High Resolution Multi-parametric Diagnostics and Therapy of Atrial Fibrillation: Chasing Arrhythmia Vulnerabilities in the Spatial Domain

Sarah Gutbrod
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High Resolution Multi-parametric Diagnostics and Therapy of Atrial Fibrillation:
Chasing Arrhythmia Vulnerabilities in the Spatial Domain
by
Sarah Renee Gutbrod

A dissertation presented to the
Graduate School of Arts and Sciences
of Washington University
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# Table of Contents

List of Tables ............................................. v

List of Figures ............................................. vi

Acknowledgments ........................................... ix

Abstract ..................................................... xii

1 Introduction ............................................. 1
   1.1 Atrial Fibrillation .................................... 1
   1.2 History of Defibrillation ............................... 5
   1.3 Shift to Low Voltage Defibrillation for Atrial Fibrillation .... 18
   1.4 Scope of Dissertation .................................. 21

2 Methods ................................................... 23
   2.1 Animal Models ......................................... 24
   2.2 Optical Mapping ....................................... 28
   2.3 Signal Processing of Arrhythmias ...................... 31

3 Quantification of the Transmural Dynamics of Atrial Fibrillation by Simultaneous Endocardial and Epicardial Optical Mapping in an Acute Sheep Model ............................................. 45
   3.1 Introduction ........................................... 45
   3.2 Methods ............................................... 48
   3.3 Results ................................................ 52
   3.4 Discussion ............................................ 60
   3.5 Limitations and Future Directions ...................... 65
   3.6 Funding Sources ...................................... 66

4 Investigating the 3D Substrate of Atrial Fibrillation in a Canine Model 67
   4.1 Introduction ........................................... 67
   4.2 Methods ............................................... 68
   4.3 Results ................................................ 72
   4.4 Discussion ............................................ 75
   4.5 Future Directions ..................................... 77
4.6 Acknowledgements ................................................................. 79
4.7 Funding Sources ................................................................. 79

5 Iterative Advancements Towards Low Voltage Defibrillation Delivery Strategies for Atrial Fibrillation .................................................. 80
  5.1 Introduction ........................................................................... 80
  5.2 Methods .............................................................................. 83
  5.3 Results .................................................................................. 87
  5.4 Discussion ............................................................................ 89
  5.5 Acknowledgements ............................................................... 91
  5.6 Funding ................................................................................. 91

6 Multifunctional Stretchable Electronics Quantify the Spatial Electrophysiology of Cardiac Tissue ......................................................... 92
  6.1 Introduction ........................................................................... 93
  6.2 Methods .............................................................................. 94
  6.3 Results .................................................................................. 100
  6.4 Discussion ............................................................................ 109
  6.5 Future Directions ................................................................. 112
  6.6 Funding ................................................................................. 113

7 Conclusions .................................................................................. 114

Appendix A Quantifying Spatial Heterogeneity of Cardiac Electrophysiology ..................................................................................... 120
  A.1 Introduction .......................................................................... 120
  A.2 Datasets .............................................................................. 122
    A.2.1 Synthetic Data ............................................................... 122
    A.2.2 Experimental Data ......................................................... 123
  A.3 Methods .............................................................................. 123
    A.3.1 Texture Analysis: Haralick Features ............................... 123
    A.3.2 Texture Analysis: Run Length Matrix ............................ 124
    A.3.3 Inhomogeneity Index: Maximum Difference Phase Map .... 125
    A.3.4 Variogram ...................................................................... 125
    A.3.5 Orthogonal Regression ................................................... 126
  A.4 Results .................................................................................. 126
  A.5 Discussion ............................................................................ 129
  A.6 Future Directions ................................................................. 129
  A.7 Acknowledgements ............................................................... 130
Appendix B    Material and Fractal Designs for 3D Multifunctional Integumentary Membranes with Capabilities in High Definition Cardiac Electrotherapy ........................................... 131
B.1 Introduction ............................................................... 131
B.2 Fabrication ............................................................... 133
B.3 Methods ................................................................. 136
B.4 Results ................................................................. 137
B.5 Conclusions ............................................................ 138

Appendix C    Protocols and Analysis Code ........................................ 140
C.1 Guide to Optical Mapping of Rabbit Heart ............................ 140
C.2 Labview code for controlling digital relay switches .............. 142

References ................................................................. 144

Vita .................................................................................. 163
List of Tables

3.1 Detailed Dynamics for Two AF Episodes .......................................................... 59
5.1 Correlations between Domination Frequency and Defibrillation Threshold for Temporal Multi-stage Therapy ................................................................. 87
## List of Figures

1.1 Historical Evolution of Electrotherapy Instrumentation .......................... 7  
1.2 Naum Gurvich’s Impulse Defibrillator .................................................. 11  
1.3 Schematic of Virtual Electrode Polarization Theory of Anodal vs. Cathodal Shocks ................................................................. 16  
1.4 Summary of Multi-Stage Electrotherapy Results in Chronic Canine Model ...... 20  

2.1 Effect of Acetylcholine on Action Potential Duration ................................. 24  
2.2 Acute Rabbit Model of Atrial Fibrillation ............................................... 25  
2.3 Acute Ovine Model of Atrial Fibrillation ................................................ 26  
2.4 Acute Canine Model of Atrial Fibrillation ............................................. 27  
2.5 Mechanism of Voltage Sensitive Dyes .................................................... 29  
2.6 Simultaneous Optical and Electrical Recordings during an Applied Shock ...... 30  
2.7 Example Atrial Fibrillation Signal Translated to Phase Space .................... 33  
2.8 Comparison of Sinusoid, Chaotic and Random Signal .............................. 34  
2.9 Definition of Phase Singularity in Experimental Data ............................... 35  
2.10 Phase Conversion using Various Second State Variable Definitions ........... 36  
2.11 Complications of Applying Phase Analysis to Electrograms ..................... 37  
2.12 Matrix of Signal Conditioning Parameters ............................................. 38  
2.13 Fractional Differences in Phase Maps by Definition ............................... 39  
2.14 Inter-item Correlation of Wavefront Count ......................................... 40  
2.15 Frequency Relationship to Inter-item Correlation ................................ 41  
2.16 Variability in Phase Dynamics by Definition ....................................... 42  
2.17 Phase Singularity Incidence Maps ....................................................... 44  

3.1 Schematic of Transillumination Mechanism ............................................ 47  
3.2 Schematic of Experimental Set-up ......................................................... 49  
3.3 Characterization of Green Excitation vs Red Excitation ............................ 53  
3.4 Action Potential Duration Differences across Acquisition Modes ................ 54  
3.5 MRI Volume Reconstructions from Sheep Left Atria. ............................... 55  
3.6 Static Atrial Fibrillation Frequency Characterization ................................ 56  
3.7 Transmural Correlation of Wavefront Count .......................................... 57  
3.8 Angle of Propagation Differences across the Transmural Wall .................. 58  
3.9 Corresponding Endocardial and Epicardial Optical Action Potentials for AF Episode 1 and 2 ......................................................... 59  
3.10 Sequential Phase Map of AF Episode 1 ............................................... 60
B.1 Representative Device Integrated on Rabbit Heart and Illustration of Functional Components ............................................. 133
B.2 Finite Element Analysis of Mechanical Properties of Fractal Electrode Patterns ................................................................. 135
B.3 Successful Shock Delivery through Fractal Electrodes ......................... 138
C.1 Front Panel for Vector Relay Control ............................................. 143
C.2 Block Diagram for Vector Relay Control ......................................... 143
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Sarah Renee Gutbrod

Washington University in Saint Louis
August 2015
This dissertation is dedicated to Jake.

On my worst day, you convinced me that I could do this. Thank you for everything.

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ABSTRACT OF THE DISSERTATION

High Resolution Multi-parametric Diagnostics and Therapy of Atrial Fibrillation:
Chasing Arrhythmia Vulnerabilities in the Spatial Domain

by

Sarah Renee Gutbrod

Doctor of Philosophy in Biomedical Engineering

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Professor Igor Efimov, Chair

After a century of research, atrial fibrillation (AF) remains a challenging disease to study and exceptionally resilient to treatment. Unfortunately, AF is becoming a massive burden on the health care system with an increasing population of susceptible elderly patients and expensive unreliable treatment options. Pharmacological therapies continue to be disappointingly ineffective or are hampered by side effects due to the ubiquitous nature of ion channel targets throughout the body. Ablative therapy for atrial tachyarrhythmias is growing in acceptance. However, ablation procedures can be complex, leading to varying levels of recurrence, and have a number of serious risks. The high recurrence rate could be due to the difficulty of accurately predicting where to draw the ablation lines in order to target the pathophysiology that initiates and maintains the arrhythmia or an inability to distinguish sub-populations of patients who would respond well to such treatments.

There are electrical cardioversion options but there is not a practical implanted deployment of this strategy. Under the current bioelectric therapy paradigm there is a trade-off between
efficacy and the pain and risk of myocardial damage, all of which are positively correlated with shock strength. Contrary to ventricular fibrillation, pain becomes a significant concern for electrical defibrillation of AF due to the fact that a patient is conscious when experiencing the arrhythmia. Limiting the risk of myocardial injury is key for both forms of fibrillation.

In this project we aim to address the limitations of current electrotherapy by diverging from traditional single shock protocols. We seek to further clarify the dynamics of arrhythmia drivers in space and to target therapy in both the temporal and spatial domain; ultimately culminating in the design of physiologically guided applied energy protocols.

In an effort to provide further characterization of the organization of AF, we used transillumination optical mapping to evaluate the presence of three-dimensional electrical substrate variations within the transmural wall during acutely induced episodes of AF. The results of this study suggest that transmural propagation may play a role in AF maintenance mechanisms, with a demonstrated range of discordance between the epicardial and endocardial dynamic propagation patterns. After confirming the presence of epi-endo dyssynchrony in multiple animal models, we further investigated the anatomical structure to look for regional trends in transmural fiber orientation that could help explain the spectrum of observed patterns. Simultaneously, we designed and optimized a multi-stage, multi-path defibrillation paradigm that can be tailored to individual AF frequency content in the spatial and temporal domain. These studies continue to drive down the defibrillation threshold of electrotherapies in an attempt to achieve a pain-free AF defibrillation solution. Finally, we designed and characterized a novel platform of stretchable electronics that provide instrumented membranes across the epicardial surface or implanted within the transmural wall to provide physiological feedback during electrotherapy beyond just the electrical state of the tissue. By combining a spatial analysis of the arrhythmia drivers, the energy delivered and the resulting damage, we hope to enhance the biophysical understanding of AF electrical cardioversion and
design an ideal targeted energy delivery protocol to improve upon all limitations of current electrotherapy.
Chapter 1

Introduction

1.1 Atrial Fibrillation

“Close attention to the muscle surface at once reveals feverish activity; the whole surface is alive with twitchings, or coarser undulatory movements... This is the full condition which is properly termed fibrillation” — Sir Thomas Lewis [109]

Portions of this chapter appear in the following original publication:


The heart is an eloquently designed organ with a simple purpose: to perform as an indefatigable pump and maintain constant blood flow to the body. Performance relies on the finely tuned interplay of electricity, energy delivery, and the relentless generation of force. The sole function of the sophisticated electrical propagation is to facilitate a reliable timing signal for a synchronous beat with the greatest efficiency. Fibrillation is a pathological condition that occurs when the integrity of the timing signal is disrupted. Chaotic electrical conduction gives rise to quivering and ineffective contraction. In the ventricles the resulting mechanical insufficiency is a hemodynamic emergency and, if sustained, it is incompatible with life. Alternatively, the lethality of fibrillation in the atria develops more slowly. The uncoordinated motion promotes the formation of blood clots and the uncontrolled electrical propagation leads to irregular conduction through the atrioventricular node, which can trigger compensatory reactions [25]. As a result, atrial fibrillation (AF) increases the risk of developing
heart failure, dementia and stroke, and has a serious impact on quality of life and morbidity. Although AF is immediately less fatal, it is a leading cause of hospitalization in the United States, accounting for 3.2 million hospital-days per year and an annual healthcare burden of $26 billion [99]. According to the Rotterdam study the lifetime risk of contracting AF is 22.2% for women and 23.8% for men of European ancestry [78]. Moreover, AF is a disease of advancing age; prevalence doubles with every decade beyond 50 years of age. Consequently, the most affected population is growing in size and projection models calculate that as many as 12 million Americans may suffer from AF by 2050 [15].

Atrial Fibrillation Drivers

Unfortunately AF is a complex, heterogeneous, and metamorphic condition. In 1925 Thomas Lewis described AF as being, “the absence of all signs of normal auricular [i.e. atrial] contraction.” The most defining characteristic of AF (and sometimes the only similarity between two individual episodes) is the fact that it doesn’t resemble normal atrial conduction. The rate, morphology and stability of the abnormal electrocardiogram vary considerably across patients and across time. AF is often defined by what it is not because it is the simplest way to broadly describe such a diverse range of the electrical manifestations. For over a century, there has been a significant effort to quantitatively dissect the underlying substrate and the biophysical mechanisms of AF initiation and perpetuation. This ongoing effort has led to several conflicting schools of thought that continue to shape the way that AF is approached therapeutically. The controversy is well stated by Carl Wiggers:

As to the fundamental mechanism of fibrillation we have plenty of theories, but none is universally accepted. ... We may note in passing that [the differing hypotheses] all center around two ideas, viz. (a) that the impulses arise from centers, or pacemakers, or (b) that the condition is caused by the re-entry of impulses and the formation of circles of excitation [189].

Remarkably, both the focal and reentrant hypotheses of AF originated concurrently. Beginning in 1907 Rothberger and Winterberg postulated that AF could be sustained by multiple, rapidly firing ectopic foci or pacemakers [200, 153]. The numerous foci-induced local contractions prevented coordinated contraction. The same year, Mayer presented an observation in the jellyfish [124] that lead to the idea of reentrant mechanisms in the heart later formulated
by Mines [127] and Garrey [61, 62]. Most of this research was conducted by meticulously observing or filming the mechanical oscillations of the fibrillating tissue. In the years to come evidence accumulated on both sides of the argument. In 1920, Lewis demonstrated electrocardiographic evidence in support of a reentrant basis of both atrial flutter (AFL) and AF using 3 vectors to calculate the electrical axis of propagation [109]. The abnormal focal activity theory also garnered empirical evidence with the use of monophasic action potential recordings in response to altering environmental conditions and pharmacological agents [161], including a notable study on the focal application of aconitine that revitalized the argument for multiple centers of local activity in experimental fibrillation [156]. As each new generation of scientists took a side in this debate, more sub-theories were spun off. Reentry branched into theories of a single mother rotor vs. multiple persisting circuits. Based on a computer model, Gordon Moe introduced the multiple wavelet hypothesis, characterized by transient reentrant circuits that quickly die off and are replaced by the generation of new pathways instead of a stable rotor [131]. Although accepted for decades, the multiple wavelet theory is losing favor to theories that focus on organizing centers with some degree of stability. Despite decades of research and substantial technological advancements to recording systems that have greatly improved AF visualization, the general sentiment expressed in 1940 by Dr. Wiggers still stands valid: no theory of fibrillation is universally accepted.

The debate over competing theories is further complicated because AF is rarely seen in isolation of other co-morbidities or cardiomyopathies including: mitral valve disease, elevated atrial pressure, or atrial extension. Additionally, the geometry of the atria introduces structural complexities that obscure the mechanism of perpetuation. Unlike the ventricles, there are abundant anatomical discontinuities for a wavefront to anchor to. It is possible that the wavefront dynamically transitions between functional and anatomical reentry.

Likely there is no overarching answer to what sustains fibrillation in the atria. Instead individual AF episodes may lie along a spectrum between the various theories. If that is the case, the field needs to focus our efforts on ways to identify sub-populations of AF in order to direct therapy appropriately. Carl Wiggers provides guidance for how to proceed, “Although we are not yet in a position to suggest any hypotheses which satisfactorily explain either the inception or the evolution of the fibrillation process, we may profitably examine the clues which recent work offers” [189]. It is my hope that this dissertation adds to the discussion.
Evolution of Atrial Fibrillation Therapy

Due to the diversity and the metamorphic nature of the disease population and the lack of clarity behind the biophysical mechanisms of the disease, AF lacks a robust therapeutic approach. The competing concepts of AF perpetuation have implications for developing therapeutic approaches. Reentry requires a substrate, whether it is anatomic or functionally based on intrinsic spatial heterogeneities in the myocardial tissue. Therefore the goal would be to extend the refractory period or create linear lines of block to disrupt the circuit. For AF driven by multiple pathways, extensive ablation functionally divides the tissue and isolates small regions incapable of maintaining a circuit. Alternatively, if AF is maintained by ectopic foci, the primary goal of a therapy is to suppress automatic impulse generation.

Pharmacologic targets for heart rhythm disorders are particularly challenging (even if the primary goal of therapy was easily identifiable) because of the ubiquitous nature of ion channels throughout the body and the delicate balance of electrophysiological parameters that allow for proper conduction in the heart [148]. For those agents with cardiac or even atrial specificity, the titration of the drug is difficult. Anti-arrhythmic agents are often pro-arrhythmic under different conditions and AF is associated with severe ionic remodeling that progressively shifts the ionic balance [79]. Therefore temporal evolution of AF further convolutes the therapy decision tree. Many of the possible agents that have shown preclinical promise have not made a widespread clinical impact or worse have had an adverse effect. There are still many groups looking to improve the atrial selectivity and effectiveness of novel drug targets. Some options include taking advantage of drugs with frequency dependent activity. Ideally these agents would not intervene when the heart is in the natural sinus rhythm but would disrupt fast fibrillatory conduction [68]. However, for now the best pharmacological option remains a prophylactic anticoagulation therapy protocol. This approach falls short of reversing the pathophysiology underlying AF, instead simply reduces the thromboembolic risk.

Electrotherapeutic approaches, including electrical cardioversion by high voltage shocks or radio frequency (RF) ablation, offer an alternative methodology but these applications are not without limitations. High voltage shock therapy is a well-developed technique for ventricular fibrillation and when applied across the atria, it is effective at terminating the fibrillatory conduction. However, the physical and psychological pain associated with shocks prevented
the adaption of this therapy in an implantable form. Ablation began as a surgical procedure and is still performed that way today. However, many clinicians opt to use endovascular catheters to transmitter RF energy to burn focal points or lines across the surface of the atrial tissue. As suggested previously, the goal is to disrupt the generating source or the driving circuit of the arrhythmia. There is a substantial push to create feedback or alternative energy sources to provide clean and controlled transmural lesions with non-surgical ablation. The results of these procedures vary widely and it is not yet clear how to identify those patients who have the greatest chance of benefiting from such a procedure.

An ablation procedure or an electrical cardioversion is deemed successful if sinus rhythm is restored; however neither approach has a long-term guarantee. AF was reported to recur in up to 71% of paroxysmal AF patients within 5-years following an RF ablation [186]. This high recurrence rate could be due to the difficulty of accurately predicting where to draw the ablation lines in order to target the pathophysiology. Electrical cardioversion is a one-time restorative therapy but without an accepted implantable option it is not a viable method to prevent sustained AF at every occurrence. Under the current bioelectric therapy paradigm there is a trade-off between efficacy and perceived pain, both of which are positively correlated with shock strength. Contrary to ventricular fibrillation, pain becomes a significant concern in the treatment of AF due to the fact that a patient is generally conscious when experiencing the arrhythmia. To improve the efficacy of both ablation and electrical cardioversion the field may need to shift from a one-size-fits-all approach to mechanism-based personalized therapeutic modalities.

1.2 History of Defibrillation

"Scientists often study the past as obsessively as historians because few other professions depend so acutely on it. Every experiment is a conversation with a prior experiment, every new theory a refutation of the old." — Siddhartha Mukherjee [136]

Portions of this chapter appear in the following original publications:

In order to move towards a mechanism-based approach to electrical cardioversion, it is critical to first review the historical evolution of electrotherapy. The road to the first successful application in a patient is tied to the plasticity of the mechanistic theory behind why a high-voltage shock restores sinus rhythm. Hidden within the theoretical understanding of defibrillation is the ability to target the applied electric field in an individualized manner and thus reduce the energy requirement for success. A mechanism-based approach bolsters the campaign for the development of a painless and harmless electrical cardioversion strategy that could be successfully implanted in AF patients. As the use of electrotherapy on AF followed the application on VF, this history refers to the use of defibrillation in general without a distinction between heart chambers.

The goal of defibrillation is to restore a synchronized rhythm to the entire myocardium. A large electrical shock has been known to successfully achieve this objective for over a century with a long circuitous history of clinical use [59]. Over several centuries the evolution of cardiac electrotherapy has traversed long geographical distances, circumvented political and cultural roadblocks, and faced bold scientific and public opposition. Despite the many external setbacks, new advances in technology have persistently driven defibrillation from the naive application of newfound electricity by amateurs in the 18th century to the powerful, life-saving, clinical tool it is today. The historical narrative navigates many seemingly unrelated fields of science and technology, including zoology, physics, power and circuitry engineering, physiology, cardiology, and computer modeling. Tenacious key individuals, who dedicated their careers to reversing sudden cardiac death and understanding the biophysical interaction between the heart and applied electric fields, propelled the study of defibrillation across generations and continents. The story of electrotherapy offers a unique perspective on the role engineering has played in shaping the optimization of delivery, the reduction in shock strength, and the ever evolving, working hypothesis of the mechanism of a successful defibrillation attempt.

**Early Naive Applications of Electrotherapy**
The therapeutic potential of applying electricity directly to the human body was recognized long before the language of physics and physiology caught up to explain the observed phenomena. In ancient times, electric fish were used in both South American and Greek societies for remedial purposes [194, 203]. These antiquated societies observed that a discharge from these fish had the ability to numb tissue. In fact the ancient Greek name for the electric fish is the root word for narcotics. The therapeutic use of the fish’s natural abilities is shrouded in myth and the efficacy of shocks delivered in such an uncontrolled manner is no longer known but there is evidence of prescribed use of the fish in the treatment of chronic pain diseases ranging from headaches to arthritis and gout from as far back as the first century [56]. Remedies involving live electric fish can be traced from antiquity to the middle ages, carried across civilizations within medical texts. However, the connection between these fish and electricity was not immediately made after the discovery of electricity in the 18th century. The first knowing medicinal application of electricity followed the invention of instrumentation that could produce electricity at will by storing the charge. The evolution of these early capacitors are shown in Figure 1.1 from the fish to Otto von Guericke’s sphere and the Leyden jar [177, 77, 176]. One simply had to charge such a device as the Leyden jar with a static generator like the sphere and then connect the circuit through the patient to deliver a large electrical shock. Although not designed with the intention of medical applications, the prospect of therapeutic electricity arose from researchers who experimented on the effects of electricity when applied to themselves. There was no physiological foundation for the use of electrotherapy, only a simple fascination with the new discovery of electricity.

![Figure 1.1: Historical Evolution of Electrotherapy Instrumentation.](image)

Figure 1.1: Historical Evolution of Electrotherapy Instrumentation. a) Electrical fish provided the first source of current for electrotherapy [56], b) Otto von Guericke’s sphere to create charge statically [177], c) the Leyden jar used to store charge [77], d) Alessandro Volta’s depiction of a voltaic pile, which would lead to modern batteries in implantable devices [176]
The first reports of electrical stimulators in hospital settings date back to 1767 and 1777 at London St. Bartholomew and King’s hospitals, respectively [37]. Electric shocks of up to 50,000V were naively applied, with a wide range of electrode placements and materials, to any number of disease states under the assumption that electricity was a cure-all. By the late 18th century there were multiple reports detailing the application of external shocks as a form of resuscitation; the first of which was a 1788 human resuscitation case reported by Dr. Charles Kite [103]. The Lancashire Society for Recovery of Apparently Dead published guidelines for attempting to revive a drowning victim stating, “let the powerful stimulus of electricity be applied by passing a shock through the heat, the balls of the electrometer being no more than a third or half and inch from each other” [1]. By 1802 the use of electrical resuscitation was endorsed by the Royal Humane Society [2].

Unfortunately, theoretical support for the use of electrical stimulation lagged behind anecdotal evidence of success and there was widespread unregulated use of electrotherapy by both amateurs and hospitals alike. Empirical animal testing conducted during this time period did not add much enlightenment to when and why electrical stimulation worked [4]. The combined lack of an understanding of electrical conduction within the cardiac tissue and the lack of an existing method for monitoring the electrical activity of the heart significantly limited these experiments. Important mechanistic strides were made in the related field of animal electricity and muscle contraction, led by Galvani and his dancing frog legs [60], but anecdotal success appeared sufficient justification for non-standardized electrotherapy in humans.

Electricity was still a new technology, whose power was only beginning to be harnessed. The next advance in electricity would come from the realization that the fish of antiquity were discharging electrical shocks without constantly shocking themselves and must therefore have a unique way of storing charge. Alessandro Volta, using John Hunter’s elegant dissections of the electric organ of the fish [56], cleverly developed the voltaic battery [176] (Figure 1.1). Volta’s invention, which he referred to as an “artificial electrical organ,” converts chemical energy into electrical energy to provide a practical continuous power source. Volta opened the door for new paradigms of electrotherapy that were not limited to a single explosive discharge. Providing an adequate and compact power source to the discharge device remains a significant aspect of the design of all defibrillators, including those used today. With the first reliable steady source of current, electrotherapists could now travel easily with their
device and carrying such a device (e.g. in a cane) became increasingly popular among amateurs and physicians in the early 1800s.

**Dark Days for Electrotherapy**

Within a century, electrotherapy would drastically fall in public opinion to such a degree that all interest in researching defibrillation would be wiped out from both lay and scientific communities. The new negative outlook on electrotherapy emerged following the widespread uneducated application of electricity to all manners of disease with outrageous claims of miraculous results. The credibility of defibrillation suffered greatly because of the implications that it could raise the dead and restore life. Such claims acquired widespread notoriety through popular literature. The public easily misunderstood and misappropriated defibrillation to sorcery and quackery. John Shoemaker wrote the following on electrotherapy:

> No remedy of equal value has been so misused; so much exaggerated as to intrinsic worth; or so greatly decried as worthless... the mechanism for operating electric force has improved much more rapidly than the intelligent use of it [163].

Ostentatious experiments such as John Aldini’s, whose studies on hanged or guillotined criminals in England and France were deemed grotesque by many, further damaged the public opinion of electrotherapy [8]. When defibrillation came back into favor in the next century it would be confined to the AC wall outlets of research laboratories without direct therapeutic intentions. Electrotherapy did not return to a public access therapy until Automated External Defibrillators were distributed in the late 1990s and early 2000s. In 1999 the American Red Cross included defibrillator training as part of the standard CPR training course to increase awareness on how to use the publicly accessible devices. Learning from past mistakes, these devices are tightly controlled with little interaction required. The success of these devices relies on sophisticated detection algorithms that do not require medical training to identify fibrillation and deliver shocks only when safe and appropriate.

Although the appeal of electrotherapy declined, research investigating the condition underlying cardiac death continued. In the second half of the 19th century, several key European physiologists including Carl Ludwig (Germany) [82], John MacWilliam (Great Britain) [126]...
and Edmé Vulpian (France) [178] published fundamental observations on the onset and progression of arrhythmias, including electrically induced ventricular fibrillation (VF) and AF. In 1899, physiologists Prevost and Batelli at the University of Geneva reported the first application of experimental defibrillation in a footnote of a paper on VF initiation by faradization. Prevost and Batelli used a capacitor discharge in the range of 2,400-4,800 V, delivered along a vector from the small intestine to the mouth, to defibrillate a canine [146]. Regrettably this important observation went largely unnoticed for a number of years because it remained unlinked to a clinical need. Defibrillation gained relevance only when electricity companies like Consolidated Edison and Bell Telephone Laboratories offered funding for research on electrocution to protect their workers [20], and doctors, whose patients were accidentally overdosed on anesthetics, began looking for ways to revive them [3].

**Geographical Divides**

At this point in history the development of defibrillation diverges geographically. For many years there are simultaneous, isolated experiments conducted in the Western world and the Soviet Union, both of which contribute to the current state of defibrillation.

In America, William Kouwenhoven and physicians at Johns Hopkins University conducted experiments delivering alternating current (AC) to electrodes in direct contact with the canine myocardium to induce fibrillation. An accidental second shock arrested the fibrillation and reproduced Prevost and Batelli’s successful defibrillation results. The American group then systematically investigated the effects of the strength (2,200V, 0.4-8A) and duration (0.1-5s) of applying 60Hz AC to canine hearts in fibrillation [85]. These experiments were conducted with transthoracic and epicardial electrodes, and concluded that transthoracic defibrillation required 5 times the current as epicardial defibrillation. They also tested the effects of the size of the electrodes on defibrillation success. Another American group, Ferris, King, Spence, and Williams, compared 60Hz AC, 25Hz AC and direct current (DC) and found that 60HZ required the least current [55]. However, they were testing long duration shocks of >1s. They hypothesized that there was no difference in defibrillation threshold (DFT) for shorter shocks, without performing the study. This oversight may be why AC shocks persisted much longer in the West. In the 1930s, defibrillation failed to restore sinus rhythm in 40-50% of the animal experiments in which it was performed. Carl Wiggers from Western Reserve University investigated the causes behind this high rate of defibrillation
failure. He meticulously described the compounding effects of VF-induced ischemia on the ability to defibrillate successfully, emphasizing the importance of early delivery. Wiggers also observed that a very brief and strong countershock was most successful and introduced an idea he called serial defibrillation, which delivered a series of 3-7 weaker AC shocks with a 1-2s interval between shocks [190].

In Russia the two critical players are Lina Shtern and her graduate student Naum Gurvich. In 1939, Gurvich published results on the superiority of monophasic DC shocks over the traditional AC, delivering shocks in a range from 2,000 to 6,000V [72, 71]. DC provided safety advantages as well as a decreased risk of re-induction. The Russian group found more pronounced shock-induced mechanical stunning after exposure to AC compared to DC [69]. Gurvich also increased the complexity of the capacitor circuit used to deliver the transthoracic shock by adding an in-line inductor. The circuit, waveforms, and the device that Gurvich used are shown in Figure 1.2 [72, 71]. This design was the first significant step in waveform optimization to reduce the DFT and cardiac damage associated with strong electric shocks. Improving the circuit in this way dispersed the energy better in time, decreasing the peak current and lengthening the duration.

![Figure 1.2: Naum Gurvich’s Impulse Defibrillator. a) Capacitor-inductor circuit implemented to create a biphasic dc discharge, b) the various waveforms used in animal experiments, and c) the transthoracic biphasic defibrillator built in 1962. All panels adapted from [69].](image)

Claude Beck and his team from Western Reserve University were the first to successfully apply defibrillation in a patient in 1946 [20]. When conducting a thoracic surgical procedure
on a 14-year-old boy, the patient’s heart began to fibrillate. Since Beck already had direct
access to the heart, he borrowed a system from the research lab down the hall and applied
110V, 1.5A AC shock, after using an ECG to confirm VF. He did not include the duration
of the shock in his case report. Upon delivery of the second shock, the heart returned to a
fast but regular rhythm and the patient made a full recovery. In 1956, Paul Zoll expanded
the successful clinical use of defibrillation to include transthoracic application on humans
with 710V, 15A AC for 0.15s [211]. The widespread use of electrocardiography provided
a method to confirm VF as well as a way to confirm successful defibrillation. The data
associated with repeated successes would promote the public perception of defibrillation as
a viable therapeutic option instead of repeating the earlier 18th and 19th century failed
attempts for legitimacy and acceptance.

With a proven clinical application, the question began to change from “can we defibrillate?”
to “how can we defibrillate better?” Defibrillation research entered an era of incremental
optimizations of the shock waveform. American researchers finally caught up to the Soviet
research, confirming that DC was superior to AC shock. Bernard Lown led the investigations
in the United States reaching the same conclusion that using an inductor to attenuate the
capacitor discharge was necessary to limit myocardial damage [118]. He proposed the Lown
waveform, an underdamped impulse with duration of 2.5ms for clinical use to reduce rise time
and pulse duration. Lown also published his ideal circuit components: a capacitance of 16µF
and an inductance of 100mH to allow for widespread replication of his results [117]. Bohumil
Peleska (Czechoslovakia) conducted rigorous instrumentation evaluations to minimize peak
voltage and current as well as minimize total energy [143]. These two goals remain the
principle aim of current defibrillation development. John Schuder from the University of
Missouri at Columbia introduced the idea of short-circuiting the capacitor after a time delay
to remove the low voltage decreasing exponential tail after observing that it could re-induce
fibrillation [159]. The truncated exponential pulse waveform gained popularity because the
relatively simple circuit lent itself to implementation of a fully implantable, automated,
catheter-based defibrillator conceived independently by both Schuder [158] and Mirowski
[129].

Theory Shapes Therapy
Defibrillation is not a therapy that was born out of a strong biophysical foundation. The mechanistic studies trailed far behind the empirical clinical use of the tool. Investigations into the interaction between tissue and applied electrical shocks were hindered by limitations in available recording instrumentation. Consequently, the precise physiological mechanism of the electrical requirements for successful defibrillation proved to be a complex problem to answer. Across decades the principle hypotheses have been shifting and sometimes even contradictory.

The original motivation behind applying a shock across the heart, based solely on observing mechanical contractions, was to incapacitate or stun the tissue. The temporary stunning hypothetically allowed the heart time to “reset”. Gurvich was the first to propose that the shock actually stimulated the heart [69] but even this description did not offer a lot of guidance for designing an optimal shock delivery system. Traditionally, defibrillator design has been driven by the need to simplify and minimize the circuitry necessary to provide a relatively safe shock that can achieve high shock strength above the DFT. Investigating factors that influence the DFT or proposing hypotheses to improve defibrillation efficacy based on biophysical mechanisms is a relatively new endeavor in the field [70].

The main premise of the early electrophysiological theories was that the electric field produced by the shock needed to be strong enough to stimulate the entire myocardium, including regions in various states of repolarization and refractoriness. This theory of total extinction states that only a homogenous depolarization interrupted the fibrillatory patterns and reset the tissue [85]. Some researchers amended this theory asserting that it was sufficient to capture enough tissue to halt all activation fronts, which did not necessarily include the entire excitable myocardium [190]. From this idea the critical mass (CM) hypothesis was formed. The CM hypothesis began as an investigation into the mechanism of maintaining continuous fibrillation but was quickly applied to achieving successful defibrillation as well. Early 20th century experiments at Washington University in Saint Louis led Garrey to postulate that the inducibility and continuation of VF was related to the mass of myocardium [62]. In 1975, Douglas Zipes presented experimental evidence in support for this theory as it applied to chemical defibrillation mechanisms. By selectively depolarizing regions of tissue chemically, Zipes demonstrated that a CM of myocardium could maintain VF and lead to defibrillation failure [210]. Correspondingly, a shock could succeed if it interrupted enough activation
fronts to isolate the patterns to small regions below the CM. Any remaining activation fronts would then self-terminate, leading to successful restoration of sinus rhythm.

Another theory born of the same early concept is the extension of refractoriness. This idea suggested that an applied shock uniformly extended the refractory period of the captured tissue. Extending action potential durations across the tissue resulted in simultaneous recovery, which wipes away the “memory” of the fibrillatory waves maintaining the arrhythmia [169]. Alternatively, the theory of upper limit of vulnerability [53, 34] supported the idea that regions of weak potential gradient were critical to providing a source for maintaining a large enough area for early activation and the continuation of fibrillation. Although these theories are not necessarily mutually exclusive, they did lead to different ideas of how to optimize defibrillation. Several groups designed defibrillation schemes and new lead configurations to target areas of low potential gradient after shock delivery or to reach the CM necessary for success [91, 187, 38]. Both of these theories supported the claim that high voltage shocks were necessary for success. However, as evidence emerged to support a delicate balance between defibrillation and myocardial damage, scientists began to look for new ways to decrease the energy required for success. Shock strength is also positively correlated with the perception of pain, which can be severe for shocks of defibrillation strength. The potential risk of myocardial damage and the pain, both physical and psychological, associated with implantable devices has driven research for lower energy defibrillation schemes.

In the 1980s and 1990s, empirical studies began to emerge, supporting the idea that Gurvich’s biphasic damped sine waveform and other biphasic waveforms were superior over monophasic pulses [87, 179, 199]. As high-power amplifier designs improved, new waveform shapes could be easily implemented in research environments; rectilinear, triangular, trapezoidal, and triphasic waveforms were tested [87, 179, 193, 86, 154]. Scientists began to probe the response to applied shocks at various scales, especially with computational studies. Using resistor-capacitor simulations to model the cardiac cell membrane’s response to applied shocks, several groups demonstrated a shape dependence on efficient delivery of energy [88, 135, 147], which was supported by empirical observations. Based on these predictions the truncated biphasic exponential waveform delivers energy inefficiently due to the time constant of the cell and an ascending trapezoidal waveform has the lowest theoretical DFT. Years later, the ascending waveform would also be shown to cause less damage independently of the decrease in efficacy [88].
Still, further advances required a more in-depth understanding of the mechanism of defibrillation failure. Fortunately, two new scientific research methodologies were introduced that revolutionized the field’s ability to study cardiac stimulation mechanistically and with high resolution. The first was a computational approach called the bidomain model [173]; the second was an experimental technique using fluorescent voltage-sensitive dyes embedded in the membrane called optical mapping [134]. Optical mapping (which will be discussed in detail in Chapter 2) allows for the uninterrupted exclusive visualization of the electrical activity of the tissue before, during, and immediately after the shock application. The path of current from an externally applied field flows through the extracellular and intracellular subdomains of the myocardium differently, creating voltage gradients that result in changes in transmembrane potentials. The transmembrane changes are the driving forces of the shock-induced restoration of sinus rhythm through initiated wavefronts. Membrane potential dyes measure the electrical activity with specificity for the signal that is mechanistically determining a successful defibrillation that cannot be achieved with extracellular electrodes. Optical mapping and bidomain models target both root causes of the failure of defibrillation: 1) the persistence of pre-shock fibrillatory waves and 2) successful defibrillation followed by re-initiation of new shock-induced wavefronts.

Both these concepts are integral in supporting a new hypothesis that emerged describing the transmembrane response to applied stimulation. This new theoretical hypothesis, called the virtual electrode polarization (VEP) theory, transformed the direction of defibrillation research [149, 48]. The theory states: the external application of an electric field induces regional polarizations known as the virtual cathodes and virtual anodes, often opposing the regions of structural or functional heterogeneity. As a secondary effect, new wavefronts and phase singularities can be initiated from these regions that can lead to defibrillation failure [49]. The theoretical foundation of this idea was formulated by Sobie et al., which was termed the “generalized activating function” [166]. The generalized activating function shows mathematically how a combination of field gradients, underlying heterogeneities in refractoriness and anatomical discontinuities in conduction properties can create neighboring regions of virtual anodes and cathodes, which simultaneously hyperpolarize and depolarize different regions of tissue. This shock-induced difference in the excitability of the tissue can form secondary sources and induce new fibrillatory wavefronts. The landmark implication of this new theory was that defibrillation efficacy was not only dependent on the properties of
the electric field but also on the intrinsic properties of the tissue structure and conductivity in a predictable fashion.

A critical computational study discovered that unequal anisotropy in tissue conduction results in a complex spatial dependence of potential distribution in response to large currents, which influences the location and the shape of the virtual electrodes [162]. Using cellular monolayers with patterned regions of discontinuity in the conduction properties, researchers were able to demonstrate how virtual electrode shapes and positions were predictably dependent on shock polarity [188]. The spatial regions of hyperpolarization and depolarization mirrored each other with reversed polarity.

![Figure 1.3: Schematic of Virtual Electrode Polarization Theory of Anodal vs. Cathodal Shocks. a) Post-shock activation of an anodal shock. b) Post-shock activation of a cathodal shock, which is more likely to sustain reentry. Modified from [207]](image)

Additional studies showed that the chirality of the VEP-induced reentrant path could be predicted from the postshock VEP pattern as the wavefront moves into the de-excited region [35]. Combining the research of polarity dependent spatial patterns of secondary sources and the reentry chirality dependence, optical mapping experiments were used to explain the mechanism for monophasic anodal shock superiority compared to cathodal shocks [207]. The VEP distribution after anodal shocks behaves like a sink; with the induced pathways traveling inward and colliding with each other to self terminate any shock-induced wavefronts. The VEP distribution of cathodal shocks behaves more like a source, leaving more spatial room to develop into a sustainable reentrant wave. In Figure 1.3 [207], panel a illustrates the sink phenomenon of anodal shocks with a schematic drawing showing a simple VEP pattern and the crowded inward propagation that is more difficult for a reentrant path to escape. Alternatively panel b shows the source behavior of cathodal shocks, illustrating the space
that the virtual anodes provide for a reentrant path to thrive and direct propagation outward to the excitable regions. The pattern in the schematic also shows how phase singularities (represented by solid black circles) can arise at the transition between the virtual electrodes with opposing polarity. In this hypothetical case, the cathodal shock induces quatrefoil reentry. The conduction velocity of the induced reentry is also dependent on the degree of polarization in the virtual anode; a more negative polarization increases conduction velocity. If the conduction is fast enough, the propagating wave will hit a line of conduction block before this tissue can recovery and an induced wavefront with self-terminate.

Furthermore, optical mapping helped reveal the mechanism behind the empirical observation that biphasic shocks were superior compared to monophasic shocks. Biphasic shocks immediately reverse the polarity and distribution of the virtual electrodes, greatly decreasing the heterogeneity in the post-shock tissue response. The mirrored VEP pattern also removes the excitable de-excited tissue, reducing the risk of VEP-induced phase singularities [49]. Empirical studies have demonstrated a low DFT for ascending waveforms. Mechanistically this is because the ascending VEP pattern achieves a greater degree of polarization, leading to a faster conduction velocity and an induced wavefront that is more susceptible to self-termination.

VEP is the most comprehensive theory describing the cardiac response to applied electric fields. It has successfully predicted a wide range of observable phenomena. Due to the diversity of arrhythmia properties, and the clear indication that DFT is dependent on intrinsic tissue conditions, it may be time for a shift in the energy delivery paradigm. The clinical field has not been quick to adopt new defibrillation strategies. Even small but powerful increments such as switching to a biphasic waveform, faced opposition to incorporation into clinical devices [198]. New methodologies that require a substantial shift in strategy also require significant redesigns of circuitry and therefore, the reduction in voltage and energy must be equally considerable without loss of safety to merit redesigns.

That is not to say that there has been no progression in implantable devices since the switch to biphasic waveforms. Clinical improvements have focused on sensing algorithms, programmability, battery miniaturization, and lead development with great success [74]. Shock delivery in both internal and external defibrillation has recently been improved by incorporating impedance compensation into the shape algorithm [94, 113]. Although simple in
concept, the idea of altering the waveform to provide more consistent current delivery was the first step in patient-tailored defibrillation strategies. By exploiting the shock-induced heterogeneities predicted by the VEP theory, we may be able to design patient-specific electrotherapies that are gentler than the gold standard approach. Continuous new advancements in monitoring and data acquisition techniques have shaped and will continue to shape the resolution and scale at which scientists can probe the state of cardiac tissue. These advances will continue to drive the mechanistic understanding of defibrillation to new depths and propel new innovations in the implementation of defibrillation. In the words of Vice-President Hubert Humphrey, who was responsible for eventually bridging the geopolitical divide between Soviet and American defibrillation groups, research on defibrillation can be described as “at least a partial conquest of death... [it is] the oldest category in the world—but one which commands our newest efforts” [89].

1.3 Shift to Low Voltage Defibrillation for Atrial Fibrillation

After the success of implanted devices for VF, researchers applied similar devices to the treatment of AF with shocks of similar magnitude. These devices, known as implanted atrial cardioverters (IAC), overlooked an important design consideration that differs from VF. Patients in AF are hemodynamically stable and therefore conscious when an implanted device responds with a high voltage shock. Initial clinical trials of early IAC devices indicated that they had high specificity and sensitivity to AF detection and delivered effective shock therapy [157]. However, they did not gain patient acceptance, primarily due to the fact that the energy level needed to achieve cardioversion by a single biphasic intracardiac shock was 1-3 J, which exceeded the pain threshold [130]. Murgatroyd and Camm reported that patients required sedation for shocks >1.1J [137]. The fear, anxiety, and psychiatric complications associated with the painful shocks severely affected patient quality of life [58].

A retrospective study conducted on 119 patients with IACs found severe descriptions of the perception of a shock including “a knife to the heart”, “being hit by a truck,” or “being kicked by a mule.” In this study 23% constantly dreaded the shock, and 5% admitted that they would rather take their chances with the IAC removed [5]. Moreover, current electrotherapies
are not entirely innocuous; many studies have demonstrated evidence of myocardial damage as a direct result of the application of high-energy shocks including the generation of free radicals, morphological destruction, and functional impairment [50, 32, 17]. In all of these studies the degree of damage was positively correlated with shock strength [205]. Several clinical trials have shown that patients with an implanted cardiac defibrillator who received either appropriate or inappropriate shocks have an increased risk of mortality compared to those that received no shock [145, 155]. This risk has also been shown to increase with the number of shocks received.

Since the biphasic capacitor discharge waveform has been incrementally optimized since its establishment in the field, it is unlikely that significant further DFT reduction can be achieved if the waveform is constrained to this traditional single shock premise. Thus, there is an acute need to explore novel physiological mechanisms, biophysical methods and engineering approaches for low energy internal atrial defibrillation to transition away from a one-size-fits-all approach [44]. Some reports suggest that high peak energy is more harmful than high cumulative energy [167]; therefore dispersing energy across a sequence of shocks may be the best option for reaching towards a tolerable pain and damage-free therapy.

For over a decade, a primary initiative of the Efimov lab has been to explore a physiological basis for a painless defibrillation strategy. The work in this dissertation builds upon previous work and continues to use the VEP hypothesis to shape shock delivery. Initial studies were based on highly organized arrhythmias (i.e. substrate based ventricular tachycardia or AFl) and the concepts gleaned have been applied to increasingly more chaotic arrhythmias. The first pivotal study observed that the shock-induced VEP pattern has a phase dependence [150, 112]. When properly timed with respect to the activation wavefront, the heterogeneous response can be taken advantage of to dramatically lower DFT [112, 12]. Since it was not straightforward to predict the ideal phase, the therapy was adapted to include multiple low voltage pulses across 1 cycle of ventricular tachycardia. Additional studies demonstrated that multiple closely timed shocks (within 1-2 cycles of arrhythmia) terminate AFl/AF with significantly lower energy than a single shock in an ex vivo rabbit model of Acetylcholine-induced AF [12]. This idea advanced from multiple shocks to a multiple-stage electrotherapy (MSE), which had a markedly decreased DFT compared to a single biphasic shock in an acute model of AF in an open-chest canine model. The feasibility of delivering the MSE from a traditional lead based approach was confirmed in a closed chest model of self-sustaining AF
in a canine model [90]. Figure 1.4 summarizes the results from the *in vivo* delivery of the MSE compared to a single biphasic shock (BPS). MSE significantly reduced the total energy (0.16 ±0.16 J vs. 1.48 ±0.91 J) and the peak shock voltage (31.1 ±19.3 V vs. 165 ± 34 V). More details on MSE and this study will be discussed in Chapter 5.

The lab has primarily focused on creating a sequence based on the temporal frequency of electrical stimulation. However, the spatial dispersion of applied energy has also been known to have a significant impact on defibrillation efficacy for nearly 40 years when Tsukerman et al. first described a rotating current field [172]. Then in the late 1980s and early 1990s there was a large push in the field to investigate new lead configurations for both atrial [38] and ventricular arrhythmias [95, 187]. Many of these studies focused on an orthogonal dual current path designed to target areas with low potential gradients after the first shock was applied, in order to achieve near uniform dispersion of applied current densities. These
studies achieved remarkable DFT reductions; however, it was still not enough to restore sinus rhythm painlessly. We believe there is room to further the understanding of how the direction of the electric field interacts with the anatomical and functional geometry of the heart. Studies have shown a clear directional sensitivity to applied electric fields on isolated single cells, where an electric field parallel to the cellular axis was superior to an electric field applied perpendicularly [174]. The organization of fibers and gap junctions on the tissue level complicates this correlation but it still supports the claim that different vectors may engage different tissue layers/regions. The increased disorder of AF and dispersion of functional and structural heterogeneities in the tissue might require several “optimal” vectors in order to target the applied electric field to the sources.

1.4 Scope of Dissertation

In this dissertation I aim to address the limitations of current AF electrotherapy by diverging from the traditional circuits driven approach. My central objective is to work towards the design of physiologically directed applied energy protocols for AF termination that are gentle on the heart. Such an approach relies on extracting detailed spatiotemporal organization of AF drivers from the chaotic electrical activity and exploiting the physiological tissue response to applied electrical shocks described by the virtual electrode hypothesis. In this dissertation I use novel optical imaging techniques and implement signal processing algorithms on a diverse array of AF animal models to characterize the spatiotemporal dynamics of AF before, during, and immediately following high voltage shock delivery. I designed and tested the efficacy of strategies targeting arrhythmias in both the temporal and spatial domain. I also developed a platform of novel instrumentation to address the lack of feedback information available to assess the immediate tissue damage incurred from a shock to further guide future therapy development. In Chapter 2, I present the disease models, experimental techniques and processing algorithms used to conduct the studies included in this dissertation. Chapter 3 follows with a study characterizing the discordance of AF dynamics across the transmural wall in an acute model of AF. In Chapter 4, I apply the same techniques to compare the transmural AF dynamics in another animal model and investigate the presence of structural discontinuities that can harbor 3D substrates in the atria. In Chapter 5, I present the evolution of the multi-stage electrotherapy including the incorporation of spatial rotation
patterns. I explore empirical reductions in DFT in two models. Finally in Chapter 6, I introduce the design and application of a stretchable electronics platform that conforms to the heart with a unique array of sensors that can assess local markers of tissue damage. Each sensor used to investigate the interplay of electrical, mechanical and energetic shock responses is accompanied with a feasibility study in an appropriate model.

It is my hope that these studies will help lead to the convergence of a successful defibrillation threshold and the pain threshold so that IAC can become a viable option to treat AF and prevent advanced remodeling. I also hope to enhance our understanding of the dynamic interaction of wavefronts that leads to termination and continue transforming the field of defibrillation into one driven by biophysics. The novel stretchable electronics platform developed and validated as part of this project revolutionizes the amount of spatial information we can gather on the effects of defibrillation shocks on tissue. By combining a spatial analysis of the arrhythmia drivers, the energy delivered and the resulting damage I hope to enhance the biophysical understanding of AF electrical cardioversion and design an ideal targeted energy delivery protocol to improve upon all limitations of current electrotherapy.
Chapter 2

Methods

Portions of this chapter appear in the following original publications:

Meyers J, Gutbrod SR, and Efimov IR. ”Tachycardia Termination by Shocks and Pacing.”
Clinical Cardiac Pacing, Defibrillation, and Resynchronization Therapy. 5th Edition.
Editors; Ellenbogen KA, Wilkoff BL, Lau CP, Auricchio A, Neal Kay G. Elsevier Health
Sciences, 2015. Ahead of Print

There are many studies that speak to the diversity of clinical arrhythmias and it is a difficult
task to replicate the pathological substrate of AF in an experimental model. Clinically, AF is
delineated by its longevity, ranging from paroxysmal to long-standing persistent. However,
a new study implies a level of uncertainty in the traditional view of the AF burden [122].
Additionally, new discordance has arisen in the field about whether all AF is a progressive
disease, continually remodeling to better preserve the arrhythmogenic substrate or if the
concept of “AF begets AF” represents only a subset of clinical episodes [191]. Where AF
is progressive, the remodeling affects many facets of atrial function including electrical and
structural changes. Moreover, the changes occur along different time scales. Electrically,
there are changes in the ionic balance and the inter-cell connectivity, manifesting in APD
reduction, loss of rate adaptation and conduction slowing. Structurally, atrial tissue in AF
is marked by increased cell size, myolysis, glycogen accumulation, increased fibrosis and
a heterogeneous distribution of gap junctions. Some researchers have speculated that the
structural remodeling is an adverse effect of high metabolic stress from sustained rapid
contraction but the full mechanism remains unknown [9]. Consequently, it is difficult to
recapitulate the spectrum of clinical AF conditions in a single model.
2.1 Animal Models

![Graph showing the effect of Acetylcholine on Action Potential Duration in Ovine Model.](image)

Figure 2.1: Effect of Acetylcholine on Action Potential Duration in Ovine Model. The reduction in APD increases the vulnerability to arrhythmia induction.

The experiments presented in this dissertation were conducted on a variety of different animal models of AF including rabbit, canine, and sheep. The rabbit is the best choice for feasibility investigations, and technology development because the cost is low, the size is ideal for whole heart optical imaging and the atrial tissue can more appropriately be approximated as 2D due to smaller tissue thickness. In parallel, the large animal models are used to test efficacy of our novel electrotherapy paradigms and signal processing techniques because these models are more accurate representations of human anatomy and physiology. Some of the models are acute, using pharmacological agents or elevated pressures to acutely decrease the action potential duration (APD) and artificially provide room for a sustained arrhythmia in otherwise healthy tissue (see Figure 2.1 for example APD after Acetylcholine). In contrast, some of the studies are conducted on chronic models that incorporate time for progressive pathological remodeling and spontaneous AF initiation. All animal experiments presented within this dissertation have been approved by one of two institutional boards, depending on where the experiments were conducted. Those that were conducted in St Louis were approved by the Washington University Animal Studies Committee and those that were conducted in Bordeaux were approved by the Université de Bordeaux ethical committee.

**Acute Animal Models**

The acute AF rabbit model is a whole heart Langendorff perfused model with pressure-loaded atria, originally developed by the Allessie group [50]. The pulmonary artery is cannulated and connected to a pressure column of 10-15 cm $H_2O$. The pulmonary and caval veins are ligated and the interatrial septum is perforated to create a single pressurized atrial chamber.
Theoretically the elevated pressure activates stretch-sensitive receptors to provide transient electrical alternations to the APD. A shortened APD combined with the obvious dilation of the atria in this model (shown in Figure 2.2) provides more “elbow-room” for a sustained arrhythmia. AF is induced using programmed stimulation of an extra beat or 100 cycles of 50 Hz burst pacing. We have used this model to empirically test the temporal parameters of the first generation MSET, the effects of cycle length on efficacy of AF defibrillation, and optimize the hardware necessary for spatial patterns. Based on the LA bipolar electrogram recordings, this model had a dominant frequency (DF) that ranged from 11.96 to 20.75 Hz with a sample mean of 15.54 Hz.

Figure 2.2: Acute Rabbit Model of Atrial Fibrillation. The left panel displays the unloaded atria. The right panel shows the increase in surface area of the inflated atria. An example electrogram from an induced AF episode is also displayed.

The acute AF ovine model is based on artificially increasing the vagal tone in an isolated left atrial preparation. The whole heart is explanted and retrogradely perfused with cold cardioplegia. The left coronary artery is cannulated for perfusion. Under constant perfusion of cold cardioplegia the right atria and the ventricles below the main branch of the circumflex artery are removed by gross dissection. All vessel branches leading to the ventricles and right atrium were ligated. The preparation is flattened by opening the right superior pulmonary vein. Figure 2.3 shows a representative example of the preparation and the distribution of frequency content for 75 episodes in this model. AF is induced by adding 0.2µM -10µM
Acetylcholine to the perfusate and using 1 second of 50Hz burst pacing delivered to either the epicardium or the exposed endocardium. Acetylcholine suppresses automaticity and shortens the APD by binding to muscarinic receptors and acetylcholine activated potassium channels. Many studies have suggested that this model induces reentrant AF. The transient substrate is formed by Acetylcholine-induced spatial heterogeneity in repolarization. The burst pacing provides the trigger. AF episodes induced in this model had a DF ranging from $3.897 \pm 0.48$ to $10.03 \pm 2.88$ Hz with a sample mean of $7.21$ Hz. AF was maintained by 1-10 transient rotors, with at least 1 rotor present an average of 70% of the time signals were recorded.

Figure 2.3: Acute Ovine Model of Atrial Fibrillation. The epicardial and endocardial field of view is displayed once the preparation is isolated and stretched on the frame. The distribution of frequency contact displays the range of AF episodes that can be induced by tuning the amount of Acetylcholine.

The canine acute AF model is similar to the sheep except that this preparation includes both atria. After explant the ventricles are removed and all vessel branches leading to the ventricles are tied off. The tissue is dual perfused through cannulas in the left and right coronary arteries. The right atrium is flattened by an incision along the vena cava and the left atrium is opened along the right superior pulmonary vein. Figure 2.4 shows a representative example of the preparation. The distribution of frequency content from 34 episodes is also included in the figure. AF is induced by adding 0.5µM -1.0µM Acetylcholine to the perfusate and using 1 second of 50Hz burst pacing. AF episodes induced in this model had a DF ranging from 5.13 Hz - 10.28 Hz and an RI ranging from 0.11 - 0.71.

Chronic Animal Models

The chronic model of AF in this dissertation is a canine tachy-paced model first described in the goat by Allessie [191]. The animals are implanted with commercial devices with the
ability to pace continuously at a high pacing rate. We used Medtronic CRT Maximo devices with the addition of a custom software high rate pacing option. The pacing lead (Medtronic 5096) is placed through the right external jugular vein in the right atrial appendage. The animal is allowed to recover for at least 1 week before continuous atrial pacing at 400 bpm is initiated for 6-8 weeks. Effective refractory period remodeling was observed a couple of weeks before spontaneous AF. Weekly interrogations are performed until AF is sustained for >30 mins. Digoxin is administered (0.25 mg/kg-0.5 mg/kg) if the ventricular rate exceeds 130 bpm with high rate pacing (HRP). This model is used for both in vivo DFT experiments as well as ex vivo optical mapping experiments. The tissue preparation for the optical mapping is the same as the acute canine model without the addition of Acetylcholine. The chronic nature of this model promotes increased heterogeneity of the electrophysiological properties over time, and in turn, alters the AF circuits. Previous studies have demonstrated that rapid-pacing induced AF has reduced L-type calcium current and intracellular Ca\(^{+2}\) overload, which manifest as a shortened APD and a loss of rate adaptation. The potassium channel function is also altered, with spontaneous \(I_{k,ach}\) opening and reduced \(I_{to}\) current. Structurally, rapid-pacing induced AF leads to an accumulation of glycogen, and a redistribution of gap junction connexins. Interstitial fibrosis development is a major architectural change in clinical AF and although some studies have suggested a mild increase in fibrosis with this model, it does not replicate the clinical level of fibrosis [141].
2.2 Optical Mapping

Optical mapping is a critical experimental technique used to study electrophysiology and the biophysical mechanisms of electrotherapy. Membrane bound potentiometric dyes translate the intrinsic electrical signal carried by ions into an optical signal that can be collected with photodetectors. Dye molecules embedded within the membrane of cardiomyocytes emit a fluorescent signal with an emission profile that is dependent on the membrane potential. When the membrane potential changes (i.e. during an action potential), the dynamic wavelength of the emitted light can be recorded as a loss in fluorescence with appropriate filter settings on the acquisition hardware. Filters are specific for particular dyes (e.g. excitation 520 ± 20nm and emission >650 nm for di-4-ANEPPS). The change in emitted light is linearly related to the membrane potential, which enables the recording of “optical action potentials”. The mechanism is illustrated in Figure 2.5. The number of myocytes that give rise to an optical action potential signal depends on the dye, the optical magnification, the focal plane and the pixel size of the sensor that is used for recording. Based on the interaction of these parameters an optical action potential is a representation of a depth-averaged transmembrane potential.

Before the development of this technique, investigators were hindered by limitations in available recording instrumentation. No other technique offers large spatial coverage and fine resolution in both the spatial and temporal domain. Additionally, electrical shocks of defibrillation strength produce large artifacts in signals recorded with extracellular and intracellular electrodes. These artifacts overwhelm all intrinsic electrical activity in the presence of the applied electric field and blind investigators to the immediate post-shock tissue interactions. The polarization of the electrodes delays the recovery of the recording integrity even after the shock is completely delivered. Some researchers developed creative attempts to minimize the time that the electrical signal is overloaded, including using a non-polarizable reference electrode (only useful if both the tissue and the field are uniform) or by switching amplifier gains or connectivity on the acquisition systems during shock delivery [33]. These techniques decreased the recovery lapse in recording time down to as low as 20ms. Unfortunately, this is still too long to observe the immediate tissue response to the shock. The translated optical signal is isolated from the applied electric field but reflects the tissue’s response.
Figure 2.5: Mechanism of Voltage Sensitive Dyes. The fractional fluorescence that is measured by the CMOS camera is a function of a conformational change in the dye in response to a change in membrane potential that shifts the emission spectrum.

Optical mapping allows for the uninterrupted visualization of the electrical activity of the tissue before, during, and immediately after the shock application. Figure 2.6 shows an example of this phenomenon from an ex vivo rabbit heart study. The optical and electrical signals are recorded simultaneously when the shock is delivered at the lightning bolt. Both the local bipolar electrogram in contact with the tissue and the far field bath electrode require >1000ms to recover with this instrumentation amplifier. Meanwhile the immediate shock-induced depolarization is visible in the optical signal morphology as well as the quiescent delay until the arrhythmia resumes. With advances in optical sensors and custom dye properties, optical mapping has become a dominant driving force of cardiac electrophysiology research and was used in every stage of this dissertation.

A Micam ULTIMA single or dual system with 100 x 100 pixel CMOS sensors was used for voltage-sensitive mapping in all experiments. Optical signals are recorded for 1-8 seconds at a sampling frequency of 1kHz. Two different voltage sensitive dyes and filter settings were used depending on the experimental objectives. For deep penetration experiments di-4ANBDQBS was used with 660nm excitation. For superficial surface mapping experiments di-4ANEPPS was chosen with 520nm excitation. Muscle contractions can lead to changes
Figure 2.6: Simultaneous Optical and Electrical Recordings during an Applied Shock. This example highlights the advantage of optical mapping during defibrillation delivery as the integrity of the signal is not compromised like the electrograms.

in emission intensity unrelated to electrical activity. To remove motion artifacts the tissue is pharmacologically uncoupled prior to acquisition. We used Blebbistatin to uncouple the mechanical contractions. Blebbistatin is a cell membrane permeable myosin II ATPase inhibitor. Myosin is an essential component in the cardiac contraction machinery, which crosslinks with actin to generate force. Once bound, blebbistatin inhibits myosin binding to actin by preventing the phosphate release and keeping the myosin in a state with low affinity for actin [104]. It is important to note that while necessary for the acquisition of clean optical signals, adding Blebbistatin does alter the energetic state of the myocardium. This is an important limitation to all optical mapping that must be acknowledged. Adaptations of the technique that are specific to each project and the details of the methodology will be addressed in each individual chapter. A detailed protocol for rabbit optical mapping is included in Appendix C.
2.3 Signal Processing of Arrhythmias

Optical mapping provides excellent signal quality and spatial coverage. However, the complex and polymorphic nature of arrhythmias makes a quantitative analysis of the spatial map of signals remarkably challenging. The goal of signal processing algorithms is to extract underlying organization out of seemingly chaotic propagation and expose spatiotemporal patterns in the recorded potential signals. If the behavior is organized on some level then it may be possible to identify zones critical to fibrillation perpetuation that can serve as targets for intervention. It is important to note that recorded signals provide a short glimpse into an arrhythmia that may persist for months (e.g. persistent AF). The algorithm must have high sensitivity to any degree of spatiotemporal organization captured within the brief recording interval. Although this dissertation focuses primarily on optical signals, this chapter considers the challenges of algorithms for both electrical and optical recordings.

The most direct approach to quantifying the primary propagation pattern is to examine a spatial map of activation times. Activation maps identify the origin of a beat as well as regions of concurrent activation, which illustrate the spread of propagation. Activation is defined as the maximum negative derivative in a unipolar electrogram or the maximum derivative of an optical upstroke. Although easily applied to paced rhythms or simple tachycardias, identifying activation time is a difficult task during AF when there are multiple simultaneous wavefronts dynamically colliding and altering the local signal morphology, along with other variables including structural interference and signal artifacts. Several algorithms implement interpolation or data fit techniques to compensate for the diversity in signal morphology and improve the accuracy of extracting activation time [24]. Even though these algorithms were tested in the best-case scenario (i.e. healthy tissue) it was still computationally intensive to differentiate the local deflections from distant activity in an automated fashion and they did not offer a method to quantify the stability of such patterns. Healthy tissue is a rare occurrence clinically and fractionated electrograms further complicate the performance of such algorithms. Bipolar electrograms remove most of the far field signal contributions but introduce a dependence on the orientation of the propagating wavefront. Due to the difficulties associated with defining activation time and assessing the reproducibility of these patterns over time, researchers began to look for alternative approaches to identifying spatiotemporal patterns.
Spectral analysis is performed in an attempt to discretize regions by the rate of excitation [21, 165]. This approach decomposes the time series into discrete frequency bands. The dominant frequency for each time series is ascertained from the power spectrum in the frequency domain: $\omega_{max s.t.} |X(\omega)|^2 = \frac{1}{n} \left| \sum_{t=1}^{n} e^{-i\omega t} x(t) \right|^2 = \text{maximum}$. This approach is appealing because it is less dependent on the peak signal amplitude and therefore less prone to signal artifacts. However, since each recorded time series is represented by a single parameter, dominant frequency is a stationary measure of organization. The regularity and organization index [18, 51] are additional parameters that can be extracted from the power spectrum to quantify the dominance of a frequency band and attempt to capture the variability of frequency within the arrhythmia. These two measures quantify how well the signal can be approximated with a sinusoid of frequency equal to the identified dominant frequency. The difference between the two parameters is the inclusion or exclusion of harmonic peaks. These parameters provide a single stationary parameter in time to describe the excitation rate and the stability of the cycle length and looks for the spatial distribution of those parameters. There are time scales of organization, many of which are important to understanding the global architecture of fibrillation. Therefore a steady-state measure of the frequency content may contain some valuable information about organization but it is not a full representation of the dynamic nature. The ideal parameter would represent the behavior of the propagating wavefronts in both the temporal and spatial domain. Some researchers are working on implementing robust time-frequency analysis methods to look for temporal variations in dominant frequency [121]. Conduction velocity vector fields are another option to combine spatiotemporal dynamics. We use these approach throughout this dissertation. However, an alternative approach exists that has the capacity to look at both the temporal and spatial domains simultaneously: phase analysis.

Phase analysis was first introduced to the cardiac field through analytical models. Art Winfree drew an analogy to the way Alan Turing’s theoretical model preceded and guided the observation of how patterns develop in biology, a theoretical approach to excitatable media directed the interpretation of rotating propagation in fibrillation. In fact, the details on how to apply this technique directly to experimental observations in a standard manner are still being worked out. In the 1970s analytical models began to address how to replicate the spatiotemporal dynamics of chemical waves [195, 196]. This led to developing conditions in the reaction-diffusion models that would manifest in stable propagating rotors in excitable media. The language used to describe this propagation varies within the field including:
vortex, critical point, rotor, spiral wave, centers of self-organization, and pivot points of reentrant circuits. However regardless of the name it is given, the behavior resembles vortices in optics, fluid dynamics, and magnetic waves.

Due to the observed similarities, researchers turned to phase analysis, a non-linear dynamic systems analysis technique often used in physics and applied mathematics to quantify oscillating behavior and turbulence stability [23]. This approach is used to differentiate deterministic behaviors in chaotic oscillators from random noise. Along the frequency spectrum, chaotic signals sit between sinusoids and noise [175]. The behavior is aperiodic, but the signal is not random so a signal processing technique that filters out the dynamic fluctuations is not appropriate. Phase analysis involves a translation from the temporal domain into the phase plane, where organized quasi-periodic signals become closed loop trajectories and the angle along the trajectory with respect to a known origin is defined as the phase. Figure 2.7 illustrates this translation using a signal obtained optically from AF in a canine model. Cardiac fibrillation is a superposition of periodic and aperiodic signals. If the signal is arbitrarily random in time, the trajectory in the phase plane is equally random, whereas the trajectory in the phase plane has a definite shape if the system is deterministic. Reconstructing the signal in the phase plane differentiates chaos from random noise where frequency analysis cannot. Figure 2.8 compares a sinusoidal signal, a signal from a chaotic oscillator circuit known as Chua’s circuits [164], and a uniform random noise signal in the time domain, the
frequency domain, and phase plane, highlighting the advantages of using phase to differentiate the signals. The definitive shape of the chaotic oscillator in the phase domain clearly distinguishes it from noise. The chaotic signal has many qualitative similarities with potential signals during fibrillation, which is why phase was considered as a method for identifying underlying organization in arrhythmias.

![Image of sinusoid, chaotic oscillator, and random noise](image)

Figure 2.8: Comparison of Sinusoid, Chaotic and Random Signal. A representative sinusoid with a single frequency (blue), a Chua’s chaotic oscillator (black), and a Gaussian white noise (red) time series are displayed along with the corresponding frequency analysis and the conversion to the phase plane to illustrate how these parameters can be used to distinguish chaos from noise. Note the spread of the frequency bands and the clear trajectory in the phase plane that are characteristic of both chaotic signals and fibrillation recordings.

In order to perform the conversion into the new coordinate system there must be two parameters that oscillate in time and are out of phase with one another. The simplest models of cardiac excitability define two parameters to characterize physiologically observed behaviors: excitability and refractoriness. Therefore the models lend themselves to be easily converted into the phase plane. A unique value now defines each point in time within the period of an oscillating signal. Critically, with this method the period is a dynamic length of time instead
of a fixed value to adjust for beat to beat variability. A topological analysis of the phase can be used to extract patterns of organization and assess the stability of these patterns in time and space. In a stable two variable system the critical points are mathematically defined as the intersection of the nullclines in space[105]. This point is defined as a “phase singularity” (PS). In stable spiral waves this is also the point in space where the spatial gradient of phase diverges. It is possible to dynamically track the core of the spatial rotation through time using the mathematical definition to identify the location in each frame. PS trajectories can be used to provide insight into the substrate that is facilitating self-sustaining arrhythmias. More recently, PS tracking has become a method of identifying targets for ablation [138, 114]. Additionally, the appearance or disappearance of PS during defibrillating shocks can be used to assess the mechanism of a successful shock.

Figure 2.9: Definition of Phase Singularity in Experimental Data. a) Phase map at an instance of time of an experimentally recorded AF from an ovine model with a single sustained rotor. b) A map displaying the gradient of phase with the red inset at the point of divergence. c) A plot of the topological charge in 3D and 2D to identify the location of the phase singularity, where the topological charge equals +1 or -1.

Unfortunately, the application of phase analysis to experimental data is less straightforward. A singularity is not as rigorously defined in physiological terms. In clinical and most research cases only one variable is recorded from each electrode, the potential. New techniques were required in order to create a second state variable and track the phase transitions and singularities in a reconstructed phase plane [13]. Additionally, algorithms were introduced to identify PS that can no longer be defined as the intersection of the nullclines from the state variables. Instead a phase singularity is defined as a point around which all phases of acyclearrepresent[26,151,49]. This is calculated using the equation for topological charge, which evaluates a contour integral of the spatial gradient of phase: $n_t = \frac{1}{2\pi} \int_C \nabla \phi \cdot dl$. A PS has a topological charge of ±1 depending on the chirality of the rotation. All other locations
will have a topological charge of 0. This definition of PS is illustrated in Figure 2.9. Panel A displays a phase map from an optical mapping experiment on an ovine model of AF that is maintained by one rotor at a single instance of time. Panel B maps the gradient of phase with an inset at the point of divergence. The spatial map of the topological charge identifies the PS at the point of divergence in Panel C.

Figure 2.10: Phase Conversion using Various Second State Variable Definitions. In experimental data, only one variable is recorded (i.e. potential). Therefore a second variable must be created in order to convert the signal to the phase domain. A representative pixel from optical recording of acute AF in an ovine model is used to highlight the differences in the phase signal between three definitions of the second state variable: Hilbert transform (green), a time delayed potential signal where tau is defined at 25% of the fibrillation cycle length (purple) and a time delayed potential signal where tau is defined as the first zero crossing of the autocorrelation of the potential signal (black).

One approach to create a second state variable is to use the potential signal offset by a time delay \( V(t + \tau) \). The phase results are dependent on the selection of \( \tau \). Several groups have suggested different definitions of \( \tau \) including a fraction of the global cycle length or the lag at the first zero-crossing of the autocorrelation of \( V(t) \) [13, 26]. The rationale behind using the zero crossing was to create a second variable that is linearly independent from the potential time series, whereas a \( \tau \) that is based on the cycle length ensures that the two state variables are out of phase. Numerical studies have shown that the choice of \( \tau \) can influence the number and stability of PS from the same dataset [27]. The Hilbert Transform provides a more robust way to create a phase-shifted signal and has gained favor because it creates a linear transition through the cycle and removes the variability of an arbitrary time delay. This technique converts a real signal to a complex signal where the imaginary portion
is instantaneously shifted by $-\frac{\pi}{2}$. Figure 2.10 shows how the definition of the second state variable influences the phase with respect to time ($\phi(t)$) for an individual optical recording in an ovine model of AF. Simultaneously plotting the phase from a single pixel calculated using two time embedding approaches and the Hilbert Transform emphasizes the morphological variability in the phase transition, especially during the second action potential.

![Diagram](image.png)

Figure 2.11: Complications of Applying Phase Analysis to Electrograms. The conversion to phase is challenging when the state variable is multiphasic as seen in a unipolar electrogram from an intracardiac catheter during human AF. Two second state variables were used a) a time delayed potential signal and b) the Hilbert transform. The Hilbert transform applied directly to the unipolar signal has many false positive phase transitions that do not correspond to activation on the electrogram.

Another challenge to overcome is the effect of the signal morphology and the signal to noise ratio of the recorded data. The Hilbert Transform performs best for monophasic zero-mean signals. When the signal morphology deviates from this shape it becomes less appropriate to directly apply this transform. When the signal that represents activation is multiphasic in nature the phase signal will transition through a cycle inappropriately with respect to the physiology. The Hilbert Transform is also sensitive to noise fluctuations in the same way. This is very easily observed by applying the transform to raw electrograms as is shown in Figure 2.11. In this figure signals from an intracardiac signal from human AF (MIT-BIH database) [66, 132] demonstrate how the direct application of phase analysis to raw
 electrograms is inappropriate. Striking differences can be observed between the trajectories in the phase domain depending of which definition of second state variable is used. However, the most significant observation is the large number of false positive deflections in phase when the Hilbert is applied to the human unipolar deflections. The phase transition does not correspond to the physiological cycle when the signals are multiphasic. Various levels of signal conditioning can be applied to the acquired signals. For example, the DC components of a signal must be removed before a Hilbert Transform can be applied, which has resulted in the introduction of empirical mode decomposition in the pipeline of signal conditioning.

![Figure 2.12: Matrix of Signal Conditioning Parameters. a) The columns represent degrees of signal processing and the rows represent definitions of the second state variable. b) Traces from a single pixel of atrial fibrillation in an ovine model highlight the effects of processing on the phase trajectories using the Hilbert Transform.](image)
Furthermore, phase analysis can be sensitive to spatial interpolation between recording sites. Since a singularity is defined based on the spatial pattern of phase it is important to choose an interpolation technique that does not artificially introduce rotation, especially along lines of conduction block. Some techniques have been introduced to limit the false positive identification of singularity points including a constraint that the phase singularity must lie along an isophase wavefront defined along $\pi/2$ with a minimum length [151]. Also groups have tried to classify PS as stable based on the waveform rotation around the point in order to separate meaningful PS from transient points or signal processing artifacts.

Figure 2.13: Fractional Differences in Phase Maps by Definition. a) Example calculation of fractional difference between baseline conditioning and temporal smoothing. b) A heat map that shows the average fractional difference compared to the baseline processing for all second state variable definitions. c) A heat map that shows the average fractional difference compared to the Hilbert Transform.

Phase analysis is a powerful technique that can be applied to cardiac fibrillation signals to look for underlying organization and determinism. In many cases the spatial pattern of phase is a cleaner representation of the potential pattern. However, there are many parameters within the application of phase algorithms that must be standardized within the field. Phase should be used in conjunction with the potential signal rather than as a replacement to be viewed in isolation. However, it is critically important to be aware that processing influences
the phase analysis results and it is difficult to perform a rigorous analysis of the accuracy of one technique over another in experimental data without a gold standard definition.

Figure 2.14: Inter-item Correlation of Wavefront Count. a) Example wavefront count traces for a single AF episode for all processing and second definition pairs. b) Measure of reliability for wavefront count. c) Heat maps for inter-item correlation for summary of all hearts tested.

To support this claim and to determine the optimal algorithm for the data in this dissertation, I developed a study to quantitatively compare different definitions of phase and look for internal consistency within methods. Using optical action potentials from a pharmacologically induced ovine model of AF, I investigated the variability of phase singularity location, stability, and trajectory when subject to increasing degrees of processing in the temporal and spatial domain and various definitions of phase variables. Once acquired, all signals were spatially filtered with a uniform 3 x 3 bin, temporally filtered with a low pass FIR digital filter with a cutoff frequency of 100Hz, normalized and detrended for baseline conditioning. Additional degrees of processing were applied to the data including: 1) a tight temporal bandpass filter from 2-10 Hz, 2) a larger spatial filter with a Gaussian 5 x 5 bin, 3) a Savitzky-Golay smoothing filter in time with a 200 ms window or 4) an aggressive combination of 1-3. Additionally, 3 definitions of the second state variable were used to calculate the phase: a) Hilbert Transform, b) a time embedded signal with a $\tau$ defined as 25% of the global dominant frequency, and c) a time embedded signal with $\tau$ defined as the first
zero crossing of the autocorrelation. The matrix of varied parameters is displayed in Figure 2.12. Phase trajectories from a single pixel with each level of processing highlight the effects of processing in the phase plane. Each $2\pi$ rotation represents a cycle of AF. The phase dynamics from 5 hearts with a total of 10 AF episodes were then quantified using each pair of variables from the matrix. PS were defined using the topological charge algorithm and considered stable when they persisted for 2 full rotations. Wavefronts were defined along the $\frac{\pi}{2}$ isophase line. Inter-item correlation tests were calculated for outcomes including, phase distribution, wavefront count, PS trajectory duration and PS spatial meandering area to assess the reliability of different tests and the internal coefficient of consistency for each parameter.

![Figure 2.15: Frequency Relationship to Inter-item Correlation. a) Dominant Frequency b) Regularity Index.](image)

The spatial phase maps were compared by calculating the fraction of the field of view that differed by greater than 5% of the phase cycle compared to the baseline condition for all 10 episodes. A representative example is shown in Figure 2.13. The autocorrelation definition was most sensitive to signal conditioning. The temporal filter and temporal smoothing were least sensitive to differing second variable definitions. Unfortunately the regions near the phase singularities were the most variable between definitions and processing conditions. The number of wavefronts were calculated at a frequency of 20 Hz for each condition based on the
isophase lines. The inter-item correlation was calculated based on the temporal wavefront count traces. All tested condition pairs failed to reach a threshold of reliability (chronbach’s alpha value of 0.8), although they were trending close to acceptable values (Figure 2.14). On the right the variability of wavefront traces are shown for each pair in the processing matrix for a single AF episode. The inter-item correlation heat maps highlight the pairs that are most correlated using the Hilbert Transform. Panel b provides a summary of the chronbach’s alpha for each definition of second state variable across all 10 AF episodes. This indicates that phase analysis outcomes are sensitive to the amount of processing applied to the data and may lead to either an overestimate or an underestimate of the number and location of critical regions contributing to the organization of AF. Figure 2.15 compares the inter-item correlation and the dominant frequency and regularity index of individual AF episodes for all 10 episodes. There was no trend between the frequency content of the arrhythmia and the sensitivity to different algorithms.

![Figure 2.15](image)

Figure 2.15: Comparing inter-item correlation and dominant frequency and regularity index of individual AF episodes for all 10 episodes. There was no trend between the frequency content of the arrhythmia and the sensitivity to different algorithms.

Figure 2.16: Variability in Phase Dynamics by Definition. a) PS trajectory spatial ratios b) Stable PS time ratio c) Stable PS count d) Average number of rotations for all PS in the field of view. Box and whisker plot overlaid with the results from 10 individual episodes of AF.
The effect on temporal and spatial stability was quantified by calculating the dynamics of all identified phase singularities. The results from each episode are shown in Figure 2.16 to highlight the variability in outcome due to arrhythmia differences using only the Hilbert Transform. Baseline filtering led to the greatest number of identified PS, the greatest spatial ratio, a high time ratio, and the lowest number of consecutive rotations per stable rotor. By investigating individual episodes, this is due to a high rate of false positive identification in the noisy boundary region even when selecting for only stable rotors. The spatial filter had the lowest PS count and the lowest time stability ratio. This pattern is because the spatial filter results in a greater percentage of time frames when no wavefront is present compared to all other levels of processing. The example with 26 consecutive rotations is parcelled into discrete rotations separated into several windows of rotation when the spatial filter is applied. Time frequency and temporal smoothing allow the most variation within the cohort of AF episodes tested. The time frequency filter was still sensitive to boundary noise but to less of a degree compared to the baseline filter. When applied to a negative control (known focal pattern) the temporal smoothing filter was the only processing group that identified the pattern incorrectly as rotation (data not shown).

Researchers and clinicians who apply phase analysis to guide patient therapies must be conscious of these effects when interpreting the results and choose appropriate filters. Perhaps for now the most appropriate answer is to use multiple algorithms and create a weighted map of the results of rotational activity (example Figure 2.17) where 2-3 regions could be identified by looking at the entire gamut of PS incidence maps). Based on the results of this pilot study we chose to condition our data with the tight temporal filter with an additional threshold map based on a signal to noise ratio (SNR) threshold to remove some of the false positive regions that were associated with low SNR.

Due to differences in morphology of unipolar electrograms, these results will not directly extend to electrical recordings, but a similar assessment of internal consistency must be done to systemically illustrate the importance of transparent and standardized preconditioning in any therapeutic phase algorithm. One approach involved using a model fit to the clinical recordings instead of a processed signal for electrogram data. For example we explored one statistical approach to the problem using a matching pursuit algorithm to compute a nonlinear approximation of the signal based on symlet wavelets. For the study in Chapter 4, we need to compare the isophase wavefronts between paced rhythms as a control and
induced AF. Paced rhythms are not as sinusoidal in shape as the AF signals and therefore they are more sensitive to noise if using the Hilbert Transform. Details of this approach are discussed in Chapter 4.

It is important to remember that while a PS is rigorously defined in mathematics, the techniques applied to experimental data and the physiological definition of such a point are less rigorous. It is not yet clear if a non-excited but excitable core exists in the physical manifestation of rotational propagation in cardiac applications. As the field becomes more familiar with tracking PS in space and time, it will help clarify the stability definitions in both domains and elucidate how this technique can be used to guide therapy most effectively.

PS identification is also an important technique for determining the mechanism of successful applications of electrotherapy. In order to terminate an arrhythmia a therapy must disrupt the circuits that are immediately sustaining the arrhythmia as well as avoid inducing new rotating waves as a result of the tissue response to a shock. Tracking the dynamics of new PS immediately following the shock is a good indication of whether the shock-induced wavefronts are at risk of re-inducing an arrhythmia.
Chapter 3

Quantification of the Transmural Dynamics of Atrial Fibrillation by Simultaneous Endocardial and Epicardial Optical Mapping in an Acute Sheep Model

Large portions of this chapter appear in the following original publication:


3.1 Introduction

Establishing a comprehensive description of AF substrates that sustain arrhythmias remains a formidable task. Unfortunately, guided treatment approaches require advancements in the way we categorize arrhythmias beyond the classical approach to differentiate cases by only
the duration of the arrhythmia. Animal models of AF offer a platform from which to investigate the relevancy of new methods of characterizing AF organization. Historically, most investigators have assumed atrial tissue is a functionally two-dimensional (2D) structure. Simulations and mapping modalities have largely ignored the role of transmural conduction during AF, emphasizing in-plane epicardial or endocardial propagation patterns as the driver circuits for the arrhythmia. In a 2D projection, transmural breakthrough can easily be misattributed to focal activity, further complicating the interplay between the multiple substrate hypotheses. However, the field is beginning to recognize that tissue thickness should not be completely ignored. Several recent animal studies have suggested that epicardial-endocardial dissociation may play an important and dynamic role in the progression of the AF substrate and the stability of the arrhythmia [46, 47, 52, 208, 22]. Although the wide diversity of the disease plays a crucial role in the mechanism controversy, it is also possible that three-dimensional (3D) conduction may account for some of the discrepancies in AF maintenance hypotheses. By taking advantage of optical tissue penetration properties, we used transillumination optical mapping to quantitatively assess the association between propagation on the epicardial and endocardial surfaces in an acute AF model compared to paced rhythms. We used these patterns to infer the 3D heterogeneity of the substrate. Using this approach we evaluated degrees of discordance in the spatiotemporal organization of individual induced AF episodes.

Traditional optical mapping is a 2D imaging technique, transducing the electrical activity of the superficial layers of cells into optical signals that linearly depend on transmembrane potential changes [28]. However, the depth of penetration is largely based on the absorption and scattering properties of the excitation and emission light and can be tuned by wavelength and camera orientations. Simultaneous imaging at two depths within the tissue is achieved by detecting fluorescence on the ipsilateral and contralateral tissue surface relative to the illumination source [19, 29]. Figure 3.1 provides for a visual schematic of the theory behind the transillumination approach [19]. Two planes of data (a reflected and transmitted plane) are collected with each illumination direction. Optical signals collected in the reflected and trans-illuminated modes are averaged signals from across depth subdivisions. Biophotonic simulations have predicted that the average depth of contribution to the reflected signal with red light is 1.5 mm, while the average depth of the contralateral signal is 4 mm from the illuminated surface. Simulations excited by green light have an average depth of 0.5 mm in the reflected configuration and 2.5 mm in the transmitted configuration [180]. Several studies
have used this technique to characterize propagation patterns in ventricular tissue [181, 182]. One additional study used the same principle to look at a small window of atrial tissue [208]. However, to our knowledge transillumination had not been applied to a large field of view of atrial tissue prior to this study. In order to optimize the methodology for this application we used a novel voltage sensitive dye [123] that is excited by a range of excitation wavelengths and a combination of emission filters to achieve the best signal quality. The flexibility of this experimental set-up allowed us to assess the feasibility of achieving two planes of data in the significantly thinner and contoured atrial tissue. We applied this technique to correlate the simultaneous propagation patterns at two different depths within the atrial wall with high spatial resolution and without having to physically expose the transmural tissue, which may disrupt the integrity of fibrillatory circuits. For paced data we were able to look at four planes by aligning two files taken with opposing illumination directions. For AF data the two configurations cannot be temporally aligned.

Figure 3.1: Schematic of Transillumination Mechanism. Distributions showing contribution to total signal in endocardial, endo-transilluminated, epi-transilluminated, and epicardial and a schematic of how those distributions cut virtual planes through a left atrial preparation. Modified from [19]

In this study we used an acute acetylcholine-induced model of AF in the isolated sheep left atrium (LA) to test the hypothesis that inherent local heterogeneities in electrophysiological
properties and anatomical discontinuities can support dyssynchronous electrical propagation in AF across the transmural wall independently of AF-induced structural remodeling. We used parameters that are commonly used for clinical characterization of AF including: dominant frequency (DF), regularity index (RI), and phase singularity (PS) analysis. As personalized therapy strategies based on high density mapping gain favor, it is increasingly important to understand the limitations of 2D mapping techniques and the subsequent effects on the efficacy of therapy paradigms. There is no clinical technique yet available to assess transmural propagation. Basket and plaque arrays only measure local surface signals. This leaves clinicians with a 2D projection of a potentially 3D substrate. It is unclear whether a 2D view is sufficient to guide directed therapeutic approaches such as Focal Impulse and Rotor Modulation [138] or rotor-guided ablation [114]. Transillumination optical mapping provides an experimental platform to investigate the spatiotemporal dynamics and infer the 3D transmural propagation patterns.

3.2 Methods

Experimental Preparation

Optical mapping experiments were conducted in isolated LA preparations from Texel cross-bred sheep (N=13 total preparations, N=5 to characterize the technique and N=8 to quantify AF dynamics) weighing 40-55 kg. The animals were treated in accordance with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the local Université de Bordeaux ethical committee. The sheep were pre-medicated with 20mg/kg ketamine and 0.02mL/kg acepromazine (Calmivet, France). Surgical plane anesthesia was induced with 10mg/kg sodium pentobarbital and maintained under isofluorane, 2% in 100% O₂. The chest was opened to provide access to the heart and the animal was euthanized by sodium pentobarbital (2000mg). The heart was rapidly excised and perfused with 200 mL of cardioplegia and heparin through an aortic cannula. The left coronary artery was isolated for cannulation. Under constant perfusion of cold cardioplegia the ventricles were removed below the main branch of the circumflex artery. All ventricular vessel branches were tied off and LA was flattened by opening the right superior pulmonary vein. The right atrium was removed via an incision down the atrial
septum and the remaining pulmonary vein ostia were inverted to maximize the exposed surface area. The LA preparation was stretched across a frame and secured to pull it flat as shown in Chapter 2. The LA preparation was perfused with methylene blue to ensure adequate perfusion and transferred to warm oxygenated Tyrode’s solution for optical mapping. Perfusion was maintained for the duration of the experiment at 20 mL/min. The tissue was suspended vertically in a bath to allow optical access to both the endocardial and epicardial surface as seen in schematic form in Figure 3.2. Two stainless steel electrodes were clipped to the endocardial surface to record a bath electrocardiogram.

![Diagram of Experimental Set-up](image)

Figure 3.2: Schematic of Experimental Set-up. a) Endocardial illumination b) Epicardial illumination.

**Optical Mapping Acquisition**

The tissue was electromechanically uncoupled with 15 μM Blebbistatin and stained with a 50 μM bolus injection of di-4 ANBDQBS or a 15 μM bolus injection of di-4 ANEPPS. Two illumination LEDs were tested to titrate the depth of signal contribution. The LEDs were either two 660 nm or two 530 nm LEDs (Cairn Research Ltd, Kent, UK). Fluorescence was filtered through a 715 nm long pass filter or a 585 ± 35 nm bandpass filter respectively.
and acquired using two CMOS cameras (MICAM Ultima, SciMedia) with 5cm x 5cm fields of view (spatial resolution 500 µM). The atria were paced at 1-5Hz from various locations with a bipolar electrode on either the epicardial and endocardial surface at twice the pacing threshold. Four-second recordings were captured at 1,000 frames per second. Acetylcholine (Sigma-Aldrich, MO) was added to the perfusate in increasing concentrations from 0.1 µM to 5 µM until AF could be induced by 50 Hz burst pacing. An AF episode was recorded if it was self-sustaining for >30 seconds.

**Magnetic Resonance Imaging (MRI)**

Limited by equipment availability, the atria from 5 hearts were imaged with MRI after optical mapping to obtain the complex anatomical geometry of the preparations. Immediately after the completion of the optical mapping, the perfusate was removed from the imaging chamber and replaced with YL VAC 14/6 Fomblin perfluoropolyether (Ausimont, Milan, Italy), an organofluorine with no 1H MRI signal. The sealed chamber was placed in a Siemens Magnetom Avanto 1.5T MRI scanner (Erlangen, Germany) with the axis of optical imaging aligned to the b0 direction. Imaging was carried out using a Siemens cardiac MRI sequence with a simulated ECG (cycle length 700ms), echo time 2.98ms, repetition time 399.19ms, flip angle 90°, matrix size of 512 x 512 x 192, spatial resolution of 0.62 x 0.62 x 0.31 mm³, for a total field of view of 316 x 316 x 60 mm³, and an acquisition time of 32 minutes. The images were segmented from the background signal using Seg3D2- 2.1.5 (Scientific Computing and Imaging Institute, University of Utah, USA). The segmentation mask was generated using a median filter and thresholding to exclude non-tissue voxels with a binary erode then dilate. The resulting segmented slices were reconstructed into a volume and further processed in Paraview using additional threshold filters and manual volume extraction to remove the ventricular tissue from the edges of the tissue preparation. Transmural thickness was estimated along a normal line through the tissue perpendicular to the CMOS camera focal plane using a custom C++ program.

**Arrhythmia Processing**

All optical signals were processed with custom MATLAB software, some of which was developed by previous students and is described in detail elsewhere [108]. Briefly, each pixel was spatially filtered with a 3 x 3 uniform average bin, every temporal sequence was low pass filtered by an FIR filter with a cutoff frequency of 100 Hz, drift in the baseline was
removed with a polynomial fit subtraction, and the magnitude of the fluorescent change was normalized to a range of [0,1]. DF was evaluated for each pixel as the frequency band with maximal power on a periodogram calculated with a Fast-Fourier transform (resolution 0.24 Hz). RI was defined as the ratio of the power within a 1 Hz band centered on the DF and the total power spectrum from 0 to 100 Hz. A perfect sine wave with a single frequency yields an RI of 1. Single parameter spatial maps were aligned using MATLAB’s intensity based image registration to account for any small translation or scaling differences in camera orientation between fields of view before statistical comparisons.

Wavefront and Phase Dynamics

Prior to transforming the optical signals into the phase domain, additional levels of preconditioning were applied to both paced and arrhythmia data as described in detail in Chapter 2. The epicardial-endocardial correlation during paced data was used as a control in these studies to account for confounding effects of optical acquisition. The signals were temporally filtered with a narrow [2,10] Hz band pass filter and were spatially masked to keep only those pixels with SNR of at least half the maximal SNR in the field of view. SNR was calculated during a 2 Hz pacing trace for each preparation and illumination direction as the ratio of the baseline amplitude to the amplitude of an optical action potential after normalization. To convert the optical signal into the phase space we applied the Hilbert Transform on the detrended processed optical signals. Wavefronts were defined as the isophase lines along $\phi(t) = \pi/2$. The number of discrete wavefronts was calculated for each field of view with a sampling frequency of 50Hz. Conduction velocity vectors were estimated from the direction of vectors normal to the curvature of the isophase wavefronts [93]. Conduction velocity maps were calculated with a 0.5s resolution. PS were calculated as non-zero topological charges constrained to the isophase wavefronts in each time frame. Static PS incidence maps were calculated by summing binary PS location maps across time. PS were considered distinct if they were >5 pixels apart in a single frame. The minimum Euclidean distance between PS on the two imaging planes was calculated using MATLAB’s nearest neighbor search algorithm. Dynamic PS stability was calculated in terms of full wavefront rotations around the PS. Stable PS were arbitrarily defined by 2 full rotations. The spatial PS ratio is defined as the fraction of the field of view through which all stable PS track. The temporal PS ratio is the fraction of the total sequence where at least one stable PS is present.
Statistical Analysis

All means that characterize the spatial average of a single AF episode are represented as sample mean ± standard deviation. All means that characterize average Epi-Endo trends across the cohort of hearts are reported as sample mean ± standard error of the mean. When comparing transilluminated vs. reflected or paced vs. AF, the data points are pooled and averaged for each heart. A statistical comparison between groups is applied to the cohort of hearts with an unpaired two-tailed t-test. When the comparison was across multiple variables, i.e. both fields of view and rhythm type, a multivariate analysis of variance was used. Significance was defined as p < 0.05. When testing whether the absolute value of the difference between the endocardial and epicardial parameter is equal to 0, a one-tailed t-test is used on the average differences per heart. Mean conduction velocity vectors and angular variance are calculated from the circular distribution of individual conduction velocity vectors from each wavefront in time [209]. The mean propagation vector is compared to the mean from the contralateral field of view for each episode. The difference in angle is averaged for each heart before a two-tailed t-test is applied between the paced and AF conditions. For comparing the wavefront count, a Pearson’s linear correlation coefficient is calculated for each episode (AF and paced). The correlation coefficients are pooled and averaged within each heart, before the two groups are compared. An average 2D correlation coefficient from MATLAB is used to compare the DF and RI intensity images from each field of view in a similar manner.

3.3 Results

Two-plane Imaging Feasibility

To characterize the application of the transillumination technique to atrial tissue, we compared the signal quality and basic electrophysiological parameters acquired for both light and filter combinations. Figure 3.3 displays representative optical action potential traces using green excitation and red excitation. The transilluminated signal is averaged across a greater depth, this manifests as an increase in upstroke duration. There is also a clear distinction between the distributions of the upstroke duration with each mode and light combination. The green reflected signal is more superficial than the red reflected signal, resulting in a shift
to shorter upstroke durations. We quantified the average signal to noise ratio and the fraction of signals with dual components in the upstroke to compare the two acquisition modes. The mean reflected SNR was $17.73 \pm 0.82$ (s.e.m) and the transilluminated SNR was $17.47 \pm 0.84$ for $N=5$ hearts with red excitation. There was no statistical difference between the mean SNR with endocardial illumination or epicardial illumination with red light ($p=0.84$). The mean reflected SNR was $11.96 \pm 0.69$ (s.e.m) and the transilluminated SNR was $8.82 \pm 0.63$ for $N=5$ hearts with green excitation. For green light 26% of the signals contained dual components in the upstroke morphology compared to 28.8% for red light. Additionally, action potential durations (APD) values were assessed at a range of pacing frequencies from both imaging planes and the observed differences are displayed in Figure 3.4. The red light configuration resulted in longer APD for all acquisition planes.

Figure 3.3: Characterization of Green Excitation vs Red Excitation. a) Representative optical action potentials with both excitation wavelengths. b) Distributions of upstroke durations with both excitation wavelengths, with a kernel distribution fit.

The transilluminated plane with red excitation light has a better signal to noise ratio. To maximize the spatial coverage of the transilluminated plane, red excitation was chosen for the cohort of AF dynamics ($N=8$ hearts, 20 AF episodes). To verify that the dual sided imaging technique was capturing information at two distinct depths within the tissue we calculated the breakthrough delay on the field of view contralateral to the pacing electrode at various locations, relative to the pacing spike. When the tissue was paced epicardially at 2Hz, the delay until breakthrough on the endocardial plane was $11.63 \pm 1.1\text{ms (s.e.m)}$ across all hearts ($N=8$ animals). Conversely, the delay for endocardial pacing until breakthrough on the epicardial plane was $9.6 \pm 2.52\text{ms}$. The breakthrough delays confirm that there is a propagation delay between the two planes.
Figure 3.4: Action Potential Duration Differences across Acquisition Modes. In all modes and across all pacing cycle lengths red excitation leads to longer action potentials.

**Transmural Heterogeneties**

From the MRI volumes we extracted local thickness parameters. A representative volume colored by thickness is displayed in Figure 3.5 to highlight the complex geometric contours of the atrial tissue in this preparation. Additionally, the tissue thickness distributions from five volumes show a wide range of variability with each preparations and across the cohort of imaging anatomies. Although the distributions are shifted within each heart, thickness ranges from $<1$ mm to $>1$ cm after excluding the ventricular edges.

Acetylcholine was added in increasing doses to each preparation, ranging from 0.1µM to 5 µM, until a sustained AF was induced. The APD after the necessary final dose of acetylcholine was given ranged from $129.73 \pm 17.3$ to $188.19 \pm 18.51$ms on the endocardial surface ($\mu \pm \sigma$) and the epicardial range was $125.14 \pm 16.46$ to $164.46 \pm 13.51$ms ($\mu \pm \sigma$) during 2Hz pacing for all 8 preparations. For paced data the imaging acquisition protocol can create 4
distinct planes through the tissue, a reflected and trans-illuminated plane for each illumination direction that have been temporally aligned. We defined the local gradient of APD as the difference from the reflected signals during endocardial illumination, to the reflected signals from epicardial illumination. The average transmural gradient in APD from endocardium to epicardium was $24.13 \pm 6.34$ ms. Finally, we registered the thickness map to the transmural APD gradient plane. Although both the thickness and the APD transmural differences were heterogeneously dispersed across the field of view there was no correlation between these two parameters (data not shown).

**Arrhythmia Static Parameters**

A total of 10 epicardially illuminated episodes and 10 endocardially illuminated episodes were included in the analysis of AF transmural discordance. The DF and spatial standard deviation, including both the endocardial and epicardial field of view, ranged from $3.90 \pm 0.48$ to $10.03 \pm 2.88$ Hz. The difference between epicardial and endocardial global DF was not significantly different from zero when assessed by a two-tailed t-test ($p=0.3516, N=8$ hearts). The global RI and spatial standard deviation ranged from $0.12 \pm 0.065$ to $0.704 \pm 0.079$, with an Epi-Endo difference that was not significantly greater than zero ($p=0.42, N=8$ hearts). The distributions in the Epi-Endo difference of these global parameters across all hearts are shown in Figure 3.6. Two representative examples from opposite sides of the spectrum are also displayed, highlighting the spatial congruency across both the imaging planes. The spatial distribution of DF across the transmural wall had an average 2D correlation across all hearts of $0.79 \pm 0.06$ (s.e.m). Likewise, the spatial distribution of the RI had an average correlation of $0.930 \pm 0.009$, with no statistical difference between illumination configurations.
PS incidence maps were created as a static characterization of each arrhythmia. The mean distance between the static PS locations and the nearest PS on the contralateral side was 5.24±0.3mm (N=8).

![Image](https://via.placeholder.com/150)

Figure 3.6: Static Atrial Fibrillation Frequency Characterization. a) Differences in global dominant frequency and regularity index across all hearts. b) Representative spatial maps of dominant frequency and regularity index for a two episodes of AF. c) Summary of spatial correlation between imaging plane for both parameters. Error bars represent s.e.m. [73]

**Arrhythmia Dynamics**

All dynamic parameters are presented in comparison to paced data as a control to account for the effect of anatomical complexities on the optical planes and SNR edge effects on the fields on view. The dynamic number of propagation wavefronts was quantified in a time series and correlated with the opposing side. Figure 3.7 illustrates the wavefront identification and displays representative wavefront count traces for a paced rhythm and an AF episode. The mean correlation between epicardial and endocardial wavefront count was 0.69±0.035 for pacing versus 0.47±0.048 for AF (p=0.0028, two-tailed t-test, N=8 hearts).

The average direction of propagation was calculated for each episode with a resolution of 0.5s with a random starting time. A representative propagation angle calculation from both the paced and AF groups is shown in Figure 3.8 along with the angular distribution. The statistically significant (p=0.044, N=8 hearts) difference in angle between the two planes was 20.37±3.04° during pacing and 61.43±12.37° during AF. A one-way multivariate analysis of variance confirmed that the angular variance did not differ between rhythms or fields of view.
The PS dynamics are specific to each heart and even vary between separate AF episodes within a single preparation. Therefore, we report the detailed observations of two AF episodes and the summary of phase tracking for the remainder of the episodes (Figure 3.12). Table 3.1 quantitatively describes two individual episodes, representing the range of observed dynamics: the first is less complex but has discordant phase dynamics across the two imaging planes while the second is more complex but displays more conserved dynamics across the transmural wall. Sample optical traces from corresponding pixels in both fields of view and electrical bath traces for each example AF episode are displayed in Figure 3.9. These highlight the temporal regions when the endocardial and epicardial propagation patterns diverge.

AF episode 1 shows a temporally stable epicardial rotor with minimal spatial meandering, while the dynamics at the endocardium are less stable in both the temporal and spatial domain. The pattern can be visualized in the sequential phase maps (Figure 3.10). The
Figure 3.8: Angle of Propagation Differences across the Transmural Wall. a)(pacing)-b) (AF): Representative propagation vector angle distributions. I) Simultaneous phase maps from endo and epi fields of view with wavefronts highlighted in white, curvature arrows are displayed along the wavefronts. II) Circular histograms of propagation vectors for endo (blue) and epi (red) fields of view. III) Average angle calculations for endo (blue) and epi (red). C: Summary of average angle differences. Error bars represent s.e.m. Statistically significant difference observed between pacing and AF. [73]

dynamic phase and potential movies are also available in the original publication (Video 1-2) There are observable spatial differences in wavefront propagation patterns at each instance of time.

This dyssynchrony manifests in an increase in the PS count with less rotations on the endocardium. The constant driver is veiled in this imaging plane (Figure 3.12). In contrast, in AF episode 2, the spatial and temporal trajectories of one imaging plane nearly trace the trajectories from the opposing plane (Figure 3.12), which is reflected in similar spatial and temporal ratios. The dynamic conservation of the propagation pattern is emulated in the small difference observed between propagation vectors (Table 3.1) and visualized in the sequence of phase maps in Figure 3.11 or dynamically in Videos 3-4 in the original publication. Despite the increase in the number of wavefronts present at each instance of time, all wavefronts track well together across the transmural wall in almost all instances of time.

Figure 3.12 extends these observations to all hearts in the cohort. The correlation coefficient between the number of PS on the endocardial imaging plane and the PS count on the epicardial plane is 0.879. The average number of stable PS was 4.04±0.66 (s.e.m, N=8 hearts)
Table 3.1 Detailed Dynamics for Two AF Episodes

<table>
<thead>
<tr>
<th>AF Episode</th>
<th>FOV</th>
<th>DF (μ±σ)</th>
<th>RI (μ±σ)</th>
<th>Stable PS Count</th>
<th>PS Rotations</th>
<th>Wavefront Count Correlation</th>
<th>Propagation angle (°)</th>
<th>PS Spatial Ratio</th>
<th>PS Time Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Endo</td>
<td>4.50±1.46</td>
<td>0.39±0.18</td>
<td>3</td>
<td>2.62±0.27</td>
<td>0.2815</td>
<td>230.47±59.95</td>
<td>0.102</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>4.79±1.58</td>
<td>0.44±0.15</td>
<td>1</td>
<td>26.02±0</td>
<td></td>
<td>305.24±14.23</td>
<td>0.011</td>
<td>0.995</td>
</tr>
<tr>
<td>2</td>
<td>Endo</td>
<td>7.78±1.78</td>
<td>0.19±0.09</td>
<td>9</td>
<td>2.97±0.23</td>
<td>0.3608</td>
<td>179.44±27.5</td>
<td>0.085</td>
<td>0.882</td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>8.88±1.64</td>
<td>0.18±0.07</td>
<td>5</td>
<td>4.60±0.80</td>
<td></td>
<td>176.37±31.89</td>
<td>0.084</td>
<td>0.899</td>
</tr>
</tbody>
</table>

Figure 3.9: Corresponding Endocardial and Epicardial Optical Action Potentials for AF Episode 1 and 2. Normalized optical signals from corresponding pixels on the endocardial (blue) and epicardial (red) imaging plane and ECG bath (black) for the two detailed AF episodes. The simultaneous potential traces highlight moments of dyssynchrony across the transmural wall. [73]

and 3.3±0.59 for the endocardial and epicardial plane respectively. The absolute value of the difference in PS count between the epicardial and endocardial plane was significantly greater than 0 with a median difference of 1.0 PS (p=1.01x10⁻⁵, 1 tailed t-test). The correlation coefficient across all hearts for the spatial stability ratio is 0.795. The average area across all hearts through which stable PS trajectories traversed was 4.63±1.05 cm² (endo) and 3.56±1.0 cm² (epi). The absolute value of the difference between the epicardial and endocardial plane was significantly greater than 0 with a median difference of 9.27% of the field of view (p=5.87x10⁻⁴, N=8 hearts). The correlation coefficient for the ratio of time that has at least one stable PS present between the imaging planes is 0.111 across all hearts. The absolute value of the difference between the epicardial and endocardial plane was significantly greater.
than 0 with a median difference of 19.75% of the total recorded sequence (p=2.78x10^{-4}, N=8 hearts).

### 3.4 Discussion

Modern clinical mapping techniques are confined to observing only the surface manifestations of 3D propagation patterns. In a manuscript describing the 3D organizing centers of chemical waves Art Winfree recognized that although a pattern could be discerned within a 2D plane, it would be nearly impossible to identify the organizing center without the complete 3D perspective [197]. Several reports have suggested that AF organizing centers may also be more completely described as 3D structures. A substantial 3D component to the drivers of AF could contribute to the complexity of personalized therapy approaches. In simulations of 3D reentry, Winfree predicted that a critical thickness exists which must be surpassed before reentry in the z-dimension is observed. This critical value was suggested and later confirmed by additional groups to be estimated by \( \frac{1}{\phi} \) times the distance a wave propagates in 1 rotation. It is important to note that the distance can be dynamic and therefore the z component of propagation may also be dynamic. Gray and Jalife eloquently articulated the role of thickness in reentry in the ventricles:
As the thickness was increased, the activity on the top and bottom surfaces near the center of rotation became discordant resulting from a curved filament whose ends remained attached to the top and bottom surfaces. As the thickness was increased further the filament first curved and then as reentry developed in the third dimension, the filament broke into two pieces... increasing the number of filaments and hence “sources of activity” [67].

However, this describes the destabilization of reentry in a uniform slab and refers to ventricular arrhythmias. The architecture of the atria is complex with structural discontinuities and sharp gradients in thickness. It is unclear how much the atrial geometry contributes to a divergence from 2D propagation patterns during AF.

Dyssynchronous transmural activation was documented with simultaneous endocardial and epicardial recordings as early as 1993 [160]. Using unipolar electrode recordings, Schuessler et al. correlated discordant activation activity with anatomic heterogeneities of the canine right atrium in acute AF. Importantly, neither surface was preferentially faster during tachyarrhythmia propagation. A recent in vivo electrical mapping study by Eckstein et al. quantified the percentage of fibrillation waves that were preceded by electrical activity on the contralateral surface [47]. The authors reported a marked increase in the incidence of breakthrough with an increase in the degree of epi-endo dissociation, suggesting that what may appear to be a focal source is actually due to 3D transmural propagation. Yamazaki et al. used simultaneous epicardial and localized endoscopically-guided endocardial optical mapping to predict the virtual transmural architecture of rotors [208]. The presented results show some rotors in phase on both planes and some with a filament twist or non-identical activation patterns. In their study the rotors were often associated with sharp transitions in tissue thickness near the thinnest regions. Computationally, a dual layer simulation by Gharaviri et al suggested that epi-endo dyssynchrony increased AF stability compared to single layer simulations [63]. The model was only a proof of principle simulation with arbitrarily placed discrete transmural connections. Even so, the incorporation of a third dimension for propagation increased the number of wavefronts at any given time and the lifespan of phase singularities without effecting the frequency content of the simulated arrhythmia, in agreement with the data observed in our study.
Our study continues the line of work towards 3D AF substrate characterization by charting the dynamic dissociation between layers and probing the transmural uniformity of potential drivers with expanded spatial coverage and resolution. The global DF and the spatial distribution of DF are both well conserved across the transmural wall. The RI is also well conserved as a global mean and a spatial pattern. However, the frequency content does not represent a complete characterization of the dynamic AF substrate. In order to further assess the transmural uniformity of the substrate we investigated individual wavefront dynamics. Compared to the paced rhythms, the number of wavefronts is less correlated during an arrhythmia. The observed correlation for paced rhythms is not 1 as might be expected. The observed value may be due to the SNR spatial mask partitioning a single wavefront into multiple segments on one imaging plane as seen in Figure 3.7. It may also be the result of breakthrough delay on the side contralateral to the pacing electrode. This unexpected outcome illustrates why we used the pacing data as a control instead of analyzing the AF episodes in isolation. We also evaluated the difference in shape of the propagating wavefront from each imaging plane, through propagation vector angles. The pacing wavefronts move in unison across the tissue, except immediately following the pacing spike (not shown in figure). However, the propagation during the arrhythmia is significantly less uniform across the transmural wall with a significantly larger difference in mean propagation angle. Figure 3.8 shows a representative example of an endocardial wavefront that is trailing behind the simultaneous epicardial pattern. The mean difference in angle shows that the organizing
centers of the AF substrate have 3D components, resulting in a difference in the propagation vector direction.

Figure 3.12: Phase Singularity Dynamics across the Wall. a) AF Episode 1, b) AF Episode 2: I) Representative phase maps with wavefronts highlighted in white. II) Spatial trajectories of all PS superimposed on the optical field of view images for endo (blue) and epi (red) imaging planes. III) Simultaneous PS trajectories in spatiotemporal domain for endo (blue) and epi (red) imaging planes. c) Summary of PS correlation data across all 20 AF episodes including PS count, spatial ratio, and temporal ratio. AF Episode 1 (green). AF Episode 2 (pink). [73]

Phase plane analysis is a technique derived from the physics of vortices (e.g. spiral waves, rotors, scrolls, etc.) and applied as a popular method to map the organizing centers of the AF substrates (See Chapter 2). Although the algorithms employed vary between institutions with various degrees of clinical efficacy, the theory behind PS tracking is sound and well established. The conversion from potential space into phase space assigns a unique
value to each instant of time within one period of a signal, which can be dynamic in fibrillation. Spatially, a PS forms at the wavebreak of reentry or wavefronts collisions. These singularity points can be localized and tracked through time as a dynamic indicator of the spatiotemporal organization and stability of an AF episode. In this study we investigated the contralateral activity at the site of stable PS to assess the transmural uniformity of the dynamic AF substrate. PS incidence maps show that the distance to the nearest neighboring PS on the contralateral surface is relatively small suggesting that PS are macroscopically localized to similar regions of the LA. In fact the distance seems to be similar in size to an ablation catheter tip, which may explain why driver ablation has been successful in some cases. However, due to tissue curvature, this observation does not allow direct inference to the transmurality of the rotor. For example, if tissue is curved and the rotor filament is perpendicular to the tissue surface (no transmural variations in rotor properties) then the PS on epicardium and endocardium will appear displaced with respect to each other in the imaging planes. Conversely, a more complex intramural filament may give rise to what would seem like perfectly aligned PS on epicardial and endocardial imaging planes. Additionally, the locations with the highest relative frequency of a PS assume each time frame is independent, which puts greater emphasis on the PS with less spatial meandering that repeatedly pass through the same pixel often. Therefore the PS trajectories may be a more important spatial measure of the transmural synchrony of organizing centers than the PS incidence maps.

Consequently, we simultaneously mapped the PS trajectories of stable PS, defined as when the wavefront passes 2 full rotations or $4\pi$ around the singularity, in both the epicardial and the endocardial imaging planes. We used the spatial ratio and the time ratio as measures of the colocalization across the transmural wall in the spatial and temporal domains. Although all three parameters we tested showed differences across the wall significantly greater than zero, the greatest measure of discordance was observed in the temporal stability of PS across surfaces. The spatial meandering and PS count are more conserved on average, although Episode 1 does display a greater degree of meandering preferential to the endocardial plane. AF Episode 1 demonstrates how non-uniform transmural propagation leads to breakthrough that disrupts the stable organized propagation pattern. The propagation videos show that the endocardial wavebreak does not occur on every pass of the stable rotation but does repeat several times within the sequence. The traces in Figure 3.9 also show propagation in the endocardial plane that is near-continuously out of phase with the epicardial trace. Detailed Episode 2 is an example of an AF substrate that is more uniform across the transmural
wall. Although not always completely synchronous, the PS track together through time and space. The video displays an obviously synchronous collective pattern. The traces in Figure 3.9 echo this pattern; there are short bursts of out of phase propagation (e.g. 1000-1500ms) but the traces track in sync for the majority of the measured sequence. The cohort of AF episodes included in this study lies along a spectrum of transmural uniformity outlined by these two examples. However, there was no correlation between frequency content and degree of dissociation that could help predict which episodes are likely to exhibit transmural differences (data not shown). There were fast and slow, disorganized and regular, single rotor and many PS arrhythmias that had varying levels of functional dissociation. A single surface manifestation of these episodes would not have captured the complete view of the dynamic 3D substrates in this study.

The acute model of AF does not incorporate pathophysiological structural remodeling, which could conceivably compound the degree of dyssynchrony observed across the transmural wall. In this model the likely sources of transmural dyssynchrony are abrupt changes in geometrical thickness due to complex contours of the atrium including the pulmonary vein ostia, trabeculations and appendage and a heterogeneous response to acetylcholine. Each heart did display regional differences in APD after acetylcholine, creating transmural dissimilarities. However, there was no correlation between the transmural repolarization gradient and thickness, nor was there a correlation with the APD differences across the wall and the spatial pattern of the frequency content. It is possible that dyssynchrony locates at the interface between these two factors, where there is substantial space anatomically and a considerable gap between repolarization to harbor transmural conduction. This study does not rigorously identify the tissue conditions that are favorable to discordant propagation patterns, instead focusing on chronicling the spatiotemporal prevalence of dissociated patterns. Future work would require transmural discontinuities to be assessed histologically to obtain further insight into the source of dyssynchrony.

3.5 Limitations and Future Directions

AF is an exceptionally diverse disease. We used a range of acetylcholine doses to encompass the variability of clinical AF with respect to frequency content and organization with the
selection of AF episodes analyzed in this study. Still, there are some limitations to extending this model to the clinical population of AF. Most notably, this is not a model that is favorable to automaticity. The depression of focal discharges by acetylcholine [42] creates a substrate that fosters re-entry and rotor drivers. Additionally, although the image acquisition technique allows for multiple planes to be imaged simultaneously, it is still not possible to fully capture out of plane rotation. The biophotonic simulations provide an estimate for the depth of the contributing signal for each illumination/optical configuration; however, in practice there are many variables that could affect this parameter including: the angle of incidence of light, the focal plane of the cameras, and the complex contours of the anatomy. We used the pacing data as a control to account for some of these effects that vary from experiment to experiment.

### 3.6 Funding Sources

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Chapter 4

Investigating the 3D Substrate of Atrial Fibrillation in a Canine Model

4.1 Introduction

The atria are complex heterogeneous structures. The twists and turns of the anatomical architecture are far more complicated than a two-dimensional sheet or a shell with uniform thickness. Intuitively, such an intricate geometry should have ensuing effects on the conduction through the volume of the atrial tissue, even disregarding any functional difference across the wall. Depth dependent distributions of electrophysiological parameters have been well studied in the ventricular tissue [65, 115, 64]; however the 3D nature of the atrial tissue has been largely ignored. Our previous study on an acute ovine model captured a range of transmural discordance in the phase dynamics of AF evaluated simultaneously on the epicardial and endocardial surface with transillumination optical mapping [73]. In addition to the functional mapping, we looked at reconstructed volumes from Magnetic Resonance Imaging as a preliminary investigation into atrial tissue structural variation. With a crude estimate of wall thickness we saw wide variety from <1 mm to >1 cm. Another study in sheep suggested that the abrupt changes in thickness, particularly the transition from thick to thin regions harbor AF drivers [208]. Although thickness alone could foster a three-dimensional substrate, we conducted a thorough investigation of structural and functional differences across the transmural atrial wall to characterize the 3D substrate in a canine model. The present
study compares the transmural electrical patterns and structural differences in an acute canine model of AF. The acute AF substrate is created by Acetylcholine-induced changes in action potential duration (APD) and induced by 50 Hz burst pacing.

Ex vivo preparations with both the left and right atria intact were optically mapped from the endocardial and epicardial surface. Two different light configurations, constant epicardial illumination and alternating epicardial and endocardial illumination, were applied to probe various planes within the depth of the tissue. The optical mapping studies were used to confirm dyssynchrony in an additional animal model and investigate electrophysiological gradients across the atrial wall. Functional studies were followed by histological analysis to quantify the pattern of structural transmural connections that could support electrical dissociation across the wall.

4.2 Methods

Experimental Preparation

All animal procedures were performed in accordance with the ethical guidelines of the National Institutes of Health and were approved by the Washington University in St Louis Animal Studies Committee. Optical mapping experiments were conducted in isolated atrial preparations from Mongrel canines (N=5) weighing 25-30 kg. Animals were initially sedated with 5-7 mg/kg propofol via the cephalic vein, intubated and mechanically ventilated. A surgical plane of anesthesia was maintained with 1-3% isoflurane. A median sternotomy was performed and the pericardium opened. The heart was arrested with cold Cardioplegia and explanted from the chest. While submerged in cold cardioplegia, both the right and left coronary arteries were isolated and cannulated. The ventricles were removed below the circumflex artery and all ventricular-descending branches were ligated. The right atrium was opened along the caval veins and the left atrium was opened along the right superior pulmonary vein, leaving most of the atrial septum and the atrial roof intact. The dual atria preparation was stretched across a frame to flatten the tissue surfaces and expose the endocardial surface (see Figure 4.1. The tissue was transferred to oxygenated Tyrode’s solution (37 °C, pH 7.4) and perfused at 60 ± 5 mm Hg pressure for the duration of the experiment. A
bath electrocardiogram was continuously monitored between 3 suspended needle electrodes, recorded with a Powerlab 26T (AD Instruments).

![Figure 4.1: Experimental Preparation for Canine Transillumination Protocol. a) Endocardial field of view. b) Epicardial field of view. c) Experimental set-up.

When spontaneous contractions resumed, 15 µM Blebbistatin was delivered in a bolus injection over 10 minutes through both coronaries, followed by a 50 µM bolus injection of Di-4 ANBDQBS. Optical mapping files were recorded for 2 or 4 seconds at 1000 Hz simultaneously from both the endocardial and epicardial cameras with a field of view of 4 cm by 4 cm. The tissue was excited by 630 nm LEDs from either the endocardial side, the epicardial side or alternating with each frame. When the light orientation alternates the effective sampling frequency is reduced to 500 Hz for each plane. The signals were processed with the custom MATLAB software described in Chapter 3. One change was made to the previous analysis for the identification of isophase wavefronts in the paced data. In this model, the Hilbert Transform was sensitive to noise and resulted in a high rate of false positive wavefronts for the paced rhythms. We added an additional layer of processing using a matching pursuit algorithm to statistically model the deflections from the paced rhythms before applying the Hilbert Transform. Figure 4.2 displays an example before and after this processing, highlighting the removal of false positive wavefronts and the conservation of the true positive wavefronts. Basic EP parameters were extracted for each plane during paced rhythms and
AF. To induce AF, 0.5-1.0 µM Acetylcholine was added to the perfusate followed by 100 cycles of 50 Hz AC burst pacing. AF was imaged if it sustained for >30 seconds. Measures of dyssynchrony including dominant frequency, regularity index, wavefront count, and primary propagation vectors were assessed using the same parameters outlined in Chapter 3. Although both atria remained intact to conserve fibrillatory patterns that involved the whole surface area, we mapped only one atria at a time due to field of view limitations.

![Figure 4.2](image)

Figure 4.2: Example of Matching Pursuit Algorithm Processing for Isophase Identification. a) Example potential before and after matching pursuit is applied. b) Phase before and after matching pursuit is applied. c) Wavefront identified in spatial map before and after matching pursuit for false wavefront. d) Wavefront identified in spatial map before and after matching pursuit for true wavefront.

**Histological Staining**

After the optical mapping protocol was completed the tissue was removed from the perfusate and fixed for 36 hours in 4% PFA solution followed by serial dehydration in ethanol (N=5 hearts). Small blocks (4cm$^2$) were removed from the pulmonary veins, left appendage, left free wall, and atrial roof for paraffin embedded sectioning along the transmural axis (Histology Core, Washington University Medical Center). Transmural sections from each block, 5 µm thick, were stained with Masson’s Trichrome (IMEB, Inc), or Hematoxylin and Eosin (H & E). All slides (N=5 hearts) were imaged under brightfield illumination using a
Olympus NanoZoomer 2.0-HT System (Hope Center, Washington University in St Louis). Fiber orientation was estimated using a custom MATLAB program based on a previously described method [11]. Images captured at 5X magnification were divided into 100 x 100 pixel non-overlapping panels. The horizontal and vertical gradients were calculated to estimate the fiber orientation. Panels with a majority of fibers oriented between 45° and 135° were identified as containing primarily transmural connection paths. Figure 4.3 demonstrates this method on an example slide from the pulmonary vein region. The red and green boxes highlight representative panels that were identified as having a transmural orientation while the blue box shows an example distribution for a non-transmural orientation. For each slide the fraction of panels that were identified as containing transmural fibers was quantified. Both Masson’s Trichrome and H & E slides were used to estimate primary fiber orientations. In addition to traditional histology we reconstructed the volume of a 5 cm by 5cm section of the pulmonary vein region using cryo-imaging technology (BioInvision, OH) to inspect the transmural transitions in fiber orientation in a region critical to AF substrates.

![Fiber Orientation Estimation Algorithm. Example slice stained with H & E. Red and green panels highlight transmural orientations. Blue highlights nontransmural distribution.](image)

**Figure 4.3:** Fiber Orientation Estimation Algorithm. Example slice stained with H & E. Red and green panels highlight transmural orientations. Blue highlights nontransmural distribution.

**Statistical Methods** Dyssynchrony parameters were compared using the methods outlined in Chapter 3. Briefly, all parameters were averaged per heart before statistical comparison to account for repeated measures in the same anatomy. Unpaired t-tests were applied to make paced vs. AF comparisons. Significance was defined as p < 0.05. Wavefront counts were compared with a Pearson’s linear correlation coefficient. The correlation coefficients are pooled and averaged within each heart, before the two groups are compared. 2D correlation
coefficients from MATLAB were used to compare the DF and RI intensity images from each field of view. The percentage of transmural fibers were compared with a two-way multivariate analysis of variance across both the location and the type of stain used to identify the fibers.

4.3 Results

AF Dyssynchrony

A total of 23 induced AF episodes were included in this experimental cohort, 15 were acquired with epicardial illumination and an additional 8 episodes were acquired with alternating illumination. The episodes had an average DF of $6.93 \pm 0.69$ Hz with a mean difference across the transmural wall of $1.06 \pm 0.28$ Hz, which is significantly greater than 0 ($p=0.0465$). Additionally, the episodes had an average RI of $0.40 \pm 0.07$ with a mean difference of $0.11 \pm 0.02$ across the wall, which is significantly greater than 0 ($p=0.0175$). The spatial distributions of the static frequency parameters were correlated across the wall for individual episodes. Figure 4.4 shows a representative example highlighting the regional similarities across the endocardial and epicardial imaging planes for DF and RI. The average 2D correlation for DF was $0.81 \pm 0.06$ and the 2D correlation for RI was $0.80 \pm 0.06$.

Contrary to the previous sheep model, AF in this model was driven by transient rotation patterns (<2 rotations) instead of persisting rotors. Therefore, we compared the temporal wavefront count and the primary propagation vectors to characterize the dynamic substrate but did not look at detailed phase singularity dynamics. For dynamic parameters, paced rhythms were used as controls. When multiple wavefronts were present simultaneously, the angular average propagation vector of each wavefront was used as the primary angle on both surfaces. Figure 4.5 shows a representative phase map in an instance of time from an AF episode with clear differences in the propagation pattern across both imaging surfaces. In this example a transient rotor is observable on the epicardial surface, while the propagation appears as a focal breakthrough on the endocardial surface. The average correlation in wavefront count was $0.57 \pm 0.027$ for AF compared to a control correlation of $0.80 \pm 0.047$, a significant reduction in correlation ($p=0.0160$). The average difference in the angle of the propagation vectors across the epicardial and endocardial surface was significantly increased.
for AF rhythms with a control difference of $16.34 \pm 4.89^\circ$ and a difference of $43.91 \pm 1.60^\circ$ for the AF condition ($p=0.0365$).

**Functional Gradients**

Before AF was induced, we looked for transmural gradients in EP parameters. We assessed average APD across both reflected imaging planes in the 4 plane acquisition. The average restitution curves from the reflected signals are displayed in Figure 4.6. The APD is relatively flat across both planes until the pacing cycle length dips below 250ms. However, the average epicardial APD is consistently shorter than the endocardial APD when the tissue is paced from the appendage, suggesting that there may be a repolarization gradient across the wall in the canine atria.

Using alternating light we extended the dyssynchrony analysis across 4 imaging planes for N=8 AF episodes. Figure 4.7 highlights the regional transitions across the transmural wall for DF and RI. The average RI and DF values were flat across the transmural wall for all episodes with no average trend between the epicardial and endocardial imaging plane.
Figure 4.5: Simultaneously Acquired Dynamic Parameters for Two-plane Imaging. a) Representative example of phase wave with dynamics that differ across the transmural wall. b) Example wavefront counts from paced and AF cases. c) Table summary of experimental cohort.

**Structure**

Looking at 4 distinct locations within the atrial geometry we found evidence of fiber bundles that seem to provide discrete fast connections between the epicardial and endocardial surface instead of a transition of fibers that all run parallel to the epicardial surface. These fibers did not make up the majority of the orientations observed in the histological sections and they were not observed at a consistent fraction across the regions sectioned. There was not a significant difference between fiber orientation measured from Masson Trichrome or H & E stains. Figure 4.8 displays example Masson Trichrome stains from each region as well as the summary data for the average fraction of transmural-orienting fibers. The two regions with the most fibers oriented through the wall were the left atrial appendage and the pulmonary vein region. Both areas had 20-25% of fibers running in a non-parallel orientation. The atrial roof and the left free-wall had <5% of fibers running through the wall to connect the epicardial and endocardial surfaces. There was no significant difference between the two stains however, there was a significant difference between the appendage and the free wall or the atrial roof, as well as a significant difference between the pulmonary vein and the
free wall or the atrial roof. There was no significant difference between the appendage and the pulmonary vein region. The reconstruction of the pulmonary vein region imaged with Bioinvision’s cryo-imaging technology can be seen in Figure 4.9 along with a 10 µM sample transmural slice to highlight the fine microstructure of the fiber orientation.

4.4 Discussion

The results of this study extend those of the previous study in an ovine model, confirming that transmural dyssynchrony is observed in multiple acute models of AF compared to paced rhythms in the same geometry. There are many differences across animal models including crude shape differences in the atria of canine and sheep. With this study, we confirmed that AF dyssynchrony was not specific to the ovine model and could be replicated in a geometry that more closely resembles human anatomy. Here, we showed that there were statistically significant differences across 2 plane and 4 plane propagation patterns during AF and paced rhythms in the canine model. Additionally, the results confirm that dynamic analysis is required to tease out spatiotemporal dissociation patterns. Histological staining of transmural
sections revealed discrete transmural pathways instead of a structured continuum between the epicardial and endocardial surfaces in the canine atrial wall that may facilitate electrical discordance across the wall and provide structural discontinuities that could harbor a 3D substrate of AF. The geometry of the atria is seemingly much more disorganized than the continual rotating fiber orientation observed across the wall in the ventricle. This may be due to mechanical differences during development. The atria are not required to generate the same degree of force and therefore tight control of the wall structure is less crucial to function. However, the resulting nonuniform distribution of transmurally-running fibers may contribute to the spectrum of functional dyssynchrony. Due to the significant longitudinal and traverse conduction velocity differences across individual cells, these fibers may serve as short circuits, rapidly connecting the epicardial and endocardial plane. Depending on the colocalization of the fibers and rotor cores, the short circuit may synchronize the functional pattern or disrupt the synchronization with rapid breakthrough. The structure alone is not enough to harbor transmural discordance because it is not observed during paced rhythms but the spatial relationship between these fibers and the AF drivers may create the discordance in abnormal patterns. In addition to non-uniform structural paths that could contribute to the spectrum of dyssynchrony, we also observed transmural differences in APD.

Figure 4.7: Simultaneously Acquired Static Parameters for Four-plane Imaging. a) Dominant frequency b) Regularity index. c) Trends across the transmural wall for all AF episodes.
Figure 4.8: Distribution of Transmural Fiber Orientation. a) Percentage of transmural fibers with respect to region. LAA and PV are statistically significantly greater than the free wall and the atrial roof. Error bars represent s.e.m (N=5 hearts) b) Example Masson Trichrome stained sections from each location.

Functional gradients may also play an important role in the 3D substrate that perpetuates stable AF. The transmural distribution of vagal innervation and receptors may provide an additional factor that amplifies the dissociation across the wall in these models that was not studied here.

Figure 4.9: Representative Reconstructed Canine Pulmonary Vein.

4.5 Future Directions

A major limitation of both this study and the previous study described in Chapter 3 is the fact that these studies were conducted on healthy tissue. The arrhythmias analyzed ignore
the temporal progression of AF in the clinical setting. There is a great deal of evidence that functional and structural alterations across different scales within the heart develop to favor AF perpetuation with increased duration [79, 9]. Several studies have begun to investigate more sustained effects of transmural differences on the progression of AF. One such study, conducted by Everett et al., performed endocardial non-contact mapping and epicardial plaque electrode mapping in 5 different models of AF in canines to assess the effect of structural remodeling on the dimensionality of the AF substrate [52]. The authors found that models with only electrical remodeling showed more dissimilarity between the endocardial and epicardial surface activation patterns than models that also incorporated structural remodeling. Conversely, Eckstein et al. charted the time course of epi-endo dissociation during pacing induced AF in goats. Their data suggests that progressive uncoupling in both the longitudinal and the transmural dimension promote stability of AF and increase the duration of AF [45]. The study also proposes that greater degrees of dissociation occur in the thicker atrial tissue. Due to this discrepancy it is critical to expand this study to include additional models of AF that develop stable substrates with some degree of pathophysiological remodeling. Infiltration of fibrosis and the redistribution of connexins have the potential to severely disrupt the epicardial to endocardial relationship observed in these models. Therefore, we have begun a preliminary study on a model of self-sustaining AF in the canine to compare directly to the results of the acute model. The chronic substrate is created by 6-8 weeks of high rate pacing-induced electrical remodeling. The chronic model, which has been used extensively by many groups including our own, results in spontaneous and self-sustaining AF. These animals were implanted with an ICD (Maximo, Medtronic) and a right atrial appendage pacing lead. After at least 1 week of recovery, high rate pacing (400 bpm) was turned on using a custom software extension. If the ventricular rate was greater than 130 bpm, 0.25-0.375 mg of digoxin was administered. The animals were checked weekly for spontaneous AF and for ventricular rate control. After AF that sustained for >24 hours the hearts underwent an unrelated defibrillation experiment and were explanted for optical mapping following the same procedure outlined above.

To clarify the structure-function relationship of the transmural fiber orientations, the anatomical architecture needs to be registered to the functional data. Analyzing both in the same coordinate system may clarify the mechanism between different levels of dyssynchrony. Due to the non-uniform distribution of these fibers, we may be able to use this analysis to identify anatomical regions that are more likely to require dual sided analysis. If we do not have
to map the complete surface area of both the epicardial and endocardial planes but instead focus on regions that are more prone to dyssynchrony we may be able to acquire this data clinically without requiring new technological advances in sensing.

4.6 Acknowledgements

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Chapter 5

Iterative Advancements Towards Low Voltage Defibrillation Delivery Strategies for Atrial Fibrillation

“When fibrillation stopped it was an accident and that [with therapy] one merely set up the conditions to permit this accident to occur more readily” - Gordon Moe [120]

Portions of this chapter appear in the following original publication:


5.1 Introduction

Electrical cardioversion of AF is effective at restoring sinus rhythm but in its current form it is not a practical continuous therapy. As long as the defibrillation threshold (DFT) exceeds the pain threshold, patients will not tolerate an implanted cardioverter. In an effort to drive down the DFT we previously developed an electrotherapy approach that shifts from a single universal waveform to an adaptable sequence tailored to the frequency of each individual episode of AF. This therapy is rooted in the initial discovery that there is a shock
strength phase dependence for successful unpinning of atrial and ventricular tachyarrhythmias [111, 150] and the notion that multiple pulses increase the probability of delivering a low-voltage shock during the optimal temporal window [12, 112]. From these initial studies, we hypothesized that a further decrease in peak voltage could be achieved if the multiple pulses were followed by anti-repinning phases to prevent the reinitiation of AF. Our novel electrotherapy consists of 3 stages of successively decreasing energy levels, which we refer to as multi-stage electrotherapy (MSE) delivered in a frequency-dependent manner as shown in Figure 5.1. We prospectively compared MSE with the gold standard single biphasic shocks (BPS) to establish the DFT in an in vivo canine model of self-sustaining AF, delivered through chronically-implanted transvenous leads. The chronic model of AF is described in 2. Briefly, a pacing lead (model number 5096; Medtronic, Inc.) was implanted into the right atrial appendage (RAA), and defibrillation leads were implanted into the RAA (model number 6935; Medtronic, Inc.), left pulmonary artery (LPA), and coronary sinus (CS) (model number 6937A; Medtronic, Inc.). The CS of canine hearts often tapers abruptly, preventing implantation of a lead in the distal CS. The inferior branch of the LPA runs adjacent to the lateral CS and was therefore used to anatomically approximate the clinical distal CS implantation. The pacing lead was connected to an implanted high-rate pacing (HRP) device (Medtronic, Inc.) programmed with custom software. The HRP at 400 beats/min began 1 week after implantation from the RAA pacing lead at twice the atrial capture threshold. For this study, AF was defined as self-sustained AF lasting >30 min during interrogation. Once this arrhythmia was observed, the animal was rechecked 1 week later to determine if AF persisted for >1 week. If AF was present defibrillation studies were performed.

A total of 32 in vivo defibrillation studies were performed in 8 dogs; 123 episodes of sustained AF were successfully converted to sinus rhythm, yielding an average of 3.8 cardioversions per study. The mean DFT of MSE was significantly lower compared with that of BPS in terms of total energy (0.16 ± 0.16 J vs. 1.48± 0.91 J, respectively; p <0.001) and peak shock voltage (31.1 ± 19.3 V vs. 165 ± 34 V, respectively; p <0.001). Importantly, these results demonstrate that atrial DFT can be effectively lowered by using commercially available transvenous implantable leads placed in RA and CS.

Subsequently, we investigated the mechanism by which MSE terminates AF by using optical mapping. The hearts of 7 dogs with self-sustaining AF induced by HRP were explanted for mechanistic studies. Due to denervation, some hearts required the perfusion of low doses of
acetylcholine to induce sustained AF *ex vivo*. A representative AF episode is shown in Figure 5.2. Sample optical recordings are shown from the highest dominant frequency locations. To illustrate a possible mechanism MSE, the spatial evolution of the tissue response after the application of MSE was contrasted between an example of successful MSE application (with a peak voltage of 7 V/cm) and an unsuccessful application (5 V/cm) from the same animal. Phase maps from these two example sequences are displayed in Figure 5.3. MSE succeeds when Stage 1 can homogenize the tissue, which allows the Stage 2 shocks to prevent any critical points that may result from the virtual electrode polarization (VEP) of the initial shocks or the original drivers to reinitiate arrhythmia. Stage 2 works by continually capturing an increasing amount of tissue with each shock. This action thus prepares the atria for the pacing spikes of Stage 3 to reset the repolarization pathway. In the unsuccessful case, the therapy fails during Stage 2. Due to the results of this biophysical investigation we designed several studies to optimize the timing of Stage 2 delivery and test the efficacy of incorporating spatial sensitivity to drive the DFT down further. The goal of these studies is to design an electrical cardioversion protocol that exploits both temporal and spatial vulnerabilities. Following a similar pattern used in earlier development we used a rabbit model to establish sensitivity and optimize parameters before implementing the therapeutic protocol in a canine model to test the feasibility of exploiting it.
Figure 5.2: Example Frequency Content of ex vivo Canine Atria. a) Optical field of view. b) Spatial dominant frequency. c) Spatial regularity index [90]

5.2 Methods

All animal procedures were performed in accordance with the ethical guidelines of the National Institutes of Health and were approved by the Washington University in St Louis Animal Studies Committee. Acute AF was induced in Langendorff-perfused, pressure-loaded rabbit hearts (N=5 per experimental condition, N=15 total). The animals were anesthetized with 80mg/kg Sodium Pentobarbitol and 400 U/kg of heparin IV through the ear vein catheter. When unresponsive to pain, the heart was removed via gross dissection and a cannula was placed in the aorta to allow retrograde perfusion of oxygenated Tyrode’s solution. Epicardial fat was removed from the atria to provide a clean field of view. The interatrial septum was perforated with access gained through the pulmonary veins, taking care not to damage the sinus node. The pulmonary and caval veins were tied off, while the pulmonary artery was cannulated and loaded with a constant elevated pressure of 10-15 cm H$_2$O. The expanded atria could then support an arrhythmia that was induced with programmed stimulation (100 cycles of 50 Hz AC pacing). A bipolar sensing electrode was placed on the left atria to estimate the frequency with Labchart’s real-time cycling algorithm (AD Instruments). The voltage delivered was recorded with a differential voltage probe (Yokagawa) and the current was recorded with a AC/DC current probe (A622, Tektronix, Inc., Beaverton, Oregon) for each defibrillation strategy. For multi-path therapies these measurements were made upstream of the digital relay gates. A CMOS camera was suspended above the preparation to view the superior aspects of the inflated atria. Blebbistatin and di-4ANEPPS were perfused to prepare the tissue for optical mapping using a 520nm excitation LED and a >650nm long pass emission filter. Due to the transparency of the inflated atrial tissue
Atrial Fibrillation

Stage 1
Stage 2
Stage 3

Figure 5.3: Comparison of Successful and Unsuccessful Multistage Electrotherapy Application. a) Successful termination with a peak shock strength of 7 V/cm displayed with representative optical trace from middle of the field of view (orange trace). The panels show the evolution of phase during therapy application, beginning with an AF example, then proceeding to Stage 1 shocks 1 and 2; Stage 2 shocks 2, 4, and 6; and first stimulus of Stage 3. The middle left panel in A shows a representative optical action potential with the definition of phase. b) Unsuccessful termination with a peak shock strength of 5 V/cm displayed with representative optical trace from the middle of the field of view (blue trace). Panels show evolution of failed response to therapy, starting from an AF example, then progressing to Stage 1 shocks 1 and 2; Stage 2 shocks 2 and 4 through 6; and first stimulus of Stage 3. [90]

the optical recordings from this perspective are a superposition of the atrial and ventricular signals. It is important to maintain sinus rhythm in the ventricles while the atria are in AF so that the signals can be separated digitally by frequency content using wavelet analysis. Therefore, the defibrillation shocks were triggered by the R-wave to prevent inducing ventricular arrhythmias. If a ventricular arrhythmia was induced, it was immediately terminated before continuing with the protocol.

Defibrillation efficacy

Three separate experimental protocols were used to test 12 different therapies. Each experiment included a control condition (BPS) to account for differences across animals. For the first study (Experiment I) the timing parameters for coupling stage 2 and stage 3 were altered. The interstage delay and the intra-stage frequency were varied compared to the AF cycle length (CL) to create the following 5 experimental conditions: 1) 50% AF CL delay + 100% AF CL frequency, 2) 80% AF CL delay + 100% AF CL frequency, 3) 100% AF
CL delay + 100% AF CL frequency, 4) 50% AF CL delay + 90% AF CL frequency, and 5) 50% AF CL delay + 70% AF CL frequency. All therapies were delivered across a single pair of mesh electrodes. For the second study (Experiment II), we tested delivery of only Stage 1 across varied shock vectors to create the following 4 experimental conditions: 1) 2 shocks delivered anterior-posterior, 2) 2 shocks delivered across lateral vector, 3) 1 shock delivered anterior-posterior followed by 1 shock lateral vector, and 4) 1 shock delivered across lateral vector followed by 1 shock delivered anterior posterior. All pairs of shocks were delivered within 1 AF CL. For the third study (Experiment III) we added more electrodes to test the following conditions: 1) 2 shocks delivered with an angle shift of 60°, 2) 2 shocks delivered with an angle shift of 120°, and 3) 3 shocks delivered with two angle shift of 60°.

Figure 5.4: a) Experimental Set-up for Rabbit Atria Defibrillation Efficacy Studies. b) Representative successful cardioversion with 2 Stage 1 shocks at 30 V each, rotated by 270°.

Custom devices with 6 cm x 1 cm stainless steel mesh electrodes were placed in the bath surrounding the whole heart. Two different devices containing 4 electrodes 90° apart or 6
electrodes 60° apart were used in these experiments. All shocks were delivered via a custom Labview based defibrillation platform developed by Cardialen, Inc that is on loan to Washington University in St Louis. Additional digital relay gates were added to the front of the system to provide the timed vector switches. The relay gates were constructed with ST1-DC5V relays (Panasonic) controlled by custom Labview code and example of which is included in Appendix C. The complete experimental set-up is displayed in Figure 5.4.

Once sustained AF (>1 min without self-terminating) was achieved, the therapies were delivered in a randomized order. Each therapy was delivered, starting at 10V and increased in increments of 10V until successful termination was achieved. Every therapy was tested at least 5 times per animal per experiment. Ten seconds were allowed to pass after a failed defibrillation attempt before another shock was delivered to prevent coupling. Five minutes passed before re-inducing AF after each successful cardioversion.

A probability of success curve with a Weibull distribution was created from the compiled series of successful defibrillations for each condition. From these distributions, the shock strength for 50% success (p50) and 80% success (p80) was determined. Figure 5.5 illustrates the process. Statistically, the Weibull distribution curves were compared within each experiment using a log-rank test for multiple conditions.

![Figure 5.5: Weibull Distribution Fit to Defibrillation Threshold Data. a) Distribution of successful BPS shocks. b) Weibull Fit to DFT data. c) Quantiles show linear trend which means that the Weibull Distribution is a good fit to the DFT data.](image)
5.3 Results

Experiment I

In this study we used the ex vivo rabbit model of AF to address the effects of the timing of the multiple components in each stage of MSE on defibrillation efficacy. The probability of success curves for all 5 conditions are displayed in Figure 5.6. Since the Weibull distribution is based on a model fit through the data, the 5% and 95% confidence intervals are also displayed for each condition. The long-rank test determined that the curves are statistically different (chi-squared 25.5, p < 0.005). The p50 and p80 values display similar trends between therapies. The minimum p50 was achieved with an inter-stage delay of 50% AF cycle length (CL) and a stimulus frequency of 100% AF CL. The mean p50 shock strength for this therapy was 2.73 V/cm (CI 2.73- 3.64 V/cm). The mean p50 shock strength for BPS was 5.45 V/cm (CI 4.55-6.36 V/cm). Elongating the inter-stage delay was detrimental to the DFT for both tested conditions. This seems to hold with the results observed optically in the chronic canine study. AF episodes in this model have a mean frequency of 15.54Hz, which is comparatively fast. We looked for a correlation between AF CL and DFT. These therapies are all dependent of frequency and it is possible that the pacing spikes in Stage 3 begin to approach the frequencies with which we induce AF in healthy tissue. Therefore we looked for a frequency limit that may lead to reinduction and drive the DFT up. Table 5.1 displays the results of the correlation with the DF assessed optically and the DFT for all therapies. Only the intra-stage frequency at 70% AF CL was negatively correlated with DFT.

<table>
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<th>50% AF CL interstage delay, 100% AF CL intrastage CL</th>
<th>80% AF CL interstage delay, 100% AF CL intrastage CL</th>
<th>100% AF CL interstage delay, 100% AF CL intrastage CL</th>
<th>50% AF CL interstage delay, 90% AF CL intrastage CL</th>
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<td>-0.3046</td>
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<td>-0.6983</td>
</tr>
</tbody>
</table>

Table 5.1: Correlations between Domination Frequency and Defibrillation Threshold for Temporal Multi-stage Therapy.

Experiment II and III
We used the *ex vivo* rabbit model of AF to address the effects of using multiple shock vectors independently or in series for Stage 1 on defibrillation efficacy. The probability of success curves for BPS compared to Stage 1 for both vectors are displayed in Figure 5.7. The long-rank test determined that the curves are statistically different (chi-squared 12.06, \( p < 0.01 \)). At p50 the lateral vector has the lowest DFT, 3.64 V/cm (CI 2.73-4.58 V/cm) compared to the BPS DFT at 5.45 (CI 4.55-6.36 V/cm). At p80 there is still an advantage to having 2 shocks but neither vector is more advantageous. Figure 5.8 shows the results of using 2 vectors with a shift in the vector by 60°, 90°, 120° or 270°. The lowest DFT is achieved with a 60° shift in Stage 1 with a P50 of 2.72 V/cm (CI 1.82-2.72), followed by a 270° shift in Stage 1 with a p50 of 3.64 V/cm (CI 3.64-4.54 V/cm). Using 3 shocks resulted in a p50 of 3.64 (CI 2.72 - 5.45 V/cm), which had the widest range in confidence intervals (data not shown).

Figure 5.7: Probability of Successful Defibrillation Sensitivity to Shock Vector a) Weibull distributions b) p50 c) p80 Errorbars represent 95-5% CI.
5.4 Discussion

In order to compare defibrillation paradigms it is imperative to have a robust method for quantifying defibrillation efficacy. In the past we have used the sample mean of the DFT as a measure of efficacy. Davy et al. evaluated using DFT in a canine model and concluded “that no unique DFT exists, . . .the relationship between the likelihood of successful defibrillation and delivered energy is best described by a ‘dose-response’ curve requiring multiple trials for evaluation” [41]. Therefore we designed a new method to use Weibull survival analysis to develop dose-response curves with respect to peak voltage and address the probabilistic nature of defibrillation. By optimizing the therapy in both the temporal and spatial domain, we found that a 60° shift between the two shocks of Stage 1 improves defibrillation efficacy. Additionally a short inter-stage delay is more critically important than cycle length of Stage 2. We did find a negative correlation between AF CL and efficacy in this model if Stage 2 and 3 are delivered faster than 90% of the AF CL. Because this model leads to AF CL that are twice what is seen clinically in humans, it is unclear if this risk persists in larger models. The ex vivo bath environment has several important limitations. The bath provides a highly conductive alternative path for the current to take through the mesh electrodes. The lack of methodology to account for the fraction of current that travels along this path makes it difficult to interpret the dose-response curves with respect to total energy delivered. Additionally the charge density from the mesh electrodes is more uniform than that created with lead-based coils.

![Graphs](image)

Figure 5.8: Probability of Successful Defibrillation Sensitivity to Vector Shift. a) Weibull distributions b) p50 c) p80. Errorbars represent 95-5% CI.
Additionally, with the myocardium exposed we have the flexibility to define shock vectors at any angle. Traditional lead based defibrillation does not provide the same degree of flexibility in available shock vectors since there are a fixed number of vessels that can accommodate shock coils. Additionally, there is some degree of variability in vascular anatomy that would make controlling for a 60° shift challenging. To test the feasibility of transferring the spatial advantage to lead-based system we have begun to test the MSE with multiple paths in the same chronic self-sustaining AF model described above (N=4). At the time of pacemaker implant we also implanted shock coils (Medtronic 6947, 6937A, or 6935) to the superior vena cava (SVC), the right atrial appendage (RAA), the proximal coronary sinus (CS), and the left pulmonary artery (LPA) under fluoroscopic guidance. We used two shock vectors that transect both the anterior-posterior and lateral orthogonal paths: SVC to CS and RAA to LPA. Unfortunately, it is extremely difficult to target the left atrium in a lead-based system.

After sustained AF (>24 hours) was achieved with high rate pacing (average DF of 8.15 ± 0.65 Hz), the animals were scheduled for a terminal defibrillation study.

![Figure 5.9: Multi-stage Multi-path Defibrillation Paradigm in Large Animal Model](image)

We tested 5 separate therapies: BPS, MSE across only the SVC-CS vector, MSE across the RAA-LPA vector, a vector shift between the shocks in Stage 1, and a repeated shift in Stage 2 where alternating Stage 2 pulses were delivered across different vectors to combine the results of the rabbit study into a multistage, multi-path therapy paradigm. In these studies, Weibull survival curves for both peak voltage and total energy can be calculated because the current path is limited to within the closed chest. Total energy is defined as: \( \int VdI \cdot dt \). The preliminary results of this pilot study are in the process of being analyzed. Preliminary results are shown in Figure 5.9. The vector switch in Stage 1 required the lowest peak voltage.
to defibrillate with 50% and 80% success rates. Although these results are based on a small N, this pilot study suggests that including a multi-path component continues to drive down the peak voltage required to defibrillate and should be investigated in a larger study.

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Chapter 6

Multifunctional Stretchable Electronics Quantify the Spatial Electrophysiology of Cardiac Tissue

Portions of this chapter appear in the following original publications:


*these authors contributed equally to the publication.
6.1 Introduction

High-density cardiac mapping has been an important experimental and clinical tool for the identification and the evolution of the understanding of normal conduction and arrhythmia mechanisms. The first electrode “heart socks” and temporary silicon electrode sheets were developed in the 1980s for global epicardial electrical mapping. As basic research tools, many of the first sock devices were handmade designs with recording electrodes mounted on synthetic fabric, sewn to loosely fit the ventricle [76, 142, 201, 202]. Studies with these devices were used to investigate local potential heterogeneities in ischemia transition regions [170], to visualize atrial activation patterns including preferential pathways [43], and to test cardiac resynchronization therapy pacing sites for mechanical resynchronization [80]. However, due to the dynamic contours of the beating heart, it is difficult to achieve uniform quality of contact consistently across the heart with the fabric socks. Additionally, the handmade assembly creates limitations in spatial coverage, array density, and scalable manufacturing. Although still used frequently in the research setting, these devices have not transitioned to clinical applications. As a result, alternative strategies based on serial mapping with point-contact catheters or imaging techniques that use fluorescence, nuclear magnetic resonance or ultrasound have emerged to attempt to replicate the spatial coverage and specificity required. Moreover, the heart is a complex electromechanical syncytium with numerous elements that work in tandem. Much has been gained through studies of each network in isolation. However, the interplay of pathophysiological remodeling across the electrical, metabolic, and mechanical machinery of the heart compels the development of tools that simultaneously probe various states and continuously provide multiparametric mapping capabilities inclusive but far beyond electrical sensing in a high resolution manner. This is a challenge with conventional materials, device technologies and imaging modalities but recent developments in material fabrication and innovative circuit design provide a stretchable electronics platform from which to integrate a range of sensors with the epicardial surface or transmural wall of the myocardium.

Using this platform, we built multifunctional semiconductor systems in lithographically defined configurations on thin elastic membranes designed to hug the curvilinear contours of the cardiac geometry or be inserted within the geometry with compatible mechanics so as not to disrupt contraction. The physical format of the 3D epicardial membrane resembles that of the
naturally occurring membrane that surrounds the heart, known as the pericardium. These systems, which we refer to as 3D multifunctional integumentary membranes (3D-MIMs) provide conformal interfaces to all points on the heart, with robust but non-restricting contacts enabled by the soft elasticity of the membrane itself. A diverse array of sensors and actuators can be placed in custom orientations across the membrane and tailored to different shapes depending on the intended implementation. Planar sheets, epicardial membranes, balloons, and ultrathin injectable needle-type sensors have all been designed and tested. Examples of each design are shown in Figure 6.1. We used ex vivo rabbit hearts to demonstrate the utility of this platform and test the feasibility of a range of sensors to simultaneously interrogate many cardiac states for research, diagnostic, or therapeutic use.

Figure 6.1: Diversity in Cardiac Specific Stretchable Electronics Platform. a) sheet, b) epicardial membrane, c) endocardial balloon, d) transmural needle

6.2 Methods

Design and Fabrication

The cardiac specific devices presented here build upon previous work at the University of Illinois- Urbana Champaign on soft-contact electronics [96, 98]. The elastomer substrate and the circuits are designed to stretch, twist, and bend to great extremes while maintaining the integrity of the circuits. The fabrication of the 3D-MIMS begins with the creation of a thin, 3D elastic membrane shaped to the heart. As shown in Figure 6.2, optical segmentation techniques first capture the full 3D geometry of a heart of interest. For the experiments presented here, a single representative rabbit geometry [116] was used in place of patient-specific imaging. A commercial 3D printer (ZPrinter 450, Z-Corporation) then
renders a solid model of the heart in a proportionally scaled form to serve as a substrate for mounting ultrathin electronic/optoelectronic sensor systems, separately prefabricated on planar substrates. The methods for creating these components exploit modern integrated circuit technologies and achieve spatial resolution far beyond that possible with manually assembled arrays. The sensors are patterned by photolithography and wet etching. The 3D-MIMs are fabricated using standard planar processing of inorganic semiconductor materials (Si, InGaN or AlInGaP) followed by transfer printing onto substrates coated either with a bilayer of polyimide (PI) on poly(methyl methacrylate) (PMMA) or poly(ethylene terephthalate) (PET) on poly(dimethylsiloxane) (PDMS) [97, 100, 101, 6]. Dissolution of the PMMA or delamination from the PDMS allows release of the devices. Metal layers (Cr/Au) are vacuum-deposited and patterned to form interconnects, resistors and electrodes. Application and patterning of a polymer encapsulation layer (PI or a photosensitive epoxy, SU8) on top of the devices completes their fabrication. Transfer printing delivers the resulting structures to a thin film of a low-modulus silicone elastomer (Ecoflex, Smooth-on). A thin layer of silicone elastomer is casted and cured on top of the heart model with these multi-functional devices on its surface. The front faces of the device components contact the model while the back faces bond to the elastomer. Removing the 3D membrane with integrated device components from the model prepares it for deployment as a type of “instrumented” artificial pericardium. Figure 6.2 shows a representative 3D-MIM that includes microscale, inorganic light-emitting diodes (µ-ILEDs) based on indium gallium nitride (InGaN) for optical mapping, silicon (Si) nanomembranes for strain gauges, gold (Au) electrodes for electrical sensing/stimulation, iridium oxide (IrOx) pads for pH sensors and Au serpentine resistors for temperature sensors/heaters. A serpentine connection design removes the rigidity of the metal wiring, allowing the circuit to stretch with the heart and the substrate on each contraction. The epicardial devices have secure holes in the substrate to prevent trapping of fluid between the device and the heart.

The 3D-MIM is engineered with overall dimensions slightly smaller than those of the real heart, to provide adequate elasticity and mechanical support for robust contact with the epicardium during diastole and systole, but with sufficiently small pressures to avoid disruption of natural behaviors of the cardiac tissue. The serpentine mesh that interconnects the device components covers the ventricle and conforms to the contours of the epicardium. Although this example is designed for research applications on rabbit hearts, the same strategies are applicable to human hearts, or even other organ systems.
Conversely for the ultra-thin needle-type sensors, Au metal film (150 nm thick) was patterned onto 6 µm thick polyethylene terephthalate needles [184, 102]. The needles were integrated with three temperature coefficient of resistance sensors, spaced 1 mm apart, each consisting of a 10 µm wide Au serpentine filament occupying 0.4 mm × 0.4 mm. A 4-wire contact configuration reduced errors caused by changes in lead wire resistances, ensuring measurement of local temperature according to the individual sensing elements. SU-8 (5 µm thick) was encapsulated over the sensor to provide electrical insulation and a moisture barrier. The needles were mounted onto 50 µm tungsten guide needles with cellulose for injection into the transmural wall. After implantation, the guide needle was removed by dissolving the cellulose adhesive, thus leaving only the ultrathin flexible temperature sensors embedded within
the myocardial tissue. A thin, flexible heat-seal conductive cable (Elform, HST- 9805-210) serves as a connection to external hardware for data acquisition, power supply and control. More details on the fabrication process that was developed by our collaborating lab can be viewed in the corresponding publications.

**Animal Experiments**

Feasibility experiments in the rabbit model were conducted in accordance with the ethical guidelines of the National Institutes of Health and with the approval of the Institutional Animal Care and Use Committee of Washington University in St Louis. Briefly, the animal is anesthetized with 80mg/kg Sodium Pentobarbitol and 400 U/kg of heparin IV through the ear vein catheter. When unresponsive to pain, the heart is removed via a thoracotomy and a cannula is placed in the aorta to allow retrograde perfusion of oxygenated Tyrode’s solution. The perfusion mimics the electrolyte balance within the animal and provides an energy substrate for the heart to continue to function normally from an electrical perspective. The heart is submerged in a perfusion chamber maintained at 37 °C with a pH in the range of 7.35-7.45. Coronary perfusion is maintained at 60-80 mm Hg with peristaltic pumps. The optical signals of transmembrane potential ($V_m$) rely on the collection of fluorescent signals from a potentiometric dye (di-4 ANEPPS) with a CMOS camera; when needed to avoid motion artifacts, an excitation-contraction uncoupler (Blebbistatin) is also added to the perfusate. 3D-MIM devices with various sensors were deployed after contraction was arrested and the heart was exposed to a number of conditions including pacing, pinacidil-induced ventricular fibrillation, cold temperatures, ischemia, reperfusion, and focal ablation with a cautery pen.

The electrical signals are recorded from the Au electrodes on the 3D-MIMs with a 240-channel unipolar EG acquisition system (Astrocard, Boston) and a custom-built interface. Both the optical and electrical signals are collected at a sampling frequency of 1 kHz, aligned with a trigger TTL pulse and post-processed separately in custom MATLAB software. The electrical signals acquired from the 3D-MIMs are first filtered with a 60 Hz notch filter internal to the acquisition software, then the electrophysiological parameter of interest (e.g. activation time) is calculated and aligned to the spatial coordinates of the electrodes based on the optical background file. The optical signals are binned, filtered and normalized as previously described in Chapter 2. The electrophysiological parameters are calculated for
Figure 6.3: Analysis Methodology for OptoElectric Spatial Comparisons. a) Electrical mapping, b) Optical mapping

the complete field of view. To create the spatial maps, the activation times are interpolated using MATLAB’s internal function for cubic interpolation of scattered data. The optical map is also sampled at the coordinates of the electrodes and the same interpolation method is applied to compare the full-resolution optical pattern with the sampled optical map and the electrical map (Figure 6.3). Data for temperature and strain sensors are acquired with measurements of resistance of each sensor using a custom-built system based on National Instruments PXI-6289 board. The resistance of each of the 16 sensors is recorded simultaneously with a 160 mA probe current, a 16-bit A/D converter and a 15 ms sampling period at each sensor.

To design the mechanical compatibility of the 3D-MIM, we deployed the devices on a left-sided working heart model instead of a Langendorff perfused model (N=3 experimental, 3 control). In this model the left atrium is cannulated through an incision in the appendage. All other outflow ostia (vena cava and pulmonary veins) are tied off. The aorta is attached to a water column that maintains an afterload of 60-80 mm Hg. A modified Krebs perfusate is used with 20 mg/L albumin to carry more oxygen to the working myocardium and 2 mM sodium pyruvate to provide a fatty acid energy source. Blebbistatin is not used in these
experiments. The performance of the cardiac contractions and the health of the heart were monitored for at least 1 hour with and without the 3D-MIM.

For the ultra-thin needle-type sensor experiments a Langendorff-perfused left ventricle (LV) flap preparation was used (N=7 hearts). After the heart was removed the distal left coronary branches were ligated and the LV free wall was dissected free by opening from apex to base along the ventricular septum. The LV free wall flap was pinned epicardial side down onto the silicone floor of the bath. Figure 6.4 illustrates the implant procedure for injecting the ultrathin temperature sensors into the myocardium. The ultrathin needle-type temperature sensors were implanted in the left ventricular wall of perfused a rabbit heart from the exposed endocardial surface. Due to the flexible nature of this device, the sensor was bonded to a tungsten guide needle (50 µm thick) using cellulose, which is a water-soluble and biodegradable natural polymer, in order to provide sufficient support for implantation. After implantation, the guide needle was removed by dissolving cellulose adhesive, thus leaving only the ultrathin flexible temperature sensors in the myocardial tissue.

Ablation lesions were drawn in close proximity to the implanted temperature sensors. For radiofrequency ablation (RF), a commercially available, non-irrigated 4 mm tip Safire catheter (St Jude Medical, Saint Paul, MN) was used for 60 seconds at varying powers (3-20W), temperature limited at 60 °C. Cryoablation was performed using an AtriCure system (AtriCure, West Chester, OH), for 120 seconds at a target temperature of -50°C. Once all endocardial surface area was exhausted, the heart was removed from the perfusion chamber and incubated in a 1% solution of triphenyltetrazolium chloride (TTC) for 20 minutes at 37C. Lesions were transected and photographed [57]. TTC-negative regions were classified as being successfully ablated while regions staining positive for TTC were considered viable. These images were converted to grayscale and analyzed using custom MATLAB code to assess depth of ablation and percent transmurality of each lesion. Temperature was determined by monitoring resistance change of implanted sensors while conducting cardiac ablation on the implantation site. The resistance change was recorded via a digital multimeter (National instruments; Austin, TX) under 4-wire resistance measurement mode with custom electronics programmed with LabVIEW software allowing simultaneous recording at 4 Hz sampling rate [184]. Resistance values were converted to temperature based on individual calibration curves for each sensor. Thermal conductivity was assessed using fabricated TCR sensors by analyzing thermal transient data while applying 10 mA current for 2 s on sensing elements,
which generated joule heating [185]. The chronothermographs recorded during RF ablations were analyzed with custom MATLAB code.

![Figure 6.4: Implant of Ultra-thin Needle Electrodes](image)

**Figure 6.4: Implant of Ultra-thin Needle Electrodes**

### 6.3 Results

**Mechanical Effects of 3D-MIM**

A chronological comparison of the pressure waveform at the aorta and electrophysiological indicators of ischemia during the working heart preparation with and without the 3D-MIMs on the heart was assessed to aid in the design of the mechanical compatibility of the device. The results can be seen in Figure 6.5. Signs of ischemia include a decrease in the pressure waveform, which is an indication of contractility, a decrease in the corrected QT interval of the far field electrogram and an elevation of the ST segment of the far-field electrogram. The temporal pattern for the control hearts indicates that the working heart preparation is not stable across the hour even without the 3D-MIM, however the pressure waveform and the ST elevation do not seem to indicate that the 3D-MIM is impairing functionality. The QTc is shortened in the 3D-MIMs group, which does suggest that there may be some impact on repolarization that would need to be investigated further.

**Spatiotemporal Cardiac Measurements and Stimulation of 3D-MIM**
To demonstrate the various functional modes of operation we conducted high-precision mapping of epicardial electrical activity. A single 3D-MIM can accommodate some range in specific sizes and shapes associated with a single type of animal model, due to its soft, elastic construction. The device here incorporates 68 Au electrodes (1 mm² surface area and spacing of 3.5 mm), distributed across both the anterior and posterior surfaces of the epicardium. The electrochemical impedances of individual electrodes are 2 kΩ at a frequency of 1 kHz, measured in phosphate-buffered saline. In the current design iteration, these devices achieve a spatial resolution of 5 mm with an average signal to noise ratio of >40 dB across both pharmaceutically arrested and beating rabbit hearts. The transparency of the membrane allows simultaneous optical mapping through voltage-dependent fluorescence, as a means for validating the electrical measurements. Signals were acquired from four hearts under a variety of conditions: normal sinus rhythm, and paced at a range of frequencies and from a range of electrode pairs to increase the variability of the propagation patterns in the spatial activation maps. The surface electrograms captured various key morphologies associated with the QRS and T waves (Figure 6.6). Representative maps and correlations between
electrical and optical activation times appear in panel c and b, respectively. The overall linear correlations between optical and electrical activation times were 0.957 for sinus data and 0.943 for paced data. Additionally, activation recovery interval and action potential duration values were correlated across recording methods. The results show a strong correlation and similar spatial patterns as seen in Figure 6.7. These studies indicate that this configuration of measurement electrodes can replicate patterns of activation to a resolution that captures the spatial variations observed optically. Figure 6.6 presents a 3D map derived from signals recorded from the anterior and posterior surfaces of the heart. Unlike optical mapping where motion artifacts dramatically impact the measurement quality requiring static heart geometries, electrophysiological mapping with 3D-MIMs can be applied under normal beating condition. The integrated sensors move synchronously with the underlying cardiac tissue. Although it is practically difficult to avoid relative lateral motion between the sensors and the epicardium during beating cycles, due to the engineered geometries of 3D-MIMs, the displacement can be minimized to be less than the characteristic sizes of the sensors and to have negligible impact to the signal quality. This feature is necessary for extending the mapping capabilities beyond laboratory studies and implementing in clinical electrophysiology.

A 3D-MIM with arrays of temperature sensors illustrates capabilities in monitoring spatial distributions of cardiac temperature. The temperature sensor elements use designs established previously, consisting of serpentine traces of gold (20 μm wide, 50 μm thick) in which changes in resistance correlate to changes in temperature. The temperature sensors exhibit linear responses over physiological range, with a measurement precision of 23 mK when sampled at 2 Hz in typical hospital settings. Figure 6.8 shows a 3D-MIM with 16 integrated temperature sensors during use on a beating heart. The sensors are calibrated in a temperature-controlled water bath before the animal experiments, exhibiting average responses of $1.23 \pm 0.05 \, \Omega/°C$ over 16 sensors. In one experiment, the temperature of the heart was changed by altering the temperature of the perfusion. The measured epicardial temperature gradually decreased by 7°C during cooling of the perfusate, with a uniform distribution of temperature across the heart. The heart rate, determined from the far-field electrocardiogram, decreased with decreasing temperature and recovered to the original value as the temperature returned to physiological levels, indicating that temperature controlled the rate of myocardial metabolism. In a second experiment, a cautery pen was used to acutely burn a small region of the epicardium, simulating clinical ablation. The associated
Figure 6.6: High-Density Electrical Mapping: Activation. a) Representative optical and electrical signals acquired simultaneously. Scale bar, 7 mm. b) Top: schematic illustration of activation time definition. Bottom: correlation of electrical and optical activation times. c) Interpolated spatial activation maps determined from the electrical and optical measurements for a paced heart and sinus rhythm. d) 3D mapping of electrical signaling from both the anterior and posterior surfaces of the heart. Scale bar, 7 mm. [206]

temperature map (Figure 6.8) shows localized elevation of temperature near the point of ablation. Such information can be used as feedback for clinical control of ablation time and size of affected area. In combination with electrical sensors, such a device could provide the real-time relation between temperature and excitation.

Transmural Spatiotemporal Temperature During Cardiac Ablation with Ultra-thin Needles

The three temperature sensing moieties on the ultrathin needle-type temperature sensors are embedded within the transmural wall such that the proximal temperature sensor (Sensor 3) is located just below the endocardial tissue surface and the implant depth of distal
Figure 6.7: High-Density Electrical Mapping: Repolarization. a) Definitions of activation recovery interval and action potential duration. b) Correlation between electrical and optical parameters. c) Example spatial patterns from electrical and optical parameters [206]

The temperature sensor is approximately 3 mm. The sensor provides real-time chronothermographs at different tissue depths during ablations (Figure 6.9). Of note, the extremely thin geometry and high degree of mechanical flexibility of the device allow great biomechanical compatibility, minimizing the risk of tissue damage (see publication for Supporting Video 1). The temperature change was monitored while conducting both cryo-ablation and RF ablation to demonstrate performance at a range of temperature values relevant to ablation. The chronothermograph was monitored at three tissue depths to characterize the distribution of thermal energy across the wall for a successful lesion.

For both cryo and low power RF ablation, the greatest and fastest temperature change was observed by the sensor located proximal to the ablation source, while those located deeper in the myocardial tissue showed progressively less and slower change as shown in (Figure 6.9). The thermographs of both ablation methods showed distinct phases, which may correspond to mechanistic changes in lesion formation. The temperature profiles from cryoablation show definitive freezing and thawing periods across all 3 sensors, while the RF ablation temperature profiles have a distinct inflection point. Mechanistically, RF ablation creates the desired tissue damage in two ways. At first the electrical current directly heats the tissue through resistive heating. The degree of resistive heating drops off with distance from the current source. The second mechanism is passive conductive heating transferring through the tissue[139]. The inflection point in the RF temperature profiles likely represents the
transition from resistive to conductive heating. The transition from resistive to conductive heating was identified by calculating the first inflection point after the start of the ablation. The second derivative of the signal was estimated using a numerical computing method for noisy data [83]. Both the timing and the temperature at this inflection point were recorded. The temperature and timing of the maximum temperature change were also extracted from the profile. Two additional parameters were defined to characterize the profile morphology: the ratio of time before the inflection point to the time the maximum temperature was achieved and the ratio of the temperature increase before the inflection point and the maximal temperature. These values were statistically compared across groups and sensor depth using 2-way unbalanced ANOVA method. Statistical significance was defined
Figure 6.9: Ultra-thin Needle Electrodes and Chronothermographs Recorded During Cryo and Radiofrequency Ablation. a) Ultra-thin needle sensor. Thermographic monitoring during both b) cryoablation and c) 5W radiofrequency by transversally implanted ultrathin and flexible temperature sensor. The sensor closest to the tissue surface and ablation source was sensor 3 (blue solid line) and the one furthest from the source was sensor 1 (black solid line), as p=0.05. A significant change in thermal conductivity was not observed after either cryo or RF ablation (results not shown).

A total of 20 experimental RF lesions were assessed to correlate chronothermographs with lesion depth. The range of ablation powers and variety in the ventricular geometry resulted in a distribution of lesion transmurality as shown in Figure 6.10. The lesions were grouped into fully transmural (N=11) and non-transmural (N=9) lesions. A representative post-processing example of a nontransmural lesion and a transmural lesion is displayed in Figure 6.10. The shaded region highlights the lesion as defined by the custom threshold program. The corresponding temperature profiles for all lesions were assessed in detail to extract parameters that may aid in the real-time identification of successful lesion attempts. A representative example of a chronothermograph recorded during a 5W RF ablation and the definitions of the parameters used to characterize the shape of the thermograph profile are shown in Figure 6.11. The first inflection point is identified in orange. The maximum temperature change is identified in green. Four parameters were evaluated: maximum temperature change, time to inflection point, ratio of temperature change at inflection point to total temperature change, and ratio of time to inflection point to time to maximum change. Each parameter was statistically compared by location within the myocardial wall and across transmurality groups. The two parameters with statistically different results across the transmural groups were the
maximum temperature change (p=0.0039) and the ratio of time in phase 1 of the temperature change (i.e. resistive heating) to the time when the maximum temperature was reached (p=0.0328). In both cases the transmural lesions were larger across all temperature sensor locations. In accordance with the analytical data (see publication for work by collaborating lab), the middle sensor on the needle-like devices observed the greatest differences between transmural and non-transmural lesions for all parameters except maximum temperature and may be the best sensor for lesion characterization. We envision real-time monitoring of the mid-myocardial temperature profile to provide feedback for increased time spent in the resistive heating phase and thus, better lesion formation. Ideally, constant power or novel current delivery strategies would be titrated while observing the temperature profile to ensure that the observed profile remained in the linear (resistive) phase for as long as possible.

Several strategies have been used to assess the thermal distribution during RF ablation on bench-top studies [30], however the dimensions and mechanical properties of these ultrathin needle type sensors provide the first technique that could be employed in vivo without restricting the contraction of the heart. Additionally, this platform could be used to mount a variety of sensors including resistivity sensors to further characterize the dynamic changes of mid-myocardial tissue during ablation and provide valuable feedback to the clinicians.

**Strain Sensors on Epicardial Surface**
Figure 6.11: Parameters for Possible Real-Time Transmurality Predictions. Comparison of chronothermograph parameters for non-transmural and transmural lesions and across sensor locations assessed by a) ratio of temperature change in phase 1 (indicated in e)) to maximum temperature change; b) maximum temperature; c) ratio of time duration in phase 1 to time maximum temperature was achieved; and d) time spent in phase 1. e) Representative chronothermograph (blue) and second derivative (gray) from which parameters were extracted. Orange identifies the inflection point and green identifies the maximum temperature. f) Differences across transmural group for each parameter and sensor location.

In addition to electrical and chemical evaluation, mechanical characteristics can be determined. Strain sensors based on piezoresistive effects in nanomembranes of Si allow monitoring of the mechanics of contractions of the heart during a variety of propagation states. Careful mechanical design of the serpentine interconnect structures allows accurate measurement in spite of the fact that typical epicardial strains [133] greatly exceed the fracture threshold of Si, as described in previously reported small-scale 2D devices [97]. In the present design, the 3D-MIM strain sensors include three p-doped Si piezoresistors in a rosette configuration. Two of the piezoresistors, with longitudinal axes perpendicular to each other, are aligned to the <110>crystalline directions of the Si, offering an effective longitudinal gauge factor of 0.33 and effective transverse gauge factor of nearly 0.06 for each piezoresistor. The piezoresistor aligned to the <100>crystalline direction exhibits relatively small change in resistance under strain, due to the intrinsic sensitivity associated with the crystalline direction as well as the overall device geometry. The piezoresistors aligned to the <110>directions
provide maximum sensitivity for characterization of mechanical rhythms of the heart while the piezoresistor aligned to the $<100>$ direction can be used to calibrate for effects of temperature. Experiments revealed the mechanical behaviors during sinus rhythm, ventricular pacing and pharmacologically induced ventricular fibrillation (VF) with Pinacidil (30 µM bulk dose). The use of Pinacidil significantly reduces the action potential duration and subsequently increases the vulnerability to reentrant arrhythmias when stimulated with 50 Hz AC burst pacing. Bath electrodes simultaneously recorded a far-field ECG to establish the temporal correlation between the electrical and mechanical behaviour. Figure 6.8 shows the response of a representative piezoresistor aligned to the $<110>$ direction. The measurements reveal mechanical rhythms of the cardiac cycles, with a frequency consistent with ECG deflections. During VF condition, both the strain gauges and ECG show that the waveform lost normal rhythm and displayed a random pattern typical for VF.

**Optical Spectroscopy with 3D-MIM Device**

The final 3D-MIM demonstration exploits arrays of µ-ILEDs to illustrate the capacity for advanced semiconductor integration and optical mapping/stimulation. Nine ultrathin (3 µm), microscale (300 x 300 mm$^2$) LEDs based on aluminium indium gallium phosphide (AlInGaP) with peak emission wavelengths of 670 nm served as local light sources for the excitation of voltage-sensitive dyes. Changes in fluorescence associated with these dyes allowed measurement of the cardiac action potential. Figure 6.8 compares signals obtained with an external light source (Prizmatix, 630 nm) and with the integrated µ-ILEDs. In spite of their small sizes, the LEDs enable recording of clear action potentials, with waveform shapes consistent with external light. The signal-to-noise ratio of the µ-ILED-excited action potentials is lower than the externally excited action potentials due to a necessary decrease in light intensity to minimize the power delivered to the device. The results demonstrate the future possibility of an in vivo optical mapping using either externally applied dyes or internal fluorescent indicators and/or stimulation system in a 3D integration format.

**6.4 Discussion**

The results presented here suggest routes for integrating active electronic materials and sensors in organ-specific designs, with potential utility in both biomedical research and clinical
applications. With attention to materials, engineering mechanics and functional devices, these systems can establish conformal interfaces with the epicardium or within the transmural wall, and perform a variety of high-density physiological multiparametric mapping and stimulation. The use of transfer printing and the reported scheme for integration onto the printed 3D heart structure allows diverse sensor/actuator devices on a single platform. Separate electrical connection, with a single trigger channel to synchronize the timing, eliminates effects of crosstalk. The devices can provide local information on the metabolic, excitable, ionic, contractile and thermal state for investigations of both the spatial and temporal responses to a variety of insults, diseases and therapies. The devices could be used to identify critical regions that indicate the origin of pathophysiological conditions such as arrhythmias, ischemia or heart failure. With future development, these membranes can be implemented as near-continuous monitors of cardiac performance, providing clinicians with a set of internal eyes guarding patients’ progression into or from disease states by tracking improvement following therapeutic intervention. Additionally, these regions could be used to guide therapeutic interventions.

The heart is a complex functional syncytium with a series of elements working in unison to reliably perform and respond to conditions of stress. The combined functional behavior on the organ level and the interaction between each subsystem is necessary to complete our understanding of disease states of the heart. AF is an excellent example of the complex network and feedback loops across systems inherent in cardiac disease states and illustrates the need for simultaneous sensing. AF is strongly associated with self-propagating electrical and structural remodeling [10]. Electrically, this is marked by changes in the conduction velocity, the action potential duration and the refractory period; however, there are many additional parameters that may contribute mechanistically to these changes. AF is often associated with atrial dilation on the cellular and the macro scale; changes in wall stress, intra-atrial pressure and activation of stretch receptors may all activate downstream cascades [192]. Additionally, AF is marked by severe metabolic changes. Studies have shown increases in oxygen consumption, decreases in atrial reserve flow, mitochondrial deletion and glycogen accumulation [171]. Together these changes significantly impact the energetic state of the atria and alter calcium handling, leading to both electrical and mechanical consequences [92]. Electrophysiological, metabolic, and mechanical changes have distinct time courses and spatial dependences, which may help separate cause from consequence and identify those changes that are reversible. A multiparametric array can locally probe these changes
simultaneously. These devices could be used to identify critical anatomical regions that indicate the origin of pathophysiological conditions such as arrhythmias, ischemia, or heart failure.

Light emitting diodes of a variety of wavelengths can be employed for optical spectroscopy of internal fluorophores and added fluorescent dyes. To date the UIUC group has created arrays of stretchable LEDs ranging from 450 to 670 nm in wavelength. With full spatial control over powering the LEDs, these devices can interrogate regional differences in fluorescence with targeted excitation. By pairing custom LEDs with stretchable silicon-based photodiodes [96] or miniature digital camera components [204] researchers can use this technique for localized diagnostics of intrinsic fluorescent indicators like NADH, which has been demonstrated as an attractive biomarker in *ex vivo* studies [14].

In addition to the numerous diagnostic applications of these devices, there are also sensing and actuating capabilities that make them a relevant therapeutic tool. The same LED arrays can be used in spatially targeted optogenetics or the release of light-activated drugs. A protocol was recently published outlining the feasible use of these LEDs in a device designed for optogenetic studies in neuroscience [125]. The wavelengths are already tailored for excitation of light sensitive channel Rhodopsin. The LEDs can also be paired with photodiodes to include feedback on the area that is receiving illumination. Using stretchable membranes in the place of fiber optics could provide an internal light source that does not impede physical behavior, a significant hurdle in *in vivo* optogenetic studies and eventual transition to clinical use of any therapy that relies on light. Furthermore, incorporating these sensors in a number of clinical procedures could provide clinicians with much-needed feedback on the instantaneous effect of therapy, such as the needle-type temperature sensors for ablation. If sensors were to remain on the patient’s heart after the procedure, the device could alert the physician when a line of block recovers. The time course of recovery may help explain the mechanism of arrhythmia recurrence after ablation procedures and guide new ablation techniques that could circumvent this occurrence.

Another clinical therapy that might benefit from the addition of such a device is the use of stimulators and defibrillators. Therapeutic electrical stimulation paradigms have long been restricted to 1 or 2 electrode sites. CRT was a sizable step forward with the introduction of simultaneous pacing at two separate sites [107]. However, not all patients respond equally to
CRT in its current form [16]. These devices offer a platform for access to an enormous increase in pacing sites and a shift from low definition to high definition electrical therapies. The square electrodes can be fabricated using platinum instead of gold for targeted pacing applications. Bipolar pacing has been achieved through these devices in the \textit{ex vivo} rabbit heart. New fractal structural designs can be implemented to build electrodes with greater surface area to deliver high voltage shocks without sacrificing the mechanical stretch (see Appendix B). With a combination of sensing and shocking electrodes, a device could be conceived for high definition therapy, targeting only the spatially vulnerable regions as identified by the sensors instead of limiting the shock to a single vector. A defibrillation protocol that takes full advantage of the spatial coverage of the electrodes can be implemented to target arrhythmias with a potential decrease in peak voltage required to successfully defibrillate. Additional sensors including pH or ionic sensors can be added to instantaneously indicate myocardial injury due to high voltage shocks or reduce the number of inappropriate shocks.

6.5 Future Directions

To increase the resolution and numbers of sensors, it may be necessary to incorporate transistors into the device to allow multiplexed addressing. Remaining challenges for use as a chronic implant include means for power supply, control/communication and encapsulation. The wired connections must be replaced with full duplex wireless communication. Work has begun to incorporate RF transmitters [125] into similarly fabricated devices. Additionally, fatigue testing has to be performed to establish the lifetime of the serpentine wired circuit, which needs to withstand the stress of a heart beating 30 million times per year. Future devices need evenly spaced sensors encompassing the entire heart surface. The complex shape of the atria, pulmonary veins, and great vessels make the development of atrial-specific devices difficult; however, the medical imaging techniques that provided the ventricular geometry can also be employed for the atria. Transitioning to \textit{in vivo} feasibility studies would also require the development of a minimally-invasive deployment strategy. Previous experience with minimally invasive implantation methodologies used for ventricular assist devices, valves and pacemakers/defibrillators will be the basis for such future development, but significant improvements will be needed for deployment of high-definition future devices. Continuing work on the substrate chemistry and material properties will also open
doors for new applications. Energy requirements are a concern for electronically implanted devices, including pacemakers and defibrillators that rely on battery power for operation. Recently, piezoelectric energy harvesting and storage have been developed through flexible and integrated systems that use the natural motion of the beating heart as an energy source. Harvesting power directly from natural processes of the body is an attractive method to reduce battery size and eliminate the need to replace them [40]. In addition to energy harvesting, transient electronics is an emerging new area in material science that has numerous cardiac applications, such as the temporary monitoring of transplanted tissue constructs to ensure proper integration. Transient circuits are built with magnesium, tungsten, zinc, and iron, all of which dissolve in physiological fluids and can be patterned onto stretchable membranes in a similar manner to the sensors and actuators mentioned previously [39]. The dissolving rates can be tuned by the addition of different metals and current circuits can remain stable and then disappear within a few hours to 20 days.

With future development, these membranes can be implemented as near-continuous monitors of cardiac function and provide necessary interventions as well as enhance current technology. Implantable defibrillators would benefit from comprehensive monitoring, where traditional low resolution electrical monitoring can lead to improper identification of arrhythmias and inappropriate, painful shocks to patients. Additionally, ablation therapy of atrial fibrillation relies on multielectrode basket catheters to locate ectopic foci or reentrant wavefronts with limited endocardial contact. Form-fitting, comprehensive diagnostic catheters could reduce the time to determine the electrical activation pattern reducing procedure time and improving patient outcomes. Combining the exciting new advances and the recent demonstrations in cardiac applications, customizable stretchable substrates for multi-parametric sensors and actuators have the potential to greatly increase the amount of data available to clinicians and researchers studying pathophysiological disease states of the heart.

6.6 Funding

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Chapter 7

Conclusions

“We can only see a short distance ahead, but we can see plenty there that needs to be done.” - Alan Turing

“We shall not cease from exploration, and the end of all our exploring will be to arrive where we started and know the place for the first time.” - T. S. Eliot

Heart rhythm disorders are the leading cause of morbidity and mortality in the developed world. Atrial fibrillation (AF), in particular, is becoming a massive and increasing burden on the health care system. Unfortunately, current therapeutic options fall short of providing relief for these patients in a consistent and reliable manner. With this dissertation, I have taken a two-tiered approach to addressing the limitations of AF therapy and diverging from the traditional electrotherapy paradigms. In order to develop therapies that are rooted in biophysical mechanisms or at least are tailored by physiological feedback, we need to develop methods to better differentiate individual episodes of AF, as well as design therapies that can better target individualized patterns. Chapter 3 and 4 are dedicated to characterizing the spatiotemporal dynamics of AF in two different species and trying to discern some organization out of the chaotic signals. By analyzing the spatiotemporal dynamics of AF propagation, I have arrived at two important conclusions. The first is that the complex atrial geometry should not be approximated as a 2D shell or even a shell with uniform thickness. The anatomical variation of the atria affects driver location, stability, and disruption in a manner that is not yet predictable due to its level of complexity. With these projects, I began to investigate how the anatomy harbors a three-dimensional substrate. The ventricular transmural wall is a tightly controlled structure, critical to function. Since the atria serve a different mechanical purpose there seems to be less order over the formation of the wall
structures, leading to areas of discrete preferential transmural paths that are not uniformly distributed throughout the atria. However, this is a significant area to expand upon. We have begun a collaboration with Dr. Christine Hendon’s laboratory to use optical coherence tomography to tease out fiber orientation and stitch together a 3D map of this orientation. I think this will be a vast improvement over the small sampling of fiber orientation patterns investigated in Chapter 4. Additionally, I think it will be important to develop a method to register the functional maps to the anatomical maps to discern the interaction between the variations in structure and the electrical substrate. In the studies presented here, I was hesitant to calculate the regional incidence of AF drivers since I thought that information should not be extrapolated from tens of animal hearts to clinical AF. However, I think if we had an anatomical map of thickness and fiber orientation that we could register directly to the driver location, it would be beneficial to look for regional correlations. The AF models used in this study are limited without structurally remodeling but they lay a good foundation before introducing the additional level of complexity resulting from pathological conduction abnormalities and the insulation of fibrosis.

My second main conclusion was that even in a controlled animal model, there was a spectrum of dissociation between animals. Although global parameters like dominant frequency and regularity index are well conserved across the transmural wall, dissociation and the role of transmural conductance are exposed with a spatiotemporal characterization of wavefront and PS dynamics in an acute model of AF that was mostly driven by short-lived meandering rotors. If we could make a differentiation along this spectrum clinically, this may provide an opportunity to distinguish a sub population of patients that are easier to target. Of course it would be beneficial to repeat the transillumination study in human atria, especially in the tissue of patients who suffer from prolonged atrial fibrillation to confirm that the spectrum exists outside of the animal models. Additionally, it remains unclear what role the dyssynchrony across the wall plays with respect to new targeted ablation approaches. I think an important next step would be to attempt to disrupt the patterns by targeting the pattern on one side of the wall. In order to complete this study, the dynamic parameter algorithms need to be adapted for quick extraction from the data. The processing would need to be performed in a near-real-time sense so that the experimenter could respond appropriately with an ablation lesion.
In Chapter 4, I began to add more optical planes through the atria to approach a 3D imaging strategy by alternating the direction of illumination. I think that with more effort and creative use of new fluorescent dye properties and alternating excitation wavelengths one could parcel the transmural wall into more planes optically and perhaps visualize out of plane rotation, if it exists. If I were to repeat these studies I would alternate the illumination between red and green light from a single side in addition to switching between the contralateral and ipsilateral direction. I might also consider adding simultaneous calcium imaging. Calcium dynamics play an important role in driving AF and it would be beneficial to simultaneously investigate the interaction between voltage and calcium in this model with both the endocardium and epicardium exposed.

There is a great deal of work to be done to bridge the characterization studies of the early chapters with the serial defibrillation paradigms discussed in Chapter 5. I think the temporal optimization studies helped to determine that it is important for Stage 2 to be tightly coupled in time to Stage 1. However, the first shock of Stage 2 can come too early, when all the tissue is refractory, which is detrimental to the therapeutic efficacy. The spatial studies included in this dissertation confirm the advantage of multi-path serial defibrillation with respect to efficacy. Surprisingly, a more acute shift decreased the threshold to a greater degree than an orthogonal shift. Understanding the mechanism of the optimal vector is a crucial next step to these studies. Ideally, I wanted to use the optical maps of the pressure-loaded atria model to look at the angle of incidence between propagating wavefronts and the shock vectors to begin to probe the spatial interaction patterns with the applied electrical field. I believe this is the ideal experimental preparation to look for the impact of different shock vectors compared to the flat atrial preps because the atria are intact and in a physiological confirmation when the shock is delivered. However, the experiment did not permit this analysis because of the superposition of ventricular and atrial signals. It was difficult to ensure that the ventricular wavefront from under the atria was sufficiently removed. I tried many things to experimentally block the ventricular signal including trying to ablate the atrioventricular node with an RF catheter and a cryocatheter. I tried injecting ethanol and formalin into the AV node to damage it. I tried using a dye or a physical material inside the volume of the atria to make the tissue less transparent and block the camera from the ventricles without consistent success. I did implement an algorithm for wavefront identification and primary conduction angle estimation for the isolated AF preps but was not able to apply it to this dataset. Therefore, I think there is a lot of work that could be done to tease out why a vector
shift decreased the defibrillation threshold, especially if this trend is confirmed in the *in vivo* canine model that is currently being conducted. Additionally, if the shifted probability of success curve is reproducible in the canine model for either Stage 1 rotation or Stage 2, there are some coupling parameters that would need to be optimized. To date we have continued to couple the Stage 2 dynamics with the Stage 3 dynamics in an effort to simplify the degrees of freedom; however, it may not be the most efficacious approach. The Labview code is written to be easily adapted to different spatial strategies. The hardware currently can dynamically shift between 6 bipolar vectors from a single current source. The relays have a hardware delay of <15ms, providing a great deal of flexibility in the shock timing. If we could add a second current source I think it would be very interesting to investigate overlapping shocks in time across separate vectors instead of discrete spatial shocks with a quiescent delay. I believe that the vulnerability within the cycle length of an arrhythmia is a spatial and temporal phenomenon, and we have yet to determine the best approach to identify and target the applied energy in both domains.

Traditionally defibrillation strategies have been guided by shock-induced electrical responses. However, it is important to remember that the electrical machinery in the heart does not function in isolation. There is a complex network of feedback loops between the metabolic, electrical, and mechanical states of the heart. All of which are affected by electrotherapy. Although we conducted many feasibility tests on a variety of high density multi-parametric sensors on the stretchable electronics platform, we did not yet use the information as a marker for myocardial damage during defibrillation. I think one of the best applications of this technology is to provide the real-time, high definition feedback on the shock-response beyond just the electrical information we can gain from optical mapping. This will greatly improve our physiological understanding of how the myocardial tissue responds energetically and mechanically to high voltage shocks. The information could be used to help design new paradigms that are truly rooted in biophysical mechanisms. As a step towards this goal, we started to design potassium sensitive electrodes to measure spatial maps of elevated extracellular potassium. We used a local and global ischemia-reperfusion model in a Langendorff-perfused rabbit heart to induce potassium release in our preliminary studies and monitored the oxygen consumption of the heart with an in-flow oxygen electrode at the inlet and outlet of the perfusion system (Strathkelvin) to correlate with the timing of spikes in potassium levels. Figure 7.1 displays the results from one of these preliminary studies. Although we were able to capture dynamics in the potassium concentration, the electrodes are
not yet stable in time. We are working on surface modifications to the potassium-sensitive membrane to remove the drift and improve sensitivity. I think this is an excellent first sensor to use to look at the spatial tissue response to high voltage shocks. Additionally, in its current form, the 3D MIM devices are designed to fit the ventricle. This was our first target because the shape is more stable and easier to conform to without deforming. Significant effort is required to design a device capable of conforming to the atria, if these devices are going to be used to provide high definition shocking vectors to the atria. By combining a spatial analysis of the arrhythmia drivers, the energy delivered and the resulting damage I hope we can enhance the biophysical understanding of AF electrical cardioversion and design an ideal targeted energy delivery protocol to improve upon all limitations of current electrotherapy.
Figure 7.1: Preliminary Study with Stretchable Potassium Sensors. a) An array of 8 K\(^+\) sensitive electrodes sutured across posterior surface of the heart, crossing tissue that is fed by both the right and left coronary arteries. The blue dye highlights the tissue that was affected when the left branch was occluded for "local ischemia." b) Temporal trace from one K\(^+\) sensor on the left side of the heart and simultaneous global outflow oxygen concentration measurements to confirm ischemic conditions during 3 different conditions: local left sided ischemia/reperfusion, global ischemia/reperfusion and a repeated local left sided ischemia-reperfusion cycle. c) Action potential shortening confirms the ischemic condition used to test the sensors.
Appendix A

Quantifying Spatial Heterogeneity of Cardiac Electrophysiology

A.1 Introduction

Although the heart is often referred to as a functional syncytium, it is a well-coordinated dynamic system of individual cells with diverse electrophysiological properties. The three-dimensional variations within the tissue properties are organized in such a fashion as to produce a single electrical circuit and a synchronous mechanical contraction when it is properly functioning. The dynamic nature allows it to uniformly respond to neural stimulation to adjust the rate to meet the circulatory demand. Due to the intricate spatial design, spatial patterns and disruptions to electrophysiological gradients cannot be neglected when investigating the cause and the effects of irregular rhythms.

Irregular propagation can be initiated at the spatial boundaries of electrophysiological properties within the tissue. The charge density is altered at sharp gradients and the path of current can be diverted. This idea is validated by computational models that have demonstrated that differences in the duration of the electrical recovery and the resistance to conduction between cells contribute to the conditions necessary for conduction block and reentrant arrhythmia [183]. Under the right conditions where the difference in electrical properties at the boundary is sufficient, the propagating wavefront results in unidirectional block at the boundary, increasing the vulnerability to an arrhythmia. The length of the cellular refractory period and how the cells are spatially dispersed is also critical for generating the entrance block that silences premature beats.
Pronounced structural and functional remodeling during arrhythmias has been extensively studied on the cellular, tissue, and intact heart level. We have discussed many of the changes associated with AF throughout this dissertation and each cardiac pathology is associated with its own course of remodeling. The changes in ion channel expression have profound effects on the temporal behavior of the action potential, affecting both depolarization and repolarization. Studies have also shown that the electrophysiology changes caused by some arrhythmias hinder or eliminate the rate adaption of the repolarization phase [191]. Temporal changes are often used as an investigative parameter to characterize arrhythmogenic conditions. Due to the complex nature of these alterations in ion channel and connexin expression it is unlikely to be a uniform change across the tissue. Therefore remodeling is likely to result in an increase in spatial heterogeneity or a transition to a disordered heterogeneity of the electrical properties with more harsh boundaries. This change in the spatial pattern may contribute to arrhythmogenic changes in addition to the temporal changes in the action potentials that are easily quantified.

Measures of variance capture some degree of these heterogeneous changes but decouple it from spatial location and therefore miss the critical changes in spatial patterns. High resolution mapping techniques offer the ability to visualize the spatial activation and repolarization patterns of isolated cardiac tissue. Despite the clear need to compare the pathological heterogeneity to that of healthy tissue and the compelling hypothesis that spatial dispersion of electrophysiological properties contributes to improper conduction within the heart, measuring cardiac heterogeneity remains largely an elusively qualitative observation. The definition of heterogeneity does not lend itself to being easily quantified, simply meaning a quality of being nonuniform. Much of the literature dedicated to developing strategies for quantifying spatial heterogeneity comes from the field of geostatistics to extract features from landscape and stream studies [110]. These strategies have only recently been applied to medical imaging for the purpose of identifying anomalies in various fields including oncology in the lungs, brain and epidermal tissue [152, 168, 81]. A clear method or set of techniques that reliably and accurately quantify the spatial heterogeneity would help to improve the understanding of the cause and consequence of electrophysiological heterogeneity and its role in arrhythmia. This study aims to apply several different statistical strategies ranging from texture analysis to orthogonal regression to identify the limitations and advantages of numerically representing the spatial heterogeneity of a map of high density simultaneous cardiac recordings.
A.2 Datasets

A.2.1 Synthetic Data

Synthetic data was used to create a framework with a controlled amount of spatial noise for initial testing and differentiating the efficacy of all methodologies. Intensity maps with a mean of 0.5 were created in a 100 by 100 array as an initial condition. Two different cases were considered: a uniform gradient from 0 to 1 or a homogenous substrate. Controlling for a constant mean, 10% of the pixels were changed to a different intensity in a random spatial pattern for each permutation. The intensity of the noise was limited to ±10% off the mean, ±25% off the mean and the full range from 0 to 1. For the uniform gradient, the standard deviation was also maintained by moving 10% of the current pixels to new arbitrary locations. Each heterogeneity strategy was then tested on the maps from 0-10 permutations to establish limitations and identify the ideal parameters to extract from each technique. Figure A.1 displays the increasing permutations of noise on the gradient template.

Figure A.1: Synthetic Data with Controlled Increases in Heterogeneity. a) random noise 0 to 1 1 perm. mu=0.5, sigma=0.29 b) 5 perm. mu=0.5, sigma=0.29 c) 10 perm. mu=0.5, sigma=0.29 d) random noise +/- 10% off mean 1 perm mu=0.5, sigma=0.28 e) 5 perm. mu=0.5, sigma=0.24 f) 10 perm. mu=0.5, sigma=0.18
A.2.2 Experimental Data

After promising techniques were identified using the control data, they were applied to experimentally collected data. A representative experiment that optically mapped cardiac tissue from a Control group (N=5) and an Experimental inflammation model (N=5) was used as a test dataset. The optical mapping system used has been described in detail in Chapter 2. Briefly, a voltage sensitive dye is used to collect fractional fluorescence changes that correspond to changes in membrane potential with a 100 x 100 pixel CMOS sensor. The tissue was externally paced at different cycle lengths ranging from 350ms to 150ms to test the hypothesis that spatial heterogeneity does not vary with the pacing rate. Some rates were excluded from this study if they produced alternans, complicating the application of the spatial processing. Spatial variation in action potential duration (APD) values was used as the target parameter. The APD was defined as the distance from the upstroke to 90% of the repolarization back to baseline (APD90). In some cases the APD50, the distance from the upstroke to 50% of the repolarization phase, was used to corroborate the findings. The data was first filtered to exclude any points beyond a physiologically relevant range of 60 to 300ms before the heterogeneity methods were applied.

A.3 Methods

All methods were implemented using custom MATLAB software.

A.3.1 Texture Analysis: Haralick Features

Texture analysis uses tone to classify the spatial dependencies of a pictorial image. First, the data must be converted to a 2D gray level intensity array with values ranging from 0 to 1. The synthetic data was built to be an intensity map of these values but the experimental data was normalized by the 99th percentile for each pixel, using each dog as its own control or normalizing all the dogs by the same average 99th percentile value. A co-occurrence matrix is created to portray the distribution of pairs of pixels. Each element of the matrix $P_{ij}$ represents the relative frequency of co-occurrence of gray levels with intensity levels i
and j separated by a given displacement. The displacement and consequent matrix has a
directionality and magnitude, which requires multiple matrices to be developed for a single
image. For this study 50 matrices were created with a displacement of 1-50 pixels in four
directions: 0°, 45°, 90°, 135°. The four directions were then averaged to ensure rotational
invariance, forming 50 individual matrices per image. A variety of statistical parameters
calculated in the spatial domain and first described by Robert Haralick can be extracted
from each matrix [75]. This study focused on the correlation (A.1), contrast (A.2), energy
(A.3), and homogeneity (A.4) parameters as defined below. The values from the matrices
created with a distance of 10-50 were then averaged to ignore nearest-neighbor distribution,
resulting in one final parameter for each image. This analysis was conducted using the
functions provided by MATLAB for creating a gray-level co-occurrence matrix (GLCM) and
calculating the Haralick features in the Statistics Toolbox.

\[
\sum_{i,j} \frac{(i - \mu_i)(j - \mu_j)p(i, j)}{\sigma_i\sigma_j} \quad (A.1)
\]

\[
\sum_{i,j} |i - j|^2 p(i, j) \quad (A.2)
\]

\[
\sum_{i,j} p(i, j)^2 \quad (A.3)
\]

\[
\sum_{i,j} \frac{p(i, j)}{1 + |i - j|} \quad (A.4)
\]

### A.3.2 Texture Analysis: Run Length Matrix

The run-length matrix method also requires a 2D gray level intensity array from 0 to 1. A
run length is a measure of the number of consecutive pixels of the same intensity calculated
along four displacement vectors: 0°, 45°, 90°, 135°. These runs are then tallied for each
intensity to create a matrix where each element P(i,j) corresponds to the relative frequency
of a run of intensity i with length j. Four matrices are calculated from each image and
the 95th percentile for the run length was extracted for each direction and averaged as the measure of heterogeneity.

### A.3.3 Inhomogeneity Index: Maximum Difference Phase Map

The inhomogeneity index calculated from a maximum difference phase map is one of the only techniques in the literature developed specifically for the cardiac application [106]. Each element of the phase map is created by determining the maximum difference between every group of four neighboring locations. A histogram is created and the inhomogeneity index, a parameter independent of the average, is extracted from the distribution using the 5th, 50th, and 95th percentile values (A.5). This method has been applied previously to conduction velocity measurements of rabbit atria to attempt to predict reentry vulnerability.

\[
I = \frac{P_5 - P_{95}}{P_{50}} \quad \text{(A.5)}
\]

### A.3.4 Variogram

A variogram is a tool often used to express spatial variation and roughness. The equation for calculating the variogram is shown in (A.6), where \( z \) is the electrophysiological parameter and \( x \) and \( y \) represent the lag vector. This method also has a directionality associated with it and must be repeated for multiple angles in order to achieve rotational invariance using the same 4 displacement vector angles. For this study an angle tolerance was not included in the lag vector. To infer this function from the observed data an approximation was used as shown in (A.7). The variogram function computed over a lag distance of 0-50 pixels was fit to a linear model because a sill was never reached. The R-squared value and slope were used as a measure of goodness-of-fit and heterogeneity.

\[
2\gamma(\Delta x, \Delta y) = E[Z(x + \Delta x, y + \Delta y) - Z(x, y)]^2 \quad \text{(A.6)}
\]
\[ \gamma(\Delta x, \Delta y) = \frac{1}{2N(\Delta x, \Delta y)} \sum_{i,j \in S(\Delta x, \Delta y)} (z_i - z_j)^2 \] (A.7)

### A.3.5 Orthogonal Regression

This method fits a 2D plane through a 3D cloud of points to minimize the orthogonal distance from the points to the plane using principal component analysis. It was applied directly to the data without any prior transformations. For the synthetic data the intensity map was multiplied by 300 to produce physiologically relevant values. The normal vector to the plane is described by the principal component analysis coefficients of the electrophysiological parameter values and the basis of the plane is spatial location of the parameter on the Cartesian map. The mean squared error of the regression analysis is used as a measure of heterogeneity.

### A.4 Results

Trends with respect to the number of permutations are displayed in Figure A.2 for a select few methods. Quantifying orthogonal regression with mean squared error (MSE) values displays a fairly linear positive correlation for both the homogenous plane and the gradient datasets. However this parameter saturates with fewer permutations of noise for the homogenous plane dataset. The data is also sensitive to the relative location of the “electrodes” to each other. Additionally this method is sensitive to the intensity of noise introduced, which is a synthetic indicator of increased heterogeneity.

The Haralick parameters do not respond to noise in a consistent manner for both the homogenuous plane and the uniform gradient. The Homogeneity and Energy parameters both decrease from 1 with increased heterogeneity in a linear fashion for the homogeneous case but the energy values stay stable for the uniform gradient case regardless of the noise introduced. The Homogeneity parameter still decreases but starts from a much lower baseline and is much less sensitive to each permutation of more spatial noise. The differences between the Homogeneity parameter with respect to distance can be used to quantitatively differentiate
Figure A.2: Synthetic Analysis of Heterogeneity Methods. Trends with respect to permutations for select methods applied to the homogeneous and gradient starting conditions. a gradient. Each direction can also be assessed independently to identify the direction of the gradient. The Contrast parameter increases with increased heterogeneity but the maximum value is unclear from this data so it may be difficult to assess absolute heterogeneity.

The run length matrix approach produced consistent results for all the tested cases with only slight variations due to noise intensity. Complete homogeneity approaches 100 and the value decreases with increases in heterogeneity. The baseline value for a homogeneous plane is much larger than the gradient but the relationship evens out as more permutations of noise are introduced. This approach is more sensitive to changes in heterogeneity since an introduction of 10% random noise drops the 95th percentile to 30% of the baseline for the homogeneous plane and near 50% of the baseline for the uniform gradient. The run length parameter then stabilizes after 5 permutations of spatial noise. This may be a good parameter to identify slight changes in the degree of heterogeneity.
The other approaches produce less promising trends. The slope of the linear fit for the variogram produces a fairly linear relationship, but the values are extremely low and the relationship does not hold for the gradient. Although the inhomogeneity index is the most consistent between the uniform gradient and homogeneous plane, there is no observable trend with respect to the permutations of noise. The 50th percentile lags behind the increases in the 5th and 95th percentile changes with respect to permutations, resulting in the observed spike at 3 permutations. There is also no discernible difference from 4 to 10 permutations.

Based on the synthetic results, the Haralick Homogeneity parameter, run length matrix and orthogonal regression approaches were applied to the experimental datasets. The results for all the tested datasets are displayed in Figure A.3. It is important to note that an increased MSE and a decreased Haralick Homogeneity or Run-length Matrix parameter refer to increased heterogeneity. All methods revealed the same trend that the heterogeneity is increased across all pacing cycle lengths for the experimental condition but the degree of increased heterogeneity varies per method.

![Orthogonal Regression Analysis for APD90](image1.png)
![Homogeneity Parameter for APD90](image2.png)
![Run Length Matrix for APD90](image3.png)

Figure A.3: Heterogeneity Methods Applied to Experimental Data. Averages for each approach to quantifying heterogeneity (N=5 for each group)
A.5 Discussion

The analysis with the synthetically created data identified several parameters as monotonic indicators of increasing spatial heterogeneity. Unfortunately there was no one parameter that captured all conditions without being influenced by the starting distribution or noise intensity. This may cloud the interpretation of each individual parameter such that a collection of methods might be the ideal approach. The run length matrix was a sensitive approach that picked up small changes in heterogeneity and may be a good starting approach. However, small changes to already heterogeneous maps may be lost using this approach. The fact that the Haralick parameters do not uniformly respond to a gradient and a planar map is concerning due to the frequent presence of both these spatial patterns in cardiac tissue. Still the correlation parameter is a measure of linear dependence and could be used to identify maps in which the homogeneity parameter fails due to the presence of a gradient. The regression analysis is also a promising approach that responds to the difference in magnitude at the borders. Using these three approaches together may help to identify spatial changes in heterogeneity within an animal.

A.6 Future Directions

Average values for all the parameters associated with a directional vector were used to create rotational invariance. However in the case of the gradient, this may mask some of the patterns. It may be worth a second look to identify different parameters for the promising methods that better capture the relationship between the parameter and the lag distance or direction. Although these techniques add spatial information in a controlled setting, it is not clear whether they can be effectively applied to a group of experimental optical maps. In this study there was no clear relationship established across cycle length or experimental group that was upheld by all the approaches. Due to anatomical variation between individuals and the heterogeneity innate in the normal structure of the heart, it is unlikely that the field of view examined between two dogs produces a similar baseline parameter for any of these approaches. This unavoidable disparity may account for a significant amount of the variation observed in the data when the mean values were compared. It is much more important from this early stage that the values calculated for the approaches are congruent
with the subjective classification of more or less heterogeneous. In most cases this seemed to be the case for the real data. It is possible that changes in heterogeneity could be assessed successfully compared to a chosen baseline condition.

The results were sensitive to the normalization process and care must be taken to establish if each dog should serve as its own control or if one normalization value is needed in order to be able to compare across dogs. Despite these shortcomings in interpreting the data across multiple dogs, this study does present some promising new ways to approach quantifying the spatial data produced from high resolution mapping of cardiac tissue. When comparing two independent maps, the approaches outlined here can successfully discriminate between levels of heterogeneity.

## A.7 Acknowledgements

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Appendix B

Material and Fractal Designs for 3D Multifunctional Integumentary Membranes with Capabilities in High Definition Cardiac Electrotherapy

This chapter appears in the following original publication:


B.1 Introduction

Physical constraints limit the nature of the interfaces between cardiac structures and the electrodes used to deliver electrotherapy. A trade-off exists between spatial control of the electrode location with respect to the local anatomy and large simultaneous coverage of the tissue. An radio frequency (RF) catheter provides precise access to anatomical regions but can only make a single point of contact and burn a small mass of tissue at a time. Defibrillation vectors simultaneously excite a large mass of tissue but the flexibility of their position is limited with respect to the anatomy. Additionally, no existing clinical method
can provide simultaneous electrotherapy with high density spatiotemporal mapping of physiological parameters for feedback control. Defibrillation strategies have remained relatively stagnant in the way in which energy is delivered. The high voltage shocks that are used in conventional procedures can be extremely painful and require a great deal of energy to be stored in the batteries of such devices. A promising direction for advanced low energy cardiac electrotherapy couples feedback-controlled, targeted defibrillation techniques with greatly reduced voltage and energy requirements [112, 90, 119, 38]. Multifunctional, conformal platforms of electrodes that can integrate over large areas of cardiac structures provide the opportunity to advance beyond traditional implantable lead-based delivery paths.

Emerging classes of materials and mechanics concepts in the field of stretchable electronics create new opportunities for integrating high performance electronics with the human body and individual organs. Devices described in Chapter 6, referred to as 3D multifunctional integumentary membranes (3D-MIM), provide conformal electronic platforms that interface with the full 3D geometry of the epicardium. 3D imaging and printing techniques enable organ-specific geometric designs with an instrumented interface. High performance electronic materials, interconnect structures, and sensors mount on this platform to yield various spatiotemporal mapping and stimulation capabilities. The results presented in this appendix expand on these concepts to demonstrate 3D-MIM devices configured for high definition cardiac electrotherapy. Here, fractal geometries yield compliant, large area, low impedance electrodes for electrical stimulation in designs that do not compromise the physical stretchability or the low effective modulus of the overall system. Advanced electrode materials, including nanotextured platinum-iridium (Pt-Ir) alloys and poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS), integrate naturally to enable low impedance interfaces that are well suited both for delivering electrical pulses and for sensing intrinsic electrophysiology. These constructs yield versatile platforms for cardiac electrotherapy. Experiments on *ex vivo* Langendorff-perfused rabbit hearts demonstrate the operational capabilities of these systems.
B.2 Fabrication

The devices reported here are compromised of an array of 8 electrodes distributed around the circumference of the heart (see Figure

![Figure B.1: Representative Device Integrated on Rabbit Heart and Illustration of Functional Components](image)

Figure B.1 also shows the materials components and illustrates a general integration scheme for functional elements of the device. A silicone elastomer (Ecoflex, Smooth-on) with low elastic modulus (60 kPa) serves as the mechanical support for the active components. This non-conductive substrate may also help contain the electrotherapy to within the boundaries of the membrane, which could potentially reduce pain associated with high-voltage shocks and improve the efficiency of defibrillation. A co-integrated array of resistive temperature sensors based on Au traces (15 µm in width and 70 nm in thickness) are integrated as a placeholder for sensors that could be used to monitor myocardial damage following defibrillation. Thin layers of Au (300 nm) and Ti (5 nm) serve as interconnects as well as conductive
surfaces for electrode deposition and processing. Polyimide (PI) layers (1.2 μm) provide electrical insulation. Low-impedance coatings of nanotextured Pt-Ir alloys or PEDOT:PSS can be electrodeposited to yield high quality electrical interfaces.

Fractal design concepts for the electrodes enable large area coverage and high filling fraction for electrically active surfaces, suitable for generating electric fields across cardiac tissue, but without compromising stretchability and compliance [54]. Here, the pattern for each electrode exploits a 2nd order iteration of the Greek cross fractal motif, with serpentine traces that fill a rectangular area of 14.5 mm x 2.5 mm. Serpentes with widths of 100 μm result in a filling fraction of 44%, thereby providing 14 mm² of geometrical surface area for each electrode. By comparison to previously reported 3D-MIM electrodes, the present configuration increases the surface area by a factor of 14 and increases the overall dimension (defined by the area of the perimeter of the electrode structure) by a factor of 36. The Greek cross involves a high degree of geometrical connectivity, to reduce the electrical resistance and provide a high degree of tolerance to defects. The unique layouts of these fractal geometries increase the ratio of the electrode-insulation edge length to the geometric surface area of the electrode, when compared to a conventional electrode of equivalent geometric area. It is well understood that current at an electrochemical electrode interface is preferentially distributed at the electrode-insulation edges [7, 140, 36]. This observation suggests that a fractal electrode of equivalent geometric area to a conventional electrode design should transfer current more efficiently from the electrode to the tissue. These improvements are particularly beneficial to the development of low power systems.

The mechanics of these structures are also critically important. Finite element analysis (FEA) illustrates the response of the fractal electrodes to applied strains, throughout a range associated with 3D integration and operation on the surface of a beating heart. The effective modulus of the fractal electrode element is 120 kPa, and it can accommodate 15% stretching in either the vertical or horizontal direction before reaching a regime of plastic deformation. By comparison, the intrinsic modulus and yield strain of the constituent metals are 100 GPa and 0.3%, respectively. The overall mechanical behavior of the integrated device must meet two requirements: i) sufficient stretchability for the device to allow deformations associated with contraction and relaxation of the heart muscle, and ii) minimal force exerted by the device on structures of the epicardial surface. FEA in combination with uniaxial and biaxial stretching experiments reveals the geometry change, strain distribution, and
effective modulus of the device, as parameters that determine the ability to meet these two requirements. Figure B.2 displays the device geometry and strain distribution in the undeformed state, with 20% uniaxial stretching and with 15% biaxial stretching, respectively. The uniaxial stretch is in the horizontal direction, corresponding to the atrioventricular plane, which makes the largest contribution to total heart volume change associated with cardiac cycles[31]. The resulting strain is accommodated by deformation of the soft elastomer substrate and buckling of the serpentine features. The maximum principal strain obtained by FEA is less than 0.3%, which is within the elastic regime for the metal and is far less than the fracture strain for all constituent materials (Ti, Au, Pt-Ir, polymer). These results suggest that the device can withstand the mounting process as well as the contraction and relaxation of the heart muscles, which result in 10-15% of tensile deformation.

The second requirement demands minimal constraint on the heart, which is equivalent to minimizing the effective modulus of the device to reduce the average pressure that it exerts on the heart. Figure B.2 shows the linear relationship between the stress and strain for the
elastomer membrane and the 3D-MIM. The effective modulus is dominated by the silicone membrane (150 µm in thickness, with modulus 60 kPa). The modulus for the 3D-MIM is only slightly larger, 71 kPa (horizontal stretching) and 74 kPa (vertical stretching). The calculated average pressure associated with integration of the device on the heart in its diastolic state (145% of the contracted volume) is 273 Pa, which is similar to the pericardial pressure under normal physiological states and well below the values associated with pericardial constraint [84].

These results indicate that the device is unlikely to cause restrictive impact on the intrinsic cardiac cycles, consistent with all of our experimental observations. Reducing the electrochemical impedance associated with the interface between the electrodes and the cardiac tissue improves the signal-to-noise ratio (SNR) for recording biopotentials and lowers the power consumption for electrical stimulation [36]. Lowering the impedance also enables reductions in electrode size without compromising charge capacity, thereby allowing improved current focusing and reduced probability of pain or other side effects caused by aberrant current, high-voltage, and high-energy electrical shocks. The large area fractal electrodes dramatically reduce the impedance compared to previous designs. Pt-Ir alloys with nanoscale surface textures and PEDOT:PSS films represent two attractive options that are compatible with the platforms and fabrication procedures reported here. The former exploits recently reported electrochemical deposition methods [144] to yield electrodes with enhanced mechanical and electrical properties compared to more conventional alternatives such as Pt, Pt black, or standard Pt-Ir alloys. We implemented this technology as a surface modification to the previously described fractal electrodes.

B.3 Methods

The animal study was approved by the Institutional Animal Care and Use Committee of Washington University School of Medicine. The heart was obtained from a New Zealand white rabbit, anesthetized with an intravenous injection of 80 mg /kg of sodium pentobarbital and 400 USP units/kg of heparin before the heart was surgical explanted. The heart was then transferred to a tissue bath that maintained the temperature (37
±1 °C) and pH (7.4 ± 0.05) to mimic physiological conditions. The heart was retrogradely perfused under a constant pressure of 60-80 mm Hg with oxygenated Tyrode’s solution (95%O2, 5%CO2, NaCl128.2 × 10^{-3}, CaCl21.3 × 10^{-3}, KCl4.7 × 10^{-3}, MgCl21.05 × 10^{-3}, NaH2PO41.19 × 10^{-3}, NaHCO320.0 × 10^{-3}, glucose11.1 × 10^{-3} M). The heart was mechanically uncoupled with 15 × 10^{-6} M Blebbistatin (Cayman Chemical, Ann Arbor, MI) and perfused with a bolus injection of di-4 ANEPPS (Life Technologies, Grand Island, NY, USA). The 3D-MIM was placed over the heart, positioned with 4 electrodes across the anterior surface and 4 electrodes on the posterior surface. The electrodes were connected in pairs to a custom defibrillator (Cardialen, Inc, St Louis, MO, USA) that delivered 50-100 V biphasic truncated exponential pulses (phase 1 duration 6 ms, phase 2 duration 4 ms, phase 2 voltage was half the peak amplitude and opposite polarity voltage of phase 1). Optical action potentials were recorded before, during, and after delivery of the shock with two CMOS cameras (SciMedia Ltd, Costa Mesa, Ca, USA) with 520 nm excitation light through a long pass emission filter with a 650 nm cutoff. The data were then processed with custom Matlab software. The VEP was determined by evaluating the sign of the optical action potential derivative from the time of the shock until 10 ms past the shock.

### B.4 Results

Animal experiments on Langendorff-perfused rabbit hearts demonstrate the multifunctional operation of the devices. Epicardial electrograms recorded with a PEDOT:PSS electrode display clear signals. To simulate defibrillation therapy, 50 V electrical shocks were applied through each pair of electrodes (with Pt coated surfaces) integrated on the epicardium to form vectors that traverse the ventricles. Optically imaged action potentials from the epicardium demonstrate an immediate tissue response to the applied electrotherapy. The video in the Supporting Information, shows the potential during a sinus beat followed by a shock induced beat to illustrate effective capture with a single vector. Figure B.3 compares the activation pattern of the sinus beat with the shock-induced activation. The varying spatial response in activation time confirms effective capture. The virtual electrode pattern (VEP) induced by high voltage shocks is an important feature that determines the mechanism of successful electrotherapy. Figure 4c illustrates this characteristic pattern following a 50 V
shock delivered from the electrode highlighted in white. This simple demonstration establishes feasibility of creating stretchable thin film electrodes that can carry high voltage and large current shocks to the cardiac tissue. Repeated high-voltage shocks have the potential to induce corrosive degradation of the thin metal layers used in these electrodes. Further investigation will be necessary for chronic operation of this type of high-voltage electrotherapy but this could be a possible device to deliver the spatially tuned electrotherapy paradigms proposed in Chapter 5.

Figure B.3: Successful Shock Delivery through Fractal Electrodes

B.5 Conclusions

In summary, advanced designs and materials approaches provide capabilities for high definition cardiac electrotherapy in an advanced 3D-MIM platform. Concepts in fractal geometry allow large area, conformal electrodes suitable for delivering cardiac electrical stimulation, and for sensing cardiac electrical activity. Surface coating materials improve the electrochemical characteristics of the electrodes, in ways that are naturally compatible with the platform and its fabrication. Integrated arrays of sensors can be used to precisely monitor the electrotherapy and other forms of intervention. Animal experiments demonstrate the
multifunctional operation of the devices. These results suggest routes for developing advanced tools with utility in both fundamental research and clinical application of cardiac electrotherapy.
Appendix C

Protocols and Analysis Code

C.1 Guide to Optical Mapping of Rabbit Heart

General Set-up
1. Make solutions: 1 Liter of stock solution prepared in advance and stored at 4 °C
Tyrode’s stock I (25X):
NaCl: 187.3 g
CaCl$_2$: 4.78g
KCl: 8.76g
MgCl$_2$: 5.335g
NaH$_2$PO$_4$: 4.105g

Tyrode’s stock II (25X):
NaHCO$_3$: 42.005g

Combine 80 mL of each stock with 4g of Glucose, fill to 2L with Ultra-Pure Water.

2. Calibrate pH probe

3. Oxygenate the solution with 10psi 95 : 5%O$_2$/CO$_2$ (adjust to maintain pH). Maintain pH=7.35±0.05, temperature 37±0.5°C in the tissue bath.
4. Prepare cannula
5. Calibrate pressure and oxygen sensor
6. Place filter in perfusion line before aortic cannula
7. Prepare camera, choose appropriate filter (650nm for di4), focus the camera.
8. Connect triggers or acquisition cables if needed.
9. Prepare small beaker of Tyrode’s for the heart

Rabbit Euthanasia
1. 80/390 ml/kg of Fatal Plus + 0.4mL/kg Heparin
2. Mix well and attach butterfly needle.
3. Trim ear hair, place clip, clean with alcohol and scratch ear to desensitize.
4. Insert butterfly needle and push drug slowly. Clear line to be sure entire volume is delivered.
5. Confirm unresponsiveness to pain, remove from restrainer
6. Locate xyphoid process, cut into skin here.
7. Cut down until the diaphragm is exposed, puncture the diaphragm and remove it from chest wall to expose the heart.
8. Cut along the ribs and spread with hemostat.
9. Scoop the heart and cut the aorta above and below the heart, continue cutting to release the heart. Place in small beaker to transport.
10. Hang heart on cannula and secure with suture.
11. Release pressure in filling pericardium with a small incision.
12. Clean the fat, lungs, etc. carefully, do not cut myocardium
13. When sufficiently clean move heart to recirculating chamber

Optical Mapping
1. Allow heart to adjust to temperature in tissue bath, add needles to check ECG. Turn off lights.
2. Dilute 0.2mL of stock BB in 1 mL and add slowly to bubble trap. Continue this until beating stops (1-2mL total BB stock).
3. Add 20-30 µL of di-4 through bubble trap.
4. Adjust light and focus until sample monitor >30.
5. Continue with experimental protocol.

Clean-Up
1. Remove all fluid from the perfusion lines. REMOVE FILTER AND CHAMBER BEFORE NEXT STEP.
2. Run alcohol through line and collect it. (must be put in hazardous waste bin for pickup)
3. Run DI water through the line.
4. Remove filters and lenses and place cover back on camera.

C.2 Labview code for controlling digital relay switches

This labview program was designed to control a front-end series of relay-gates between the Cardiaelen defibrillator and the subject. It was designed in Labview 2012 with a compactRIO ADC with an Analog Input and a Digital Output module (National Instruments). The software is designed to receive a constantly wait for a trigger to signal that the shock is going to be delivered in 10 ms. Upon receiving the trigger, the compactRIO will deliver a series of signals to turn on and off the relay gates in a manner defined by the user. In the current design, the user can choose 1) Rotate Shock 1 based on the AF CL with no rotation in Shock 2, 2) Rotate Shock 2 based on the AF CL with no rotation in Shock 1, or 3) No rotation with the whole therapy delivered to either Vector 1 or Vector 2. Additional vector options are easily added by adding more frames to the sequence structures. The code will alert the user when a trigger is received and highlights the vectors that are turned on with virtual LEDs.
Figure C.1: Front Panel for Vector Relay Control

Figure C.2: Block Diagram for Vector Relay Control
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