DYNAMIC VIEWS OF STRUCTURE AND FUNCTION DURING HEART MORPHOGENESIS

Thesis by

Arian S. Forouhar

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2006

(Defended May 12, 2006)

Acknowledgments

The work presented here was generated from the efforts of an All-Star team. And since the game of basketball has taught me so much…here is my way of saying thank you.

The front office: Mory Gharib (*Donald Sterling*), Scott Fraser (*John Wooden*), Mary Dickinson (*Shaquille O'Neal*), Jay Hove (*Antoine Walker*), and Michael Dickinson (*Rick Fox*).

The starting lineup: Michael Liebling (*Steve Nash*), Julien Vermot (*Manu Ginobili*), John Dabiri (*LeBron James*), Reinhard Koster (*Scottie Pippen*), and Aaron Hawkey (*Tim Duncan*).

The bench: Mehrdad Zarandi (*John Stockton*), Anna Hickerson (*Tracy McGrady*), Michele Milano (*Ron Artest*), Derek Rinderknecht (*Gary Payton*), Arash Kheradvar (*Dirk Nowitzki*), Sean Megason (*Reggie Miller*), David Koos (*Ray Allen*), Le Trinh (*Karl Malone*), Luca Caneparo (*Jason Williams*), Jian Lu (*Smush Parker*), Abbas Moghaddam (*Vlade Divac*), Ying Gong (*Ed O'Bannon*), Magdalena Bak (*Gilbert Arenas*), Chris Waters (*Tony Parker*), and Gabriel Acevedo-Bolton (*Marcus Camby*).

The assistant coaches: Kristy Hilands (*Maurice Williams*), Martha Salcedo (*B.J. Armstrong*), Kathleen Hamilton (*Jeff Hornacek*), and Linda Scott (*Derek Fisher*).

In the stands: Brian Zid (*Dennis Rodman*), Armin Sorooshian (*Jason Terry*), Danson Njoroge (*Vince Carter*), John Bird (*Ben Wallace*), Jordan Carlson (*Jerry West*), Marie Giron (*Jason Richardson*), Yezdan Badrakhan (*Chris Anderson*), and David Zito (*Stephon Marbury*)

In the suite: My wife Parvin (*Baron Davis*), Mom (*Michael Jordan*), Dad (*Elton Brand*), and Pamon (*Dwayne Wade*). Manijeh (*Rasheed Wallace*), Guiv (*Magic Johnson*), Bahram (*Charles Barkley*), Meech (*Sam Cassell*), and Kat (*Dikembe Mutombo*).

Dedication

My Dad.

Abstract

Congenital heart defects remain the most common birth defect in humans, occurring in over 1% of live births. The high prevalence of cardiac malformations can be partially attributed to limited knowledge regarding the embryonic roots of the disease. A variety of congenital heart defects are thought to arise from combinations of genetic and epigenetic factors. In an effort to better understand this dynamic relationship, our study explores the structure and function of the developing heart and valves and examines hemodynamic factors influencing valvulogenesis. In order to study cardiac mechanics, we employed novel high-speed confocal microscopy and four-dimensional visualization techniques. A dynamic four-dimensional dataset describing heart and valve development along with blood flow patterns throughout cardiac morphogenesis is presented. Utilizing newly developed tools, we propose a novel pumping mechanism in the valveless embryonic heart tube via elastic wave propagation and reflection. We show that this form of pumping leads to oscillatory shear stresses in the developing atrio-ventricular canal, a phenomenon that had not previously been documented. An *in vivo* method to modulate trans-valvular oscillatory flows is described and used to test our hypothesis that oscillatory shear stress across the primitive valve cushions stimulates heart valve leaflet formation. Our results suggest hemodynamic forces contribute to valvulogenesis and enhance our understanding of normal and abnormal heart valve development.

Table of Contents

Acknowledgements iii
Dedication v
Abstract vi
Table of Contents vii
List of Figures x
CHAPTER 1: Prologue 1
1.1 Introduction 1
1.2 Organization 3
1.3 Goals 5
1.4 Chapter References 7

CHAPTER 2: Vertebrate Heart Development 8
2.1 Introduction 8
2.2 Vertebrate Heart Morphogenesis 8
2.3 Embryonic Zebrafish as a Model 9
2.4 Zebrafish Heart Morphogenesis 11
2.5 Factors Influencing Heart Morphogenesis 13
2.5.1 Genetic Contributions 13
2.5.2 Epigenetic Contributions 14
2.6 Discussion 17
2.7 Chapter References 19

CHAPTER 3: Zebrafish Cardiac Imaging Tools and Techniques 22
3.1 Introduction 22
3.2 Zebrafish Preparation 23
3.3 Fluorescent Contrast Agents 26
3.3.1 Transgenic Lines 26
3.3.1.1 *Tg(gata1:GFP)* 27
3.3.1.2 *Tg(cmcl2:GFP)* 28
3.3.1.3 *Tg(tie2:GFP)* 29
3.3.2 Vital Dyes 31
3.4 High Speed Confocal Microscopy 32
3.5 Four-Dimensional Reconstructions 34
3.5.1 Data Collection 35
3.5.2 Algorithm 36
3.5.3 Limitations 38
3.5.4 Conclusions 40
3.6 Four-Dimensional Data Analysis 41
3.6.1 Volume Measurements 41
3.6.2 Cardiac Cell Tracking 43
3.6.3 Qualitative Flow Analysis 45
3.7 Blood Flow Visualization 46
3.7.1 Digital Particle Imaging Velocimetry 47
3.7.2 Particle Tracking 49

3.8 Chapter References 51

CHAPTER 4: Embryonic Heart Tube Biomechanics 53
4.1 Introduction 54
4.2 Embryonic Heart Tube is Not Peristaltic 55
4.2.1 Bidirectional Wave 55
4.2.2 Blood Velocity Exceeds Heart Wall Wave Speed 57
4.2.3 Nonlinear Frequency-Flow Relationship 58
4.3 Embryonic Heart Tube is a Dynamic Suction Pump 59
4.3.1 Resonance Peaks in Frequency-Flow Relationship 59
4.3.2 Reflections at Mismatched Impedance Sites 60
4.3.3 Pressure-Flow Relationship 61
4.3.4 Net Flow Reversal 62
4.4 Materials and Methods 64
4.4.1 Imaging Parameters 64
4.4.2 Quantitative Flow Analysis 64
4.4.3 Pressure Variation Estimates 65
4.5 Discussion and Perspectives 68
4.6 Chapter References 71

Chapter 5: Oscillatory Flow and Valvulogenesis 72
5.1 Introduction 72
5.2 Methods 75
5.2.1 High-Speed Confocal Imaging 75
5.2.2 Brightfield Imaging 75
5.2.3 Discrete Flow Representation 75
5.2.4 Methods to Control Heart Rate 76
5.2.4.1 Lidocaine Treatment 76
5.2.4.2 Temperature 76
5.2.5 Valve Development Assay 76
5.3 Intracardiac Flow Patterns 77
5.4 Zebrafish Valvulogenesis 80
5.4.1 Valve Morphogenesis 80
5.4.2 Valve Dynamics 83
5.5 Frequency and Flow 89
5.5.1 Decreased Heart Rate 89
5.5.2 Oscillatory Flow Reduction Mechanism 91
5.5.2 Lidocaine Treatment 92
5.6 Reduced Oscillatory Flow Induces Valve Defects 93
5.6.1 Range of Valve Defects 94
5.6.2 Incidence of Valve Dysmorphology 95
5.7 Control Experiments 96
5.8 Discussion and Perspectives 99
5.9 Chapter References 102
Chapter 6: Conclusions 105
6.1 Primary Contributions 105

6.2 Challenges 107
6.3 Future Work 108

Appendix 110
A. Intracardiac Fluid Forces Are an Essential Epigentic Factor for Embryonic Cardiogenesis 110
B. Viewing Angles for Cardiac Imaging 131
C. Shear Stress Sensitive Genes Involved in Valve Formation 147

List of Figures

Figure 2.1 Embryonic zebrafish and heart development 11
Figure 2.2 Hemodynamic forces are essential for proper cardiogenesis 17
Figure 3.1 Blocked pigment formation in PTU-treated embryos 24
Figure 3.2 Heart morphogenesis in *Tg(gata1:GFP)* embryos 28
Figure 3.3 Heart morphogenesis in *Tg(cmcl2:GFP)* embryos 29
Figure 3.4 Heart morphogenesis in *Tg(tie2:GFP)* embryos 30
Figure 3.5 BODIPY-ceramdie stained embryos reveal non-tissue-specific fluorescent contrast 32
Figure 3.6 Acquiring and synchronizing nongated motions in sequential optical planes 36
Figure 3.7 Period determination in the zebrafish heart 37
Figure 3.8 Realignment artifacts due to non-periodic cardiac cycles 39
Figure 3.9 Four-dimensional data of heart contractions in *Tg(cmlc2:GFP)* embryos 40
Figure 3.10 Embryonic cardiac volume renderings 43
Figure 3.11 Cardiac cell tracking in *Tg(cmlc2:GFP)* embryos 45
Figure 3.12 Quantitative description of endocardial cushion dynamics 45
Figure 3.13 First use of DPIV to characterize blood flow in the embryonic zebrafish 48
Figure 3.14 Intracardiac blood flow characterization utilizing DPIV 49
Figure 3.15 Blood cell tracking through a *Tg(gata1:GFP)* heart tube 50
Figure 4.1 Biomechanics of embryonic heart tube contractions contradicts peristalsis as the main pumping mechanism 56
Figure 4.2 Endocardial cell trajectories during heart tube contractions contradict peristalsis 57
Figure 4.3 Blood cell velocities greatly exceed the traveling wave velocity 58
Figure 4.4 Non-linear frequency flow relationship for 26 hpf zebrafish heart tube despite similar contraction amplitudes 59
Figure 4.5 Hydroelastic nature of the embryonic heart tube wall 61
Figure 4.6 Pressure gradient estimations 62
Figure 4.7 Net flow reversal in the heart tube at different contractile frequencies 63
Figure 4.8 Blood cell velocity measurements over a range of frequencies 65
Figure 5.1 Silent heart mutants undergo incomplete cardiogenesis 74
Figure 5.2 Oscillatory flow across the developing valve 79
Figure 5.3 AV valve morphogenesis in BODIPY-ceramide stained embryos 82
Figure 5.4 Temporal asymmetry in valve leaflet formation 83
Figure 5.5 Valve dynamics and blood flow in 36 hpf embryos 84
Figure 5.6 Valve dynamics and blood flow in 72 hpf embryos 86
Figure 5.7 Valve dynamics and blood flow in 84 hpf embryo 87
Figure 5.8 Valve dynamics and blood flow in 120 hpf embryos 89
Figure 5.9 The duration of retrograde flow decreases with decreased heart rate 90
Figure 5.10 Oscillatory flow reduction mechanism 92
Figure 5.11 Lidocaine decreases heart rate 93
Figure 5.12 Range of valve dysmorphology at 96 hpf 95

Figure 5.13 Incidence of valve dysmorphology 96
Figure 5.14 Valve dysmorphology rescue with elevated temperature 98
Figure 5.15 Heart valve dysmorphology rescue in embryos treated with 0.15% lidocaine 98
Figure 5.16 Heart valve dysmorphology resuce in embryos treated with 0.09% lidocaine 99
Figure A.1 Cardiac dynamics in the zebrafish embryonic heart at 4.5 dpf 125
Figure A.2 High-velocity, high-shear conditions generated in the 4.5 dpf embryonic zebrafish heart 126
Figure A.3 Dynamics of valveless atrio-ventricular junction in the 37 hpf embryonic zebrafish heart 128
Figure A.4 Impaired blood flow influences cardiogenesis 129
Figure B.1 30 hpf stack 132
Figure B.2 30 hpf left lateral progression 133
Figure B.3 30 hpf right lateral progression 134
Figure B.4 48 hpf stack 135
Figure B.5 48 hpf left lateral progression 136
Figure B.6 48 hpf right lateral progression 137
Figure B.7 72 hpf stack 138
Figure B.8 72 hpf left lateral progression 139
Figure B.9 72 hpf right lateral progression 140
Figure B.10 96 hpf stack 141
Figure B.11 96 hpf left lateral progression 142
Figure B.12 96 hpf right lateral progression 143
Figure B.13 144 hpf stack 144
Figure B.14 144 hpf left lateral progression 145
Figure B.15 144 hpf right lateral progression 146