [53], [54]. The present study uses a PES protocol that varies different pacing parameters, such as rate of extra-stimulus, cycle length of fixed rate stimuli, and cycle length of burst or extra-stimuli pacing. Gillis et al. [55] showed that the a reduction in the coupling intervals between fixed rate stimuli in turn decreased the attainable coupling interval between extra-stimuli, indicating a shortened ventricular effective refractory period. Therefore, in this study, various rates of extra-stimuli were delivered following various cycle lengths of the fixed rate stimuli.

1.6 Objectives

The purpose of this study is to investigate potentially adverse effects of the injectable materials on the electrophysiology of the heart post-MI, and to explore the effects on properties of cardiomyocyte electrical propagation when the cells interface with the different materials. Looking into these aspects of injectable materials will provide some insight on the effects of the materials on the electrophysiology when they are interfaced with the whole heart and cardiomyocytes.

Cell and material transplantations in the whole heart have been areas of research interest for their potential in treatment for heart failure. Both have shown improvements in different aspects of heart failure, such as improved heart function and neovascularization. The inducibility of arrhythmias has often been used as a parameter to evaluate the safety of injected cells in an infarcted heart, and has shown an increased arrhythmogenicity in some cell types but fewer risks in other types. However, this approach, or any other ways to study electrophysiological safety, has not be used with

any injectable materials to this day. Thus, the arrhythmogenesis of the heart with injected materials is used in this study to determine the safety of using injectable materials as a treatment for heart failure. To do this, we used the programmed electrical stimulation technique, often used to study inducibility of arrhythmias through rapid pacing of the heart. This *in vivo* approach can provide comparisons of arrhythmogenicity between different types of injectable materials.

Cell retention and survival have been shown to increase when they are injected with materials as a scaffold. Moreover, the added advantages of injecting both materials and cells increases the benefits of the treatment for heart failure. Therefore, injectable materials directly interact with the injected cell types. In this study, the effects of materials on the electrophysiology of cardiomyocytes are explored. To accomplish this, optical mapping (OM) of monolayers are used to provide a non-invasive, and relatively simple, 2D model that can be used to investigate cell electrophysiology. This aspect of the study can shed insight on how the electrophysiological properties of injected cells would be affected (and thus possibly leading to increased arrhythmia risks). Thus it is critical to know how the electrophysiology of cells differs when they are cultured in different environments. The electrical propagation properties of cells growing while interfacing with the materials may be different with different materials, as shown in Miskon et al. [56]. Electrical parameters such as action potential and beating frequency have been shown to differ in cell monolayers cultured on different types of coating.

The combination of these two aspects, arrhythmia inducibility of the whole heart with injected materials and effects on electrical properties when cardiomyocytes are interfaced with the materials, allows for a thorough understanding of the effects on heart electrophysiology following treatment for heart failure.

2. STUDY OF ARRHYTHMIA INDUCIBILITY TO ASSESS SAFETY OF INJECTABLE MATERIALS

2. STUDY OF ARRHYTHMIA INDUCIBILITY TO ASSESS SAFETY OF INJECTABLE MATERIALS

2.1 Introduction

Programmed electrical stimulation (PES) protocols have been used to study arrhythmia inducibility after cell transplantation in animals post-myocardial infarction. In general, animals and patients transplanted with skeletal myoblasts [30], [40] were observed to be more prone to having arrhythmias. PES studies were able to induce non-sustained VT in infarcted, vehicle-injected animals, whereas SkM injected hearts showed significant increase in incidence of sustained VT. This may be an attribute of SkMs possibly due to their inability to electrically couple with the host myocardium [40], [57]. Due to this concern, SkM transplantation was recommended to be used for patients with an implanted cardioverter defibrillator [58].

Biomaterials have been shown to improve heart function post-MI, may improve cell retention, and therefore may be transplanted along with cells. However, the material's effects on the heart electrophysiology have not been studied as well as it has been with cell transplantation. In this study, pacing (PES) protocols have been applied to study a rat occlusion-reperfusion model that has been injected with three different types of materials.

The control used was 1X concentration of Phosphate Buffered Saline (PBS). Several pacing studies conducted with cell transplanted rat MI models have used PBS

as the control. PBS injected infarct hearts were shown not to be any more prone to arrhythmias than were infarcted hearts without any injections. The experimental groups include myocardial matrix (ECM), which was first described in Singelyn et al [1], and Polyethylene Glycol (PEG), a synthetic polymer. Myocardial matrix is a digested, solubilized form of the extracellular matrix surrounding the ventricular myocardium (decellularized muscle). It has shown great promise for use as treatment for heart failure, as described previously. Briefly, it has been shown to promote vascular cell migration into the material, significantly increase arteriole density, and markedly improve differentiability of cells cultured on this material. Being a weak gel, the myocardial matrix tends to spread more in the heart than does PEG, which tends to be less viscous.

2.2 Materials and Methods

2.2.1 Myocardial Infarction

The female Sprague-Dawley rat (Harlan Sprague-Dawley, 225-250g) was placed under inhalative anesthetics. ECG leads were inserted at various places on the rat's body - one at the joint of each arm and one into each leg. The leads were connected to an ECG recorder, which displayed the ECG data on the monitor. This provided information regarding arrhythmias during the infarction procedure. Via sternectomy, suture and catheters were used to tie off the descending left coronary artery, effectively blocking blood flow past that point in the artery (Figure 2). After a 25-minute occlusion period, the catheter and sutures were removed, allowing the heart to re-perfuse.

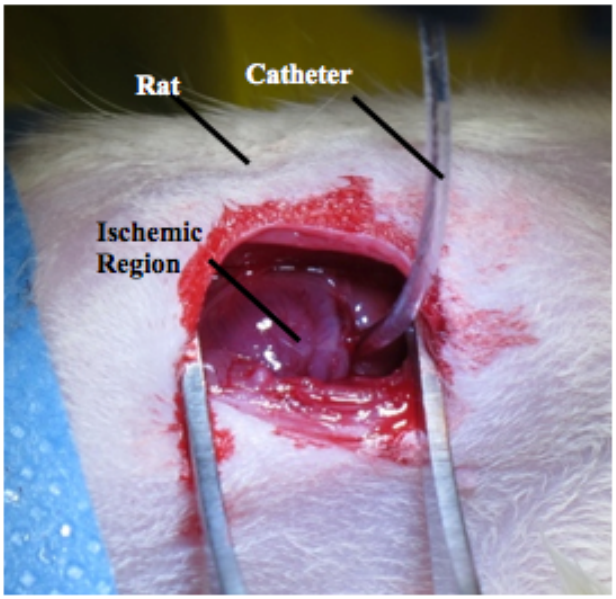


Figure 2: To induce myocardial infarction, a catheter is used to block blood flow.

2.2.2 Biomaterial Injection

Two weeks following myocardial infarction (Figure 5), the rat was again put under inhalative anesthetics and prepared for material injection (Figure 6a,b) from below the diaphragm.

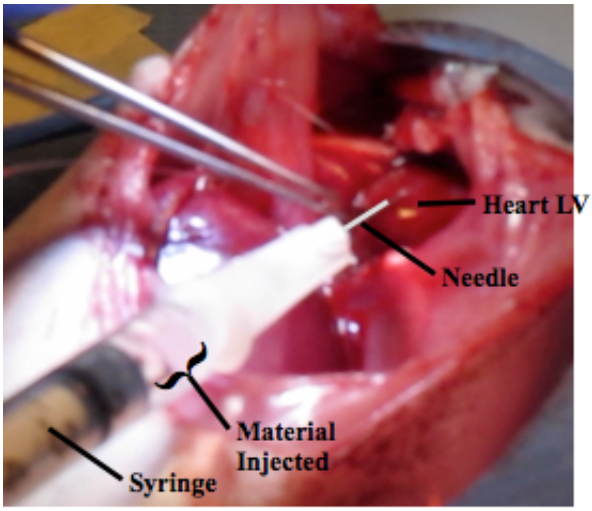


Figure 3: Injection of material into left ventricle of heart.

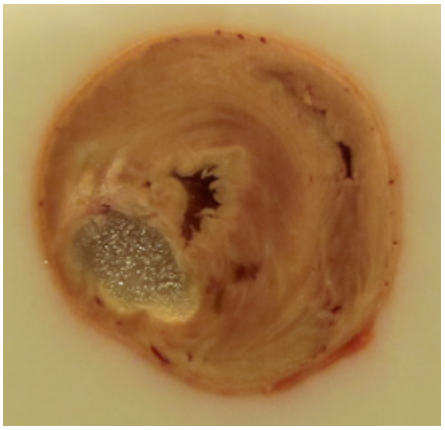


Figure 4: Cross-section of PEG-injected heart.

1X Phosphate buffered saline (PBS) was sterile filtered using a 0.22μm filter. Vials were filled with 135μL of PBS (only 75μL was injected but the extra was needed due to dead space within the needle and hub). The vials were placed on ice in a styrofoam container until injection.

Myocardial matrix (ECM) at concentration of 6mg/mL was solubilized by adding appropriate amount of sterilized MilliPore water. The solution was vortexed until mostly dissolved and clear. To rid the solution of remaining undissolved particles, the solution was taken into a syringe using a 22 gauge needle, and injected out into a sterile scintillation vial through a 30 gauge needle. Vials were filled with 135μL of ECM solution and were kept on ice until injection.

Polyethylene Glycol (PEG) gels by cross-linking with trilysine of concentration 1mg/mL. Therefore the different components needed to create a PEG gel were kept separately in ice and were combined only seconds before injection. About 20mg of PEG was weighed out, and a 10μL/mg ratio with sterile phosphate buffer at neutral pH was

combined, vortexed, and kept on ice. 2mg/mL of trilysine solution was made using sterile borate buffer at 8.4 pH. This mixture was vortexed and kept on ice.

For PBS and ECM injections, 45μL of the solution was pipetted into the hub of a 30 gauge needle. A 0.1mL glass syringe was fit tightly into the hub of the needle. Then the material solution was drawn into the syringe through the needle until it filled 75μL of the syringe lumen. The syringe was given to the surgeon to inject the material into the infarct region in the left ventricle (with the needle's bevel facing left).

For PEG injections, an amount of trilysine equal to the amount of PEG-phosphate buffer solution made was drawn into a pipette tip. Starting a timer for 45 seconds (by which time the PEG would have cross-linked with trilysine and gelled), the trilysine was pipetted into the PEG solution. A 1mL syringe without needle was used to draw in the PEG-trilysine mixture. A 27 gauge needle was attached to the syringe and handed to the surgeon. All this was ideally done in 25-30 seconds. Starting at 35 seconds, the surgeon was asked to start injecting, with bevel facing left. The injection was performed over a 10 second period, and was completed at 45 seconds from the time when the PEG and trilysine were combined. Refer to Figure 4 for the cross-section of a PEG-injected heart.

2.2.3 Programmed Electrical Stimulation

PES study was conducted at 1 week post injection (3 weeks post-MI). Refer to Figure 5 for timeline from MI to PES study.

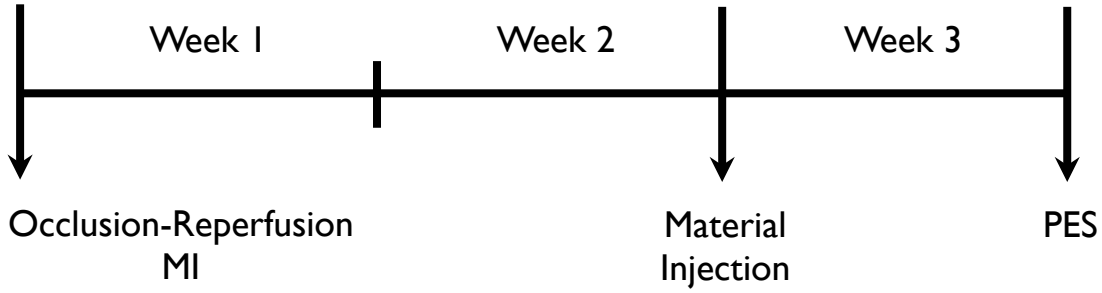


Figure 5: Time interval between each phase in PES experiment.

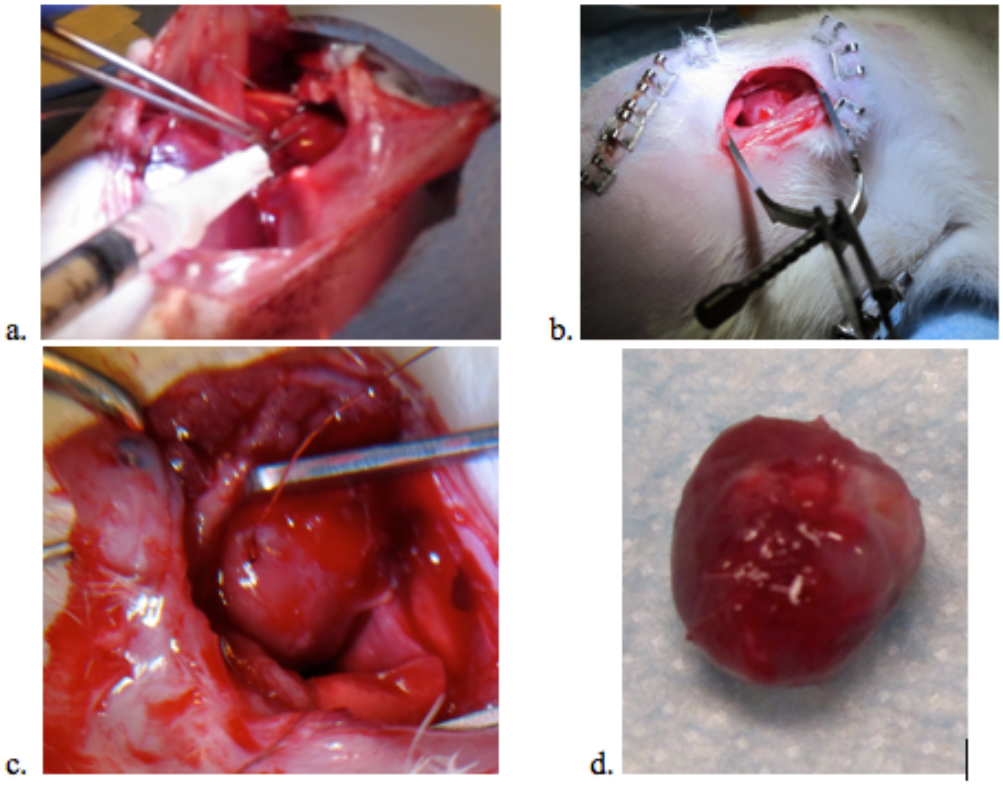


Figure 6: Material injection into rat's left ventricle (a). Injected material appears pale in heart (b). Pacing study requires pacing lead inserted into LV wall and ground lead placed below heart (c). Excised heart with injected material in the infarct zone (d).

2.2.3.1 Materials

1. Stimulator (DS8000, World Precision Instruments)

2. Isolator (DLS100, World Precision Instruments)

3. Unipolar leads

    1. Pacing lead (positive connection on BNC cable)

    2. Reference lead (ground connection on BNC cable)

2. BNC cable/lead interface between stimulator and DAQ Board

    1. Ground lead

    2. Positive lead

3. DI-148 DATAQ ECG system

2.2.3.2 Animal Preparation and Lead Placement

The rat was anesthetized in the gas chamber and its chest was opened. The ECG leads were inserted and fed into an ECG signal recorder (using DATAQ di-148 system). The positive (pacing) electrode was inserted gently into the viable region of the left ventricle (LV) wall (such that the exposed electrode tip came in contact with the interior of the LV wall). The reference lead (ground) was placed in the chest cavity right underneath the heart such that the heart rested over it and kept it in place.

2.2.3.3 Stimulator Setup

Appendix A includes the entire detailed protocol on the stimulator setup and pacing procedure involved in the PES study. The following setup is a brief overview. The BNC end of the pacing and reference electrode wire was connected to the isolator, which plugs into the stimulator. To provide for visible stimulus spikes on the ECG

recording, the stimulus signal was input into a channel on the DAQ board. The BNC end connects to the Combined Analog of Channel 4 on the stimulator.

Before beginning the pacing for arrhythmia inducibility, fixed rate pacing at pulse width of 1ms, cycle length (CL) of 120ms, and at an initial voltage was attempted to ensure capture of the pacing stimuli. On the ECG display monitor pace-ability was checked. Voltage was increased until the heart was able to be paced. This voltage is the capture threshold needed for pace-ability. The voltage was then doubled for the rest of the pacing protocol for the rat.

2.2.3.4 Programmed Electrical Stimulation

ECG recording was set for 1 hour, and begun when pace-ability was being determined (to ensure recording of entire procedure and any arrhythmias). Refer to Figure 6c.

Table 1: Abbreviation and description of protocols used in programmed electrical stimulation.

Term	**Definition**
PES	Programmable electrical stimulation. A method of using a stimulator to provide electrical stimuli and manually pace the heart.
Extra Stimuli	1, 2, or 3 high frequency pulses applied at the end of a train of pulses at a slower, fixed frequency.
Burst Stimuli	High frequency pulses applied without following a previous train of pulses as in extra stimuli.

Table 2: Procedure for PES shown in tabulated form (top and bottom).

Burst Stimulus (BS)
Cycle length (CL) (ms)
50
40
30
20
10

Extra-stimulus (ES)											
	1	2	3		1	2	3		1	2	3
Fixed rate	Extra-stimuli CL			Fixed rate	Extra-stimuli CL			Fixed rate	Extra-stimuli CL		
120 ms	**80**	**80**	**80**	**110 ms**	**80**	**80**	**80**	**80 ms**	**80**	**80**	**80**
	70	**70**	**70**		**70**	**70**	**70**		**70**	**70**	**70**
	60	**60**	**60**		**60**	**60**	**60**		**60**	**60**	**60**
	50	**50**	**50**		**50**	**50**	**50**		**50**	**50**	**50**
	40	**40**	**40**		**40**	**40**	**40**		**40**	**40**	**40**
	30	**30**	**30**		**30**	**30**	**30**		**30**	**30**	**30**
	20	**20**	**20**		**20**	**20**	**20**		**20**	**20**	**20**

Refer to Table 2 for a tabulated form of PES procedure. First, burst pacing (Table 1, Figure 8) was done at CL of 50 ms for 15 seconds [59] and ECG was observed for induced arrhythmias (ventricular tachycardia and fibrillation). If sustained VT (≥ 30 seconds of consecutive ventricular beats [60]) was induced, that cycle length was recorded in notes. If arrhythmia ceases and heart rate returns to normal intrinsic rhythm, burst pacing was continued at the next step (CL of 40 ms). The procedure for burst stimulus was completed after CL of 10 ms. NOTE: Between stimulations, a recovery period of at least 15 seconds was provided.

After burst pacing procedures, extra-stimuli pacing (Table 1, Figures 9-12) was done. The stimulator was configured to apply first set of extra stimuli: single extra stimulus (starting CL of 80 ms) following 15 seconds of fixed rate pacing at CL of 120 ms. ECG was observed for induced arrhythmias and the fixed rate CL and extra-stimulus CL were noted. If no VT was induced or any sustained VT ceased and heart rate returned to intrinsic rhythm, continue to reduce extra-stimulus CL by 10ms stepwise reductions (until the ventricular effective refractory period, VERP, of 20 ms is reached). If sustained VT was induced and unable to be stopped, pacing procedures were stopped until heart recovered to normal rate or rat died of arrhythmia. Otherwise, at the same fixed rate and extra-stimulus CL, the number of extra-stimuli was increased by one and procedure was repeated (from extra-stimulus CL of 80 ms down to 20 ms). The number of extra-stimuli was increased each round by one until a maximum of three extra-stimuli (three rounds).

Then, fixed rate CL was reduced to 110 ms and extra-stimulus CL was set to 80 ms and procedure was repeated as described above. Extra-stimulus CL was reduced again in 10 ms stepwise reductions, and number of extra-stimuli was increased by one, for a maximum of three extra-stimuli. Then, fixed rate CL was reduced to 80 ms and extra-stimulus CL was set back up to 80 ms and procedure was repeated as described above. After each pacing test, the ECG was observed for any induced arrhythmias (Figures 12 and 13). At the end of the entire burst pacing and extra-stimulus pacing procedures for the rat, the ECG recording was stopped and saved. The rat heart was excised, washed in saline to remove blood, and frozen in OCT Tissue-Tek.

2.3 Results

2.3.1 Arrhythmia Inducibility in Relation to Material Injected

Arrhythmia inducibility was assessed using Programmed Electrical Stimulation (PES) *in vivo* by burst (Figure 8) and extra-stimulus (Figures 9-12) pacing protocols in rats with LV infarcts 21 days after injury (7 days after injection of PBS, ECM, or PEG). *In vivo* pacing protocols were able to induce non-sustained ventricular tachycardia (monomorphic or polymorphic, self-terminating VT as in Figure 12) in 37.5%, 35%, and 33.33% of PBS (negative control, n=16), ECM (n=20), and PEG (n=12) injected rats respectively. Sustained VT (Figure 13) was induced once during burst pacing in an ECM injected rat (5% occurrence rate in the ECM group), and once during extra-

stimulus pacing in a PBS injected rat (6.25% occurrence rate in the PBS group). Refer to Figure 7 for ECG recording of the intrinsic rhythm of a rat heart.

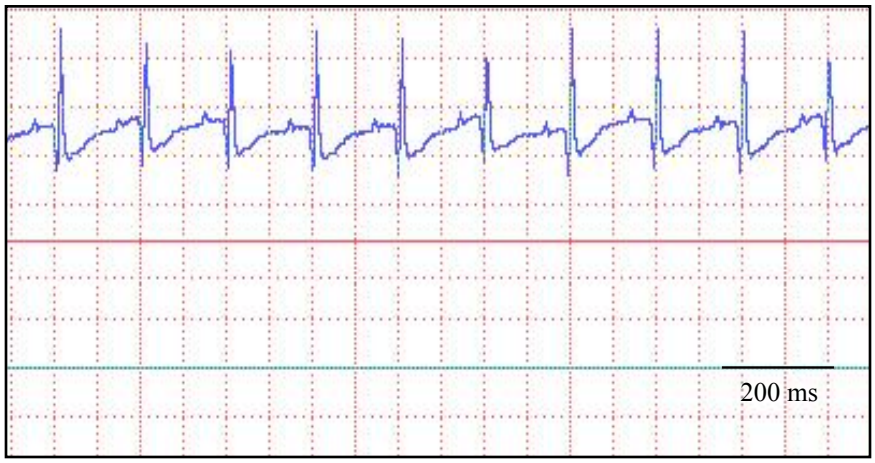


Figure 7: Intrinsic rhythm of rat heart recorded with ECG.

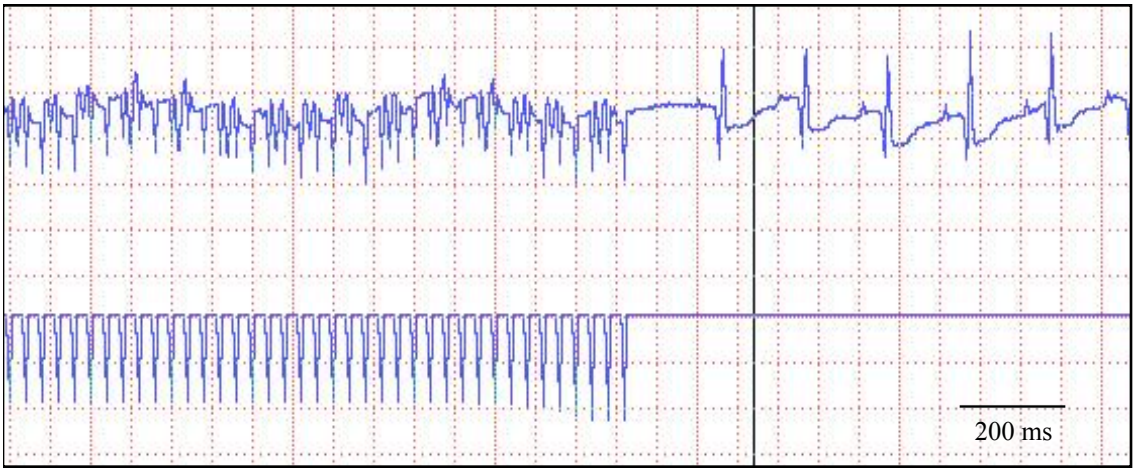


Figure 8: Burst stimulation pacing captured by heart. Returns to intrinsic rhythm when stopped pacing. Recorded with ECG.

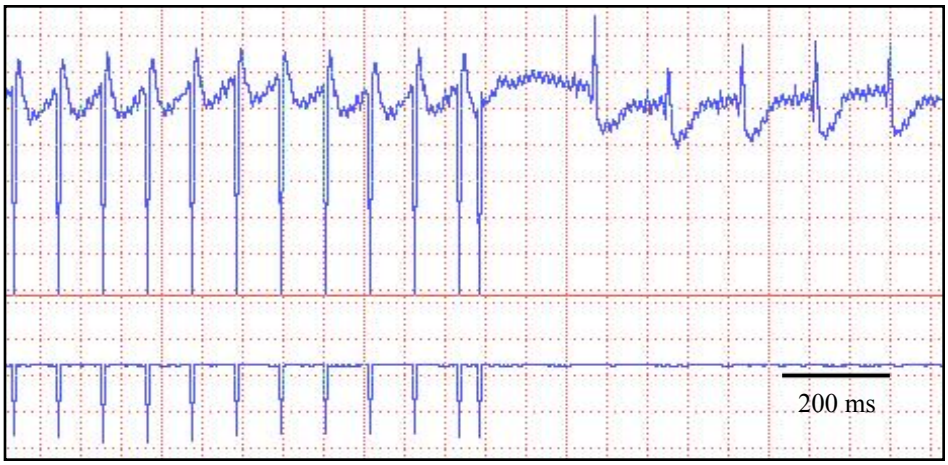


Figure 9: Extra-stimulus protocol captured by heart. Single extra-stimulus is delivered, and is recorded with ECG.

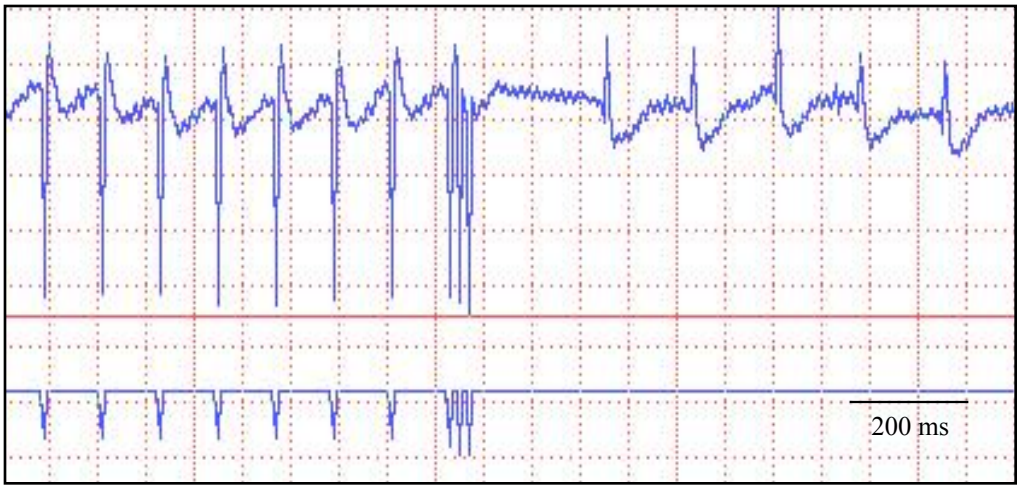


Figure 10: Extra-stimulus protocol captured by heart. Double extra-stimuli are delivered, and is recorded with ECG.

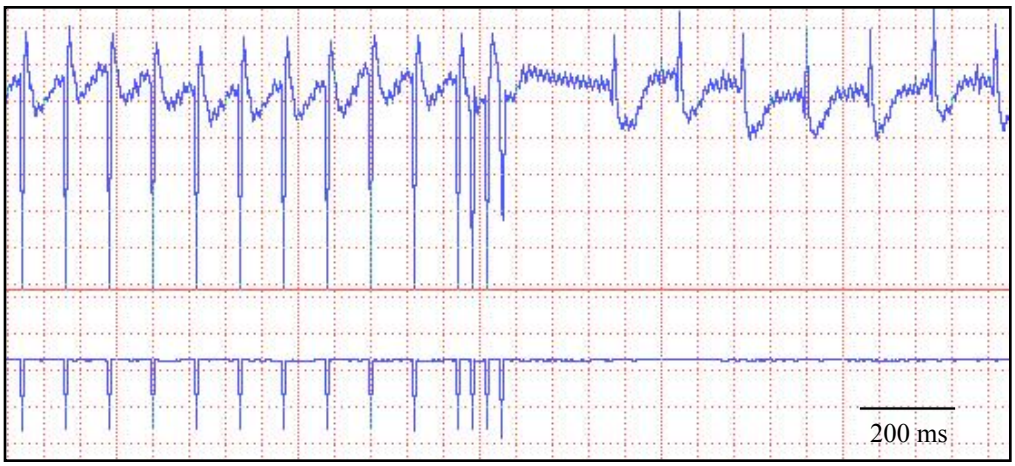


Figure 11: Extra-stimulus protocol captured by heart. Triple extra-stimuli are delivered, and is recorded with ECG.

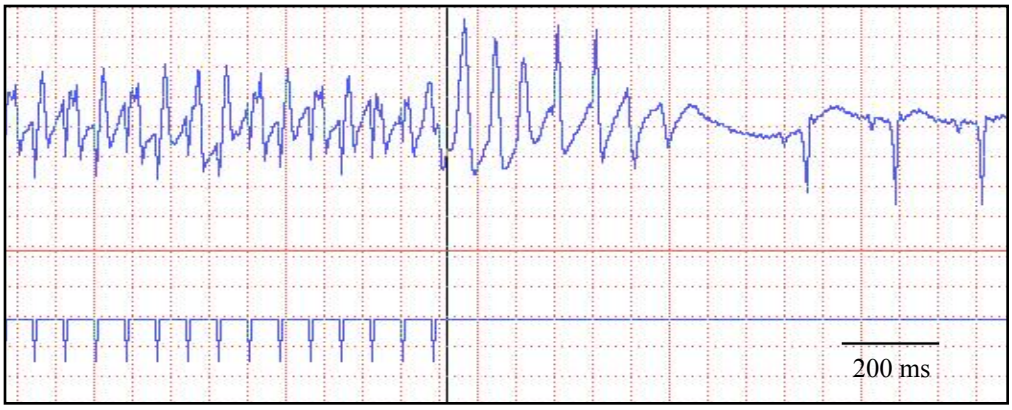


Figure 12: Extra-stimulus protocol captured by heart. Single extra-stimulus is delivered (cycle length matches that of fixed rate stimuli) and induces VT with seven ventricular contractions, and is recorded with ECG.

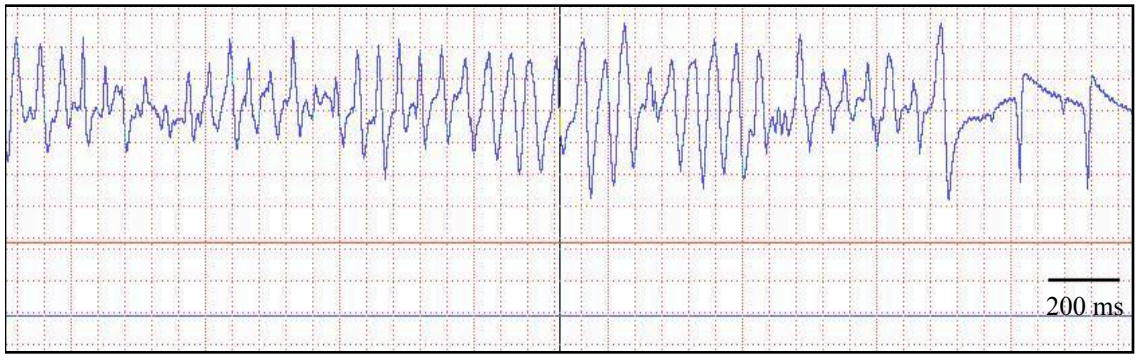
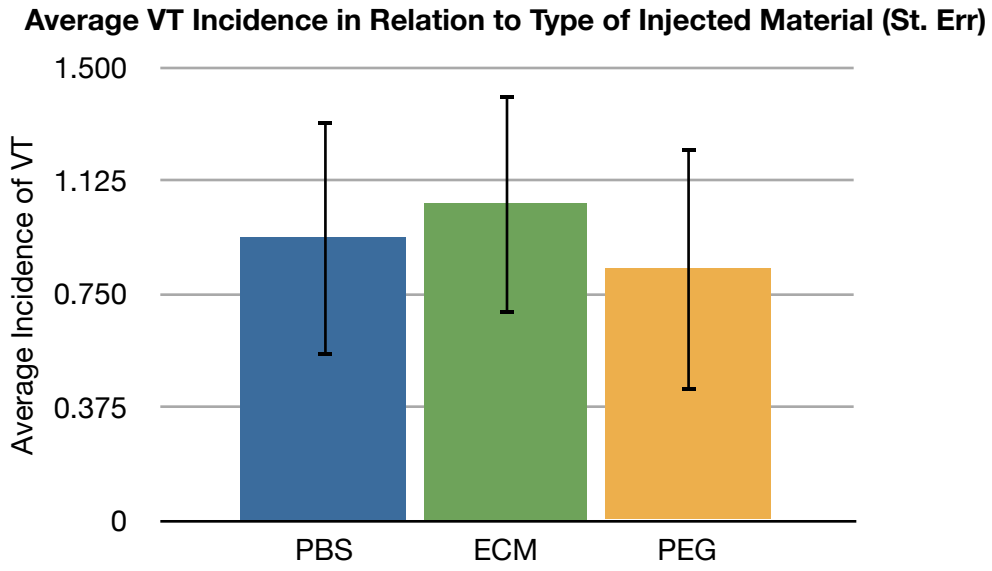


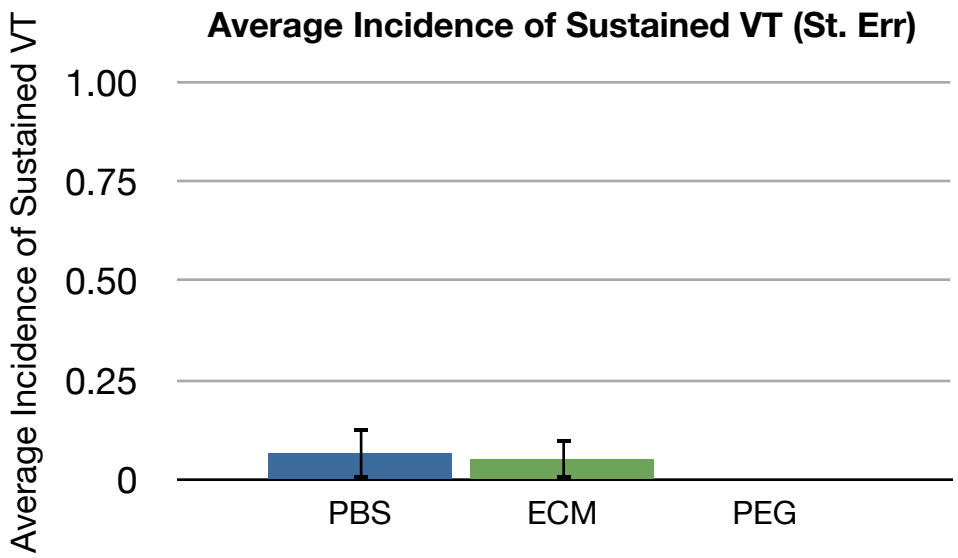
Figure 13: Induced sustained VT that self-terminated.

The average number of occurrence (with standard deviation) of VTs in each experimental group were 0.94 ± 1.57, 1.05 ± 1.64, and 0.83 ± 1.40 in PBS, ECM, and PEG injected rats respectively. These values include VT incidence under both burst and extra-stimulus pacing protocols. Graph 1 depicts VT incidence in relation to type of injected material. Displayed error bars are of standard error values. Graph 2 shows average occurrence of sustained VT separately in burst pacing and extra-stimulus pacing. Hence, there seems to be no statistically significant difference in VT inducibility ($p>0.1$ between all pairs of groups) in rats based on the type of material injected into their LV wall after the localized myocardial infarction.

Graph 1: Average incidence of VT depending on the type of material injected in heart (PBS, n = 16; ECM, n = 20; PEG, n=12).

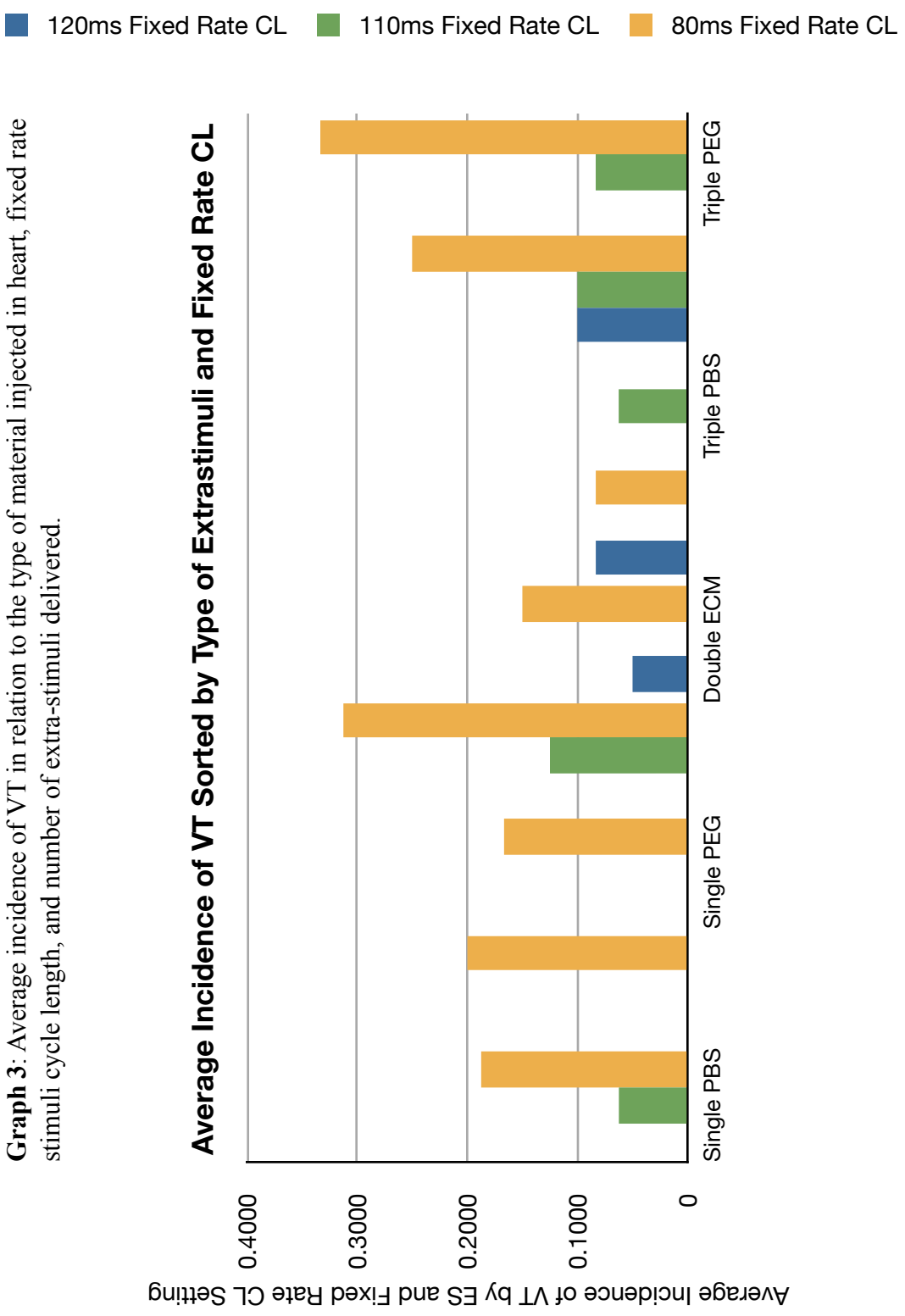


Graph 2: Average incidence of sustained VT in relation to the type of material injected in heart (PBS, n = 16; ECM, n = 20; PEG, n=12).



2.3.2 Arrhythmia Inducibility Affected by Fixed Rate Cycle Length and Number of Extra-Stimuli Delivered

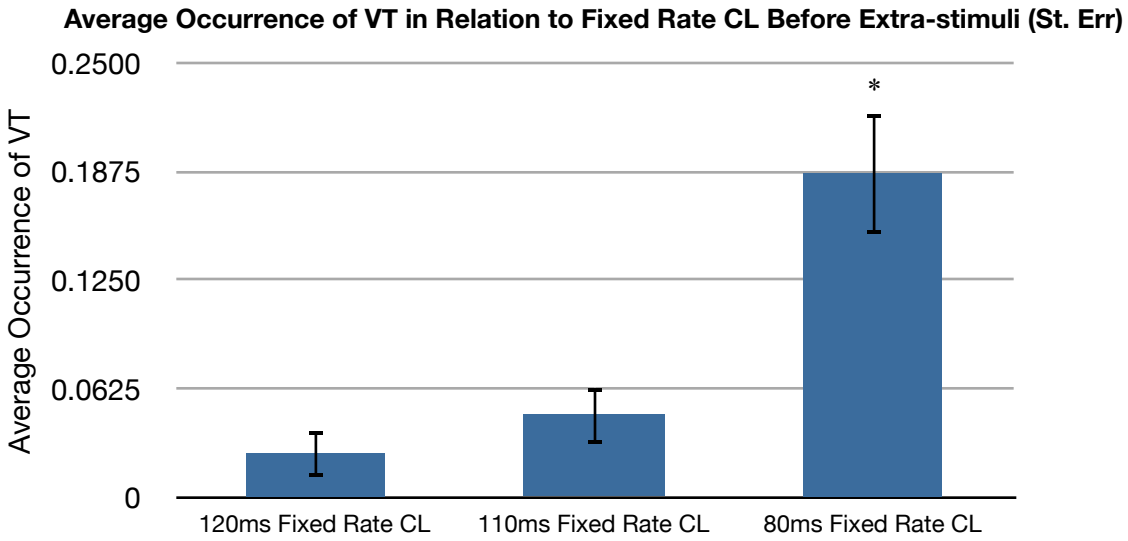
Graph 3 shows the relationship between VT occurrence, the fixed rate cycle length, and the number of extra stimuli. It is a breakdown of VT incidence during extra-stimulus pacing and shows the relationship between incidence and the number of extra-stimuli provided at the end of the fixed rate pacing at different cycle lengths. Here, "Single PBS" refers to a PBS injected rat provided with a single extra stimulus, and likewise for each set.



Graph 3: Average incidence of VT in relation to the type of material injected in heart, fixed rate stimuli cycle length, and number of extra-stimuli delivered.

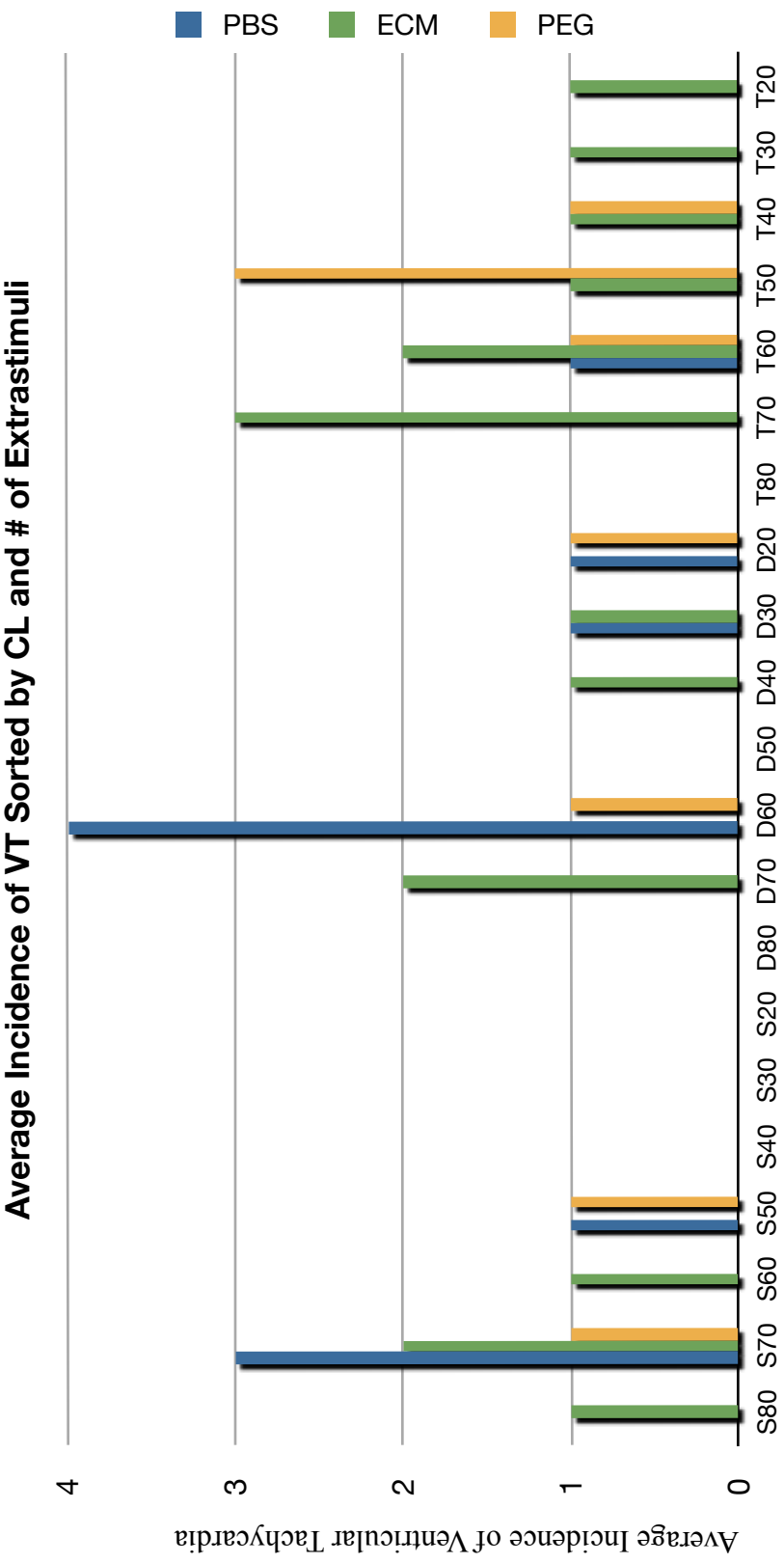
Graph 4 shows the average occurrence of VT in relation to the value of fixed rate cycle length before the application of extra-stimuli during the extra-stimulus pacing protocol. There seems to be a statistically significant difference in VT incidence between 120ms and 80ms fixed rate CL ($p<0.001$) and between 110ms and 80ms fixed rate CL ($p<0.01$) but no difference between 120ms and 110ms fixed rate CL ($p>0.1$).

Graph 4: Average incidence of VT in relation to the fixed rate cycle length preceding extra-stimuli.



2.3.3 Arrhythmia Inducibility Affected by Extra-Stimulus Cycle Length and Number of Extra-Stimulus Delivered

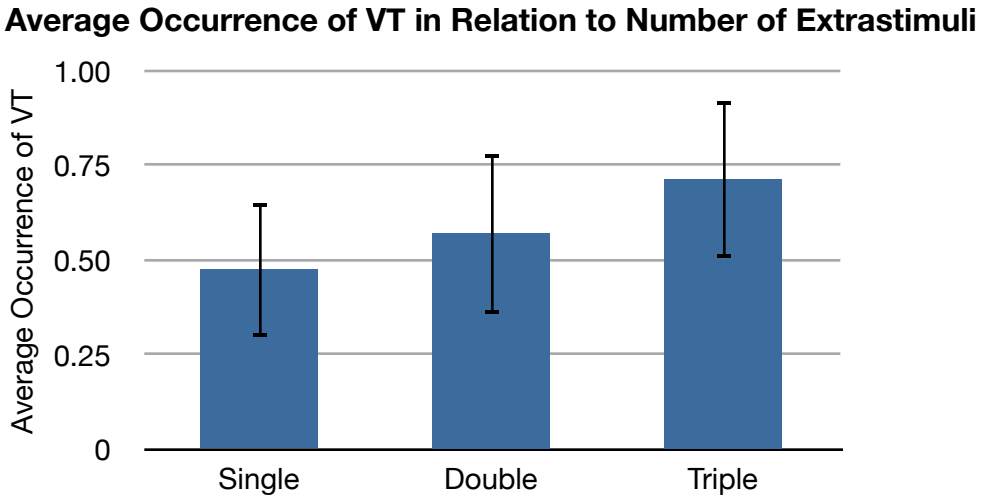
An even finer breakdown is shown in Graph 5, where the average VT incidence is shown in relation to the extra-stimulus cycle length and number of extra-stimuli. Here, "S80" refers to a single extra-stimulus at extra-stimulus CL of 80ms, "D80" refers to double extra-stimuli at extra-stimulus CL of 80ms, "T80" refers to triple extra-stimuli at extra-stimulus CL of 80ms, and likewise for the other groups.



Graph 5: Average VT incidence in relation to extra-stimulus cycle length and number of extra-stimuli delivered.

Graph 6 shows the average occurrence of VT in relation to number of extra-stimuli provided. There seems to be a slight trend showing an increase in incidence with number of extra-stimuli but the difference is not statistically significant ($p>0.1$ between all pairs of groups).

Graph 6: Average incidence of VT in relation to the number of extra-stimuli delivered.



2.4 Discussion

Altogether, the results from the *in vivo* electrophysiological study using programmed electrical stimulation protocols show that material injections (PEG and ECM) confer a degree of arrhythmogenicity comparable to that of saline (vehicle) injections. The percent incidence of non-sustained VT in PBS injected hearts is similar to the value reported by Li et al., who also used PES on a rat MI model [61]. The percent occurrence of VT in ECM and PEG injected hearts is similar to that in PBS injected hearts, indicating that the material injections do not increase risk of arrhythmia

more than vehicle-injections. This promising result could mean that materials can be safely used with cell-transplantations to help improve heart function and increase cell retention without the added risk of inducing arrhythmias.

PEG is a synthetic polymer that does not spread much in the heart, while ECM spreads more and was derived from a de-celled myocardium. Therefore it was expected that PEG increase risk of arrhythmias relative to that in ECM injected hearts. However there is no difference in the average incidence between the groups. This may indicate the presence of a characteristic of cell transplantations (that increases and is critical to arrhythmogenicity), that is missing in materials. Electrical coupling between native cardiomyocytes and transplanted cells that has the potential to create reentrant signals [62], automaticity of cells versus the inactive nature of the materials [62], or paracrine factors produced by transplanted cells are possibilities [41].

Figure 10 and 11 show two perspectives of Figure 8 data: VT occurrence in relation to 1. fixed rate cycle length, and 2. number of extra stimuli. The increase in occurrence of VT with reduced fixed rate CL was also observed in a patient study using PES [55]. It was reported that the addition of ventricular extra-stimulus played a larger role in the induction of VTs compared to reducing the fixed rate CL, though in Estes et al. [63] shortening of fixed rate CL was more effective in inducing VTs. In the present study, the addition of extra-stimuli following fixed rate stimuli showed a strong trend, but not a statistically significant difference, in increase in VT inducibility, but the reduction of fixed rate CL from 120 ms to 80 ms did show a statistically significant

difference. PES studies with rat models generally used a single fixed rate cycle length of 100 ms [64]. This study went further to include an 80 ms fixed rate CL, which was used in a mice model [58]. This indicates that a fixed rate cycle length as low as 80 ms may contribute to a statistically significant increase in VT inducibility, being much closer to the effective refractory period of the rat heart.

As reported in [55] and shown in Figure 9, the average VT incidence was not affected significantly by the decrease in CL of the extra-stimulus. Though the addition of extra-stimuli aides in the induction of VTs as also reported in [65], shortening of the CL of extra-stimuli does not seem to be as significant of a contributor.

As the material was injected largely into the infarct zone where there are few viable host cardiomyocytes, not much contact or interaction occurs with the viable electrical components of the heart, and therefore the heart was observed to have similar and low arrhythmia inducibility with material compared to saline injections. Furthermore, a material injection in the infarct zone would not cause a large degree of delay in conduction around the injection. This possibly removes the risk of reentrant arrhythmias.

Comparing between possible mechanism of arrhythmia in cell versus material transplantations: The tendency for increased inducibility of VT after cell transplantation possibly arises from their heterogeneous integration in the host myocardium, conflicting electrophysiology, or automaticity [62], [66]. Injections of materials alone, however, may not contribute to as large of an increase in VT occurrence because they lack the

above described nature of cells. Moreover, the materials do not form cellular junctions to electrically couple with the native cardiomyocytes, preventing reentrant circuits that may be created due to injected cells interfacing with cardiomyocytes. Paracrine factors released by myoblasts or bone marrow-derived cells (but not materials) may also have an effect on the development or electrical activity of surrounding native myocardium, adding to increased inducibility of VTs with cell transplantations [41].

2.5 Limitations

The pacing lead used to deliver the stimuli was inserted into the left ventricular wall such that the electrode tip contacted the inner section of the wall. However, there could be several variations in the lead location in the rats used for this study. The same length of the pacing lead was inserted into the heart wall, but depending on the thickness of that heart's wall, the exact point of contact in the wall could have varied. Moreover, the lead was inserted into the viable region of the left ventricle, away from the infarct and injected zones. Depending on the size of the infarct and spread of the injection, the lead may not have been inserted the same distance from the infarct zone or from the apex (reference point) of the heart.

In using the programmed electrical stimulation protocol, the sequence of the various parts of the protocol was not randomized. The initial application of burst stimuli pacing may have altered some temporal factors related to repetitive ventricular pacing. This includes neurohumoral-mediated changes in ventricular refractory period or local conduction velocity. Moreover, the fixed rate pacing CLs were introduced in decreasing

order, as were the CL of extra-stimulus under each specified fixed rate pacing CL. Delivering these stimuli in order of decreasing CL might have posed some adverse effects on the heart. However, Gillis et al. showed that no differences were found in results when reverting to the large CL at the end of each step of the protocol [55].

3. STUDY OF ELECTROPHYSIOLOGICAL CHANGES IN CARDIOMYOCYTES WHEN INTERFACED WITH MATERIALS

3 STUDY OF ELECTROPHYSIOLOGICAL CHANGES IN CARDIOMYOCYTES WHEN INTERFACED WITH MATERIALS

3.1 Introduction

Optical mapping (OM) is a technique that uses voltage-sensitive fluorescing dyes to track the electrical propagation spreading via cardiomyocytes in monolayer (and can also be used in whole-heart systems). The imaging uses light-tissue interaction, which depends on the light wavelength used to excite the fluorescent dye. Depending on the voltage measured during a specific time, the dye emits a light of a different wavelength, which is recorded. As such, OM allows for the non-invasive recording of APs from multiple sites and generation of maps of AP propagation (compared to using surface electrodes, which come in contact with cells).

The variability of cell development and differentiation on dishes coated with different materials has been studied by many groups. It has often been suggested that the elasticity and storage modulus of the materials can affect differentiation speed and rate of cell proliferation. The environment affects the types and degree of expression of channels critical in electrical conduction, leading to direct effects on maturation and differentiation of the cells [67], [68]. The cell development and excitability may also

depend on the substrates they express and the interactions they form with their surrounding environment (depends on substrate-environment binding strength).

The optical mapping technique can be utilized to study the cell monolayer system non-invasively (without electrodes contacting the cells). By using a voltage-sensitive dye and making use of the light-tissue interactions, this method allows for the recording of electrical signal propagation from a point or line stimulus on the monolayer. The speed at which the signal propagates across the plate of cells and the AP duration at different levels of repolarization can be measured using the fluorescing intensity of the voltage-sensitive dye.

It is expected that the myocardial matrix (ECM) coated plates will exhibit a more matured or healthy culture of cells because it is derived from swine heart myocardium and consists purely of the extracellular components that surround the cardiomyocytes. The culture grown on collagen coated plates may not be as highly matured or healthy at the same time point due to the fact that it is a single component and does not represent the complex make-up of the myocardial extracellular matrix. The maturity of the cells cultured on these plate coatings can be indicated by the conduction velocity or AP duration measured during the OM experiment.

For OM studies with cardiomyocytes, fluorescent probes are generally used due to their higher fractional yield in signal variation with minute changes in voltage. Fast and slow dyes can be used, depending on the optimal response time and molecular mechanism for measuring voltage that are studied or needed for the experiment [69].

Fast dyes are used in cardiac studies to enable detection of voltage changes on a time scale of microseconds. Styryl dyes, such as di-4-ANEPPS, can be excited using visible light and are thus widely used [70]. Di-4-ANEPPS can be characterized by its 10% change in fluorescence intensity for every 100 mV change in membrane potential [71]. No calibration of the optical signal for voltage-sensitive experiments using dye is required since APs have a constant amplitude after reaching threshold. The change in fluorescence is taken and normalized over time to account for the drift in fluorescence intensity with photobleaching.

3.2 Materials and Methods

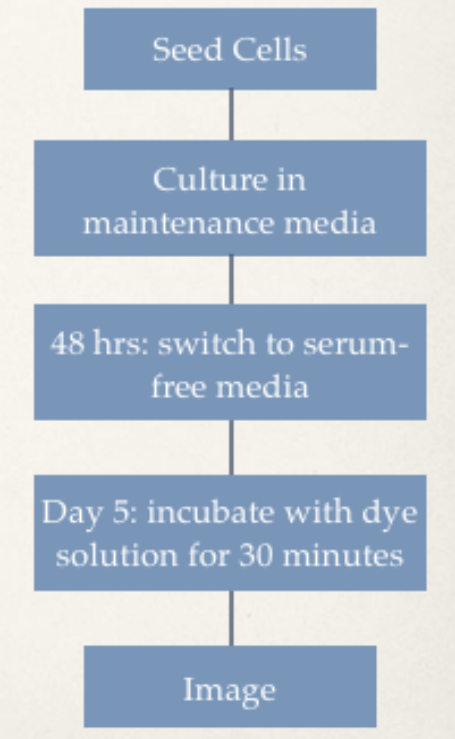


Figure 14: Flowchart of protocol from seeding cells to imaging.

3.2.1 Coating Plates

Refer to Figure 14 for a flowchart of the processes from cell seeding to imaging. Plates for one experimental group were coated with collagen digested in acetic acid, for a 1mg/mL concentration. The second experimental group required plates coated with ECM solubilized in acetic acid, for a 1mg/mL concentration. The plates were kept in the incubator to allow for the collagen and myocardial matrix to adhere to the bottom surface of the plate. After at least one hour in the incubator, the solutions were aspirated and the plates were rinsed with 1X PBS. The plates were then stored for a maximum of one week before they were used for culturing cardiomyocytes.

3.2.2 Cardiomyocyte Monolayer

3.2.2.1 Materials

1. 2- to 3-day old Sprague-Dawley neonatal rats
2. Cardiomyocyte isolation kit (Cellutron)
3. Cell maintenance media containing penicillin-streptomycin
4. Serum-free media (maintenance media without horse or fetal bovine serum)
5. Coated plates (with collagen or myocardial matrix)

3.2.2.2 Methods

The following procedure for cell isolation was performed by Michael Angelo, and follows the “Neonatal Myocyte Isolation Protocol” [72].

Neonatal rat ventricular cardiomyocytes were dissociated from 2- to 3-day old Sprague-Dawley rats and pre-plated in two steps for purification as described previously in Rohr et al [73]. After cardiomyocyte isolation the cells were plated on collagen or myocardial matrix coated 55 mm diameter plates (each isolation was evenly distributed among collagen and ECM coated plates) at a seeding density of 2300 cells/ mm^2 and cultured for 5 days to yield confluent and contractile cardiac monolayers [74]. Forty-eight hours after seeding, the maintenance media was switched to serum-free media. This media was used throughout the rest of experiment, and was changed every two days.

3.2.3 Electrode Design and Stimulation Protocol

3.2.3.1 Materials

1. Platinum wire with 0.125 mm diameter (World Precision Instruments Inc.)
2. Coated cable (Belden)
3. Shrink tubing (Alpha Wire Company)
4. Heated air blower
5. Soldering iron
6. Metal resin
7. Plastic culture dish lid
8. Rectangular slab of acrylic
9. BNC cable and connectors

10. Digital stimulator

3.2.3.2 Methods

Line electrodes were made using platinum wires. Two pieces of 2-cm long Platinum wire were cut and soldered to the free end of a BNC connector cable. One electrode was soldered to the main positive wire (used as pacing electrode) and the other was soldered to the ground wire (used as the ground/reference wire). The electrodes were positioned parallel to each other, separated by 3 mm, and glued to a small slab of acrylic using electric liquid glue [75]. This piece of acrylic was attached to the inside of a plastic culture dish such that when the lid was placed over the culture dish, the electrode projected into the media, and was 1 mm above the cardiomyocyte layer at the bottom surface of the dish.

Unipolar line electrodes were used to deliver stimuli at the side of the culture plate such that the stimulus line lies immediately to the left of the field of view on the plate. The electrode is connected to the stimulator and was triggered by a computer-controlled pacing sequence. The cells were paced with a monophasic signal at 2 Hz frequency with stimulus width of 10ms.

3.2.4 Staining Procedure

3.2.4.1 Materials

1. di-4-ANEPPS dye (Invitrogen)
2. Pluronic F-127 (20% solution in DMSO) (Invitrogen)

3. Sterile PBS (1X, Gibco)

3.2.4.2 Methods

The cells were stained using the following protocol [76]. In 5 mL of 1X PBS, 17.79 μL of di-4-ANEPPS stock (final concentration of 30 μM) and 25 μL of Pluronic solution (final concentration of 0.1%) were added and vortexed in a foil-wrapped 15 mL conical tube. Media from 5-day old culture dishes was aspirated and staining solution was added, enough to cover the layer of cells (about 3 mL in a 55 mm diameter plate). The culture dishes were wrapped in foil to prevent photobleaching of the voltage sensitive di-4-ANEPPS dye and placed on the shaker for 15 minutes and in the incubator for 25 minutes.

3.2.5 Acquisition System Setup

3.2.5.1 Materials

1. MiCam Ultima power box
2. MiCam Ultima acquisition box
3. Ultima acquisition software
4. Voltage divider

3.2.5.2 Methods

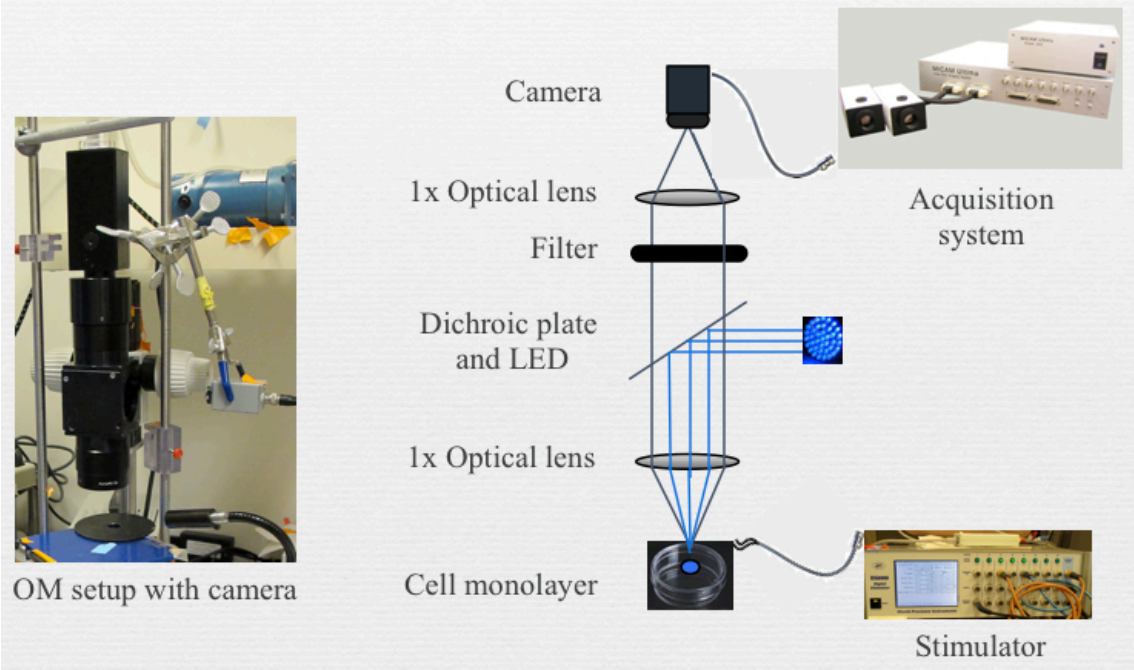


Figure 15: Acquisition imaging setup shows camera (left), stimulator with pacing leads, and LED excitation light.

The optical mapping setup is shown in Figure 15. The stimulator is linked to the MiCam Ultima acquisition box, which feeds into the computer for processing and analysis. The parameter setup for the acquisition software was as follows: the sample acquisition speed was set to 2 ms/frame at 2048 frames/pages. A three-second acquisition duration was chosen.

3.2.6 Optical Mapping Setup

3.2.6.1 Materials

1. CMOS camera with 1 cm x 1 cm chip adopted with 100 x 100 pixels (Ultima Master 6013)
2. LED light source
3. Objectives with 1X lenses (2) (Planapo/Leica)
4. 500 nm dichroic mirror (500 DRLP 69326, Omega Optical)
5. Long-pass emission filter

3.2.6.2 Methods

After staining, the staining solution was replaced with serum-free media. Now the cells were ready to be optically mapped. The optical mapping experiment setup works by recording the fluorescent image from the cells (excited by LED fluorescent lights) and transferring the data to a processing computer. Here the action potential and other electrophysiological data were extracted and used to determine quantitative properties of the plate of cells.

To do this, the culture plate was placed on a heating pad. The top lid was removed and replaced with the lid that was adopted with the line electrode. The line electrode was adjusted to sit 1mm from the bottom of the culture plate for proper pace-ability. The stimulator was setup to provide stimuli at a period of 500ms and with width of 10ms. The voltage of the stimulus was increased until threshold capture was

obtained, then the voltage was increased 1.3X threshold voltage. The rest of the experiments were done at this voltage.

An acquisition software was used to analyze the data obtained from pacing the plate of cells (Figure 17). The plate was rotated between tests and was optically mapped at different regions. Matlab codes were used to process and analysis the plate of cells for conduction velocity and action potential duration values. The values were compared between the two groups, collagen coated plates and myocardial matrix coated plates.

3.2.7 Analysis

3.2.7.1 Materials

1. Matlab
2. Matlab scripts
3. Cluster access (granite.ucsd.edu)
4. Cyberduck and Terminal (Mac)

3.2.7.2 Methods

Following acquisition of data via the optical mapping, .rsh, .rsm, and .rsd data files from the processing computer were saved in specified location for access by Cluster for analysis. Matlab scripts used for processing were submitted on Cluster and data processing was thus conducted. “Raw” and “vars” matlab files were created and used for analysis and quantification of data. Activation time (time duration for activating the entire imaged field of view in the CM monolayer, Figure 16),

repolarization, and AP duration (at 20% and 80% repolarization) values were extracted from the data and were used to determine conduction velocity (CV) of the CM monolayer. Refer to Figure 16.

CV was calculated for each beat of each run using conduction velocity vector fields obtained (Figure 16). The vector field magnitudes were obtained by taking the derivative of the upstroke slope (maximal upstroke velocity). Then the median of the magnitude of all velocity vectors bound within a specified region of interest was calculated for each beat. The CV values quantified in this study were determined by taking the mean of the resulting magnitudes over all beats in a specific run, then averaging the CV over all runs for a specific plate, then averaging the CV over all the plates in each group. AP duration at 20% and 80% repolarization were determined by taking the mean of the median values obtained by the processing, and averaged similarly.

3.3 Results

The effects of plate coating on electrical propagation properties of cardiomyocyte monolayers were studied using optical mapping. Conduction velocity (CV) and action potential duration (APD) values were able to be determined using this technique.

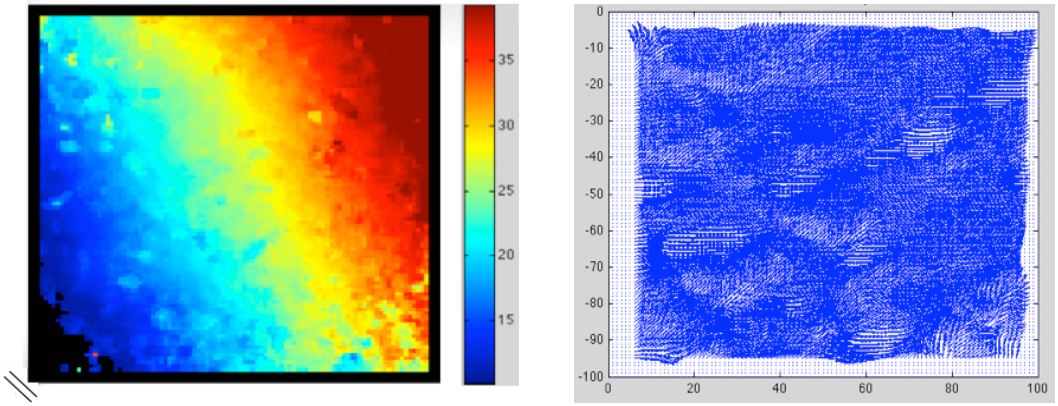


Figure 16: Activation time color map with line stimulus at lower left corner (left). Conduction velocity vector gradient (right).

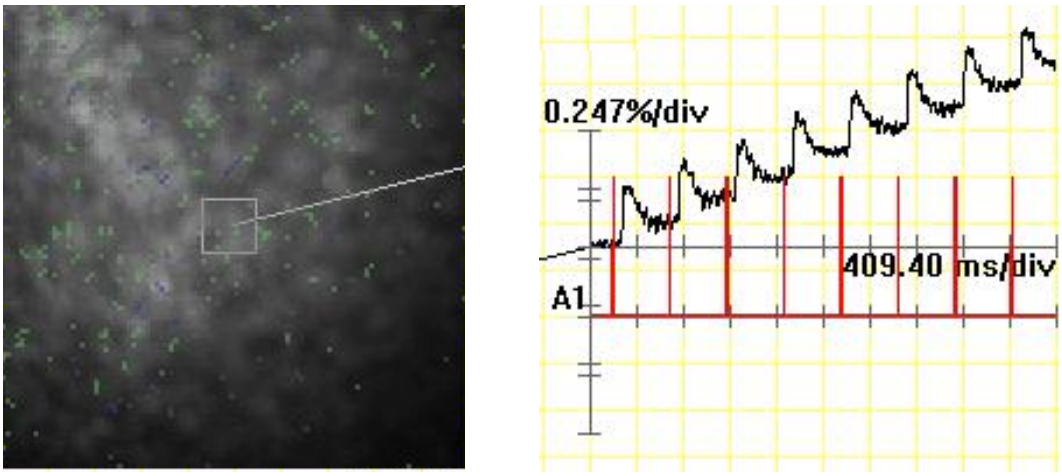


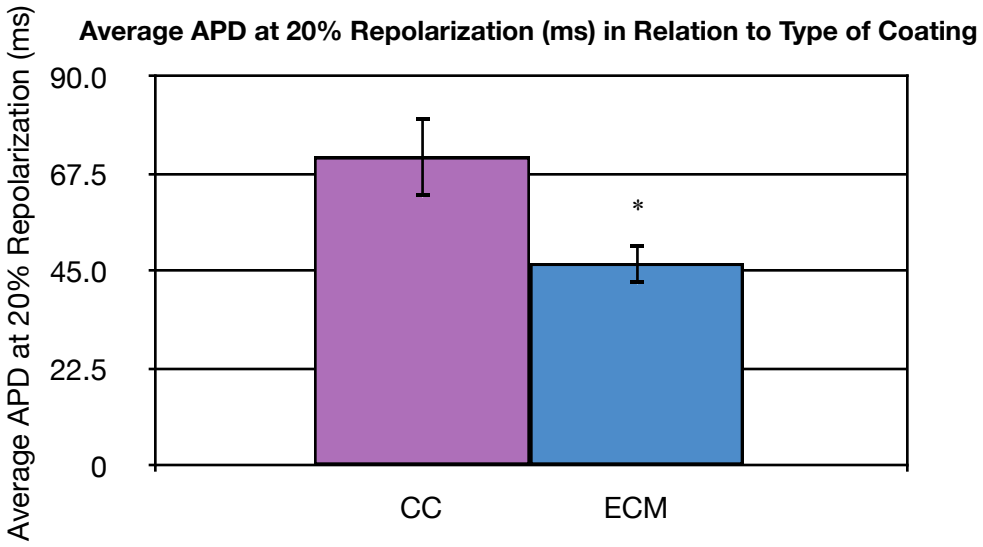
Figure 17: Raw data of monolayer field of view recorded by camera (left). APs measured over time from square region shown in raw-image figure (right). Red lines indicate stimulus spikes. Synchronization with recorded APs indicate that stimulus is captured by cardiomyocytes.

3.3.1 Action Potential Duration at 20% Repolarization

The average (with standard deviation) APD value at 20% repolarization was 71.52 ms ± 29.66 ms for collagen coated plates and 46.91 ms ± 14.54 ms for ECM coated plates. Graph 7 depicts this data in a bar graph. There is statistically significant

difference between the groups in this category ($p < 0.05$) with the lower APD value for ECM coated plates compared to the value for collagen coated plates.

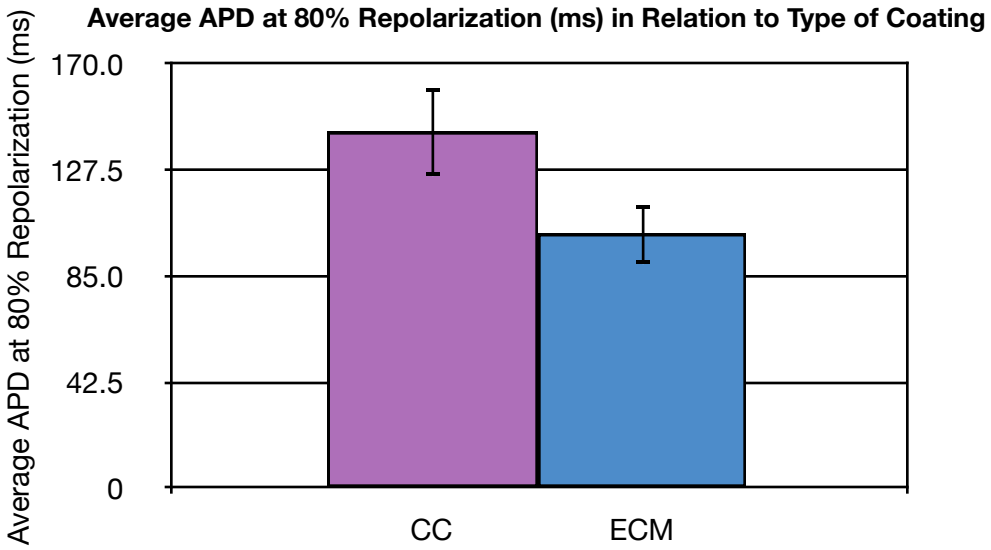
Graph 7: Average action potential duration at 20% repolarization in relation type of coating on plate (collagen, n = 10; myocardial matrix (ECM), n = 9). Standard error shown.



3.3.2 Action Potential Duration at 80% Repolarization

At 80% repolarization, average (with standard deviation) APD value for collagen coated plates was 142.87 ms ± 56.04 ms and that for ECM coated plates was 102.05 ms ± 36.48 ms. This set of data is shown by the bar graph in Graph 8. The difference between the groups in this case is not statistically significant but there seems to be a very strong trend with the lower APD value for ECM coated plates as compared to collagen coated plates.

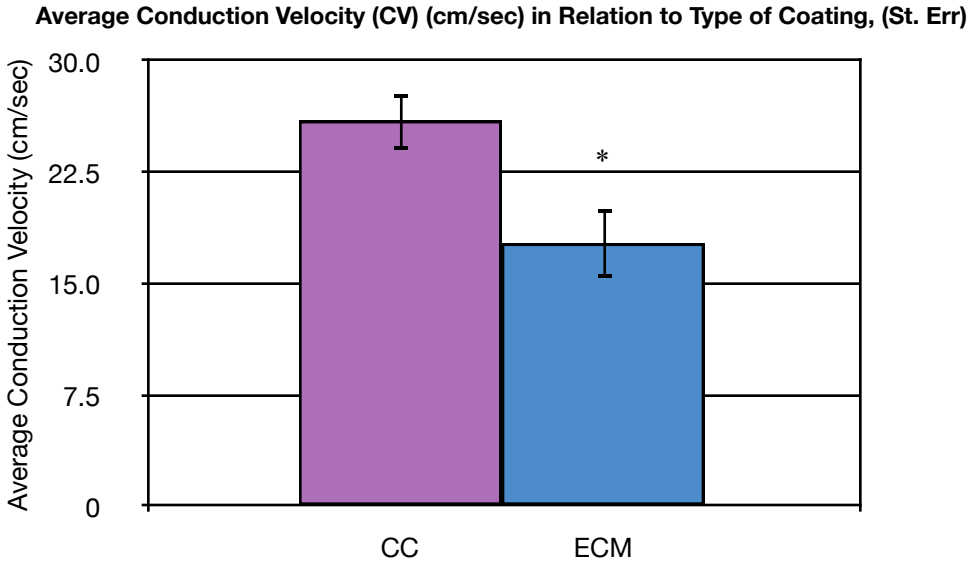
Graph 8: Average action potential duration (APD) at 80% repolarization in relation type of coating on plate (collagen, n = 10; myocardial matrix (ECM), n = 9). Standard error shown.



3.3.3 Conduction Velocity

In collagen coated plates (n=10) the average (with standard deviation) conduction velocity of signal propagation in the cardiomyocyte monolayers was 25.97 cm/sec ± 6.20 cm/sec, whereas for ECM coated plates (n=9) it was measured as 17.71 cm/sec ± 7.05 cm/sec. Graph 9 shows a bar graph of collagen vs. ECM coated plates. Statistical analysis with Student t-test shows the data to be significantly different between the two groups ($p<0.05$).

Graph 9: Average conduction velocity (cm/sec) in relation to type of coating on plate (collagen, n = 10; myocardial matrix (ECM), n = 9).



3.4 Discussion

As was expected, there is a definite difference in conduction velocity and APD values between the two material coatings. As expected, APD values at 20% and 80% repolarization show a strong trend in being lower in ECM coated plates compared to that in collagen coated plates although there isn't a statistically significant difference. APD values at 20% and 80% repolarization are often used when studying cardiac electrophysiology as they signify the initial and final points of the repolarization segment of the AP curve and are useful in determining any changes to the AP wave pattern. Shorter APD values are shown to signify more developed or matured cardiomyocytes [77]. That the cultured cells showed shorter APD when cultured on

ECM coated plates is a sign that they were able to mature better than those cultured on collagen coated plates.

On the other hand, CV values are significantly lower in cells cultured on ECM than those in cells cultured on collagen coated plates. A slower CV indicates a reduced expression of connexin gap junctions in the cell type, as shown in Fijnvandraat et al. [77]. This was unexpected since it was thought that cells cultured on ECM coated plates would electrically couple with their neighbors better than those cultured on collagen coated plates since the myocardial matrix would provide an environment more closely representing the ECM that cardiomyocytes are surrounded by in the natural myocardium. The processed myocardial matrix used to coat the plates may have altered gene expression in the CM such that it reduces connexin expression, known to lower CV [78], [79], [80], [81], [82], [83], [84]. The connexins form hemichannels, which connect to hemichannels expressed by an adjacent cells. This junction formed between CMs allow for exchange of ions used in propagation of the electrical signal. The degree of electrical coupling between cells is determined by the number of channels active and the channel's conductance when in its open state [74]. Moreover, the intrinsic excitability of the CMs may vary since it depends on the type of channel expressed. Sodium channels enable fast upstroke of the AP in mature hearts. In less developed hearts the depolarization mainly depends on calcium channels (with slower upstroke velocity) [85].

Though connexin expression can affect CV, de Boer et al. stated that a large safety factor lies between gap junction expression and CV values such that a very large decrease in connexin expression is required to reduce CV by a small amount. A fifty percent decrease in connexin expression does not affect CV, though the same reduction in sodium channel expression reduces CV by eighteen percent. Therefore it is also possible that sodium channel expression plays a large role in affecting CV [85].

In addition, the decrease in CV could be because of changes in resting potential and decreased excitability of the cells grown on ECM coating [79], [86]. Propagation of the signal across the layer of cells can also be affected by factors related to the spread characteristics of the cell on the culture dish. The size and shape of the CMs in the culture affects signal propagation by altering cytoplasmic resistance - larger cell size (due to cell swelling and decreased extracellular volume loss) leads to reduced CV [85], [87].

3.5 Limitations

This *in vitro* model allows for a high-resolution two-dimensional analysis of the electrophysiological system of the heart. While it provides for a non-invasive and straightforward method to study electrical propagation, the model does not replace the whole-heart models in terms of the complexity of the tissue structure. The adult heart is not isotropic and each layer has its own fiber orientation and therefore data from this simplified model can only be used to complement that from the whole heart models.

The purpose of studying electrical characteristics of cells grown on different coatings is to study how their interaction with each type of environment affects electrical propagation. The necessity to look into interactions and their effects arises in the case where cells are transplanted with materials into the heart post-MI. In that scenario, there are more types of cells involved in interaction with the materials than only cardiomyocytes. Therefore, a more thorough model of the system would involve a combination of a variety of cells, including those involved in the immune and inflammatory response post-MI.

4. CONCLUSION AND FUTURE DIRECTIONS

4 Conclusion and Future Directions

4.1 Conclusion

Several causes of arrhythmogenesis exists but in regards to myocardial infarction and heart failure the cause is likely to be reentrant conduction circuits. In treatments where cells are injected, these arrhythmias may be induced by the abnormal electrically coupled junctions or the lack of enough channels formed by the transplanted cells. To date, biomaterial injections into the infarct zone have been studied as a potential treatment by studying its effects on heart function post-MI, but the arrhythmogenicity in hearts where these polymeric biomaterials are injected has not been investigated.

This study explores that aspect of material injections as treatment for HF. The fact that there was no statistically significant difference in the arrhythmogenicity in relation to the material injected indicates that it is likely due to the inert nature of materials (when compared to cells) and the lack of increased number of abnormal electrical junctions formed (which was suggested to be the reason behind arrhythmia induction in cell-transplanted hearts).

To further look into effects on electrophysiology at the cardiomyocyte-material interface, a 2D optical mapping technique was utilized. While the conduction velocity decreases in the myocardial matrix coated plates (indicating a possible reduction in

connexin gap junctions in these cells), the action potential duration at 20% repolarization is also observed to decrease (as is found in maturing cardiomyocytes). A further look into how changes in electrophysiology of the cardiomyocytes interacting with injected material could shed light over better use of materials and cells as treatment for HF.

4.2 Future Directions

Programmed Electrical Stimulation is a very powerful and useful technique and can be used to explore the risks of injectable substances as treatment for heart failure. It can provide critical information on arrhythmia inducibility and therefore can be an important tool for newly developed materials. However, the variability in experimental setup needs to be limited to better perform the experiments. Variability in factors such as infarct size and location of injected material should be better controlled.

The use of PES where materials are injected largely in the border zone where they can be more interspersed between healthy cardiomyocytes can provide useful insight on how arrhythmia inducibility is affected. Furthermore, PES can be applied to injections of cells together with materials. This can allow us to evaluate how the combined benefits of both substances fares against any new risks in arrhythmogenesis.

The 2D monolayer optical mapping was very convenient to measure electrical propagation properties of cells interfaced with materials. However, the ideal situation would be whole heart optical mapping with injected material to see how the global

electrical properties are altered and to study the exact mechanism of arrhythmia formation.

Using larger sample sizes can help obtain significance in data that showed strong trends. For future 2D and whole heart OM studies, the processing done on the raw data must be bettered to allow for faster analysis and less noisy data. Many other parameters can be gathered from optical mapping if the right programs are used -- action potential amplitude, maximum capture rate, resting potential, and so forth can also provide more clues to lead us toward an optimized treatment for heart failure.

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