BIOMECHANICS OF AORTIC VALVE LEAFLET FUSION AND STIFFENING

A Thesis
Presented to the
Faculty of
San Diego State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Bioengineering

by
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Summer 2013
SAN DIEGO STATE UNIVERSITY

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DEDICATION

Dedicated to my family for their unconditional patience, love and support.
ABSTRACT OF THE THESIS

Biomechanics of Aortic Valve Leaflet Fusion and Stiffening
by
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San Diego State University, 2013

Calcific Aortic Stenosis (CAS) involves narrowed aortic valve (AoV) orifice, fibrotic thickening and is clinically presented with a high transvalvular gradient and jet velocity. By Echocardiography (EC) derived opening area (EOA) estimation is used as an index for accurate assessment of stenotic severity. However, there are often discrepancies between EC derived EOA and invasive indices of CAS, thus leading to incorrect assessment of CAS severity and clinical decisions. There exists a need for an alternative non-invasive quantification method for analyzing the AoV dysfunction which serves as a predictor of CAS onset and progression of AoV. It is generally agreed upon that CAS progression is influenced by alterations in the valve hemodynamics, wherein it is continually subjected to cyclic stretches, bending, pressures, and shear stresses.

The objective of this study is to measure the effect of leaflet fusion and stiffening on geometric opening area (GOA), hemodynamics (pressure and flow) and mechanical strain response in the AoV using a cardiac simulator. Seven bioprosthetic porcine AoVs were tested under controlled matched conditions. Fusion was simulated by suturing the leaflet edges together along one (F1) or two of the commissures (F2) and fibrosis by applying a thin layer of cyanoacrylate adhesive on the aortic face of one of the valve leaflets (F1S, F2S). The cardiac simulator is a mock circulatory loop that has preprogrammed settings of cardiac contractility (Off, Low, Medium) at a heart rate of 72 bpm. Pressure and flow are measured at several points in the system and a single CCD camera mounted under the simulator records images of the valve as it opens and closes in response to biomechanical changes. Images are analyzed to obtain the GOA values during the cycle.

The mean GOA decreases due to commissural fusion and stiffening. Statistical analysis has confirmed that level of cardiac support and fusion (P < 0.01) are the most significant contributors to GOA reductions, which are indicators of stenosis. Fibrotic stiffening along with commissural fusion of the AoV contributes to reduced distensibility as seen by the strain response; this in turn is believed to lead to an increase in the internal stress. Additional studies are required to confirm this finding.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT .............................................................</td>
</tr>
<tr>
<td>LIST OF TABLES .........................................................</td>
</tr>
<tr>
<td>LIST OF FIGURES ...........................................................</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS ..........................................................</td>
</tr>
<tr>
<td>CHAPTER</td>
</tr>
<tr>
<td>1 INTRODUCTION .............................................................</td>
</tr>
<tr>
<td>2 BACKGROUND ..............................................................</td>
</tr>
<tr>
<td>2.1 Cardiovascular Anatomy and Physiology ................................</td>
</tr>
<tr>
<td>2.2 Anatomy and Physiology of the Aortic Valve ...........................</td>
</tr>
<tr>
<td>2.3 Valvular Heart Diseases ..............................................</td>
</tr>
<tr>
<td>2.3.1 Valvular Calcific Aortic Stenosis (Cas): Etiology, Morphology and Pathobiology</td>
</tr>
<tr>
<td>2.3.2 Aortic Regurgitation (AR) ..........................................</td>
</tr>
<tr>
<td>2.4 Biomechanics of the AoV Tissue ......................................</td>
</tr>
<tr>
<td>3 METHODS .................................................................</td>
</tr>
<tr>
<td>3.1 Overview .................................................................</td>
</tr>
<tr>
<td>3.2 Imaging and Strain Analysis ..........................................</td>
</tr>
<tr>
<td>3.2.1 Reference State Image Selection ...................................</td>
</tr>
<tr>
<td>3.2.2 Image Analysis for Valve GOA ......................................</td>
</tr>
<tr>
<td>3.3 Image Analysis ...........................................................</td>
</tr>
<tr>
<td>4 RESULTS .................................................................</td>
</tr>
<tr>
<td>4.1 Strain Analysis ..........................................................</td>
</tr>
<tr>
<td>4.2 Opening Area Analysis ..................................................</td>
</tr>
<tr>
<td>4.3 Hemodynamic Analysis ..................................................</td>
</tr>
<tr>
<td>5 DISCUSSION ...............................................................</td>
</tr>
<tr>
<td>5.1 Detection of Cas: Role of Current Study ..............................</td>
</tr>
<tr>
<td>5.2 Summary of Findings ....................................................</td>
</tr>
</tbody>
</table>
5.3 Aortic Valve Mechanobiology and Structural Analysis ..............................................54
5.4 Proposed AoV Disease Model for CAS .....................................................................60
5.5 Limitations .................................................................................................................61
5.6 Future Scope ..............................................................................................................62
REFERENCES ...................................................................................................................63
APPENDIX
A  IMAGE J:MACROS .........................................................................................................67
B  STRAIN, HEMODYNAMIC PROFILE : MAXIMUM VALUES ........................................71
LIST OF TABLES

Table 3.1. Experimental Variables for Valve Studies .......................................................... 22
Table 4.1. Mean Pressure- Strain Response for Medium Cardiac Support (M7) .................. 32
Table 4.2. Average Circumferential & Radial Strains in the ROIs (M6) ............................ 33
Table 4.3. Mean Circumferential and Radial Strain (M7) .................................................. 34
Table 4.4. Average and Maximum GOA, Duration and Integrated GOA (M6) ............... 38
Table 4.5. Average and Maximum GOA, Average Duration of Opening and Integrated Area (M7) ............................................................................................................. 38
Table 4.6. Comparison of Average Opening Area, Integrated Area and Flow (M7) .......... 42
Table 4.7. Factors used in One-way ANOVA for GOA Analysis ......................................... 42
Table 4.8. Hemodynamic Profile for M7 .............................................................................. 44
Table 4.9. Hemodynamic Profile (M6) ................................................................................ 45
Table 5.1. Limitations of Doppler Echocardiography in Detection and Assessment of CAS Severity .................................................................................................................. 52
Table 5.2. Summary of Change in Leaflet Strain Response due to Fusion and Stiffening .......................................................... 53
Table 5.3. Summary of Change in AoV Opening Area due to Fusion and Stiffening .......... 53
Table 5.4. Summary of Change in Hemodynamic Profiles Due to Fusion and Stiffening .......................................................................................................................... 54
Table A.1. Crop.ijm Is Used to Automate Image J Analysis for M6. This Helps Cropping Images with Precise x,y,z Co-ordinates ................................................................. 68
Table A.2. EDGE.IJM Is Used for Automated Detection of Edges for GOA Analysis ....... 69
Table A.3. Analyze.ijm Is Used to Automate Image J Analysis. The Surface Area Is Calculated by Edge Detection by Image J .................................................................................. 70
Table B.1. Maximum Values of Circumferential and Radial Strain for Low and Medium Cardiac Support (M6) .................................................................................... 72
Table B.2. Maximum Values of Circumferential and Radial Strain for Low and Medium Cardiac Support (M7) .................................................................................... 72
Table B.3. Summary of Mean and Maximum Data for Hemodynamic Profile and GOA Parameters for Medium Cardiac Support (M6) ................................................................. 73
Table B.4. Summary of Mean and Maximum Data for Hemodynamic Profile and GOA Parameters for Medium Cardiac Support (M7)..........................73
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Deoxygenated blood (blue) flows into the right atrium, pumped into the right ventricle and the pulmonary artery to the lungs. Oxygenated blood (red) flows to the left atrium, ventricles and rest of the body.</td>
<td>4</td>
</tr>
<tr>
<td>2.2</td>
<td>Aortic valve and the left ventricular outflow tract (LVOT).</td>
<td>5</td>
</tr>
<tr>
<td>2.3</td>
<td>Valves of the human heart.</td>
<td>6</td>
</tr>
<tr>
<td>2.4</td>
<td>Distribution of coronary arteries (R- Right, LM- Left Main, LC- left Circumflex, LAD- Left Anterior Descending)(Hurt’s Heart).</td>
<td>6</td>
</tr>
<tr>
<td>2.5</td>
<td>Aortic root: aerial view.</td>
<td>7</td>
</tr>
<tr>
<td>2.6</td>
<td>Aortic root: sinotubular junction, sinuses (Hurt’s Heart).</td>
<td>7</td>
</tr>
<tr>
<td>2.7</td>
<td>Anatomy of the AoV.</td>
<td>8</td>
</tr>
<tr>
<td>2.8</td>
<td>Principal layers of the AoV leaflet tissue.</td>
<td>9</td>
</tr>
<tr>
<td>2.9</td>
<td>Bicuspid AoV.</td>
<td>11</td>
</tr>
<tr>
<td>2.10</td>
<td>Commissural fusion and cuspal thickening of AoV.</td>
<td>12</td>
</tr>
<tr>
<td>2.11</td>
<td>Tricuspid AoV with calcium deposition as seen in the longitudinal section from a 89 year old patient.</td>
<td>12</td>
</tr>
<tr>
<td>2.12</td>
<td>Pathogenesis of aortic stenosis.</td>
<td>14</td>
</tr>
<tr>
<td>2.13</td>
<td>ECM remodeling and role of VICs in CAS progression.</td>
<td>15</td>
</tr>
<tr>
<td>2.14</td>
<td>Cardiac cycle - changes aortic pressure (AP/AoP), left ventricular pressure (LVP), left atrial pressure (LAP), left ventricular volume (LV Vol).</td>
<td>17</td>
</tr>
<tr>
<td>2.15</td>
<td>AoV dynamics during systole and diastole.</td>
<td>17</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic of cardiac simulator system with AoV.</td>
<td>20</td>
</tr>
<tr>
<td>3.2</td>
<td>Porcine bioprosthetic AoV with surface markers.</td>
<td>22</td>
</tr>
<tr>
<td>3.3</td>
<td>Difference in the location of the pixels correlates deformation.</td>
<td>24</td>
</tr>
<tr>
<td>3.4</td>
<td>Aortic valve leaflet showing circumferential, radial directions.</td>
<td>24</td>
</tr>
<tr>
<td>3.5</td>
<td>ROI with mask for estimation of strain using DaVIS.</td>
<td>25</td>
</tr>
<tr>
<td>3.6</td>
<td>Calibration image used for setting the scale and unit conversions.</td>
<td>25</td>
</tr>
<tr>
<td>3.7</td>
<td>Valve GOA measurement and analysis.</td>
<td>26</td>
</tr>
<tr>
<td>3.8</td>
<td>GOA detection by image J using thresholding.</td>
<td>26</td>
</tr>
<tr>
<td>4.1</td>
<td>ROI (W) for whole strain gradients.</td>
<td>28</td>
</tr>
</tbody>
</table>
Figure 4.2. Circumferential and radial strain gradients. ..............................................................28
Figure 4.3. Strain cycle for unfused (U) CSMed (M6). .................................................................29
Figure 4.4. Strain cycle for unfused (U) CSMed (M7). .................................................................29
Figure 4.5. Circumferential strain for leaflet fusion with CSMed (M6). ......................................30
Figure 4.6. Circumferential Strain for leaflet fusion with CSMed (M7). .....................................30
Figure 4.7. Average circumferential and radial strain (M7). .......................................................30
Figure 4.8. Maximum circumferential and radial strain (M7). .....................................................31
Figure 4.9. Average circumferential strain (M6, M7). .................................................................31
Figure 4.10. Average radial strain (M6, M7) ................................................................................32
Figure 4.11. Correlation of TVP and strain, fusion for CSMed (M7). ............................................33
Figure 4.12. Strain gradient: ROIs used for measurement ............................................................33
Figure 4.13. Strain gradients along the radial direction (Exx) (M6). ............................................34
Figure 4.14. Strain gradients along the radial direction (Eyy). .....................................................35
Figure 4.15. Circumferential strain gradients for Exx (M6). .........................................................35
Figure 4.16. Radial strain gradients for Eyy (M6). .......................................................................36
Figure 4.17. Radial strain gradients for Exx (M7). .......................................................................36
Figure 4.18. Radial strain gradients for Eyy (M7). .......................................................................36
Figure 4.19. Circumferential strain gradients for Exx (M7). .........................................................37
Figure 4.20. Circumferential strain gradients for Eyy (M7). .........................................................37
Figure 4.21. Effect of fusion on GOA for medium cardiac function (M6). .................................39
Figure 4.22. Effect of fusion on GOA for medium cardiac support (M6). .................................39
Figure 4.23. Effect of stiffening on GOA for low cardiac function (M7). .................................40
Figure 4.24. Effect of stiffening on GOA for medium cardiac function (M7). .............................40
Figure 4.25. Reduction of GOA due to fusion (M6). .................................................................41
Figure 4.26. Reduction in GOA due to stiffening (M6). ...............................................................41
Figure 4.27. Reduction in GOA due to fusion (M7) ...................................................................41
Figure 4.28. Reduction in GOA due to stiffening (M7). ...............................................................41
Figure 4.29. Statistical significance of average GOA and fusion ..............................................43
Figure 4.30. Statistical significance of integrated GOA and fusion ...........................................43
Figure 4.31. Effect of Fusion on TVP time series (M7). ...............................................................45
Figure 4.32. Effect of stiffening on TVP time series (M7). ..........................................................45
Figure 4.33. Variation of TVP with low cardiac support (M7). ......................................................46
Figure 4.34. Variation of TVP with medium cardiac contractility (M7).................................46
Figure 4.35. The variation of LVP with fusion.................................................................47
Figure 4.36. The variation of AoP with fusion.................................................................47
Figure 4.37. The variation of AoQ with fusion.................................................................47
Figure 4.38. Effect of stiffening on LVP (M7).................................................................48
Figure 4.39. Effect of stiffening on AoP (M7).................................................................48
Figure 4.40. Effect of stiffening on AoQ (M7).................................................................48
Figure 5.1. Continuous wave spectral recording from a patient with CAS.........................50
Figure 5.2. Simplified continuity equation used to determine EOA.................................51
Figure 5.3. Estimation of velocity and area for the simplified continuity equation..............51
Figure 5.4. Schematic of biomechanical function and interactions.....................................55
Figure 5.5. Proposed model for AoV pathogenesis............................................................60
ACKNOWLEDGEMENTS

My sincerest thanks and regards to members of my thesis defense committee, Dr. Karen May-Newman, Dr. Samuel Kassegne and Dr. Michael Buono for insightful pointers and unending support.

I would like to express my sincere gratitude to Dr. Karen May-Newman for her expert guidance every step of the way, as a professor and my thesis advisor. I would not have completed this work without her perseverance and belief in me.

I would like to express my heartfelt thanks to Dr. Samuel Kassegne for having been so patient, understanding and encouraging. I consider myself lucky to have cross roads with a professor who truly inspires his students to go beyond the obvious, challenge their beliefs and only be satisfied when one learns to apply their knowledge to real life problems.

I would like to thank Pratit Bastola for his help and support with the project esp. DaVIS and clarifying numerous doubts.

I would like to thank Phanthiwa Posuwattanakul for her help with the project.
CHAPTER 1

INTRODUCTION

Aortic valve calcification is a prevalent condition found in 21% to 26% of the adult population over 65 years of age [44]. The onset, degree and severity of calcification is a strong cardiovascular risk factor and influences the progression and outcome of calcific aortic stenosis (AS). In developed nations, AS is a progressive and degenerative disease, and with a prevalence of 3% to 9%, is the primary cause of valve replacement. Although this disease is characterized by a long asymptomatic phase, severe symptomatic AS is associated with a life expectancy of 5, 3 and 2 years or less with the onsets of angina, syncope, and heart failure, respectively [44] [10], AS involves narrowing of the aortic valve orifice and is clinically presented with narrowing of the valve area, a higher transvalvular gradient or jet velocity, a harsh systolic murmur, increased exercise stress and so on. The severity of AS at baseline is determined by the markers using echocardiogram or electrocardiogram and is critical in the prediction of progression and outcome of the disease [10].

Several studies have identified the onset and degree of aortic tissue remodeling due to commissural fusion and calcification as strong predictors both for the progression and outcome of aortic Stenosis [10] [26] [29] [44]. Most of the clinical studies in this arena, currently rely heavily on echocardiographic valve morphology. Therefore, there exists a need for accurate and reliable method for quantification of the extent of aortic valve fusion and calcification and its influence on parameters such as the valve area, transvalvular gradient, and stress response.

The current research has been undertaken with an objective to understand the biomechanical effect of remodeling and commissural fusion of the aortic valve leaflet tissue and its correlation to the pathogenesis of AS under in-vitro conditions in the biomechanical laboratory at San Diego State University. It involves studying the alteration in strain, hemodynamic properties and valve orifice area in bioprosthetic aortic valves subjected to stresses caused by alteration of the inherent material properties. Structural remodeling is introduced by means of fusion of the valve leaflets along the commissures by suturing either
one or two of the leaflets. Additionally, stiffening of one of the valve leaflets to simulate the pathophysiological calcification of the valve leaflet is used as an experimental variable. The in-vitro cardiac simulator is a mock circulation loop that simulates normal physiological conditions. Variation in cardiac contraction levels are designed to simulate low and moderate heart failure conditions with stroke volume (mL) and cardiac output (L/min) values of 35 ml, 2.5 L/min and 49 mL, 3.5 L/min, respectively which are the experimental settings.

The governing hypothesis is that a positive feedback loop influences the pathogenesis of AoV dysfunction and disease due to pathological remodeling of the extracellular matrix (ECM) of the valve tissue. While, the exact mechanisms are yet to be understood, it is believed that a loss of equilibrium between the synthesis of the ECM components and their degradation leads to its disruption and dysfunction which translates to structural remodeling in terms of loss of elasticity and fibrosis/thickening due to collagen deposition and elastin fragmentation [7] [12].

In order to test this hypothesis the Geometric Opening Area (GOA), hemodynamic data (pressure, flow) and mechanical strain response data have been examined closely to understand the underlying biologics due to simulated structural remodeling. Structural remodeling has been introduced in the study by means of commissural fusion and stiffening of the porcine bioprosthetic AoV leaflet tissue. Significant effects and interactions are brought into light by undertaking the statistical analysis of desired parameters across valve studies that have been published previously. Finally, an attempt at the comparison of the in-vitro data in the light of established clinical paramaters, has helped us understand its relevance.
CHAPTER 2

BACKGROUND

2.1 Cardiovascular Anatomy and Physiology

The human cardiovascular system can be aptly described as a biomechanical structure consisting of a pump and a series of closed branched tubes that cycle a fluid in endless loops, unidirectionally. The pressure generated in the pump (heart), propels the fluid (blood) to circulate continuously, enabling the exchange of nutrients, electrolytes, dissolved gases and waste products with the surrounding tissues. Thus the heart and the vasculature along with the cells and plasma form a very efficient transport system.

The mammalian heart, a cone shaped, hollow muscular organ, consists of two separate functional units, interconnected by a conduction system and can be effectively visualized a structure-function continuum. Situated in the thoracic cavity, it is encased within the visceral layer of the pericardium, which is attached to the walls of the great vessels and the diaphragm. The pericardium consists of a fibrous tissue and a serous membrane layer on the outer and the inner side, respectively. When viewed from the apex, the human heart, resembles a pyramid, that has one-third of its mass to the right of the midline, and two thirds to the left. While anteriorly, it is protected by the sternum and the costal cartilages of the third, fourth, and fifth ribs, posteriorly, it is cushioned by the esophagus and the tracheal bifurcation, and the bronchi. The right lung overlies the right surface of the heart and reaches to the midline of the thoracic cavity, while the left lung retracts from the midline to the cardiac notch. Figure 2.1 depicts the anatomy and physiology of the heart.

The wall of the heart is comprised of the pericardium (connective and adipose tissue layers), myocardium (cardiac muscle tissue supplied by blood capillaries and nerve fibers) and endocardium (epithelium and connective tissue layers with elastic and collagenous fibers) on the outer, middle and inner side respectively.

The septum, composed of myocardium (cardiac muscle tissue), divides the heart into right and left halves. Each half is provided with an atrioventricular (AV) valve, that further divides it into atria and ventricles and ensure unidirectional flow. The atria receive blood
Figure 2.1. Deoxygenated blood (blue) flows into the right atrium, pumped into the right ventricle and the pulmonary artery to the lungs. Oxygenated blood (red) flows to the left atrium, ventricles and rest of the body. Source: "Anatomy and Physiology of the Cardiovascular System." Accessed December 2, 2012. http://samples.jbpub.com/9781449652609/99069_ch05_6101.pdf.

returning to the heart, while the ventricles pump the blood into the arteries. The left ventricular wall is thicker than the right in order to withstand and pump blood against a higher resistance to the flow.

The AV valves (tricuspid valve on the right and mitral on the left) open and close according to changes in pressure in the respective chambers and maintain unidirectional flow. The pulmonary valve is located at the opening of the pulmonary artery to prevent the backflow of the blood into the right ventricle. The mitral valve is located at the inlet to the left ventricle. The chordae tendineae and the papillary muscles act as tension apparatus and help in maintaining the valve’s closed state [1] [22] [27] [39].

The outflow tract of the left ventricle (LVOT) (Figure 2.2 [27]) consists of both muscular and fibrous portions. The septal portion, though primarily muscular, also includes the membranous portion of the ventricular septum.

The left bundle of the cardiac conduction system enters the left ventricular outflow tract posterior to the membranous septum and immediately beneath the commissure between
the right and noncoronary leaflets of the aortic valve. After traveling a short distance down the septum, the left bundle divides into anterior, septal, and posterior divisions [27].

The tricuspid and pulmonary valves on the right side are widely separated by the inner curvature of the heart; conversely, the mitral and aortic valves lie adjacent to one another, with fibrous continuity of their leaflets. The aortic valve occupies a central position, wedged between the tricuspid and pulmonary valves as seen in Figure 2.3 [27].

**2.2 Anatomy and Physiology of the Aortic Valve**

The Aortic Valve (AoV) is located centrally and its relationship with the other cardiac chambers is an essential key to understanding the pathology and various congenital cardiac malformations and dysfunctions. It consists primarily of three semilunar leaflets or cusp - the left coronary (LC), the right coronary (RC), and the non-coronary (NC) cusp [27].

Each leaflet of the AoV is attached to the aorta and behind it, the aortic wall bulges outward to form the sinuses of Valsalva. The inlets to the coronary artery system lie in the the sinus of Valsalva, superior to the the leaflet attachments and inferior to the sinotubular junction. Figure 2.4 shows the distribution of the coronary arteries.

Figure 2.4. Distribution of coronary arteries (R- Right, LM- Left Main, LC- left Circumflex, LAD- Left Anterior Descending)(Hurt’s Heart).

The aortic root in Figure 2.5 comprises the proximal portion of the aorta and consists of sinuses of Valsalva, the interleaflet triangles and the AoV leaflets. It is delineated by the sinotubular ridge superiorly and the base of the AoV leaflets, inferiorly [35].

The sinotubular ridge, seen in the cross-sectional view in Figure 2.6, is a circular structure that delineates the beginning of the ascending aorta. It is observed to be thicker than the sinuses that lie beneath it.

Figure 2.6. Aortic root: sinotubular junction, sinuses (Hurt’s Heart).

Figure 2.7 [21] shows the anatomical view of a single AoV leaflet. The leaflets of the AoV meet/unite centrally along the line/edge of coaptation, at the center of which is a thickened nodule, called the nodule of Arantius.

Peripherally, adjacent to the commissures, the line of coaptation is thinner and normally may contain small perforations. During systole, the leaflets are thrust upward and away from the center of the aortic lumen, whereas, during diastole, they fall passively into the center of the aorta. With normal morphology, all three leaflets meet along lines of coaptation and support the column of blood within the aorta to prevent regurgitation into the ventricle [17] [27].
The AoV leaflets, are primarily composed of collagen, elastin, and glycosaminoglycans (GAGs); each of these leaflets consist of three principal layers (Figure 2.8 [26]) - the ventricularis, the spongiosa, and the fibrosa on the outer, middle and inner sides, respectively. These layers are responsible for imparting biomechanical properties to the AoV, which regulates its function.

The outer layers of the leaflet form a continuum with the aortic or ventricular endothelium. The ventricular side contains collagen coupled elastin-rich fibers, aligned in the radial direction, which is perpendicular to the leaflet margin. Elastin helps maintain structural integrity and ensure that the fibers return to their original state once the external forces are removed; it also helps maintain a specific collagen fiber configuration. The collagen fibers are aligned in the circumferential direction, parallel to the free edge.

The inner layer of corrugated fibrosa on the aortic side contains collagen fibers aligned in the circumferential direction and, in a relaxed state, assume a waveform pattern. The spongiosa, consist of loose connective tissue or mucopolysaccharides. These principal layers of the aortic leaflet provide the necessary biomechanical properties for proper valve function.
The endothelial cells that tend to align along the direction of stress (e.g. flow stress) are present in the fibrosa layer of the AoV leaflet tissue. However, in this case, the cells are arranged in a circumferential pattern, which results in major stress occurring in that direction, which is perpendicular to the direction of the flow of the blood [11] [26].

The AoV consists of four types of cells – the endothelial (VEC), the interstitial (VIC), the cardiac muscle, and the smooth muscle cells. The AoV surface is lined by a confluent monolayer of VECs while the VICs are distributed across the three layers; the other cells are present towards the basal region of the valve [20]. The extracellular matrix (ECM), histologically distributed in the three avascular layers, (discussed above) helps maintain the spatial structure and regulates complex functions that ultimately regulate the physical and mechanical properties of the AoV.

The cells synthesize the essential components of the ECM including collagen, elastin, proteoglycans, glycoproteins, cytokines as well as matrix metalloproteinases (MMP) and tissue inhibitors (TIMP) that are responsible for degradation of the ECM components. The VECs contribute to valve homeostasis and ECM remodeling through regulation of permeability, adhesiveness to inflammatory cells, and paracrine signaling to circulating cells.
and VICs, which are responsible for normal AoV structure and function [7] [12]. The cell surface proteins such as cadherins, desmogleins, connexins, integrins help in intracellular as well as intercellular (with the ECM) communication and are believed to be expressed by the VICs. The communication network, particularly the integrins, behaves as sensors and transducers and enables the response to the external mechanical forces/stimuli on the AoV throughout the cardiac cycle [8].

The ECM communication network influences the modulation of various biochemical and biomechanical signals, thereby regulating the activity of various growth factors and cytokines. During early embryogenesis, Twist1, a transcription factor, facilitates ECM remodeling by converting unassembled precursors of collagen, elastin and proteoglycans into a laminar structure. Twist1 is downregulated thereafter, but is expressed during ECM disintegration [20].

2.3 Valvular Heart Diseases

The aortic valve is positioned strategically at the end of the left ventricular outflow tract (LVOT) and its normal functioning is critical to maintaining efficient cardiac function. Valvular aortic stenosis and aortic regurgitation are the most prevalent valvular diseases.

2.3.1 Valvular Calcific Aortic Stenosis (Cas): Etiology, Morphology and Pathobiology

The most common cause of AS is degenerative calcification of the AoV leaflets, leading to immobilization of the cusps. This may be congenital or acquired. Pathogenesis of CAS leads to progressive restriction to the LVOT. This condition is reached with either an increase in the peak systolic pressure gradient (< 50 mm Hg), with normal cardiac output (CO) or with a decrease in effective aortic orifice area (EOA) (< 0.8 cm²). The CO may be maintained with the onset of left ventricular hypertrophy (LVH), resulting in a large pressure gradient across the stenotic valve. As the left ventricle becomes less compliant, an onset of atrial fibrillation leads to further ventricular decompensation [26].

Congenital AS or a calcified bicuspid aortic valve (Figure 2.9 [32]) is one of the most commonly occurring form and results in significant stenosis, early in life.
It is observed to be present in approximately 2% of the general population. The abnormal architecture of the bicuspid aortic valve induces turbulent flow, which injures the leaflets and leads to fibrosis, increased rigidity, leaflet calcification, and narrowing of the aortic valve orifice. Bicuspid valves often are associated with dilatation of the ascending aorta related to accelerated degeneration of the aortic medium that in some cases may progress to aneurysm formation.

In case of acquired AS, the extension of the degenerative calcification into the aortic sinuses and the ascending aorta and of the focal sites of the lesions into the aortic annulus and calcification sites is commonly observed. It is a dynamic inflammatory disease process, in which the valve doesn’t open completely, thereby impeding forward flow. The common risk factors for the acquired AS may be elevated serum levels of low-density lipoprotein (LDL) cholesterol, or diabetes, or smoking, and/or hypertension. AoV Sclerosis (AVS) with focal, irregular thickening of the cusps, formation of calcific nodules, commissural fusion and impaired leaflet motion are usually observed in older patient populations (> 65 years) and precedes the onset of calcification [15] [31] [32].

Additionally, CAS may also be observed in cases of rheumatic fever, Paget disease (bone) and end-stage renal disease. Ochronosis with alkaptonuria occurs rarely, leading to a greenish discoloration of the aortic valve. Rheumatic AS usually occurs in conjunction with mitral valve stenosis and characterized by the diffuse fibrous leaflet thickening of the tricuspid valve with leaflet commissural fusion.

The morphologic hallmarks of non-rheumatic CAS with either the tricuspid or bicuspid AoV, are the calcified lesions within the cusps that deform the leaflet architecture and often protrude through the outflow surfaces into the Sinuses of Valsalva, preventing
normal function. The calcification is observed to originate at the points of maximal cusp flexion along the margins of attachment in the fibrosa, and the microscopic layered architecture is largely preserved. In patients with CAS of rheumatic etiology, however one of more of the commissures are often fused as seen in Figure 2.10 [36] along with fibrosis.

![Figure 2.10. Commissural fusion and cuspal thickening of AoV. Source: Tan, Carmela D., and E. R. Ridriguez. "Valvular Diseases." Accessed May, 6 2013. http://www.e-heart.org/index.htm.](image)

The stenotic tricuspid aortic valve in older persons contains calcific deposits on the aortic surfaces, involving the sites of cuspal attachments, and the commissars characteristically may not be fused shown in Figure 2.11 [32].

![Figure 2.11. Tricuspid AoV with calcium deposition as seen in the longitudinal section from a 89 year old patient. Source: Roberts, William C., and Jong M. Ko. "Some Observations on Mitral and Aortic Valve Disease." Baylor University Medical Center Proceedings 21 (2008): 282-299.](image)

Acquired CAS is usually associated with aortic valve malformation involving commissural fusion that sometimes results in a bicuspid AoV. The morphologies are presented by fusion of the RC, LC cusps (~ 70%) and RC, NC cusps (~ 30%); fusions of the
LC and NC cusps are rare. The AoV remodeling is often associated with maladaptive ECM remodeling due to activated metalloproteinases (MMPs) triggered by a possible deficiency of ECM components such as elastin, fibrillin, emilin [15].

While the malformed valve tissue is observed to be associated with altered hemodynamics and manifestation of thoracic aortic aneurysm (TAA), the exact pathobiology is yet to be understood. The evidence for concluding that altered hemodynamics and asymmetric blood flow patterns resulting from the deformed AoV are responsible for triggering TAA in patients, is currently insufficient [15].

The degree of CAS severity is primarily evaluated by the degree of measurable obstruction to the blood flow which contributes to an increase its velocity, corresponding to the systolic pressure gradient across the LV. The secondary effects of the decrease in the functional valve area, is observed on the flow through the LV and its vasculature, which progressively leads to pressure overload. Both the TVP and the velocity are observed to increase with decrease in the valve GOA [33].

Despite the high prevalence and mortality associated with both aortic valve calcification and stenosis, the exact pathobiology involving the signal transduction and/or molecular mechanisms are yet to be understood clearly. A number of mechanisms have been proposed for AoV calcification including osteogenic differentiation of VICs, calcification secondary to cellular apoptosis, and necrosis-related deposition of calcium [3].

One of the theories, put forth by Yu et al, is that, the three primary processes (schematic shown in Figure 2.12 [44]) that drive the pathogenesis of AS are lipid accumulation, inflammation and calcification. In the osteogenic driven pathway for AoV dysfunction, the glycoprophosphoprotein osteopontin (OPN), involved and upregulated during cell-mediated inflammation and biomineralization is the key mediator of AoV calcification and tissue remodeling leading to valve dysfunction. Additionally, it was observed during clinical trials that ‘Plasma OPN’ levels were higher in patients diagnosed with moderate-to-severe aortic valve calcification [44].

Another study was conducted [12] linking inflammation and ECM remodeling to aortic valve diseases especially CAS by means of extensive histological and biochemical quantitative analysis. It was noted that the disorganization of collagen bundles, fragmentation
and stratification of elastic fibres were significantly increased in AS as compared to the control. Additionally, inflammatory cell infiltration, mainly composed of plasmocytes, and the process of angiogenesis were also observed in AS [12].

A ‘response to injury’ mechanism activated and regulated by the VICs (Figure 2.13 [20]) for the structural remodeling of the AoV has been put forth by Li et al. [20]. The valve dysfunction and structural remodeling is attributed to the overactivation and expression of the VICs involved in a natural injury response mode that activates inflammation, neovascularization, oxidative stress, ECM remodeling, calcification, osteogenesis and so on. The VIC proliferation has also been linked to disruption of communication pathways with the endothelium (VECs) or an injury to the endothelium [20].

The inflammatory cell adhesion pathway is believed to be upregulated, primarily in the fibrosa along with pro-inflammatory cytokines (TNF2, HLA-DR, BMP-2/4/7 etc.) and the anti-inflammatory mediator (TGF-β). While TGF-β attempts to repair the fibrosis, it
promotes VIC differentiation and secretion of MMPs and TIMPs, promoting scar tissue formation.

Additionally, in a dysfunctional/diseased mode, the upregulation of angiogenic factor VEGF and MMPs promote neovascularization and tissue remodeling primarily due to the downregulation of chondromodulin-I [7] [20].

The ECM is believed to regulate various active cellular processes by means of modulating the activation of cytokines, growth factors, hemodynamic forces on the AoV. As discussed earlier, any physical/mechanical shear stress/injury to the endothelium triggers VIC proliferation in the AoV; however, this event induces damage to the protective ECM and thereby triggers an additional stress response from the VICs that initiate structural remodeling and stiffness by rearrangement of its cytoskeleton. As a consequence, the biomechanical properties of the AoV leaflets are altered which influence the hemodynamics which in turn induces fibrosis by expression of paracrine factors and additional VIC proliferation. The TGF-β activity is upregulated promoting myofibroblast differentiation, as
well as the contractile activity of VICs. MMPs and TIMPs are in turn upregulated in an effort to modulate the signals and biomechanical activity, ultimately resulting in a stiffened and disintegrated ECM, which initiates a cascade of molecular signaling events that contribute to fibrosis, and thickening of the valve leaflets in a self-propagating feedback loop [7] [12] [16] [20].

### 2.3.2 Aortic Regurgitation (AR)

AR is a diastolic reflux of blood from the aorta into the left ventricle owing to failure of coaptation of the valve leaflets during diastole. The presentation varies depending on the acuity of onset, severity of regurgitation, compliance of the ventricle and aorta, and hemodynamic conditions prevalent at the time. The pathophysiology of the AR varies according to the onset and duration of the disease process and may be classified as chronic or acute.

### 2.4 Biomechanics of the AoV Tissue

The mechanisms governing the proper structure and function of the heart valves (AoV) are essentially controlled by the interactions between the valve, its cells, and the surrounding hemodynamic environment. The AoV interacts closely with the dynamic fluidic environment facilitating unidirectional blood flow while maximizing the flow rate and minimizing the resistance. Understanding the effect of the surrounding mechanical environment on the AoV biology or pathology is essential to understand normal valve function and disease progression.

The AoV opens during systole when the ventricle contracts and closes during diastole as the ventricle relaxes, as seen in Figure 2.14 [19] and 2.15 [2], during the cardiac cycle [19].

Under normal physiological circumstances, the specific gravity of the leaflets is equal to that of blood and there is no resistance to the flow. The blood rapidly accelerates through the AoV during the systolic ejection phase, when the AoV leaflets are fully open and reaches a peak velocity. This peak velocity is reached rapidly during the first part of the systole ejection period and thereafter the flow begins to decelerate rapidly (Figure. 2.15).

The valve closure mechanism involves the development of vortices within the aortic sinuses during the diastolic ventricular ejection phase (Figure 2.15). As the pressure across the LVOT and the aorta equalizes, the deceleration of the ejected blood leads to reversal of the direction of the flow. This is due to formation of vortices (eddy currents) in the space between the edge of the AoV leaflets and the aortic wall. The adverse pressure gradient has a higher impact on the fluid along the aortic wall. As the flow gradually decelerates, the adverse axial pressure difference across the previously open leaflets decreases and leads to low inertia flow in the developing boundary layer along the aortic wall. At the end of ejection and prior to valve closure, the transverse pressure created by the vortices, push the leaflets towards the center. This flexural point moves along the valve leaflet and terminates at the free edge. This helps in acceleration of the closing of the aortic valves (Figure 2.15) [2] [14] [25] [26] [35].

The axial force along with the vortices that push the leaflet tips prove to be a very efficient and fast system. While, some studies have proven shown that the axial pressure gradient is sufficient to close the valve, without the presence of the vortices in the sinuses, the closure is not very efficient [2].

During the course of the cardiac cycle, the opening and closing mechanisms (Figure 2.15), causes the AoV leaflets to undergo physiological loading and unloading, reversal of curvature and flexion in the region of leaflet attachments. The leaflet tissues are remarkably durable and able to withstand complex stresses and strains that are potentially damaging and undergo billions of cycles [37]. The valve tissue is in reality inhomogeneous, anisotropic, nonlinear, and viscoelastic with a complex geometry and pronounced mechanical coupling between the circumferential and radial directions [34] [43] however for simplicity and understanding, the valve tissue is usually assumed to be homogeneous. The variation in the AoV fiber structure and composition across the leaflets determine the valve anisotropy and inhomogenous material properties. Although the tissue is asymmetric in terms of distensibility, the basal region tends to be relatively more isotropic than the central region. The collagen fibers are aligned along the circumferential direction and the elastin fibers, which help maintain the specific collagen fiber configuration, are oriented in the radial direction. In terms of composition, both the ventricularis and fibrosa are stiffer in the circumferential direction than the radial direction. However, the ventricularis is more
extensible radially than circumferentially, while the fibrosa had uniform extensibility in both directions [41].

The AoV tissue biomechanics, imposed cyclically, can be broadly demarcated into flexure as the valve opens, shear, as the blood flows, flexure as the valve closes, and tension, which prevents the reverse flow of the blood, coaptation and full physiological loading. The complex mechanics can be attributed to the changes in the tissue structure with strain which involves straightening of highly crimped collagen fibers and rotation of these fibers toward the stretch axis [34].

The variation in pressure or the estimated stress exerted on the AoV leaflets determines the strain response or elongation during the various phases of the cardiac cycle. The TVP or normal stress during diastole, acting in a perpendicular direction, is usually about 80–120 mmHg under normal physiological conditions as seen in Figure 2.15. This force is supported by the fibrosa and thereafter transmitted from the collagen fibers to the cells that are aligned with the collagen fibers [2]. Shear stress is experienced by the ventricular surface, as the blood flows during systole and on the aortic surface when blood accumulates into the sinuses during diastole. Shear stress is lead to elongation and realignment of endothelial cells (VEC), perpendicular to the direction of the flow. Actin stress fibers in the cytoskeleton are believed to increase in number with increasing shear stress as well as realign with the flow. The shear stress also influences cell adhesion of macrophages and other factors in the bloodstream [3].

The change in leaflet curvature, due to loading and unloading during the cardiac cycle, gives rise to bending stresses in the circumferential direction, which tend to be both tensile and compressive in nature. During diastole, when the valve is closed, the leaflets are convex towards the ventricular side and this curvature changes to concave when the valve opens during systole [41]. The bending is localized on the cusp near the wall of the aorta with the zone of attachment acting as a hinge, thereby facilitating movement; the valve tissue on the convex side undergoes tensile stress while the concave side experiences compressive stress. The collagen fibers are free to bend in the circumferential direction without significant resistance from the elastin fibers aligned in the radial direction. The bending stresses are believed to increase with AoV leaflet stiffness [2].
CHAPTER 3

METHODS

3.1 OVERVIEW

The objective of the research is to study the biomechanics of commissural fusion and leaflet stiffening in the AoV. The approach was to use the SDSU cardiac simulator, a mock circulatory loop, to measure hemodynamics and aortic valve biomechanics before and following fusion and stiffening. Valve biomechanics were analyzed including GOA (geometric opening area), leaflet strain and hemodynamics.

The cardiac simulator is an *in-vitro* mock loop of the human cardiovascular system (Figure 3.1) equipped with the left atrium (LA), left ventricle (LV), aorta and the aortic (AoV) and mitral valves (MV). The apparatus consists of acrylic chambers and tygon tubing used to simulate the left side of the heart, consisting of ventricular, atrial chambers, and blood vessels. A continuous-flow LVAD (Micromed Debakey, TX), a stepper motor (Industrial Devices Inc, NC) controlled by BioBench and LabView (National Instruments, TX), with flow and pressure sensors are a part of the mock loop system. A CCD camera is mounted underneath the LV chamber (LaVision, MN) for capturing images. A 0.9% saline solution is used as the circulating fluid.

![Figure 3.1. Schematic of cardiac simulator system with AoV.](image)
The pressure and resistance generated due to the right atrium (RA), right ventricle (RV), and peripheral resistance of systemic circulation are controlled by a compliance chamber and adjustable resistance components. Bioprosthetic valves from Medtronic (Medtronic, Minneapolis, MN) have been used as the MV, which separates the LA and the LV chambers and the AoV to separate the aorta from the LV. A MicroMed DeBakey (MicroMed, Petaluma, CA) continuous-flow LVAD, placed outside the chamber, can be used for cardiac support (Figure 3.1). Three levels of LVAD speeds that have been used in previous experiments are 7.6, 10.0 and 12.2 krpm. The simulated heart beat is generated by a piston pump driven by a stepper motor at the bottom of the LV chamber is controlled by Labview. The resistance and compliance of the cardiac simulator circuit are preset at the beginning, such that the target range of pressure and flows are acquired and continued for the entire duration of the experiment [30].

The stepper motor, programmed to create a specific displacement profile, produces the volume change thus creating the normal pressure-volume loop that controls the Stroke Volume (SV) displaced from the LV to the Aorta, while generating a heartbeat (72 bbpm) and the Cardiac Output (CO). The flow and pressure are monitored by flow meters and pressure sensors. Two flow meters (Transonic Systems, Inc.) measure the aortic flow (AoQ) and LVAD flow (LVADQ) while the three pressure sensors (Abbott Transpak IV) measure the pressure level at the LA (LAP), LV (LVP) and aortic root (AoP). Two levels of cardiac contraction, low and medium, were used to simulate heart failure for severe and moderate conditions, respectively. The values of the stroke volume (SV) and average cardiac output (CO) for low and med ranges of cardiac contractions were 35 ml, 2.5 L/min and 49 ml, 3.5 L/min, respectively. These settings represent ventricles with compromised contractile function, characteristic of patients with cardiac failure [23] [30].

The hemodynamic profiles for all the valve studies (M1-M7) have been obtained as described above; however, for this study LVADQ = 0, representing pre-VAD conditions, as the LVAD support was turned off and the conduit was clamped for the specific datasets in consideration (M6, M7) (Table 3.1). The valve images have been recorded by a CCD camera mounted underneath the LV chamber, such that the ventricular sides of the leaflets were visible [23].
Table 3.1. Experimental Variables for Valve Studies

<table>
<thead>
<tr>
<th>Valve Study</th>
<th>M6, M7</th>
<th>M6, M7</th>
<th>M6, M7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion</td>
<td>Unfused</td>
<td>F1(Single)</td>
<td>F2 (Double)</td>
</tr>
<tr>
<td>Stiffening</td>
<td>-</td>
<td>F1S</td>
<td>F2S</td>
</tr>
<tr>
<td>Cardiac Contractility</td>
<td>Low, Med</td>
<td>Low, Med</td>
<td>Low, Med</td>
</tr>
</tbody>
</table>

Approximately, 50-60 markers with a diameter of 0.25 mm were attached to the ventricular surface of each valve leaflet by means of a small amount of cyanoacrylate adhesive so as to obtain an even distribution as seen in Figure 3.2; for imaging purposes, the markers were concentrated in the belly region as compared to the edges of the leaflet. The strain in the valve leaflets during various conditions was calculated from the displacement of the markers wrt the reference image in a particular of interest (ROI); this process is further explained in detail in the subsequent imaging section. Commissural fusion was identified as “binding of tissue between the aortic leaflets at a commissure in-vitro and the length of the fusion was measured previously [23]. Commissural fusion was simulated by suturing from the annulus towards the center, along the edges for approximately 10 mm, similar to the clinically observed fusion length. The current study used three conditions of fusion – Control/ Unfused (U) (Figure 3.1), one (F1) and two (F2) fused commissures (Figure 3.1) under various flow conditions.

In order to simulate stiffening (S) in the leaflet, a thin layer of cyanoacrylate glue was applied on the aortic side of the valve and the corresponding valves were denoted as F1(S), F2(S) as seen in Figure 3.1 and 3.2 [30].

A total of seven valve experiments/studies have been performed (M1-M7) in the laboratory, to investigate commissural fusion, out of which only M6 and M7 (Table 3.1) incorporated the study of valve stiffness.

### 3.2 Imaging and Strain Analysis

The images are obtained with a CCD camera situated below the AoV. The AoV biomechanics and strain are measured by digital image correlation (DIC) imaging technique during various flow conditions. A sequence of 90 images are captured at a frequency of 9.8 Hz over 10 seconds with a CCD camera controlled by DaVIS (LaVision, MI) [30].

The 2-D (in plane) strain measurement consists of strain measured in two directions (x,y) concurrently and requires a minimum displacement of atleast three points or markers. The 2-D strain tensor (E) given by

\[
E = \begin{bmatrix} \varepsilon_x & \varepsilon_{xy} \\ \varepsilon_{xy} & \varepsilon_y \end{bmatrix}
\]

The strain tensor (E) is defined in terms of displacement gradients (\(\delta u/\delta X\)) in

\[
\varepsilon_{ij} = \frac{1}{2} \left( \frac{\delta u_i}{\delta X_j} + \frac{\delta u_j}{\delta X_i} + \frac{\delta u_i}{\delta X_j} \frac{\delta u_j}{\delta X_i} \right)
\]

where \(u_i = x_i - X_i\) are the material coordinates, \(x_i\) are the space coordinates (position vectors), \(u_i\) are the displacement vectors, and \(\varepsilon_{ij}\) are the strain components. The indices i, j represent mutually perpendicular directions [30].

The DIC is a non-contact optical measurement method wherein consecutive images during the deformation period are used to evaluate the change in (surface) characteristics and understand the specimen behavior. Initially, a correlation field is computed using the surface markers by means Fast Fourier Transformation algorithm; this serves as the reference state image (Figure 3.3 [9]). Thereafter the cross-correlation between the reference state image and deformed images (a series of 90 (dot) patterned images) are computed. The deformed images show a different pattern of surface markers relative to the initial undeformed reference state as seen in Figure 3.3. As high correlation corresponds to high similarity, the maximum/peak correlation indicates the position of the same group of markers. The resultant displacement is

represented in the form of strain vectors and is computed across the entire valve surface. The local strain is measured by means of a strain gauge window on the regions of interest (ROI). In this study, the ROIs on the leaflets are defined, and the average (non-rotational) strain in the x and y directions, corresponding to the alignment of the leaflet fibers, is calculated [9] [23] [30].

The stretch of the anisotropic valve leaflet (Figure 3.4) is estimated in terms of its mechanical strain response.

*Figure 3.4. Aortic valve leaflet showing circumferential, radial directions.*

The leaflet stretch along both the circumferential and radial directions, (Exx, Eyy) or (Ecirc, Erad), is determined (Figure 3.4) for all the experimental factors, i.e. the fusion states (Unfused, F1, F2) and stiffened states (Unfused, F1S, F2S) for valve study sets M6 and M7. The Unfused state is used as control for comparison of leaflet elongation in all the other states.

The rectangular ROIs are selected such that, an average of at least 12 markers are visible in the selected region (Figure 3.5); additionally, it is ensured that the edges of the ROIs do not coincide with those of the commissure or the leaflet itself.
3.2.1 Reference State Image Selection

The reference state image is the initial undeformed image. The selection of the reference state image, is based on the value of the TVP. The AoV is observed to open and close at about 0 mmHg and 2-3 mm Hg, TVP, respectively. The reference state is the moment between valve opening and closure at which there is the maximum possible contact between leaflets, i.e. the valve is completely closed. At this point the leaflets are relaxed because they are not subjected to the flexure of fluid flow or the stress of holding pressure due to the valve opening or closing. The reference image is appended to the beginning of the data set for batch processing in DaVIS.

3.2.2 Image Analysis for Valve GOA

The images are calibrated by a grid of 2 mm spaced markers for used unit conversion (pixel to mm²) as seen in Figure 3.6.

The AoV GOA is digitized by an open source software Image J (NIH, MD), a Java-based image processing program. The images recorded by the CCD camera are converted to
.BMP files and used for analysis of area biomechanics. The edges of the valve orifice are traced as it opens and the embedded area is computed (Figure 3.7). Depending on the original set of images, the brightness or contrast can be adjusted for the manual computation of the valve opening area. Additionally, the software is capable of detecting the GOA by means of edge detection and thresholding, as in Figure 3.8, in certain cases where the image can be converted to 8 bit binary and the information from the .bmp image is preserved. Macros can be written to partially automate the analysis. Macros used in the present study are described in the Appendix A (Tables A.1, A.2, A.3).

![Figure 3.7. Valve GOA measurement and analysis.](image)

![Figure 3.8. GOA detection by image J using thresholding.](image)

### 3.3 Image Analysis

Analysis for the opening area measurement has been carried out for each set of 90 images from each flow condition and the values of maximum and average GOA and duration of opening was obtained and the integrated area was computed. Using Simpson’s Trapezoidal rule, the ROI is divided into a number of individual trapezoids whose area are determined and integrated to obtain the total area under the curve.

Statistical Analysis for GOA was carried out using Minitab (Minitab Inc., PA), using one way ANOVA followed by post-hoc (Tukey’s) test for significance. The data from earlier valve studies M2, M3, M5 were used to determine trends of behavior and understand the relevance of valvular dysfunction to the GOA parameters.
The hemodynamic data along with the time was analyzed by Labchart (AD Instruments, CO), wherein specific sequences/cycles (~ 10 cycles) were identified, averaged and plotted [23] [30].

As described earlier, the mechanical strain measured using the DIC technique, followed by vector postprocessing produced a 2D displacement map of the markers in the ROI form which the strain components in the circumferential and radial direction were calculated. The time series data was analyzed using MS Excel, averaged (~ 10 cycles) and plotted.
CHAPTER 4

RESULTS

4.1 STRAIN ANALYSIS

The mechanical strain response developed in the AoV leaflet (NC) tissue is measured using the help of StrainMaster, DaVis, using the principle of DIC. Rectangular ROIs, as seen in Figure 4.1, are used to define the area of strain measurement on a specific leaflet aligned with circumferential and radial directions.

![Figure 4.1. ROI (W) for whole strain gradients.](image1)

The average leaflet strain is calculated from ROIs by using larger Rectangles (W) (Figure 4.1), spanning the entire area of the leaflet surface area, which were subdivided for the circumferential and longitudinal directions (C2, C3, C4, R1, R2) (Figure 4.2) to assess regional variations.

![Figure 4.2. Circumferential and radial strain gradients.](image2)
The strain responses from the Unfused (U) states (Figure 4.3) are used as controls and baselines for comparison to all the other factors. The level of cardiac support (Low or Med) is another experimental factor that is analyzed in the current datasets. Figure 4.4 show the variation of strain as a function of time for the Unfused (control) from M6 and M7 using medium cardiac contractility.

![Figure 4.3. Strain cycle for unfused (U) CSMed (M6).](image)

![Figure 4.4. Strain cycle for unfused (U) CSMed (M7).](image)

The leaflet is anistotropic and the strain is observed to be higher in the radial direction as compared to the circumferential. The magnitude of strain increases with higher cardiac support (Figure 4.5). The variation in the magnitude of circumferential strain with time is used to compare the leaflet elongation during remodeling i.e. for fusion states (F1, F2) with medium cardiac contraction (low, med) in M6 and M7 as seen in Figures 4.6 and 4.7.
The average and maximum values of strain are considered for comparison across the fusion states. Figures 4.8 and 4.9 show the average and maximum values, respectively, of the circumferential and radial strain across the fused and unfused states for M7. The reference state for the individual strain categories (U, F1, F1S, F2S) is selected such that the
Figure 4.8. Maximum circumferential and radial strain (M7).

Figure 4.9. Average circumferential strain (M6, M7).

valve is completely closed corresponding to a TVP of 2-3 mm Hg and appended in DaVIS before the other images.

From the mean values, we can see that the strain increases initially with fusion of one commissure (F1) in the circumferential direction, and thereafter decreases with the fusion of two commissures (F2) and/or stiffening (F1S/F2S). In the radial direction, the magnitude of strain is higher and it decreases from the control (unfused) to F1, F2, F2S and so on.

The maximum strain values for M6, M7 are presented in Appendix B (Tables B.1-B.2). Figures 4.9 and 4.10 show the mean values of strain for studies M6 and M7 in both the circumferential and radial direction.

A correlation between for the valve hemodynamics and biomechanics is tabulated below. A comparison between the trans-valvular pressure (TVP), the difference between the aortic and the left ventricular pressure, and the leaflet elongation along the circumferential and radial directions is shown in Table 4.1.
Table 4.1. Mean Pressure-Strain Response for Medium Cardiac Support (M7)

<table>
<thead>
<tr>
<th>Fusion/CS Contraction</th>
<th>TVP</th>
<th>Circumferential Strain</th>
<th>Radial Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>U, CSLow</td>
<td>10.54</td>
<td>0.83 ± 0.64</td>
<td>3.85 ± 2.93</td>
</tr>
<tr>
<td>U, CSMed</td>
<td>20.18</td>
<td>1.06 ± 0.85</td>
<td>4.76 ± 3.49</td>
</tr>
<tr>
<td>F1, CSLow</td>
<td>10.62</td>
<td>1.04 ± 0.91</td>
<td>3.44 ± 2.87</td>
</tr>
<tr>
<td>F1, CSMed</td>
<td>20.24</td>
<td>1.49 ± 1.09</td>
<td>4.25 ± 3.24</td>
</tr>
<tr>
<td>F1S, CSLow</td>
<td>6.90</td>
<td>0.1 ± 0.21</td>
<td>0.48 ± 0.81</td>
</tr>
<tr>
<td>F2S, CSMed</td>
<td>10.07</td>
<td>0.36 ± 0.38</td>
<td>1.4 ± 1.31</td>
</tr>
<tr>
<td>F2S, CSLow</td>
<td>3.99</td>
<td>0.28 ± 0.45</td>
<td>0.83 ± 1.05</td>
</tr>
</tbody>
</table>

The magnitude of the radial strain is higher than the circumferential as seen in Figure 4.11. The effect of fusion on the strain seems to be minimal as it transitions from U to F1, however the effect of stiffening on leaflet distensibility is prominent (F1 to F1S). Coupling of two factors (fusion and stiffening as in F1S, F2S), represents the in-vivo CAS onset shows a significant drop in the strain and the TVP.

The strain distribution in the AoV leaflet tissue is determined by the circumferential and radial strain gradients. Strain gradients are estimated by dividing the larger ROI into smaller rectangular ROIs horizontally (C1, C2, C3, C4) and vertically (R1, R2) (Figure 4.12) from the center to the edge and calculating the strain in each region. C1 lies in a section close to the center (free edge) and strain being a relative spatial measurement of the movement of
the markers, C1 is not always visible during when the valve opens. Therefore it is not included in the current section.

The circumferential and radial strains for each of these sections are measured and averaged in Tables 4.2 and 4.3 for M6 and M7, respectively.

Table 4.2. Average Circumferential & Radial Strains in the ROIs (M6)

<table>
<thead>
<tr>
<th>Category / Direction</th>
<th>Circumferential ROI</th>
<th>Radial ROI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2</td>
<td>C3</td>
</tr>
<tr>
<td>Unfused Exx</td>
<td>0.89</td>
<td>1.21</td>
</tr>
<tr>
<td>Unfused Eyy</td>
<td>1.22</td>
<td>3.49</td>
</tr>
<tr>
<td>F1 Exx</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>F1 Eyy</td>
<td>2.40</td>
<td>2.93</td>
</tr>
<tr>
<td>F2 Exx</td>
<td>0.34</td>
<td>0.41</td>
</tr>
<tr>
<td>F2 Eyy</td>
<td>4.97</td>
<td>5.53</td>
</tr>
<tr>
<td>F2S Exx</td>
<td>0.25</td>
<td>0.37</td>
</tr>
<tr>
<td>F2S Eyy</td>
<td>5.26</td>
<td>2.90</td>
</tr>
</tbody>
</table>
Table 4.3. Mean Circumferential and Radial Strain (M7)

<table>
<thead>
<tr>
<th>Fusion/Direction</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfused Exx</td>
<td>2.97</td>
<td>0.87</td>
<td>0.35</td>
<td>0.33</td>
<td>1.63</td>
</tr>
<tr>
<td>Unfused Eyy</td>
<td>10.27</td>
<td>4.53</td>
<td>1.08</td>
<td>4.06</td>
<td>4.89</td>
</tr>
<tr>
<td>F1 Exx</td>
<td>1.88</td>
<td>0.57</td>
<td>0.49</td>
<td>0.53</td>
<td>1.98</td>
</tr>
<tr>
<td>F1 Eyy</td>
<td>7.98</td>
<td>3.00</td>
<td>0.48</td>
<td>3.95</td>
<td>4.92</td>
</tr>
<tr>
<td>F1S Exx</td>
<td>0.74</td>
<td>0.49</td>
<td>0.38</td>
<td>0.14</td>
<td>1.26</td>
</tr>
<tr>
<td>F1S Eyy</td>
<td>4.93</td>
<td>2.52</td>
<td>0.70</td>
<td>2.78</td>
<td>3.50</td>
</tr>
<tr>
<td>F2S Exx</td>
<td>0.73</td>
<td>0.29</td>
<td>0.27</td>
<td>0.28</td>
<td>0.72</td>
</tr>
<tr>
<td>F2S Eyy</td>
<td>3.30</td>
<td>1.18</td>
<td>0.23</td>
<td>1.82</td>
<td>2.75</td>
</tr>
</tbody>
</table>

The CS speed is directly proportional to the magnitude of the strain, irrespective of the direction and the presented strain gradient data, is only for CS Med. Centrally located C2 & C3 (Figures 4.13 and 4.14) display increased strain as compared to C4, both circumferentially and radially, whereas for R1 and R2, the strain distribution is homogenous.

![Figure 4.13. Strain gradients along the radial direction (Exx) (M6).](image)

The radial strain gradients are observed to increase with fusion and decrease with stiffening for Exx (Figure 4.13).

The radial strain gradients are observed to increase with fusion and decrease with stiffening for Eyy (Figure 4.14).

The circumferential strain gradients for Exx don’t show any change for fusion or stiffening in M6 (Figure 4.15).
Figure 4.14. Strain gradients along the radial direction (Eyy).

The gradients increase with double commissural fusion (F1 to F2), however there is no change with stiffening (Figure 4.16).

The gradients are observed to flatten out with both fusion and stiffening as seen in Figure 4.17.

The strain gradients progressively decrease with fusion from unfused to single commissure as well as with stiffening from F1 to F1S and to F2S (Figure 4.18).

The strain gradients along the circumferential direction increase with fusion from the unfused to F1 and decreases with stiffening from F1 to F1S and to the F2S state as seen in Figure 4.19.

Figure 4.20 shows that the circumferential strain gradients are observed to increase with fusion and decrease with stiffening.
Figure 4.16. Radial strain gradients for Eyy (M6).

Figure 4.17. Radial strain gradients for Exx (M7).

Figure 4.18. Radial strain gradients for Eyy (M7).
4.2 OPENING AREA ANALYSIS

The valve opening area is dependent on fusion and cardiac contractility as seen in Table 4.4 and 4.5 for M6 and M7, respectively. Figures 4.21-4.24 show the GOA plotted against time for the fusion and CS speed (Low, Med) for M6 and M7.

The effect of stiffening (F1S, F2S) and decrease in GOA as compared to unfused is seen in Figures 4.23 and 4.24 for M7.

The average and maximum and integrated values of GOA along with the mean duration data for M7 is shown in Table 4.5.
Table 4.4. Average and Maximum GOA, Duration and Integrated GOA (M6)

<table>
<thead>
<tr>
<th>Category</th>
<th>Average GOA</th>
<th>Maximum GOA</th>
<th>Average Duration</th>
<th>Integrated GOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Med</td>
<td>Low</td>
<td>Med</td>
</tr>
<tr>
<td>Fusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>89.15</td>
<td>100.56</td>
<td>132.86</td>
<td>130.69</td>
</tr>
<tr>
<td>F1</td>
<td>64.00</td>
<td>79.30</td>
<td>90.16</td>
<td>95.64</td>
</tr>
<tr>
<td>F2</td>
<td>31.66</td>
<td>48.57</td>
<td>45.83</td>
<td>71.98</td>
</tr>
<tr>
<td>F2S</td>
<td>31.32</td>
<td>52.38</td>
<td>45.39</td>
<td>78.19</td>
</tr>
</tbody>
</table>

Table 4.5. Average and Maximum GOA, Average Duration of Opening and Integrated Area (M7)

<table>
<thead>
<tr>
<th>Category</th>
<th>Average GOA</th>
<th>Maximum GOA</th>
<th>Average Duration</th>
<th>Integrated GOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Med</td>
<td>Low</td>
<td>Med</td>
</tr>
<tr>
<td>Fusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfused</td>
<td>100.16</td>
<td>121.52</td>
<td>118.21</td>
<td>152.69</td>
</tr>
<tr>
<td>F1</td>
<td>121.37</td>
<td>115.48</td>
<td>139.80</td>
<td>151.29</td>
</tr>
<tr>
<td>F2</td>
<td>80.47</td>
<td>84.70</td>
<td>95.62</td>
<td>100.79</td>
</tr>
<tr>
<td>F2S</td>
<td>67.59</td>
<td>77.55</td>
<td>80.95</td>
<td>88.99</td>
</tr>
</tbody>
</table>
The average and maximum GOA do show a change with increased cardiac contractility, however it is not as significant as fusion. The average and maximum area of opening show a marked decrease with fusion of one and two commissures as compared to the unfused control. In case of M7, the F1 GOA parameters (average, max, integrated) increases in magnitude as compared to the control and then decreases with tissue remodeling during stiffening and double commissural fusion.
Figure 4.23. Effect of stiffening on GOA for low cardiac function (M7).

Figure 4.24. Effect of stiffening on GOA for medium cardiac function (M7).

The effect of stiffening reduces the GOA from F1 to F1S and F2 to F2S. The integrated area increases with greater cardiac pumping and decreases with fusion.

The effect of the reduction in GOA in M6 is shown in Figures 4.25 and 4.26. The effect of commissural fusion and stiffening on GOA size and shape is evident.

The reduction of GOA and effect of fusion and stiffness for M7 are seen in Figures 4.27 and 4.28.

The average flow across the AoV (Table 4.6) is obtained by subtracting the LVAD Flow from the Aortic Flow in the hemodynamic data for M7. The flow rate increases with cardiac contractility and decreases with tissue remodeling in the AoV.
Figure 4.25. Reduction of GOA due to fusion (M6).

Figure 4.16. Reduction in GOA due to stiffening (M6).

Figure 4.27. Reduction in GOA due to fusion (M7).
Table 4.6. Comparison of Average Opening Area, Integrated Area and Flow (M7)

<table>
<thead>
<tr>
<th>Category</th>
<th>Opening Area</th>
<th>Integrated Area</th>
<th>AoV Flow (AoVQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion state/CS Speed</td>
<td>Low  Med</td>
<td>Low  Med</td>
<td>Low  Med</td>
</tr>
<tr>
<td>Unfused</td>
<td>100.15 121.52</td>
<td>26.72 32.09</td>
<td>2.22 3.07</td>
</tr>
<tr>
<td>F1</td>
<td>121.37 115.48</td>
<td>36.54 42.39</td>
<td>2.14 3.30</td>
</tr>
<tr>
<td>F1S</td>
<td>80.47 84.70</td>
<td>25.50 28.59</td>
<td>2.09 2.95</td>
</tr>
<tr>
<td>F2S</td>
<td>67.586 77.55</td>
<td>23.23 24.67</td>
<td>1.79 2.80</td>
</tr>
</tbody>
</table>

A comparison of certain parameters of the AoV opening area such as the average duration of opening, integrated GOA, the average GOA (Table 4.7) carried out across the current (M6, M7) and previous (M3, M4, M5) data sets.

Table 4.7. Factors used in One-way ANOVA for GOA Analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Type</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valve</td>
<td>fixed</td>
<td>5</td>
<td>M2, M3, M5, M6, M7</td>
</tr>
<tr>
<td>CS Speed</td>
<td>fixed</td>
<td>2</td>
<td>Low, Med</td>
</tr>
<tr>
<td>Fusion</td>
<td>fixed</td>
<td>3</td>
<td>U, F1, F2</td>
</tr>
</tbody>
</table>

One way ANOVA and post hoc (Tukey’s test) analysis has shown mean and Integrated GOA to be significant across the valve studies. The results are shown in Figures 4.29 and 4.30.
The letters (A,B) are used to indicate statistical significance in the average GOA and the effect of commissural fusion between as well as among the valve study groups M2, M3, M5, M6, M7. A letter (A or B) shared in common between or amongst the groups would indicate no significant difference. Different letters show significant difference between categories. In case of M2, M3, M5, M6, M7, the effect of fusion is significant between Unfused and F1, but not between F1 and F2.

The effect of fusion on Integrated GOA and its statistical significance is shown below. The values of integrated GOA is significant between the valve study groups M2, M3 and M5, M6, M7. There is no statistical significance attached to fusion.
4.3 HEMODYNAMIC ANALYSIS

The hemodynamic data is obtained by means of LabChart (Tables 4.8 and 4.9) in terms of Left Ventricular Pressure (LVP), Aortic Pressure (AoP), Aortic Flow (AoQ) and LVAD Flow (LVADQ). The TVP (transvalvular pressure) and AoVQ (Flow across the AoV) are calculated from the data obtained. The value of flow ratio determined by the ratio of LVAD and Aortic Flow, is used to predict the type of flow in terms of Normal (present condition), Series (LVAD only) or Parallel (both the AoV and the LVAD). In the above profile, the blood flows through the AoV into the aorta, and this being a preVAD condition, the LVADQ returns a flow ratio of 0. Additional hemodynamic data for M6, M7 with medium cardiac support are presented in Appendix B (Tables B.3, B.4).

Table 4.8. Hemodynamic Profile for M7

<table>
<thead>
<tr>
<th>Fusion</th>
<th>CS Speed</th>
<th>LVP (mmHg)</th>
<th>AoP (mmHg)</th>
<th>TVP (AoP - LVP) (mm Hg)</th>
<th>Post-LVAD Pressure (mmHg)</th>
<th>Aortic Flow (AoQ) L/min</th>
<th>LVAD Flow (LVADQ) L/min</th>
<th>AoV Flow (AoQ - LVADQ) L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfused Low</td>
<td>21.44</td>
<td>31.98</td>
<td>10.54</td>
<td>36.98</td>
<td>2.23</td>
<td>0</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>Unfused Med</td>
<td>27.48</td>
<td>47.66</td>
<td>20.17</td>
<td>52.38</td>
<td>3.07</td>
<td>0</td>
<td>3.07</td>
<td></td>
</tr>
<tr>
<td>F1 Low</td>
<td>21.31</td>
<td>31.93</td>
<td>10.611</td>
<td>34.97</td>
<td>2.14</td>
<td>0</td>
<td>2.14</td>
<td></td>
</tr>
<tr>
<td>F1 Med</td>
<td>28.38</td>
<td>48.62</td>
<td>20.24</td>
<td>51.69</td>
<td>3.30</td>
<td>0</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td>F1S Low</td>
<td>22.14</td>
<td>29.03</td>
<td>6.89</td>
<td>31.52</td>
<td>2.09</td>
<td>0</td>
<td>2.09</td>
<td></td>
</tr>
<tr>
<td>F1S Med</td>
<td>30.22</td>
<td>42.48</td>
<td>12.25</td>
<td>45.27</td>
<td>2.95</td>
<td>0</td>
<td>2.95</td>
<td></td>
</tr>
<tr>
<td>F2S Low</td>
<td>22.27</td>
<td>26.26</td>
<td>3.99</td>
<td>30.91</td>
<td>1.79</td>
<td>0</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>F2S Med</td>
<td>30.65</td>
<td>40.72</td>
<td>10.07</td>
<td>45.50</td>
<td>2.79</td>
<td>0</td>
<td>2.79</td>
<td></td>
</tr>
</tbody>
</table>

The effects of valvular tissue remodeling (fusion and stiffening) on TVP for the M7 dataset are seen in Figures 4.31 and 4.32.

Both fusion and stiffening contribute towards reduction in the TVP magnitude as seen on the time series data.

Cardiac contractility affects the pressure values across the hemodynamic profile as evident in the values of LVP and AoP and consequently the TVP (Figures 4.33 and 4.34).

The amount of cardiac support as well as fusion influences the TVP values. The TVP value increases with cardiac contractility and decreases with fusion and stiffening.

The hemodynamic profile for M6 is shown in Table 4.9.
Table 4.9. Hemodynamic Profile (M6)

<table>
<thead>
<tr>
<th>Fusion</th>
<th>CS Speed</th>
<th>LVP (mmHg)</th>
<th>AoP (mmHg)</th>
<th>TVP (AoP - LVP) (mm Hg)</th>
<th>Post-LVAD Pressure (mm Hg)</th>
<th>Aortic Flow (L/min)</th>
<th>LVAD Flow (L/min)</th>
<th>AoV Flow (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfused</td>
<td>Low</td>
<td>22.54</td>
<td>8.55</td>
<td>22.17</td>
<td>53.6</td>
<td>2.84</td>
<td>0</td>
<td>2.84</td>
</tr>
<tr>
<td>Unfused</td>
<td>Med</td>
<td>30.10</td>
<td>8.35</td>
<td>14.48</td>
<td>53.43</td>
<td>3.86</td>
<td>0</td>
<td>3.86</td>
</tr>
<tr>
<td>F1</td>
<td>Low</td>
<td>23.94</td>
<td>23.30</td>
<td>14.23</td>
<td>46.26</td>
<td>2.61</td>
<td>0</td>
<td>2.61</td>
</tr>
<tr>
<td>F1</td>
<td>Med</td>
<td>31.84</td>
<td>33.05</td>
<td>6.00</td>
<td>45.9</td>
<td>3.60</td>
<td>0</td>
<td>3.60</td>
</tr>
<tr>
<td>F2</td>
<td>Low</td>
<td>25.98</td>
<td>19.71</td>
<td>N/A</td>
<td>31.0</td>
<td>1.99</td>
<td>0</td>
<td>1.99</td>
</tr>
<tr>
<td>F2</td>
<td>Med</td>
<td>36.22</td>
<td>28.12</td>
<td>N/A</td>
<td>41.17</td>
<td>3.22</td>
<td>0</td>
<td>3.22</td>
</tr>
<tr>
<td>F2S</td>
<td>Low</td>
<td>26.24</td>
<td>11.63</td>
<td>N/A</td>
<td>27.55</td>
<td>2.10</td>
<td>0</td>
<td>2.10</td>
</tr>
<tr>
<td>F2S</td>
<td>Med</td>
<td>34.52</td>
<td>11.9</td>
<td>N/A</td>
<td>33.17</td>
<td>3.14</td>
<td>0</td>
<td>3.14</td>
</tr>
</tbody>
</table>

Figure 4.31. Effect of Fusion on TVP time series (M7).

Figure 4.32. Effect of stiffening on TVP time series (M7).
The value of LVADQ is 0 as there is no LVAD support for the current dataset. Due to an error with pressure transducer, the values of TVP for F2 and F2S are not shown in the table.

The Figures 4.35, 4.36, and 4.37 show the effect of fusion on hemodynamics (LVP, AoP, AoQ) for M2, M3, M5, M6, M7.

It can be seen from the figures that the LVP increases with fusion, while the AoP, AoQ decreases with fusion.

The effect of stiffening on LVP, AoP, AoQ for medium cardiac support (M7) are seen in the Figures 4.38-4.40.

The LVP increases, while the AoP and AoQ decreases with stiffening as seen in the figures.
Figure 4.35. The variation of LVP with fusion.

Figure 4.36. The variation of AoP with fusion.

Figure 4.37. The variation of AoQ with fusion.
Figure 4.38. Effect of stiffening on LVP (M7).

Figure 4.39. Effect of stiffening on AoP (M7).

Figure 4.40. Effect of stiffening on AoQ (M7).
CHAPTER 5

DISCUSSION

5.1 DETECTION OF CAS: ROLE OF CURRENT STUDY

The current research is an in-vitro macro-scale approach towards the understanding of AoV mechanics, function and the effect of the complex hemodynamical environment pertaining to valvular tissue remodeling, dysfunction and calcific AoV disease (CAVD). CAVD includes calcific aortic stenosis (CAS) in which thickened, fibrotic and stiffened leaflets resulting from ECM remodeling impact the valve biomechanics, hemodynamics and cardiac function [7]. Despite significant work in this area, the mechanobiology of the disease pathogenesis and progression is not fully understood.

Due to its non-invasive, radiation-free, low-cost, and versatility, Doppler-echocardiography is currently the primary non-invasive imaging method for the diagnosis and evaluation of valvular diseases. CAS is clinically presented with a higher transvalvular gradient and aortic jet velocity detected by echocardiographic morphology which forms the basis of clinical decision-making of the severity of valvular stenosis [4] [13]. Doppler echocardiography is used to detect the direction and velocity of blood flow, evaluate the AoV anatomy to identify the number of leaflets, describe leaflet mobility, thickness, calcification and the level of obstruction. Accurate data recording requires multiple acoustic windows in order to determine the highest velocity. Careful patient positioning and adjustment of transducer position and angle are crucial as the velocity measurement assumes a parallel intercept angle between the ultrasound beam and direction of blood flow (Figure 5.1 [18]) [4] [18].

The presence of a lesion or CAS is determined by examining the changes in the continuous and pulsed wave spectral velocities caused by the resultant turbulent systolic flow in the ascending aorta. The hallmarks of CAS seen in the echocardiocardiographic morphology are usually marked spectral broadening, delayed systolic peaking and increased peak velocity. The shape of the Continuous Wave Doppler velocity curve is helpful in distinguishing the level and severity of obstruction. The difference in pressure between the
left ventricle and the aorta in systole or TVP is a standard measure of stenosis severity [4] [13].

The TVP is estimated indirectly, using the simplified Bernoulli equation, namely, $p_1 - p_2 = 4V^2$, where $p_1 - p_2$ corresponds to the value of the TVP, while $V$ stands for the peak velocity. TVP fluctuates with flow across the valve and cardiac output, especially during physical movement or exercise. The TVP estimation is dependent on the accuracy of the velocity data and the shape of the velocity curve which varies with stenosis severity and flow obtained from the aortic jet profile. Any human error on the part of the technician can result in an incomplete hemodynamic profile (pressure, flow) of the AoV.

The AoV area or Effective Orifice Area (EOA), calculated by the simplified continuity equation (Figure 5.2 [18]), is prone to measurement errors.

The flow velocity is equated to the peak velocity profile as the spectral recordings are simplified to triangles (Figure 5.3 [18]) and requires typical or standard velocity profiles.

The limitations of Doppler Echocardiography as a diagnostic and assessment tool for CAS, has been evaluated and is shown in Table 5.1.

Thus, it can be easily observed that despite being a non-invasive, versatile, tool, clinical decisions based on only echocardiographic morphology can be potentially erroneous.
There exists a need for an alternate system for characterization of CAS and quantitative assessment of disease parameters such as tissue remodeling which can serve as predictors of the progression and outcome of CAS. We have attempted to narrow this gap by trying to develop a bench-top study that explores some fundamental relationships linking valvular tissue remodeling to changes in the valve biomechanics, and hemodynamics in a controlled and systematic *in-vitro* environment.
Table 5.1. Limitations of Doppler Echocardiography in Detection and Assessment of CAS Severity

<table>
<thead>
<tr>
<th>Sources Of Error</th>
<th>Measurement Variability (%)</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misalignment of Doppler Beam</td>
<td>3-4</td>
<td>5% underestimation of flow velocity for 150° angle change</td>
</tr>
<tr>
<td>Measurement error for LVOT diameter</td>
<td>5-8</td>
<td>EOA estimation error</td>
</tr>
<tr>
<td>Aortic Jet Velocity</td>
<td>3-4</td>
<td>EOA estimation error</td>
</tr>
<tr>
<td>Underestimation of aortic velocity</td>
<td>NA</td>
<td>Underestimation of mean pressure gradient</td>
</tr>
<tr>
<td>Inadequate Acoustic Window</td>
<td>NA</td>
<td>EOA estimation error</td>
</tr>
<tr>
<td>Discordant relations between EOA, TVP</td>
<td>NA</td>
<td>Incorrect clinical decisions about severity of CAS, AoV Replacement</td>
</tr>
</tbody>
</table>


### 5.2 SUMMARY OF FINDINGS

In this project, we have attempted to simulate tissue remodeling in a controlled and systematic ex-vivo environment. The AoV leaflet stretch is measured on the leaflet, wherein the collagen fiber orientation is parallel to the circumferential direction. The inherent valve tissue anisotropy constrains the valve extensibility circumferentially and the observed strain response is higher in the radial direction. Comparison of the TVP and strain response has revealed that, as the TVP increases, the strain response increases along both circumferential and radial directions. The strain responses of the stenosed valves are characteristically similar to the normal, in terms of higher values in the radial direction. Stiffness of the valve is observed to be more prominent over fusion in terms of effects of tissue remodeling on the leaflet elongation. Thus the stiffened valve tissue is less distensible than single or double commissural fusion. Also, the effects of fusion seem to be variable and need to explored further (Table 5.2).
Table 5.2. Summary of Change in Leaflet Strain Response due to Fusion and Stiffening

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change from Unfused State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Circumferential Strain</td>
<td>NC</td>
</tr>
<tr>
<td>Radial Strain</td>
<td>NC</td>
</tr>
</tbody>
</table>

The distribution of strain appears to be concentrated in the center/belly region from the strain gradient result analysis. The vertical strain gradients (circumferential strain gradients) along the radial directions are bigger than the horizontal strain gradients. Also Eyy is greater in magnitude than Exx amongst each category, i.e. (U, Eyy) > (U, Exx).

The GOA for the various fusion and stiffening states with the free edges aligned together (of the NC cusp) has clearly demonstrated the leaflet deformations due to higher external bending stresses imposed by tissue remodeling. The reduction in the GOA due to fusion and stiffening is clearly evident. The mean and maximum values of the GOA as well as that of the integrated area are observed to reduce with commissural fusion. It is a graded response, where double commissural fusion contributes to a further reduction in the flow through the valve. Stiffening of the fused leaflets leads to a greater decrease in the GOA. In the stenosed valve, the decrease in flow through the valve is evident. There is no change in the opening duration, except during F2. Increased cardiac support leads to an increased response in the GOA and duration (Table 5.3).

Table 5.3. Summary of Change in AoV Opening Area due to Fusion and Stiffening

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change from Unfused State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Mean GOA</td>
<td>-</td>
</tr>
<tr>
<td>Integrated GOA</td>
<td>-</td>
</tr>
<tr>
<td>Duration</td>
<td>-</td>
</tr>
</tbody>
</table>

The hemodynamical profile for M7 is shown in Table 5.4. The TVP (given by the difference in the LVP and AoP), increases with single commissural fusion but decreases due to stiffening. This can be attributed to the decrease in the aortic pressure during F1 and S.
Table 5.4. Summary of Change in Hemodynamic Profiles Due to Fusion and Stiffening

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change from Unfused State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>AoP</td>
<td>-</td>
</tr>
<tr>
<td>LVP</td>
<td>+</td>
</tr>
<tr>
<td>TVP</td>
<td>-</td>
</tr>
<tr>
<td>AoQ</td>
<td>-</td>
</tr>
</tbody>
</table>

TVP from M6 is unavailable due to an error in the pressure transducer while conducting the experiment.

Overall, the results indicate stiffness being a dominant tissue remodeling effect in comparison with commissural fusion on the valve GOA and strain. This needs to be explored further by conducting more experiments.

5.3 AORTIC VALVE MECHANOBIOLGY AND STRUCTURAL ANALYSIS

The normal structure and function of the AoV is regulated by means of its interaction with the surrounding biomechanical environment and therefore a thorough understanding is essential to understand the pathobiology of AoV dysfunction and disease such as calcific AS (Figure 5.4). As a sophisticated check valve, the AoV allows the blood to flow unidirectionally into the aorta, while maximizing its flow rate and minimizing the resistance to flow. The tri-layered AoV leaflets are predominantly composed of fibrillar collagen in the fibrosa layer, GAGs in the spongiosa, and elastin in the ventricularis. The structural arrangement of the aligned collagen network defines the valve biomechanics [25].

The ventricularis of the AoV is exposed to unidirectional pulsatile shear stress due to the relative motion between the surface and the blood flow, while the fibrosa experiences a low bidirectional oscillatory shear stress on the aortic surface [2] [41]. While, the oscillatory shear stress doesn’t necessarily lead to any pathophysiological response in the tissue, it is believed to initiate VEC injury and consequent ECM remodeling due to initiation of a signal transduction pathway for an inflammatory response and the action of the VICs; the exact mechanism of this signaling event is yet to be identified.
ECM proteins such as collagen, elastin and GAGs are present in the normal human AoV tissue and play important roles in maintaining the ECM structure and organization and function.

Collagen types I and III are abundantly present in the fibrosa layer, aligned along the circumferential direction in the normal AoV. In case of AoV dysfunction, an alteration in the collagen content and organization, leads to fibrosis, leaflet thickening and ECM disarray mediated by the action of the MMPs [7] [12].

GAGs are abundantly present in the spongiosa in the normal valve, and there occurs an alteration in its content and presence in the pathogenesis of CAS. GAGs are associated with areas of lipid accumulation, inflammation and sites for formation of calcific nodules and regulating signal transduction through TGF-β signaling pathways in CAVD [2] [7].

Elastin is predominantly associated with the ventricularis; it provides structural support and elastic recoil. AoV disease leads to fluctuations in elastin content and organization leading to reduction in the amount, fragmentation and disarray. This increases
the ECM stiffness, and triggers signaling events leading to myofibroblaast and osteoblast differentiation [2] [7] [20].

In our study, we have attempted to mimic the structural remodeling of the AoV that occurs during the pathogenesis of CAS, in a controlled and systematic environment. Our findings have confirmed that a definite cause and effect relationship does exist between the tissue level dysfunction and organ level biomechanics, and hemodynamics.

While, a number of bench-top research has been conducted [5] [38] [43] on CAS disease models and various aspects of valvular stenosis, none of them have addressed the stiffness and fusion associated with ECM remodeling as a study of integrated functional biomechanics.

A fluid mechanical flow-mapping study had been undertaken by [42], wherein in-vitro flow visualization and Doppler anemometry measurements were carried out on various bioprosthetic stenosed valves. The stenotic valve had a characteristic angulated jet-type flow field with highly turbulent shear layers as compared to the evenly distributed flow field obtained at peak systole in the normal AoV. The flow field continued to be more chaotic as the degree of CAS increased; the elevated levels of turbulence measured downstream of the stenotic valves were believed to be potentially damaging for the RBCs and the platelets [41].

In 2000, Billiar and Sacks studied the micromechanics and structural-functional relationship of the porcine bioprosthetic AoV and developed a constitutive model [5] [6]. The biaxial mechanical properties of the excised AoV were studied for the very first time by comparing the data from native and chemically (glutaraldehyde) fixed valves. Green’s strains in both the circumferential and radial directions and index of anisotropy, defined by the peak strains were used as mechanical indices of deformation of the AoV. The parameters obtained were the pre strains (reference states), peak strains and midstrains due to specimen mounting with a certain preload, peak diastolic load, and equibiaxial membrane stress, respectively.

The fiber architecture of the AoV was studied and used to map and correlate to the strain response using an optical non-contact small angle light scattering (SALS) device.

Billiar et al, have characterized the strain distribution in the cusps of the valve tissue as being more homogenous around the central regions, which is in line with our conclusions from the strain gradient studies. An important conclusion of this study was that the higher radial extensibility due to the directional alignment of the collagen fibers, coincides with our
data and conclusions from the study of the mechanical strain response in the native and deformed AoV tissues. Higher radial extensibility of the cusps are essential for preventing retrograde flow of blood by forming a coaptive seal.

Planar biaxial testing is used to fully characterize the inherent anisotropic properties of soft tissues and obtain homogenous specimen deformation for accurate in-vivo simulation and development of constitutive models. Additionally, biaxial testing mode prevents realignment of the fibers along the test axis and consequent alterations in the mechanical properties, damage to the tissue or stress concentrations alongwith unrestricted lateral deformations. Constitutive modeling helps describe the responses of biological materials to various loading conditions mathematically for integration into finite element models. Combined with a structural constitutive model, tissue biaxial testing can help identify the specific structural mechanisms underlying the AoV’s two-dimensional mechanical behavior.

The constitutive model developed by Biliar et al, following the biaxial study, describes the complex anisotropic, non-linear in plane behavior of the AoV leaflets and aids in understanding the fatigue damage; the difference in structure between the native and chemically fixed deformed valves was attributed to the alterations in the fiber alignment. The model, based on a single tissue layer with homogenous fiber distribution, takes into account shear stresses and uses a fiber angular distribution to understand the structural-functional relationship [5].

May-Newman et al have used strain energy function \( (w) \) of two strain invariants \( (I_1, I_4) \) to accurately describe the nonlinear anisotropic behavior of the aortic valve tissue. \( I_1 \) is the first invariant of the left Cauchy-Green deformation tensor given by \( \text{tr}(C) \); \( I_4 \) is the fourth invariant for a transversely isotropic material defined by \( N\cdot C \cdot N \). \( N \) defines the direction of the collagen fiber and \( w = c_0 (\exp^Q - 1) \) [24].

The biaxial testing of the porcine AoV leaflet tissue was based on constant invariant protocols such that one of the strain invariants was held constant, while varying the other.

The testing protocol also included preconditioning of the tissue and obtaining the reference states prior to, during and post testing. The strain values obtained by the marker displacements on the ventricular surface; these along with the stress are used to calculate derivatives of the strain energy function, with respect to each invariant. The stress was predicted for the various stretch protocols (equibiaxial, off-biaxial, strip-biaxial). Nonlinear
regression of the combined data set of about 3-4 biaxial test protocols was used to determine the coefficient values for each specimen which was compared to the predicted stress values. Based on the stress-strain behavior for the various specimens, the aortic valve tissue was described as a hyperelastic transversely isotropic material with a three coefficient (C₀, C₁, C₂) strain-energy function [24].

Mirnajafi et al. have simplified the study of the AoV commissural region by configuring them as cantilever beams in order to understand the tissue stiffness and the flexural deformations occurring in the region [28]. The effective elastic modulus helped elucidate some of the difference in stiffness between the belly region of the leaflets and the commissures. Additionally, it was understood the natural fiber structure and configuration enabled unidirectional forward flexure in the AoV. The commissural region supports high tensile stresses and flexural deformations that the valve undergoes during opening and closing. In our study, we study the effective stiffness and consequent deformation by means of the valve GOA and mechanical strain response. This aids in understanding the flexural deformations and specific bending behaviors of the AoV native tissue as compared to the test specimen.

More recently, Sacks et al. [34], have studied the effect of AoV leaflet stiffness, due to chemical fixation of the bioprosthetic AoV, to the valve geometry and flexure. They have attempted to quantify leaflet geometries, motion and examine change in shapes using a laser-light imaging system, 3D reconstruction of the images, overall change in commissural angle and leaflet excursion. This study helped bring out the difference in leaflet dynamics such as increased localized bending, impaired flexure between the normal and stiffened (chemically fixed) valves. Despite some similarities, the primary difference between our research and that of Sugimoto et al., is that we have considered a more holistic and integrated approach to understand the effect of tissue remodeling on valve dynamics and flexure. Our approach of quantifying AoV opening area biomechanics in terms of average, maximum, integrated GOA, duration of opening coupled with the 2D visualization of the GOA shape, size comparisons for various fused and stiffened conditions provide the complete picture required to understand the complex GOA biomechanics, leaflets deformations, flexure and effects of bending stress.
The dynamic deformation of the AoV leaflets under varied mechanical conditions due to elevated hemodynamics (pressures, flow), as observed in diseased (hypertensive, severely hypertensive) valves were simulated and characterized in a mock physiological flow loop [40]. The 3D shape functions obtained from transformation of the 2D co-ordinates (using dual camera photogrammetry) were used to obtain indices in terms of stretch ratios and strains during the cardiac cycle; the discrete differentiation of the stretch ratios wrt time was used for obtaining the peak stretch rates. The leaflet deformation kinematics were studied using the TVP data calculated from the pressure-stretch energy parameter, where the in-plane stress was substituted for the TVP. During diastole, the high transvalvular pressure induces a stretch waveform which plateaus in both circumferential and radial directions and increased with pressure; during systole, the leaflet stretches in the radial direction due to forward flow drag forces but compresses in the circumferential direction.

The primary difference observed in the systolic and diastolic phases were the influence of hemodynamics on strain response. While, the diastolic stretch ratios were influenced by pressure, effects of flow were pronounced in systole. The diastolic phase was characterized by uniform surface pressure and high stiffness. The stretch ratio was observed to increase with higher stress attributed to the hypertensive conditions, whereas during systole, the flow was observed to play a vital role with radial elongation and circumferential compression.

We have attempted to study the AoV deformation in our laboratory, under very similar conditions using a non-contact optical 2D strain measurement system along with valve GOA characteristics. We have simulated the diseased AoV by varying the cardiac contractility signifying heart failure as well as tissue remodeling. Higher cardiac support associated with higher stress elicits a greater strain response that in turn triggers adverse biological responses in the AoV tissue associated with inflammation, calcification and ECM remodeling cascade [2] [7] [40].

A frictional drag force is generated due to the flow of the blood across the AoV, determined by the flow rate, leaflet flutter and valve geometry. In a normal valve, this force is low whereas during CAS it is much higher. However, during the systolic phase, the AoV is less stiffer and more distensible in the radial direction and the stress exerted by the drag force leads to the radial leaflet elongation [40]. Consequently, there exist elevated radial strains
during systole even in native, normal AoV tissue. The collagen fibers are crimped in the circumferential direction and the strain response is low.

AoV tissues are complex, nonlinear, and anisotropic in nature, which can be understood from the small orthogonal stress-strain response, despite large leaflet deformations/stretch. This is due to the aligned collagen architecture, circumferentially. During diastole, the pressure gradient across the valve leaflets causes bending and tensile strain; the low velocity of the flow leads to the development of vortices in the aortic sinuses that lead to an efficient closing mechanism, that prevents the retrograde flow of the blood and are able to withstand large transvalvular pressure gradients [25]. The diastolic pressure results in a stress-strain curve that is representative of the biaxial planar stretch of the leaflets [23].

5.4 PROPOSED AOV DISEASE MODEL FOR CAS

Based on our findings and literature review, we propose a AoV disease (CAVD) model in the Figure 5.5 for the pathogenesis of calcific AS and its implications.
The onset and progression of CAS is associated with a number of cardiovascular risk factors such as hypertension, lipids, oxidative stress, injury and so on. CAS is one of the most leading causes of valve replacement surgeries in the developed countries. Certain clinical factors such as age (>65 years), sex (male), LDL, metabolic syndrome, genetics (predisposition, polymorphisms), smoking are also associated with CAS. Pathologically, CAS presents with symptoms as valve thickening, formation of calcific nodules, ECM remodeling, collagen, elastin fragmentation, inflammation, fibrosis and so on. A number of signaling pathways including calcification with osteogenic differentiation of valvular interstitial cells, calcification secondary to cellular apoptosis, and necrosis-related deposition of calcium could be initiated for calcification in the AoV. Of a number of mechanisms involved in the pathobiology of AoV dysfunction, our model assumes that ECM remodeling is the central phenomena that dictates other events and outcomes. ECM disarray gives rise to a separate signaling cascade on the right hand side, that is associate with changes at a multi length scale, wherein mechanical stimuli dictate physiological responses and functions and vice versa. Even though studies of CAS mechanobiology usually involves the study, molecular mechanisms of the TGF-B signaling pathway, it is not included as a part of our study, but a separate and essential pathway that is linked to this mechanism. Ultimately, our model predicts a self propagating feedback loop that takes into account a number of factors and mechanisms, the details of which are yet to be unexplored.

5.5 LIMITATIONS

The current laboratory set-up is used to study the alterations in biomechanics and hemodynamics of the AoV leaflet due to tissue deformation in a porcine bioprosthetic valve. This allows the study to be conducted under pathophysiological conditions that are difficult to mimic in-vivo. However, in this environment, one is unable to simulate the exact physiological interactions and specific environments that influence a native tissue. The simulation of tissue remodeling and calcification is carried out by suturing the valve leaflets and by stiffening. While this method allows one to study the overall effects and deleterious trends in the biomechanical behavior, the molecular signal transduction mechanism that affects the pathogenesis of calcified tissues and the consequential initiation of several other signal transduction pathways and upregulation of synthesis of other enzymes/factors are
impossible to emulate and incorporate. Additionally, the suturing and stiffening might not be a very accurate physiological replica of the in-vivo fusion and calcification process. The bioprosthetic valve requires chemical fixation such as glutaraldehyde fixation that may contribute to undesirable stiffening of the tissue. The CS simulator uses stented bioprosthetic valves for the study. This may lead to constriction of movement and thus might not be an accurate representation of the tissue deformation, in-vivo. The use of DI water in the flow loop may lead to altered flow rates as compared to blood due to lower viscosity. The strain measurement uses DIC, that essentially require the markers to be visible at all times to correlate their positions. Thus selecting the ROI close to the free edge may lead to erroneous results. In summary, this study was able to establish a relationship between the AoV altered tissue dynamics and biomechanical and hemodynamical parameters. The use of a parameter such as the valve orifice area as a key predictor in the onset and clinical presentation of calcific AS, and a correlation to the current research has been identified. Though no mechanisms have been identified, one may be able to link the onset of valvular dysfunction and disease to induced aortic tissue remodeling, which in turn accelerates the strain and pressure on the valve that impair its normal function.

5.6 **Future Scope**

One of the future directions/additions to the current work would be that of an electrophysiological computational modeling approach for exploring the AoV cellular and molecular interactions. This would enable investigations that are currently unachievable by in-vivo methods. Understanding the key molecular events and signaling pathways are critical to any study of the valvular disease. Additionally, a computational model based on the key findings and constitutive relationships would allow us to develop a computational model for CAS in the AoV tissue. This would accelerate the understanding of key complex anisotropic, non-linear behaviors of the AoV under pathophysiological conditions that are hard to simulate in a laboratory environment.
REFERENCES


APPENDIX A

IMAGE J:MACROS
Automated detection by Image J consists of three distinct steps. The first step is to crop the image to enable batch processing followed by edge detection and analysis. Edge detection essentially consists of converting the image to ‘binary’ to enable the process. Thereafter, ‘thresholding’ is used for calculating the surface area of the ROIs and analysis. The macros used for this process are listed in Table A.1 - A.3.

**Table A.1. Crop.ijm Is Used to Automate Image J Analysis for M6. This Helps Cropping Images with Precise x,y,z Co-ordinates**

```java
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");
list = getFileList(dir1);
setBatchMode(true);
for (i=0; i<list.length; i++) {
    showProgress(i+1, list.length);
    open(dir1+list[i]);
    makeRectangle(71, 52, 346, 311);
    run("Crop");
    saveAs("BMP", dir2+list[i]);
    run("Close");
}
```
Table A.2. EDGE.IJM Is Used for Automated Detection of Edges for GOA Analysis

```java
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");

list = getFileList(dir1);
setBatchMode(true);
for (i=0; i<list.length; i++) {
    showProgress(i+1, list.length);
    open(dir1+list[i]);
    run("Make Binary");
    run("Find Edges");
    saveAs("BMP", dir2+list[i]);
    run("Close");
}
```
Table A.3. Analyze.ijm Is Used to Automate Image J Analysis. The Surface Area Is Calculated by Edge Detection by Image J

| GOA MACROS | dir1 = getDirectory ("Choose Source Directory ");  
|------------|------------------------------------------------------
|            | list = getFileList (dir1);                           
|            | setBatchMode (true);                                 
|            | for (i=0; i<list.length; i++) {                     
|            | showProgress(i+1, list.length);                     
|            | open(dir1+list[i]);                                 
|            | run("8-bit");                                      
|            | setAutoThreshold("Default");                      
|            | //run("Threshold...");                             
|            | setThreshold(0, 60);                                
|            | run("Convert to Mask");                           
|            | run("Find Edges");                                
|            | run("Analyze Particles...", "size=10-300 circularity=0.00-1.00 show=Nothing display clear include");  
|            | run("Summarize");                                 
|            | Dialog.create("save the results manually");       
|            | Dialog.show();                                      
|            | run("Close");                                     
|            | close();                                            
|            | }                                                   


APPENDIX B

STRAIN, HEMODYNAMIC PROFILE :
MAXIMUM VALUES
Table B.1. Maximum Values of Circumferential and Radial Strain for Low and Medium Cardiac Support (M6)

<table>
<thead>
<tr>
<th>Cardiac Contractility</th>
<th>Circumferential Strain</th>
<th>Radial Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Med</td>
<td>Low</td>
</tr>
<tr>
<td>Unfused</td>
<td>1.99</td>
<td>4.32</td>
</tr>
<tr>
<td>F1</td>
<td>5.27</td>
<td>11.35</td>
</tr>
<tr>
<td>F2</td>
<td>2.73</td>
<td>8.96</td>
</tr>
<tr>
<td>F2S</td>
<td>2.00</td>
<td>9.81</td>
</tr>
</tbody>
</table>

Table B.2. Maximum Values of Circumferential and Radial Strain for Low and Medium Cardiac Support (M7)

<table>
<thead>
<tr>
<th>Cardiac Contractility</th>
<th>Circumferential Strain</th>
<th>Radial Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Med</td>
<td>Med</td>
</tr>
<tr>
<td>Unfused</td>
<td>2.00</td>
<td>8.05</td>
</tr>
<tr>
<td>F1</td>
<td>4.59</td>
<td>9.05</td>
</tr>
<tr>
<td>F1S</td>
<td>0.88</td>
<td>3.19</td>
</tr>
<tr>
<td>F2S</td>
<td>1.90</td>
<td>4.82</td>
</tr>
</tbody>
</table>
Table B.3. Summary of Mean and Maximum Data for Hemodynamic Profile and GOA Parameters for Medium Cardiac Support (M6)

<table>
<thead>
<tr>
<th>Category</th>
<th>Unfused</th>
<th>F1</th>
<th>F2</th>
<th>F2S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean LVP</td>
<td>30.09</td>
<td>31.84</td>
<td>36.22</td>
<td>34.52</td>
</tr>
<tr>
<td>Max LVP</td>
<td>191.42</td>
<td>162.05</td>
<td>160.22</td>
<td>163.60</td>
</tr>
<tr>
<td>Mean AoP</td>
<td>8.35</td>
<td>33.05</td>
<td>28.12</td>
<td>11.90</td>
</tr>
<tr>
<td>Max AoP</td>
<td>10.15</td>
<td>108.27</td>
<td>91.70</td>
<td>12.62</td>
</tr>
<tr>
<td>Mean AoQ</td>
<td>3.86</td>
<td>3.60</td>
<td>3.23</td>
<td>3.14</td>
</tr>
<tr>
<td>Max AoQ</td>
<td>27.80</td>
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<td>23.21</td>
<td>20.16</td>
</tr>
<tr>
<td>Mean TVP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Max TVP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean GOA</td>
<td>100.56</td>
<td>79.30</td>
<td>48.58</td>
<td>52.38</td>
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<tr>
<td>Max GOA</td>
<td>130.70</td>
<td>95.64</td>
<td>71.98</td>
<td>78.19</td>
</tr>
<tr>
<td>Mean Integrated GOA</td>
<td>37.80</td>
<td>24.48</td>
<td>18.11</td>
<td>20.94</td>
</tr>
</tbody>
</table>

Table B.4. Summary of Mean and Maximum Data for Hemodynamic Profile and GOA Parameters for Medium Cardiac Support (M7)

<table>
<thead>
<tr>
<th>Category</th>
<th>Unfused</th>
<th>F1</th>
<th>F1S</th>
<th>F2S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean LVP</td>
<td>27.56</td>
<td>27.44</td>
<td>29.84</td>
<td>30.96</td>
</tr>
<tr>
<td>Max LVP</td>
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<td>116.89</td>
<td>117.90</td>
<td>120.70</td>
</tr>
<tr>
<td>Mean AoP</td>
<td>47.60</td>
<td>48.62</td>
<td>42.55</td>
<td>40.85</td>
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<tr>
<td>Max AoP</td>
<td>128.14</td>
<td>131.71</td>
<td>116.31</td>
<td>110.18</td>
</tr>
<tr>
<td>Mean AoQ</td>
<td>3.09</td>
<td>3.14</td>
<td>2.96</td>
<td>2.82</td>
</tr>
<tr>
<td>Max AoQ</td>
<td>17.91</td>
<td>19.53</td>
<td>19.53</td>
<td>17.05</td>
</tr>
<tr>
<td>Mean TVP</td>
<td>20.05</td>
<td>21.19</td>
<td>12.71</td>
<td>9.89</td>
</tr>
<tr>
<td>Max TVP</td>
<td>13.23</td>
<td>14.82</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean GOA</td>
<td>121.52</td>
<td>115.48</td>
<td>84.70</td>
<td>77.56</td>
</tr>
<tr>
<td>Max GOA</td>
<td>152.70</td>
<td>151.29</td>
<td>100.79</td>
<td>88.99</td>
</tr>
<tr>
<td>Mean Integrated GOA</td>
<td>32.09</td>
<td>42.39</td>
<td>28.59</td>
<td>24.68</td>
</tr>
</tbody>
</table>