1-1-2013

The Influence of Cyclic Pressure and Angiotensin II on the Biomechanical Properties of Aortic Heart Valves

Valtresa Shena Myles

The influence of cyclic pressure and angiotensin II on the biomechanical properties of aortic heart valves

By

Valtresa Shena Myles

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biomedical Engineering
in the Department of Agricultural and Biological Engineering

Mississippi State, Mississippi

May 2013

The influence of cyclic pressure and angiotensin II on the biomechanical properties of aortic heart valves

By

Valtresa Shena Myles

Approved:

James Neill Warnock Associate Professor of Agricultural and Biological Engineering (Major Professor)	Jun Liao Assistant Professor of Agricultural and Biological Engineering (Committee Member)
John Allen Crow Associate Research Professor of Basic Science (Committee Member)	Steven H. Elder Professor and Graduate Coordinator of Agricultural and Biological Engineering (Committee Member)
Sarah A Rajala Dean of the Bagley College of Engineering	

Name: Valtresa Shena Myles

Date of Degree: May 10, 2013

Institution: Mississippi State University

Major Field: Biomedical Engineering

Major Professor: Dr. James N. Warnock

Title of Study: The influence of cyclic pressure and angiotensin II on the biomechanical properties of aortic heart valves

Pages in Study: 72

Candidate for Degree of Master of Science

Hypertension, a risk factor for aortic valve stenosis, increases transvalvular load and can elicit extracellular matrix (ECM) remodeling. Elevated cyclic pressure and the vasoactive agent angiotensin II (Ang II) both promote collagen synthesis, an early hallmark of aortic sclerosis. It was hypothesized that increased collagen production induced by elevated pressure conditions or the presence of Ang II would affect the mechanical properties of leaflet tissue by decreasing extensibility. Porcine aortic valve leaflets were exposed to pressure conditions of increasing magnitude with and without Ang II. Biaxial mechanical testing was performed to determine peak stretch. Collagen content was determined using a quantitative dye-binding method. The results demonstrated Ang II and elevated pressure decrease the extensibility of leaflet tissue and increase the collagen content in the ECM. *In conclusion*, the results demonstrated that both elevated pressure and Ang II play a role in altering the biomechanical properties of aortic valve leaflets.

DEDICATION

This work is dedicated to my mother, Janet. This would not be possible without your continuous sacrifice and unwavering dedication. Thank you for your endless encouragement, support, and love. To my father, Wilbert, thank you for teaching me the value of hard work. To my younger siblings, Anthony, Shelby, Stephanie, and Aleah, thank you for giving me a reason to aspire. To my friends and the rest of my family, thank you for your constant support and inspiration.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Dr. James N. Warnock. Thank you for your unwavering patience, support, and guidance. I have learned a lot over the past few years and have you to thank for this invaluable experience. To Dr. Jun Liao, Dr. Allen Crow, and Dr. Steve Elder, thank you for your guidance while serving on my thesis committee. To Chelsea, Divina, and Paloria, thank you for all your help and support in the lab. I would also like to thank Dr. I-Wei Chu at the Electron Microscope Center and everyone at Sansing Meats.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

TV	Tricuspid Valve
PV	Pulmonary Valve
MV	Mitral Valve
AV	Aortic Valve
VECs	Valvular Endothelial Cells
ECM	Extracellular Matrix
VICs	Valvular Interstitial Cells
qVICs	Quiescent VICs
aVICs	Activated VICs
pVICs	Progenitor VICs
obVICs	Osteoblastic VICs
MMPs	Matrix Metalloproteinases
TVP	Transvalvular Pressure
CVD	Cardiovascular Disease
TGF-β	Transforming Growth Factor
TIMPs	Tissue Inhibitors of Metalloproteinases
RAS	Renin-Angiotensin System
Ang II	Angiotensin II
ACE	Angiotensin-Converting Enzyme

AT_1	Ang II Type 1 Receptors
PAVR	Percutaneous Aortic Valve Replacement
ANOVA	Analysis Of Variance

CHAPTER I

INTRODUCTION

Human Heart Physiology

The heart consists of four chambers: two atria and two ventricles. Their primary function is to ensure the proper circulation of blood throughout the body through repeated rhythmic contractions. Deoxygenated blood travels from the body via the vena cava and collects in the right atrium, which directs blood into the right ventricle. The right ventricle contracts, pumping deoxygenated blood through the pulmonary arteries and into the lungs, where it is oxygenated. The newly oxygenated blood collects in the left atrium via pulmonary veins and is directed into the left ventricle. The left ventricle contracts, ejecting oxygenated blood through the aorta to the systemic circulation to continue the cycle. The heart pumps an average of 1-20L of blood per minute [1]. A diagram of the human heart and blood flow is shown in Figure 1.

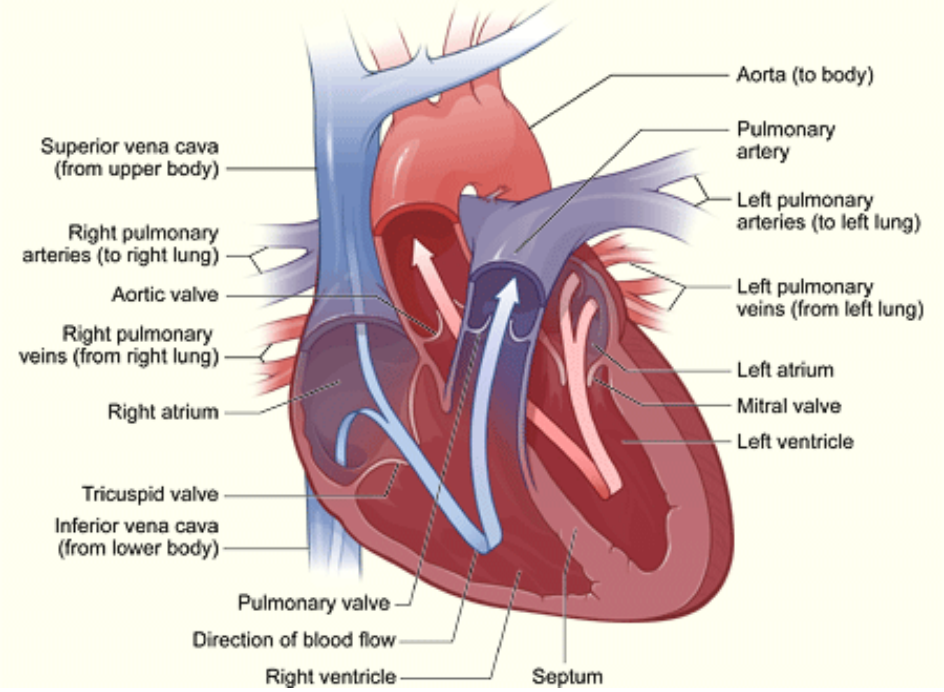

Figure 1.1 Human heart anatomy and blood flow. (cardiachealth.org).

The cardiac cycle consists of two phases: a single contraction and a single dilation. Systole refers to ventricular contraction, which causes the ejection of blood, as shown in Figure 1.2. When ventricular pressure exceeds the pressure in the aorta and pulmonary artery, blood is forced out of the ventricles through the semilunar valves. As pressure decreases in the ventricles and becomes greater in the aorta and pulmonary artery, the semilunar valves close and prevent the backflow of blood into the ventricles. The closing of the valves signify the end of systole and the beginning of diastole. Diastole refers to the dilation of the ventricles. During this phase the ventricles relax and the atrioventricular valves are open, allowing the ventricles to fill with blood from the atria. When the atrioventricular valves close and pressure begins to build in the ventricles, the cycle is restarted.

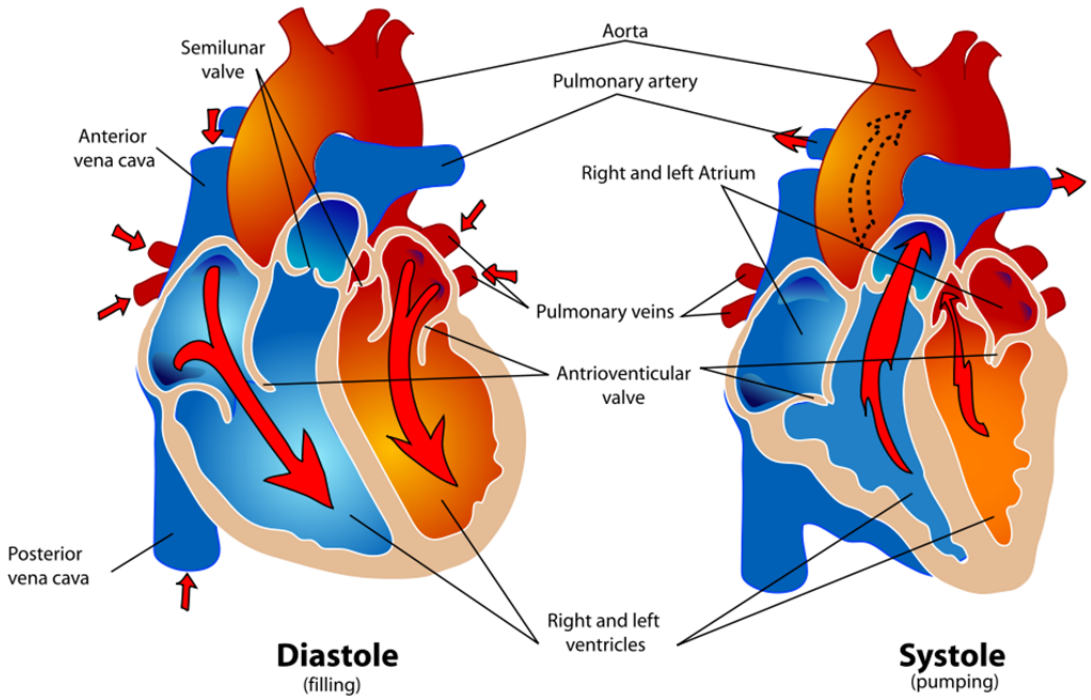

Figure 1.2 The heart in diastole and systole

(interactive-biology.com)

The unidirectional flow of blood is achieved by a series of four valves composed of fibrous tissue: tricuspid valve (TV), pulmonary valve (PV), mitral valve (MV), and aortic valve (AV). Heart valves are generally believed to open and close passively in response to the flow of blood and changes in pressure. However, recent evidence has suggested that the movements of the valve are controlled by interactions between the components of the valve and the surrounding hemodynamic environment [2]. The atrioventricular valves, named for their location between the atria and ventricles, consist of the tricuspid and mitral valves. Collectively known as the semilunar valves, the pulmonary and aortic valves each consist of three crescent shaped cusps. The pulmonary valve is located between the right ventricle and pulmonary artery. The aortic valve is located between the left ventricle and aorta. Of the four heart valves, the aortic valve

experiences the harshest hemodynamic environment and is most prone to disease [3,4]. Its unique structure is designed to withstand the demands of the surrounding environment while sustaining proper function [1].

Aortic Valve Structure

The aortic valve is contained by the aortic root. The aortic root is composed of the crown-shaped annulus, three sinuses of Valsalva, sinotubular junction, commissures, and valve leaflets. Each component of the root has its own histological profile and anatomical design. The coordinated structures function to maintain specific blood flow characteristics, coronary perfusion, and left ventricular function [1,5]. The area between each edge of the valve leaflet and aorta is known as the sinus of Valsalva [5]. The leaflets are referred to as the right coronary, the left coronary, and the non-coronary leaflet based on their relation to the coronary ostia found in two of the sinuses. Adjacent leaflets meet at the commissures [6], as shown in Figure 3. Together, the leaflets act as a hemomodynamic junction between the left ventricle and the aorta [6]. Located below the free edge of the leaflet is the line of closure and the coapting surface. The lanula is located above the coapting surface and contains a nodule of fibrous tissue near its center known as the nodule of Arantius. When the valve closes, the suspended area of the leaflets flatten and the coapting surfaces meet to separate the left ventricle and the aorta [5,7,8].

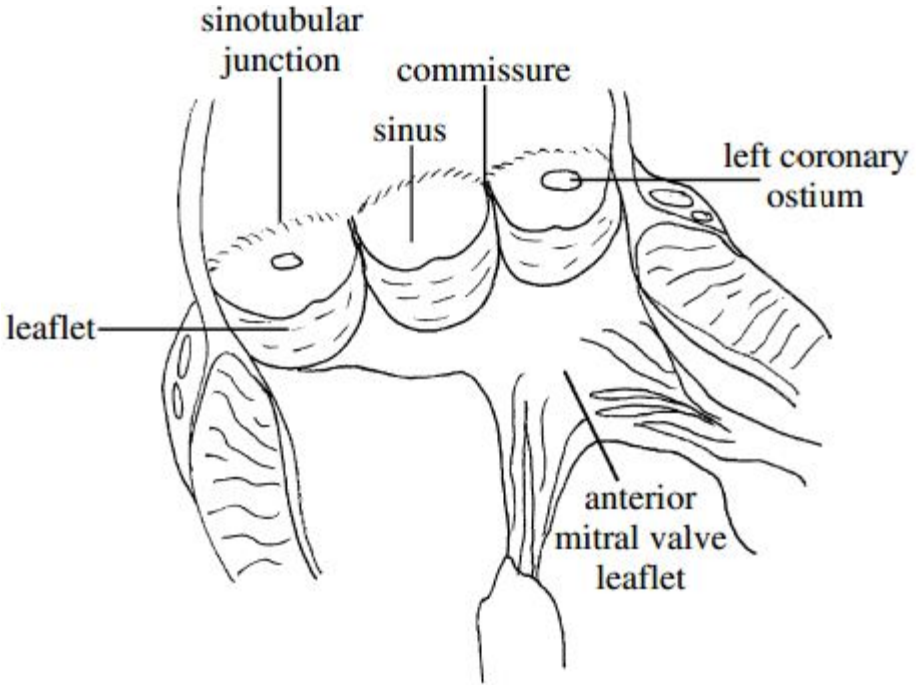

Figure 1.3 Schematic drawing of aortic root and valve [1].

Aortic valve leaflets are composed of mainly collagen, elastin, and glycosaminoglycans, which are organized into three layers: the fibrosa, ventricularis, and spongiosa. Each layer has distinctive mechanical properties and matrix composition. The fibrosa layer lies adjacent to the aorta and is primarily composed of circumferentially-oriented collagen. The fibrosa displays great tensile strength and adopts a folded or crimped formation while under compressive forces. The folds in the fibrosa are preserved by elastin fibers which encompass and connect collagen fibers [8,9]. The fibrosa provides most of the tensile strength to the leaflet as the dominant load bearing layer [10] and constitutes approximately 40% of total leaflet thickness [11]. The ventricularis is primarily composed of radially-aligned elastin, which contributes to the elasticity of the valve [10] as shown in Figure 4. The layer is adjacent to the left ventricle

and provides a smooth surface for laminar blood flow during systole [8]. The ventricularis constitutes approximately 30% of the total leaflet thickness [11]. Located between the fibrosa and ventricularis is the spongiosa and is primarily composed of loose connective tissue, glycosaminoglycans, and proteoglycans. The spongiosa acts as a shock absorber and lubrication between the fibrosa and ventricularis during various parts of the cardiac cycle [9,10,12]. The mechanical characteristics of collagen and elastin contribute to the continuous preloaded state of the leaflet. The fibrosa layer is under compression while the ventricularis is under tension. During diastole, the collagen fibers in the fibrosa unfold and elastin fibers allow the leaflet to extend radially to close the valve. During systole, the original wavy, folded configuration and orientation of the collagen fibers is restored as the elastin fibers recoil to open the valve [9].

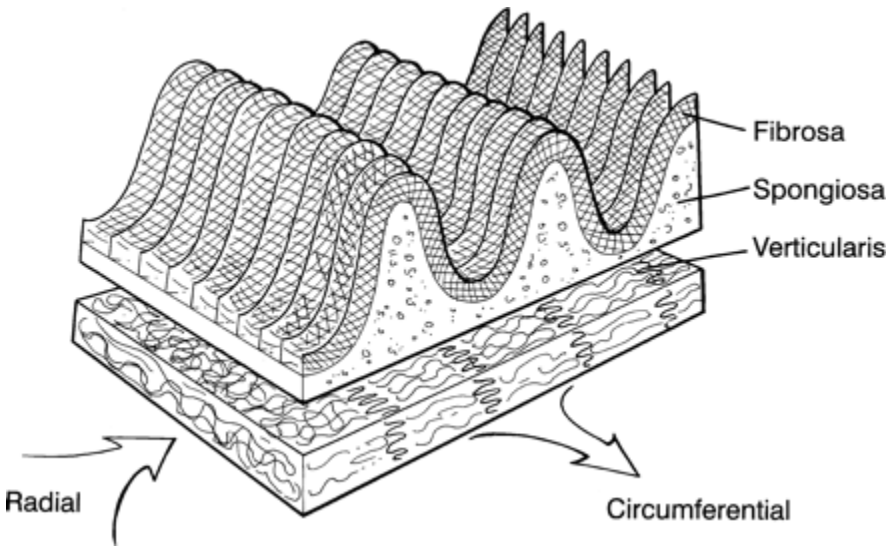

Figure 1.4 Schematic of aortic valve leaflet layers [5].

Aortic Valve Cells

The aortic valve leaflet is primarily composed of two cell types; endothelial cells and interstitial cells. Valvular endothelial cells (VECs) sheath the surface of each leaflet in a monolayer. The cells are aligned circumferentially and perpendicular to blood flow [12] and function as an interface between the blood and the leaflets. They are able to detect variations in shear stress and translate mechanical stimuli into biological responses [10]. VEC phenotypes are aortic and ventricular -side-dependent and may be determined by a combination of intrinsic and spatial factors: namely pre-natal development and local environmental stimuli, such as blood flow characteristics [13,14].

Positioned within the extracellular matrix (ECM) of the leaflets are the valvular interstitial cells (VICs). VICs are primarily composed of a heterogeneous population of fibroblasts, myofibroblasts, and smooth muscle cells [10]. These cells are responsible for organizing and remodeling the leaflet by regulating cell proliferation, migration, synthesis, and degradation of ECM components that provide its vital mechanical characteristics [15–17]. Innervation has been observed in the proximal medial sections of the leaflet [16]. Studies suggest the nerves transmit information between the VECs and the VICs via signaling mechanisms that are sensitive to changes in the mechanical environment [16,18]. Five VIC phenotypes have been identified including embryonic progenitor endothelial/mesenchymal cells, quiescent VICs (qVICs), activated VICs (aVICs), progenitor VICs (pVICs), and osteoblastic VICs (obVICs) [19]. The qVICs are fibroblast-like cells that synthesize collagen, elastin, proteoglycans, fibronectin, growth factors, and matrix metalloproteinases (MMPs). MMPs contribute to maintaining the structural integrity of the ECM by degrading collagen, elastin, and other proteins. The

aVICs are of a myofibroblast phenotype and are characterized by prominent stress fibers [19,20] and have been implicated in the remodeling of the ECM. Studies have shown in the ECM of diseased or remodeling leaflets myofibroblasts have significantly increased in number [17,20]. The pVICs are derived from qVICs and are associated with repair. The obVICs regulate osteogenesis and are associated with the mediation of calcification and valve disease [19]. Smooth muscle cells are capable of contraction and contain secretory properties responsible for maintenance and repair of the ECM [10]. Another cell phenotype has been shown to actively synthesize collagen as it contains secretory and synthetic organelles associated with an essential enzyme for stabilizing the collagen triple helix [20].

Aortic Valve Biomechanics

Although valves are thought to be generally passive structures driven by surrounding hemodynamics, they also exhibit sophisticated and complex biomechanical functions. Its structure directly relates to its biomechanical functions. The valve is composed of resilient and pliable fibrous tissue and designed to meet the demands of mechanical loading during the cardiac cycle [11].

As the valve opens during systole, leaflets experience flexion as they bend toward the aortic valve sinuses [21,22]. Elastin fibers in the ventricularis recoil, allowing the valve to open efficiently [23,24]. Bending stiffness of the leaflet is related to its ability to effectively open during systole and create a copative seal while closing during diastole [21,25,26]. When the valve closes during diastole, the leaflets experience biaxial stretch and compressive strain as a result of the increasing pressure. While under pressure, the collagen fibers in the fibrosa uncrimp and further align toward the axis of stretch [11,27].

Each layer of the leaflet has a distinct response to mechanical loading during the cardiac cycle. The fibrosa and ventricularis exhibit vastly different mechanical properties, resulting in an anisotropic tissue behavior [11]. Bending stress during systole is primarily transmitted by radially-aligned elastin fibers in the ventricularis, while tension is transmitted by circumferentially-oriented collagen fibers in the fibrosa [11,12]. These side-specific responses to mechanical loading contribute to the non-linear stress behavior, viscoelasticity, and biaxial coupling characteristics of the leaflet [28].

When tension is applied in the circumferential direction, the leaflet displays an elastic behavior as the collagen fibers forgo their wavy configuration and uncrimp (Figure 1.5). A transitional phase follows and is characterized by the strain as all of the fibers uncrimp. The final phase is characterized by the total uncrimping and extension of the collagen fibers. The stiff response in the circumferential direction allows the leaflet to withstand the increased transvalvular load during diastole [11,29,30]. The response to loading in the radial direction is dominated by elastin fibers as it contains much fewer circumferentially-aligned collagen fibers [23]. As a result, the radial direction exhibits a more gradual response to loading [29,31]. Biaxial mechanical testing has shown that both the fibrosa and ventricularis are characterized by distinct non-linear, anisotropic mechanical behaviors. The collagen fibers in the fibrosa dominate the mechanical loading response to loading while the ventricularis provided a significant contribution to radial tension [1,11].

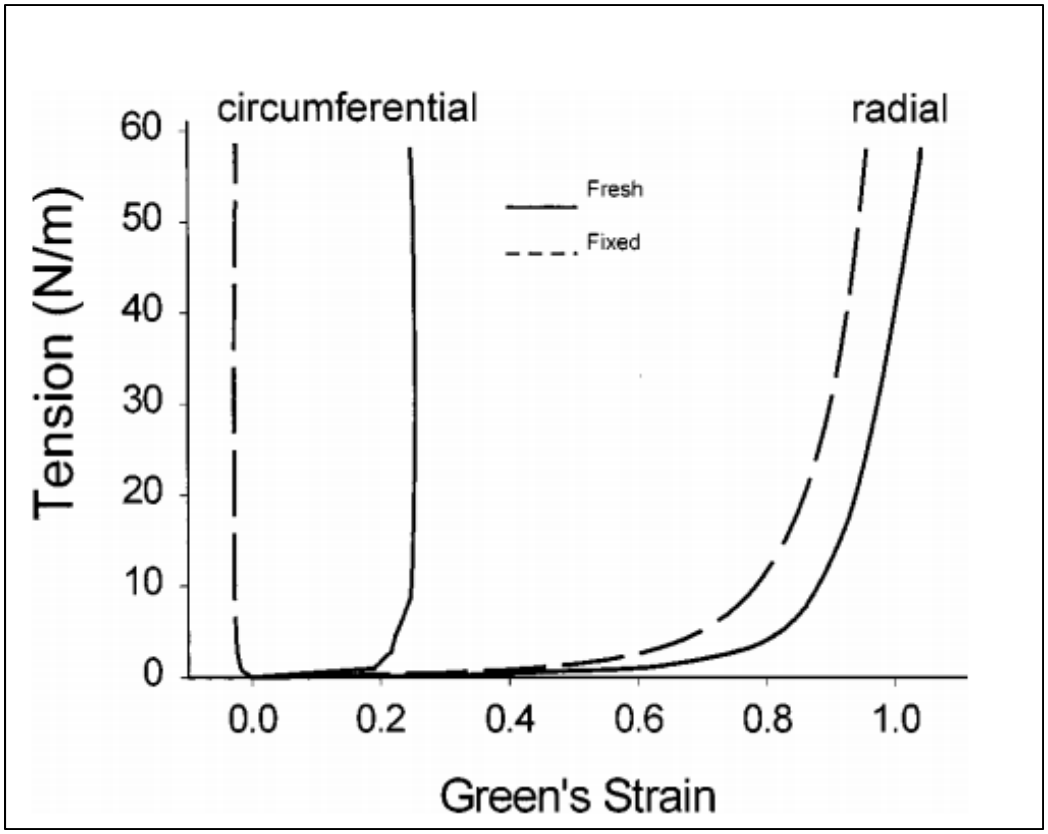

Figure 1.5 Representative circumferential and radial stress–strain curves from a fresh and fixed aortic valve leaflet, demonstrating the pronounced mechanical anisotropy of both tissues [31].

Stress relaxation characterizes the transmission of diastolic stress from the leaflet to the aortic root and influences its fatigue characteristics [32,33]. In addition, it demonstrates the leaflet's ability to reorganize its microstructure dynamically. Studies indicate that aortic leaflet tissue displays a direction-dependent response to stress relaxation, which is likely a result of structural anisotropy [34].

In diseased valves, the trilayer composition of the leaflet is disrupted as a result of disorganized protein synthesis and degradation in the ECM [35]. Biomechanical properties are potentially compromised when structural changes occur as a result of

collagen and elastin fiber disorganization and fragmentation [35,36]. In addition, biological consequences occur as the disorganization of the ECM promotes further remodeling and repair [35,37].

Mechanical Environment

The aortic valve experiences the harshest mechanical environment of the heart valves. The intense mechanical environment is regulated by the surrounding hemodynamic environment. The total stress on the leaflet is tensile throughout the cardiac cycle [22]. However, during various stages of the cardiac cycle, the aortic leaflets experience tension, compression, shear, pulsatile, and flexure forces.

The leaflet layers vary in ECM composition; as a result, each layer exhibits different responses to the application of force. During the cardiac cycle, the belly of leaflet undergoes changes in three dimensional curvatures, resulting in bending stress. As the valve begins to close, vortices develop in the sinuses of the leaflet as a result of the abrupt change in the direction of blood. These vortices and increasing pressure force the leaflets away from the sinus wall as the leaflets align to form a seal, preventing the backflow of blood into the left ventricle [28]. The total stress experienced varies throughout the thickness of the leaflet. The stress in the circumferential direction is maximal on the ventricular side during diastole at 36 to 75 gm/mm^2 and minimal on the aortic side at 9 to 15 gm/mm^2 [22]. During systole, stress in the circumferential direction is compressive on the ventricular surface at 0.3 to 0.8 gm/mm^2 and tensile on the aortic surface at 4 to 9 gm/mm^2 [22].

Leaflets experience side-specific shear stresses during the cardiac cycle [38]. The ventricular surface is exposed to unidirectional pulsatile shear stress during systole as

blood is ejected out of the left ventricle at a peak rate of 1.35 ± 0.35 m/s [39]. During diastole, the aortic surface experiences oscillatory shear stress as the blood pools into the sinuses [38,40]. Determining wall and leaflet shear stress values remains a challenge. As a result, estimates range as low as 15 dynes/cm^2 to as high as 1800 dynes/cm^2 [40,41].

Strain experienced by the leaflets is dictated by transvalvular pressure (TVP). TVP acts perpendicular to the leaflet area and is transmitted from the collagen fibers to the cells in the ECM [2]. The average diastolic TVP under normal hemodynamic conditions is 80mmHg. TVPs of 100 and 120mmHg signify the classifications of Stage I and Stage II hypertension, respectively [42]. Strain varies in magnitude and occurs in the both the radial and circumferential directions.

The aortic valve opens and closes approximately 3x10^9 times in an average lifetime [12]. The leaflets undergo stretch in both the radial and circumferential directions during diastole as they stretch to form a coaptive seal [11,43]. The average elongation is three times higher in the radial direction than in the circumferential [44].

Altered hemodynamic forces, such as increased shear stress and blood pressure, have been implicated in the initiation and advancement of aortic valve disease. These conditions potentially lead to compromised valve structure and function [45].

Cardiovascular Disease

Cardiovascular disease (CVD) affects 1 in 3 Americans and is the leading cause of death in the United States. CVD encompasses multiple pathologies and ailments including heart failure, myocardial infarction, stroke, hypertension, arthrosclerosis, and congenital heart defects [3]. One of the most common surgeries associated with CVD is heart valve replacement; most notably, aortic valve replacement [3,4,19]. The number of

replacement surgeries in the aortic position has steadily increased over the last two decades [4].

Valvular disease can be acquired or congenital. Acquired disease refers to conditions that develop in previously healthy valves. Most acquired valve diseases are age-related degenerative diseases or conditions caused by lifestyle choices [5,12,46]. Congenital conditions develop before birth. These defects include improper leaflet attachment, incorrect valve size, or malformed leaflets. Bicuspid aortic valves are the most common congenital heart anomaly in the general population [47]. Approximately 40,000 infants are born with a bicuspid aortic valve each year [48]. The malformation results in altered hemodynamics and mechanical stress distributions. The altered mechanical stresses on the valve can eventually lead to calcific deterioration [5,47]. The average bicuspid valve functions successfully 20 years less than a normal tricuspid valve [48].

Aortic valve abnormalities are classified as regurgitation, stenosis, or a combination of both conditions. Regurgitation occurs when the valve is unable to close completely, resulting in retrograde blood flow into the left ventricle. Aortic valve sclerosis is a precursor to stenosis. It is described as calcification and thickening of the valve without a hindrance of blood flow. When aortic sclerosis is present, the risk of myocardial infarction and death increases by 50% [3]. Aortic valve stenosis refers to the significant narrowing or obstruction of the valve caused by calcification or scar tissue (Figure 1.6). In a 2010 study, aortic stenosis was found in 43% of patients with valvular heart disease [3]. Prevalence of aortic stenosis has shown a strong positive correlation

with increasing age, high cholesterol levels, smoking, elevated body mass index, and diabetes [3,49].

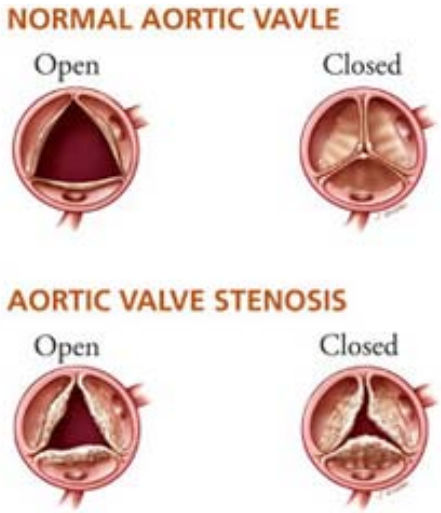

Figure 1.6 Normal aortic valve and aortic valve stenosis.
Adapted from intermountainhealthcare.org.

Aortic Valve Disease Pathology

Valvular disease is characterized by inefficiency of the valve due to stenosis and/or regurgitation. Originally, aortic valve disease was thought to be a passive process of fatigue and degeneration over time [49]. However, recent studies show that the pathogenesis of aortic valve disease is regulated by active cell-based processes involving ECM remodeling, inflammation, lipid deposition, and potential calcification [19,50,51] (Figure 1.7).

Evidence has shown that calcified aortic valve experience significant ECM remodeling, regulated by VECs and VICs [19]. Repair and remodeling of the ECM has been associated with an up-regulation of transforming growth factor (TGF-β) [19]. Homeostasis of the aortic valve ECM is dependent upon the balance between matrix

degradation and synthesis maintained by proteolytic enzymes such as MMPs, their tissue inhibitors (TIMPs), and cathepsins. MMPs regulate collagen turnover, TIMPs inhibit the effects of MMPs, and cathepsins are associated with the degradation of collagen and elastin fibers in addition to atherosclerotic plaque progression [52,53]. A disruption in the delicate balance of these enzymes as a result of altered biomechanical and biochemical signals can affect the remodeling of the ECM and lead to compromised valve function [19].

Inflammatory mediators regulate the active process of valvular calcification. Healthy valves do not contain inflammatory cells such as macrophages and lymphocytes [49]. Histological studies have confirmed their presence in early calcified lesions associated with valvular disease, which indicates the initiation of an injury response mechanism. Proinflammatory cytokines have also been observed in diseased valves [19].

The presence and accumulation of lipids have also been observed in calcific aortic valve disease [54]. Lipoproteins deposits in aortic lesion and increased aortic valve cholesterol content have also been associated with the disease. The accumulation of ECM proteoglycans plays a role in mediating lipoprotein depositions [50].

Calcification contributes to lesion stiffness, potentially compromising the efficiency of the aortic valve by obstructing blood flow from the left ventricle [54]. Calcification is characterized by calcific deposits of calcium-phosphate minerals, the accumulation of ECM proteins such as collagen, and the presence of osteopontin, bone morphogenic proteins, and tenascin C [19,50,55]. Recent studies suggest that the predominant form of calcification in calcific aortic valve disease is osteogenic calcification, rather than the passive degenerative process of dystrophic calcification.

Osteogenic calcification is an active process regulated by genes associated with cartilage and bone development [19].

Figure 1.7 Theory of the pathogenesis of calcific aortic valve disease [19].

Hypertension and Angiotensin II

Studies have shown a positive correlation between hypertension and aortic stenosis and calcification [42,46,56]. Hypertension is defined as a chronic elevation of blood pressure caused by a narrowing of arterial walls; this can be caused by blockage, thickening, or by altered contractile and stiffness responses [42]. The increased mechanical load hypertension places on the aortic valve has been associated with the development of aortic calcification and sclerosis [42,57].

It affects approximately one third of the industrialized population [3]. The development of hypertension may be a result of factors including abnormal functioning of the heart, vasculature, kidneys, improper diet, smoking, obesity, genetics, or a combination of multiple factors. Hypertension is a risk factor for other cardiovascular conditions, such as stroke, heart failure, atherosclerosis, and intracranial hemorrhage [3,42].

Hypertension can be classified as primary or secondary based on the cause of the disease [57]. Primary hypertension accounts for 95% of occurrences and etiology remains unknown [58,59]. Secondary hypertension affects approximately 5% of the population and has an identifiable cause [60]. The most common causes of secondary hypertension are endocrine and renal disease. Hypertension is classified by severity in stages: stage 1 hypertension consists of diastolic pressures of 90-99 mmHg, stage II of pressures 100-109 mmHg, and stage III exceeds 110 mmHg [61].

Hypertension is associated with multiple organs; however the kidneys play a major role in the development of the disease [60,62]. Multiple types of renal diseases and hypertension are commonly linked to an increase in renin secretion. Renal complications

and its connection to the renin-angiotensin system (RAS) have been credited as both a cause and consequence of hypertension [59,63]. The RAS contributes to the regulation of fluid and blood pressure. Kidney damage compromises its ability to excrete salt and excess fluid, causing a decrease in renin. A decrease in renin allows for the advancement of hypertension. An increase in blood pressure can potentially cause further kidney damage, creating a detrimental cycle of kidney damage and increased blood pressure [59,63,64]. High levels of renin have been associated with renovascular hypertension [63]. Renovascualar hypertension causes a decrease in blood flow to the kidneys, resulting in an increase in blood pressure and potential advancement of hypertension.

In addition to hypertension and renal disease, the RAS has also been implicated in the progression of atherosclerosis and calcific valve stenosis [64–67]. Angiotensin II (Ang II) is a potent vasoactive agent and the primary effector of the RAS. The octopeptide is formed from its precursor Angiotensin I following the cleavage of two terminal amino acids by the angiotensin-converting enzyme (ACE) [59,68]. Ang II has been shown to mediate the regulation of vasoconstriction and blood pressure. The elevated TVP on the leaflet caused by hypertension can activate the renin-angiotensin system. Ang II has been shown to stimulate collagen type I production in hypertension-associated aortic diseases [69]. Locally, the physiological effects of Ang II on the AV are mediated by transforming growth factors such as TGF-β1[70,71] and angiotensin type I receptors (AT_1) [72].

Treatment Options

Typically, by the time valvular disease is clinically detected, it can only be treated by surgical repair or replacement. The life expectancy of most individuals with severe

aortic valve stenosis is less than 10 years if left untreated [3]. Valvular repair is a treatment option that most commonly involves replacing components of the aortic valve with allogeneic or autologous tissues. The vast majority of treatment methods require replacing the aortic valve leaflets and/or root altogether. These replacements fall into two divisions: mechanical and tissue. Mechanical replacements are composed of various materials but are generally constructed with pyrolytic carbon coated metal [48,73]. While these replacements are structurally durable, they are not without limitations. Mechanical replacements contain components and geometries that potentially cause platelet lysis and protein aggregation on the surface of the valve. The patient incurs considerable lifestyle restrictions in addition to taking anticoagulant medication for the rest of their lives. Patients are also at a higher risk for stroke, hemorrhage, and myocardial infarction [74].

Tissue replacement options include xenogenic and allogenic valves. Xenografts are most commonly obtained from bovine and porcine tissue. These replacements offer similar physiological hemodynamics, but contain no living cells [48]. Without living cells, the tissue replacements cannot regenerate or maintain the ECM and may potentially degenerate over time. Structural deterioration of tissue replacements after implantation may be affected by tissue preservation, fabrication, chemical fixation, and insertion techniques of the valve replacements [74].

The Ross Procedure is an autologous tissue replacement option. In this procedure, the aortic valve or aortic root is replaced with the patient's own pulmonary valve, while the patient's pulmonary valve is replaced with a prosthetic valve. It is commonly performed in children and young adults (72,73). The pulmonary valve provides suitable hemodynamic characteristics and low prevalence for thromboembolism and infection

[75,76]. However, there are considerable limitations. The procedure is extremely demanding and commonly requires an extensive operation time. Reoperation rates of 15-52% beyond 10 years have also been documented [75,77]. In addition, distortion and malposition of the mobilized coronary arteries have also been reported [75].

Minimally invasive procedures are also becoming common treatment options for aortic valve disease. For many patients, invasive surgery many not be an option due to their age, general health, progression of disease, or various other factors. Others may simply not wish to have surgery for personal reasons [48,78].

Percutaneous aortic valve replacement (PAVR) is a minimally invasive and relatively new technique, with the first successful implantation reported in 2002 [78]. Implanted valves using this technique usually incorporate a stainless steel or titanium alloy stent and bovine or porcine pericardial tissue. The valves are most commonly balloon-expandable or self-expanding. The PAVR is delivered via a catheter guided through the femoral artery. When the PAVR reaches the aortic valve, the stent expands and displaces the diseased leaflets (Figure 1.8). In their place lies the new functional valve [79,80]. Complications with PAVRs include the risk of arterial perforation or dissection while maneuvering the catheter through vasculature. In addition, embolic stroke, aortic disruption, and coronary obstruction caused by the native or prosthetic valve may also occur [79,81,82].

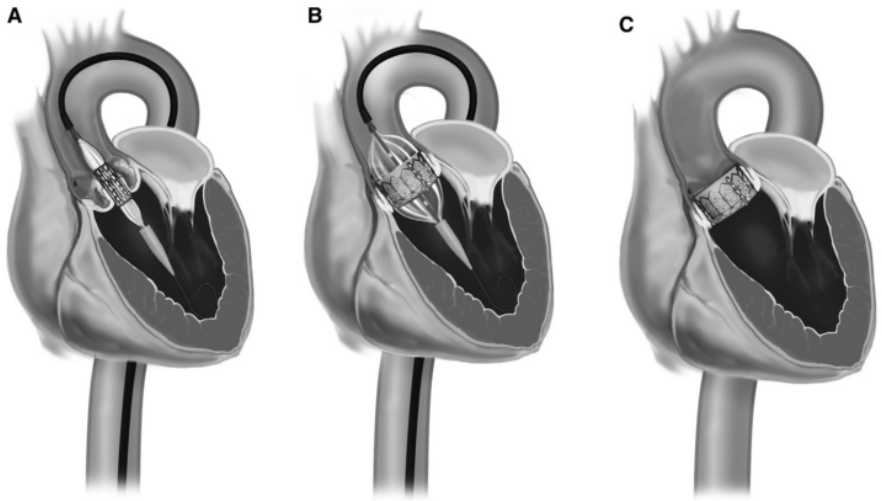

Figure 1.8 Schematic drawing of the percutaneous aortic valve replacement

A) Positioning of the catheter-mounted valve. B) Dilatation of the device dilatation with a balloon. C) Correct positioning at the end of the procedure. Adapted from Heinze et al [80].

Motivation

Aortic valve disease can potentially cause significant problems for not only the heart, but the entire body. Currently, surgery is the primary treatment option [3,48]. Multiple epidemiological studies have shown a correlation between hypertension and aortic valve disease [42]. Hypertension affects approximately one third of the industrialized population [3]. By 2025, the number of adults with hypertension is expected to increase by 60% [60]. It has been suggested that the excessive pressure caused by hypertension inflicts additional tension on the leaflets. Over time, the constant excessive pressure may elicit active cellular responses that promote calcification. The changes that result in the calcification and thickening of the aortic valve occur in the ECM. They are caused by active cellular processes that lead to lipid and calcium deposition and collagen synthesis [36,54]. Multiple studies have investigated the

structural adaptations in the ECM of the leaflet [17,43,83]. However, the intrinsic regulations of the biological and mechanical properties that lead to compromised valvular function are poorly understood. While there are medications on the market to treat hypertension, current pharmacological approaches to prevent and/or treat aortic valve disease have not shown consistent success. The results of the current study will help to provide a better understanding of the biological responses of aortic valves to mechanical and biochemical changes that occur under hypertensive conditions.

CHAPTER II

HYPOTHESIS AND SPECIFIC AIMS

Hypothesis

The current study hypothesizes that hypertensive cyclic pressure and/or angiotensin II decreases extensibility of aortic valve leaflets in a time dependent manner due to an increase in collagen content and/or interstitial cell stiffness.

Specific Aims

Specific Aim 1: Determine the effects of mechanical and biochemical hypertensive conditions on the mechanical properties of aortic valve leaflets.

Hypertension and Ang II have both been implicated in aortic valve sclerosis. Hypertensive pressures and Ang II have been shown to independently increase collagen synthesis in previous studies [84,85]. As collagen fibers are the main load-bearing component of the leaflet, hypertensive conditions may affect leaflet extensibility. Determining the mechanical properties of the leaflets after exposure to hypertensive conditions will elucidate if hypertensive conditions cause functional changes within the valve.

Specific Aim 2: Determine the biological effects of mechanical and biochemical hypertensive conditions on the ECM that cause the functional changes observed in SA1.

Thickening and stiffening of the leaflet are signs of aortic valve sclerosis and are associated with hypertensive conditions. An increase in net collagen content in the ECM may account for the thickening and dysfunction of the leaflet [43,85]. VICs respond to altered local stresses transferred from the surrounding ECM and remodel accordingly. A change in the mechanical properties of the VICs may lead to malfunction in the aortic valve. Investigating the collagen content and mechanical properties of aortic valve leaflets may provide a better understanding of the changes in the ECM, resulting in a better understanding of the changes in mechanical properties observed in *Specific Aim 1*.

CHAPTER III

METHODS AND MATERIALS

Tissue Extraction and Culture

Porcine hearts were obtained within 30 minutes of slaughter from a local slaughterhouse (Sansing Meat Service, Maben, MS). Female Yorkshire/Hampshire pigs were slaughtered before 6 months of age with a post-slaughter weight of less than 190 lbs. The aortic valve leaflets were excised and transported to the laboratory on ice in sterile Dulbecco's phosphate-buffered saline (DPBS, Sigma, St. Louis, MO).

Upon arrival at the lab, leaflets were placed in six well plates (one leaflet per well) and incubated at 37°C overnight in 3mL (in each well) of Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% Fetal Bovine Serum (FBS; Invitrogen, Carlsbad, CA) and 1% Anti-biotic/Anti-mycotic solution (ABAM; Invitrogen, Carlsbad, CA). The overnight incubation step was required to insure that all the cells were synchronized.

Mechanical and Biochemical Studies

Leaflets were randomly assigned to one of six groups as shown in Table 3.1.

Table 3.1 Aortic Valve Leaflet Experimental Groups

Pressure / Ang II	0 mmHg	80 mmHg	120 mmHg
Ang II -ve	Group 1	Group 2	Group 3
Ang II +ve	Group 4	Group 5	Group 6

Group 1 represents static conditions. Group 2 represents normotensive conditions. Group 3 represents hypertensive mechanical conditions, group 4 static mechanical and hypertensive biochemical conditions, group 5 hypertensive biochemical conditions, and group 6 hypertensive mechanical and biochemical conditions. To mimic a biochemical hypertensive environment, culture media was replaced with fresh media supplemented with 10^{-6}M Ang II (Sigma). A custom-designed pressure system was utilized to generate cyclic pressures representative of *in vivo* normotensive and hypertensive conditions (Appendix A). The media used in the 6-well plates entering the pressure system was supplemented with HEPES 25mM (Sigma) to stabilize pH. The leaflets were exposed to the experimental conditions for 24 and 48 hours and media was replaced every 24 hours. These durations were chosen as previous studies have shown this to be sufficient time to observe biomechanical and/or biochemical changes in valve leaflets [84–86].

Biaxial Testing

Biaxial mechanical properties were characterized with a biaxial mechanical testing system (Figure 3.1). 10mm x 10mm samples of tissue were dissected from the

central region of the leaflets (Figure 3.2). Four fiducial graphite markers arranged in an approximately 4 mm x 4 mm square were placed in the center of the extracted portion of the leaflets to track tissue strain using a CCD camera. Two loops of 000 nylon sutures of equal length were attached to each side of the samples with four stainless steel hooks. The samples were mounted on the biaxial device with the radial and circumferential directions aligned with the x_1 and x_2 stretch axes of the device, respectively. A membrane tension (applied force/unit length) was applied along each axis and increased slowly from a pre-stress tension of ~0.5 N/m to a peak tension of 60 N/m. A peak tension of 60 N/m was used as it corresponds to the *in vivo* diastolic pressure.

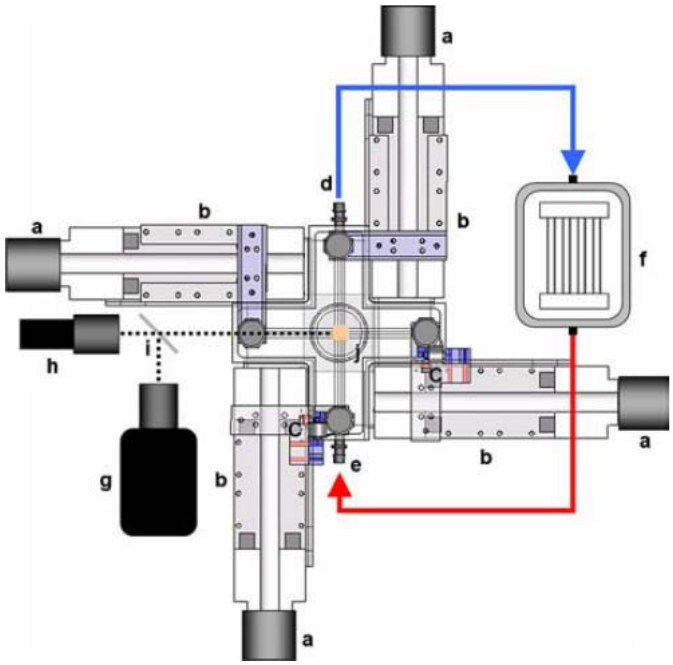

Figure 3.1 Overhead schematic of a high-speed biaxial testing device

(a) stepper motors; (b) screw-driven linear actuators; (c) load cells; (d) specimen bath outlet; (e) specimen bath inlet; (f) heating element maintained bath temperature at 37°C; (g) high-speed digital camera; (h) standard digital camera; (i) beam splitter; (j) sub-specimen mirror[87].

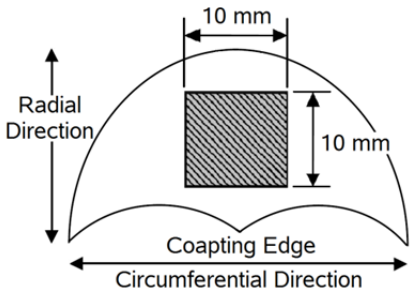

Figure 3.2 Portion of leaflet used for biaxial testing

The samples were preconditioned for ten contiguous cycles, following an equibiaxial protocol of T_{CC}:T_{RR} = 60:60 N/m, where T_{CC} and T_{RR} are the tensions applied in the circumferential and radial directions, respectively at 15 seconds per cycle. Tissue extensibility was characterized by maximum stretch along the circumferential direction (λ_{CC}) and maximum stretch along the radial direction (λ_{RR}), at an equibiaxial tension of 60 N/m (A representative tension vs. stretch curve shown in Appendix A).

Collagen Assay

To determine changes in collagen content following exposure to elevated pressure and/or Ang II, leaflets were prepared as previously discussed with the exception that leaflets were cut in halves before exposure to the experimental conditions. Paired leaflets were exposed to –ve and +ve Ang II biochemical conditions and the same pressure conditions. Following the end of the experiments, the leaflet halves were dehydrated in an oven at 50°C for 48 hours and the dry masses were recorded. The tissue was dissolved in pepsin (Sigma) at a concentration of 0.1 mg/ml of 0.5 M acetic acid (Sigma) at 4°C for 48 hours with occasional agitation. The Sircol Collagen Assay (Accurate Chemicals, Westbury, NY) was used to measure the pepsin-soluble collagen content in the leaflets

using a quantitative dye-binding method. After the assay was performed, a standard curve was plotted using the absorbance data from the blank and collagen standard samples. The slope of the standard curve was used to calculate the collagen content of the test samples. An R^2 value of 0.95 was used as the calibration equation. If R^2 value of < 0.95, the assay was repeated.

Aortic Valve Interstitial Cell Isolation and Culture

Porcine aortic valve leaflets were obtained as previously described. Upon arrival at the laboratory, leaflets were incubated in sterile collagenase type II (Worthington, 300u/mg) for 10 minutes at 37°C and 5% CO_2. Endothelial cells were removed by gently scraping the leaflet surface with sterile cotton swabs. Remaining leaflet portions were immediately digested for 8-10 hours in collagenase type II and DMEM with constant agitation. Digested tissue fragments and cells were centrifuged at 1000 rpm for 5 minutes and plated in T12.5 cm^2 tissue culture flasks. The leaflets were incubated in Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum and 1% Anti-biotic/Anti-mycotic solution using standard tissue culture methods. Cells were passaged at 75-85% confluencey and maintained in T75 cm^2 flasks. Cells were frozen for long-term storage and recovered as needed for experiments (Protocol outlined in Appendix B).

Cell Mechanics

The interstitial cells were plated into 60 x 15mm petri dishes and exposed to the 6 experimental groups in table 1 for 24 hours. Media was replaced with new media supplemented with HEPES 25mM to stabilize pH during experimentation. An atomic force microscope (AFM) was used to evaluate the stiffness of VICs (Veeco Instraments,

Bioscope Catalyst AFM). Cells were not fixed. Fixation methods potentially kill the cell and distort its mechanical properties. Measurements were made using a 'Point-and-shoot' method in contact mode (Veeco Software). Tip-less cantilevers mounted with 5 μm diameter polystyrene spheres (Novascan Technologies, Ames, IA) were used. Cantilever probes were calibrated by thermal vibrations using the manufacture's software. Spring constants were calculated as having values between 0.06294 and 0.07148 N/m. Measurements were performed on 6 cells from each experimental condition, for a total of 36 cells measured. For each VIC, 72 indentions in an 8x9 rectangular grid pattern were made over the surface of the cell. The Young's modulus, E, of the cell was calculated using the Hertz contact model:

$$E = \frac{3}{4}\frac{(1-\nu^2)}{\sqrt{R\delta^3}}F \tag{3.1}$$

where E is the Young's modulus, ν is the Poisson's ratio, R is the radius of the spherical indenter, δ is the depth of indentation, and F is the force of indentation. Poisson's ratio was set to 0.5 as the cell was assumed to be incompressible.

The relationship between applied force of indentation and indentation depth (Eq.3.1 and Figure A.4) was fit using the linearized model method for fitting parameters to a nonlinear equation (NanoScope Analysis, Bruker). Measurement locations with fits resulting with R^2 values of 0.90 were included in analysis. A target depth of approximately 600nm was used for indentations.

Statistical Analysis

Biaxial mechanical testing and collagen assays were performed 6 times and is represented as mean ± SD. For all biaxial mechanical testing, the circumferential and

radial data groups were considered separately. Data underwent two-way analysis of variance (ANOVA), one-way ANOVA, and *t*-tests for pairwise comparisons where appropriate. A *P*-value of less than 0.05 was considered statistically significant.

CHAPTER IV

RESULTS

Biaxial Peak Stretch

The results demonstrate that leaflet extensibility of aortic valve leaflet tissue was greater in the radial direction than in the circumferential direction, which is consistent with previous studies [30,85].The peak stretches of fresh tissue tested 4 hours after slaughter were calculated as 1.065±0.031 and 1.399±0.017 in the circumferential and radial directions, respectively (Figure 4.1). Peak stretch between different time intervals were statistically similar ($p<0.05$) in the circumferential direction, as well as in the radial direction. These results validated the control as peak stretch did not significantly change during the experiment.

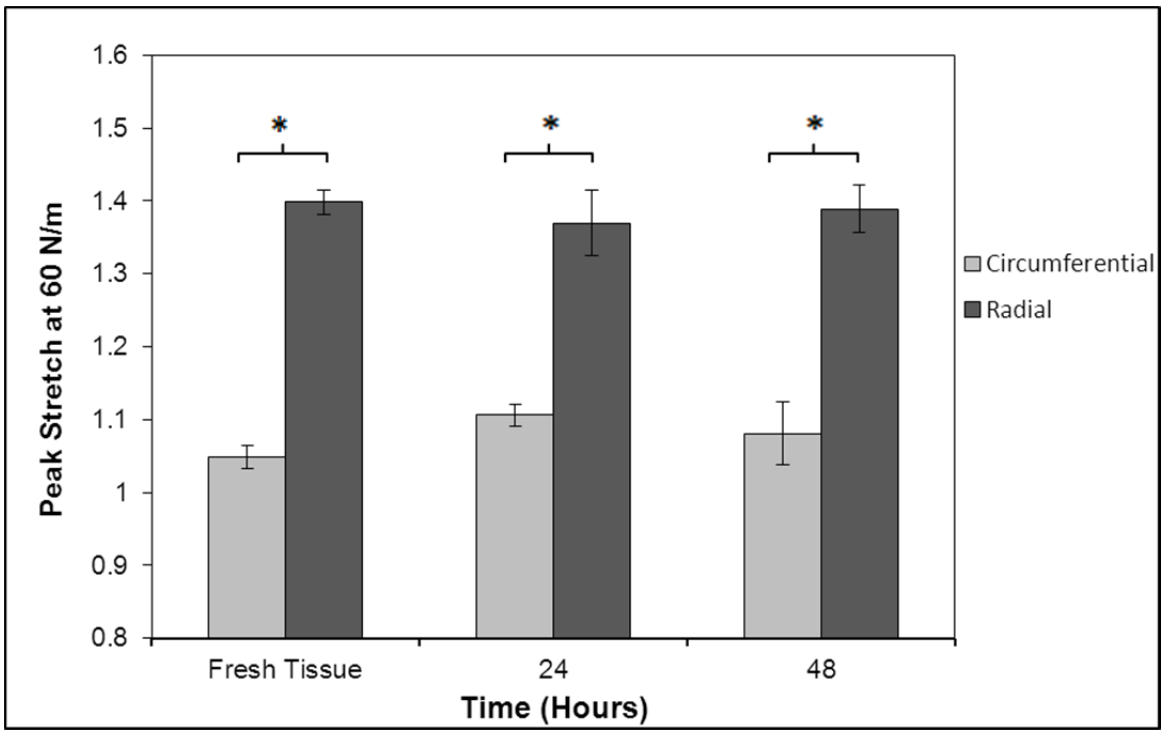

Figure 4.1 Peak stretch of aortic valve leaflets at various time intervals. Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

When leaflets were incubated with 10^{-6}M Ang II, there was no significant difference when compared to controls in circumferential direction at either 24 or 48 hours as shown in Figure 4.2A. However, Ang II significantly reduced peak stretch in the radial direction at both time intervals (Figure 4.2B).

The elevated pressure of 120mmHg reduced peak stretch in both the circumferential and radial directions at both time points as shown in Figure 4.3. After 24 hours, the peak stretch of leaflets incubated at both 80 and 120 mmHg was significantly less than those at 0 mmHg in the circumferential direction (Figure 4.3A). No significant difference occurred between 80 and 120 mmHg in the circumferential direction. After 48 hours, the peak stretch of leaflets incubated at 120 mmHg was significantly less than

those at 0 and 80 mmHg. There was no significant difference in leaflets incubated at 0 and 80 mmHg in the circumferential direction after 48 hours.

As can be seen in Figure 4.3B, the peak stretch of leaflets incubated at 120 mmHg was significantly less than those incubated 0 and 80 mmHg in the radial direction after 24 hours. Leaflets incubated at 0 mmHg were statistically similar ($p<0.05$) to those incubated at 80 mmHg. After 48 hours, the peak stretch in leaflets incubated at 120 mmHg was significantly less than those incubated at both 0 and 80 mmHg in the radial direction. When compared to 80 mmHg, there was no significant difference in leaflets incubated at 0 mmHg. There was no significant difference between time intervals of leaflets incubated at the same pressure.

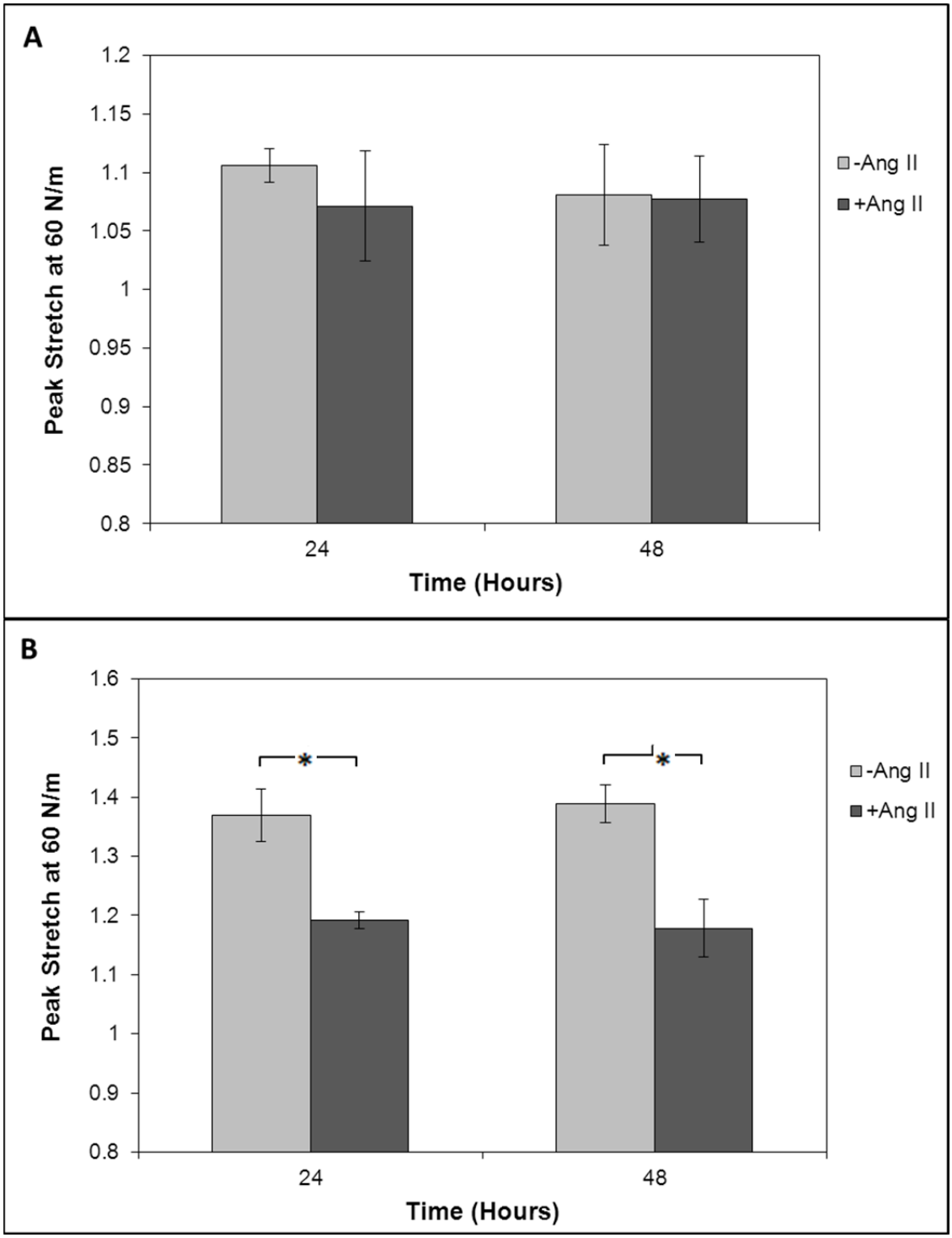

Figure 4.2 Peak stretch of aortic valve leaflet tissue with and without the presence of 10^{-6}M Ang II at 0 mmHg at various time intervals

A) Circumferential direction. B) Radial direction. Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

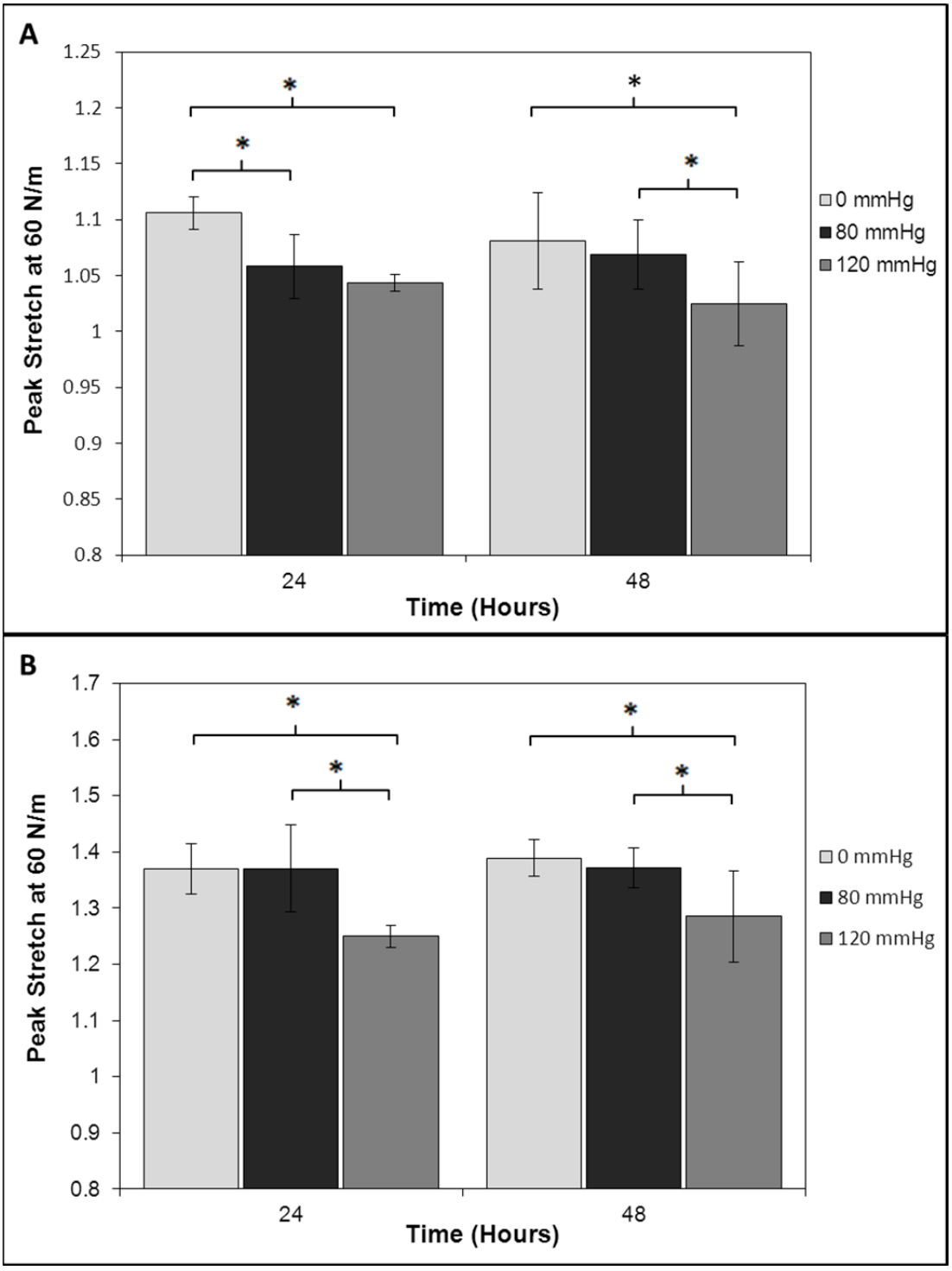

Figure 4.3 Peak stretch of aortic valve leaflet tissue at 0, 80, and 120 mmHg at various time intervals

A) Circumferential direction. B) Radial direction. Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

The combination of Ang II and the elevated pressure of 120 mmHg significantly reduced peak stretch in the circumferential and radial directions at both time intervals. In the circumferential direction, no significant difference in peak stretch occurred between the control and leaflets incubated with Ang II at 80 mmHg at either time intervals (Figure 4.4A). In addition, no significant difference was observed in leaflets incubated with Ang II between 80 and 120 mmHg at either time interval in the circumferential direction.

As can be observed in Figure 4.4B, both 80 and 120 mmHg in combination with Ang II significantly reduced peak stretch in the radial direction when compared to the control group at both time intervals. There was no significant difference between leaflets incubated with Ang II in tandem with 120 mmHg when compared to those with Ang II in combination with 80 mmHg.

In the circumferential direction, there was no significant interaction between Ang II and pressure. However, an interaction in between Ang II and pressure was found in the radial direction as an increase in pressure had an attenuating effect on Ang II.

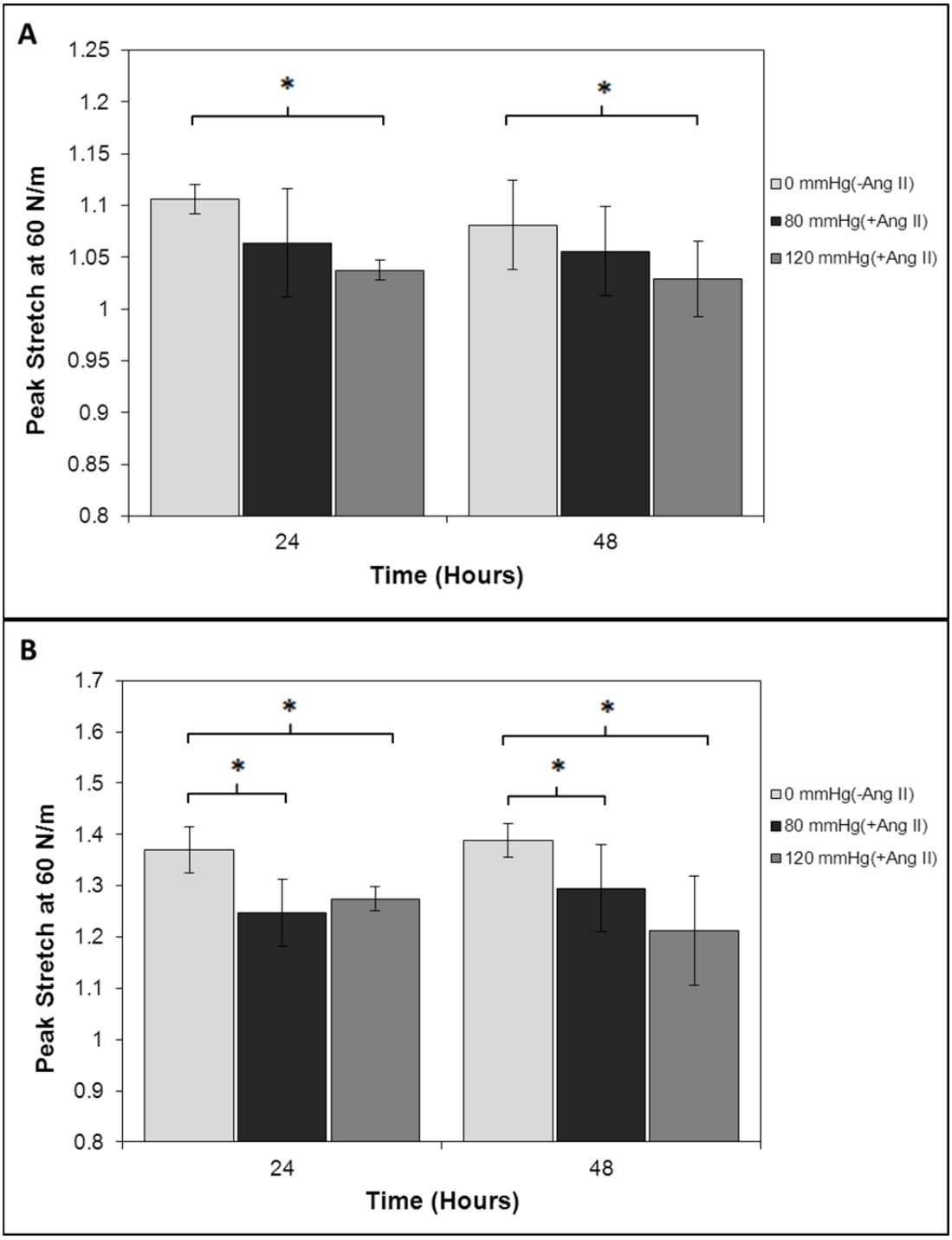

Figure 4.4 Peak stretch of aortic valve leaflet tissue at 80 and 120 mmHg incubated with Ang II compared to control incubated at 0 mmHg without Ang II at various time intervals

A) Circumferential direction. B) Radial direction. Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

Collagen Content

Leaflets incubated at 0 mmHg and 80 mmHg had statistically similar ($p<0.05$) collagen content when compared to fresh controls (21.77±4.84 µg/mg of dry weight) at both time intervals as shown in Figure 4.5.

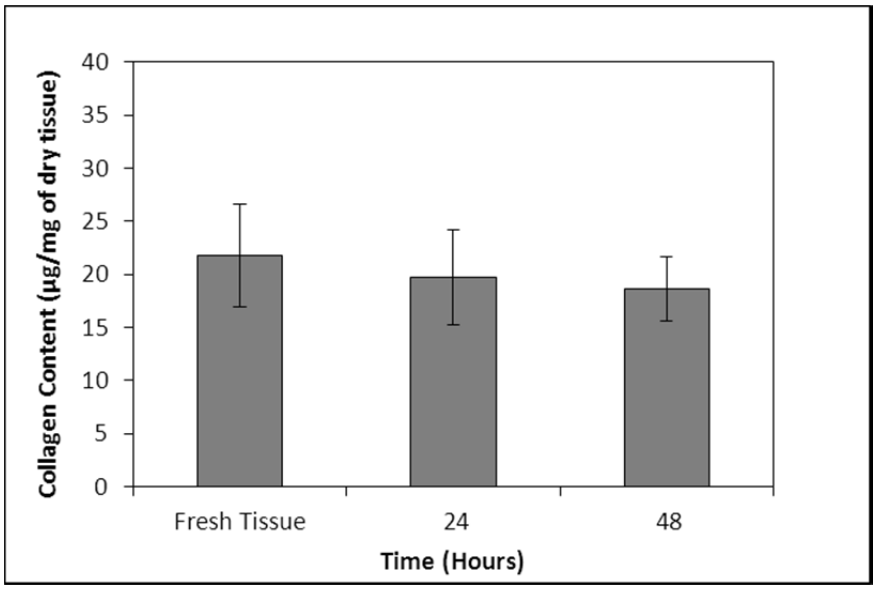

Figure 4.5 Collagen content of aortic valve leaflets at various time intervals Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

When leaflets were incubated with 10^{-6}M Ang II, the collagen content was significantly greater than those incubated under the same pressure condition without Ang II at both time intervals (Figure 4.6). At both time points, the collagen contents of leaflets incubated with Ang II at 80 mmHg and 120 mmHg were significantly greater than those at 0 mmHg without Ang II. The collagen content of leaflets incubated with Ang II at 24 hours was significantly similar to those without Ang II under the same pressure condition after 48 hours.

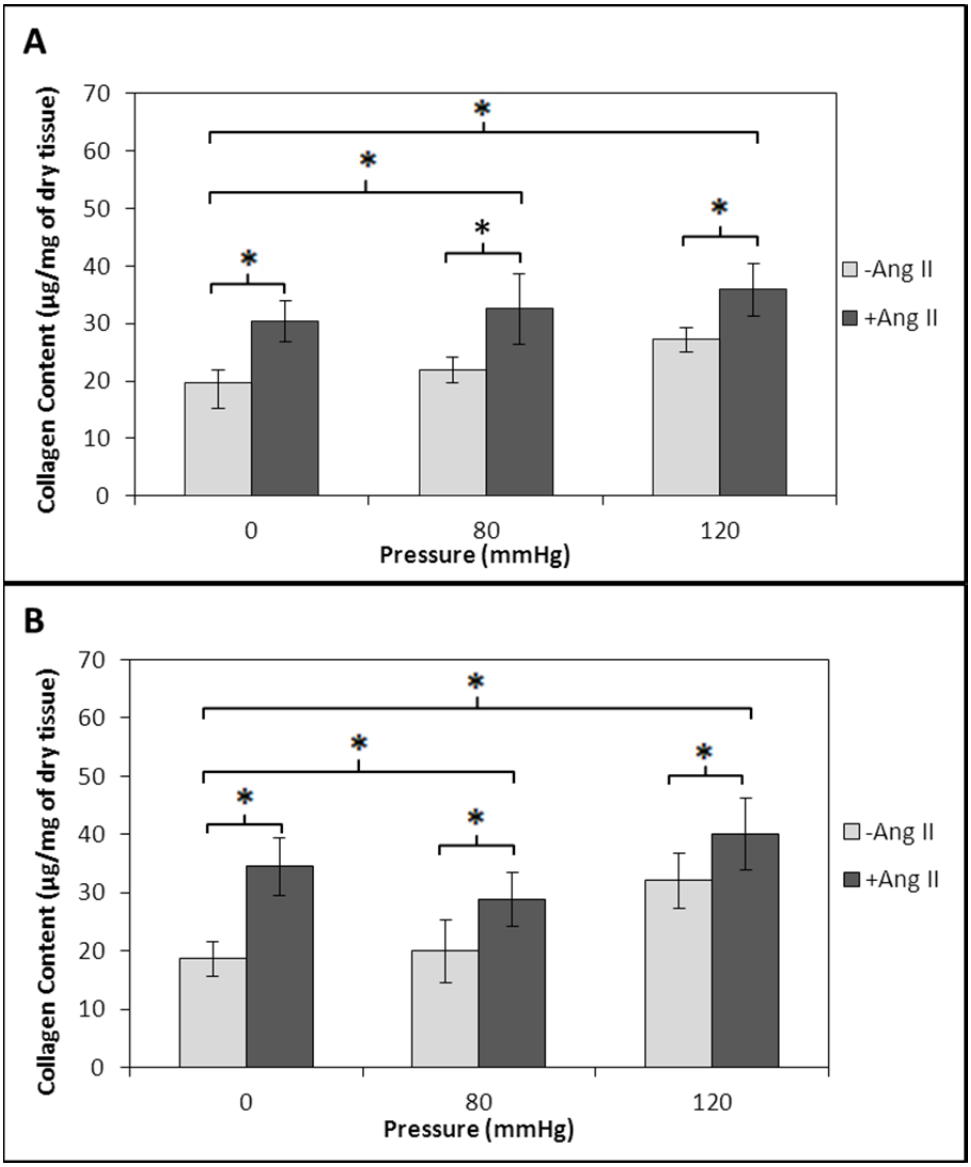

Figure 4.6 Collagen content of leaflet tissue at 0, 80 and 120 mmHg incubated with Ang II compared to those incubated without Ang II under same pressure condition and to 0 mmHg (-Ang II) controls

A) 24 hour interval. B) 48 hour interval. Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

As can be seen in Figure 4.7, the collagen content of leaflet tissue incubated at 80 mmHg were stastically simimlar ($p<0.05$) to those at 0 mmHg at 24 and 48 hours. There was a significant increase in collagen content in tissuse incubated at 120 mmHg when

compared to those at 0 and 80 mmHg at both time intervals. At 48 hours, there was a significant increase in tissue incubated at 120 mmHg when compared to 24 hours under the same pressure conditon. No significant interaction was observed between Ang II and pressure.

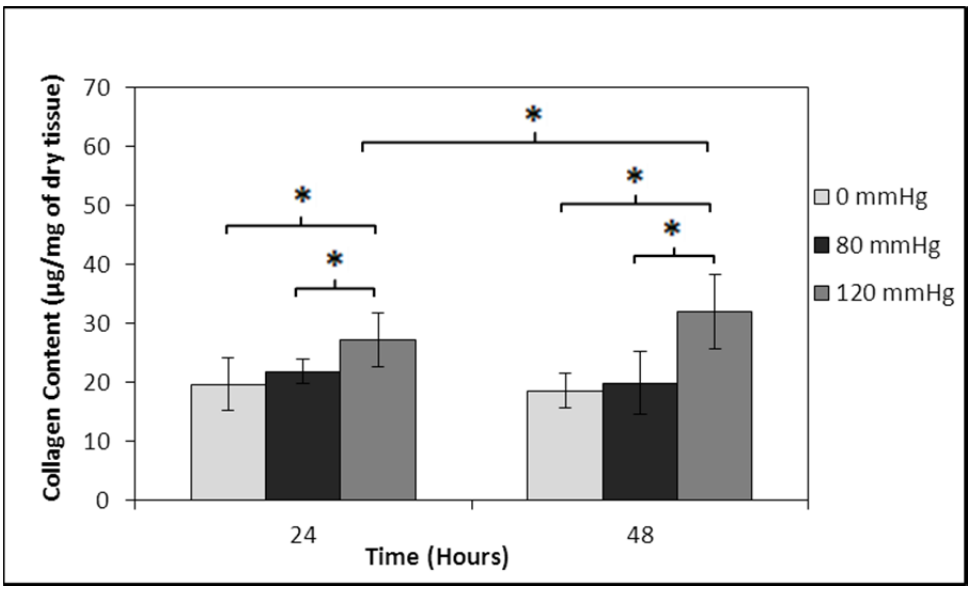

Figure 4.7 Collagen content of leaflet tissue at 0, 80, and 120 mmHg at 24 and 48 hour intervals

Error bars represent standard deviation (n=6). *denotes significant difference ($p<0.05$).

Cell Mechanics

As can be observed in Figure 4.8, the Young's modulus of VICs incubated at 120 mmHg was significantly lower than those incubated at 0 and 80 mmHg. There was no significant difference between cells incubated at 0 and 80 mmHg.

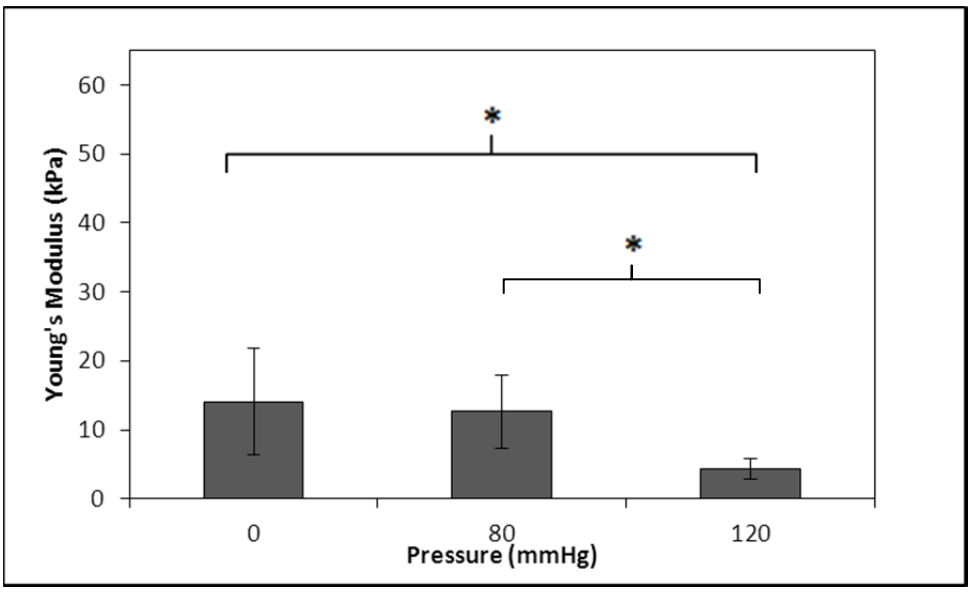

Figure 4.8 Young's moduli of aortic valve interstitial cells after 24 at 0, 80, and 120 mmHg

Error bars represent standard deviation (n=7). *denotes a significant difference ($p<0.05$).

ANOVA results demonstrate that VICs incubated with Ang II had a significantly higher Young's modulus than those without Ang II at the same pressures (Figure 4.9). VICs incubated in Ang II had a significantly higher modulus than those without the vasoactive agent at 0 mmHg and 80 mmHg after 24 hours. There was no significant difference between VICs incubated with Ang II at 80 mmHg verses those incubated without Ang II at the same pressure. A significant interaction was observed between Ang II and pressure as normal pressure had an attenuating effect on Ang II.

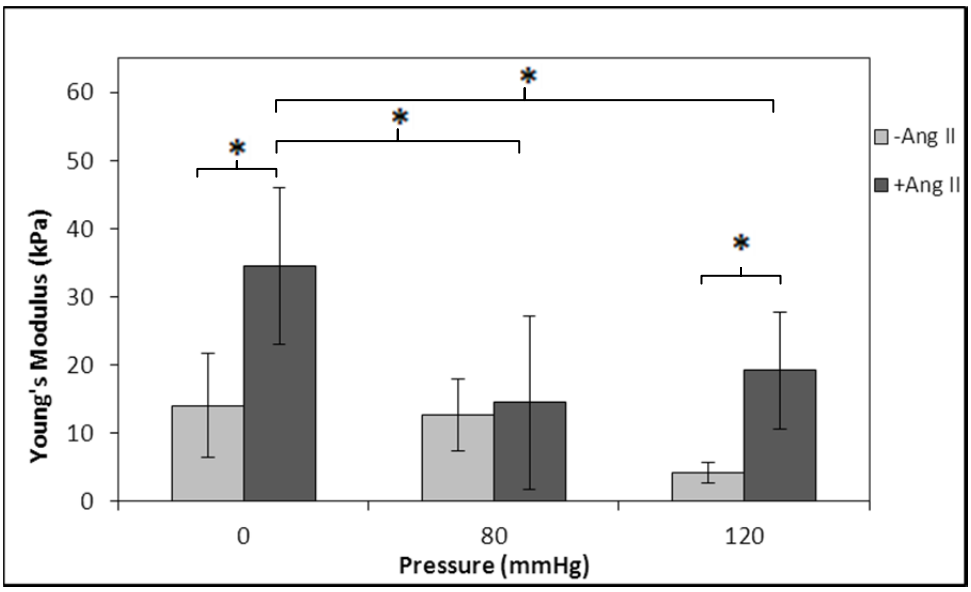

Figure 4.9 Young's moduli of aortic valve interstitial cells after 24 hours at 0, 80, and 120 mmHg with and without the presence of Ang II

Error bars represent standard deviation (n=7). *denotes significant difference ($p<0.05$).

CHAPTER V

DISCUSSION

Blood pressure is a critical factor in the harsh mechanical environment of the aortic valve. Valve leaflets must endure dynamic and complex mechanical forces generated by TVP gradients during the cardiac cycle. The biomechanical properties of the aortic valve leaflet are therefore critical for proper valve function. A decrease in leaflet extensibility may provide protection from prolapse that often occurs as a result of hypertension and aortic valve disease. However, an excessive increase in collagen production may be damaging to the valve and cause a progression of valve disease. Ang II and elevated pressure have both been implicated in valve disease and collagen synthesis. The results demonstrate that elevated pressure and Ang II are critical to and can alter the biomechanical properties of aortic valve leaflet tissue.

The results of the current study support previous findings [11,44,88] as the peak stretch of fresh leaflet tissue at 60 N/m was greater in the radial direction than in the circumferential direction. Primarily circumferentially-oriented collagen fibers in the fibrosa layer are the major load-bearing component of the leaflet. When the leaflet is stretched in the circumferential direction, collagen fibers align and the tension in the fibers resist further stretching. When the leaflet is stretched in the radial direction, collagen fibers separate and provide less resistance [88]. It is this architecture that largely

defines the distinct responses to biaxial tension in the circumferential and radial directions.

After 24 hours, leaflets incubated with 10^{-6} M Ang II at 0 mmHg (static atmospheric pressure) had a significantly reduced peak stretch in the radial direction at both time intervals when compared to leaflets incubated without the vasoactive agent. The reduction in peak stretch shows a reduction of extensibility of the leaflet due to the presence of Ang II. In previous studies [71,89], Ang II has been shown to increase collagen synthesis. Ang II exerts its effects on interstitial fibroblasts in the leaflet through AT_1 receptors [49] and the induction of the transforming growth factor, TGF-β1 [70,90]. Fibroblasts in healthy leaflets do not express AT_1 receptors; the receptors are expressed by fibroblast lesions. The receptor is activated when Ang II is present [49]. The decrease in extensibility exhibited by the leaflet inversely relates to the significant increase in collagen content observed in leaflets incubated with 10^{-6} M Ang II at static atmospheric pressure when compared to those without Ang II. In addition to collagen synthesis, Ang II has also been shown to mediate collagen degradation in human [91] and rat [92] cardiac fibroblasts by decelerating interstitial collagenase activity via TIMP-1 production in endothelial cells [93]. This finding implies that an increase in collagen content caused the decrease in leaflet extensibility by initiating collagen synthesis and/or inhibiting collagenase activity.

Although extensibility decreased in the radial direction after 24 hours, leaflets incubated with and without Ang II were statistically similar in the circumferential direction. A previous study reported that the vasoactive agent, 5-hydroxytryptamine, increased the elastic modulus of aortic valve leaflet tissue in the circumferential direction,

while Ang II caused an increase in the radial direction [84]. In addition, a study by Simmons et al., demonstrated that gene expression can be side-dependent on porcine aortic valves. The results of the current study may be related to side-specific response mechanisms of Ang II on the leaflet tissue. The effect of Ang II on collagen production may be specific to the ventricularis layer of the valve. The collagen fibers in the fibrosa are primary oriented in the circumferential direction, however, the ventricularis contains radially-aligned collagen fibers [24,27]. In addition, calcific aortic valve disease may cause the disorganization and fragmentation of collagen fibers in the ventricularis and fibrosa layers [35]. Disorganization and/or an increase in collagen production in radially-oriented fibers in the ventricularis may explain the decrease in extensibility found only in the radial direction.

Biaxial testing shows that the cyclic pressure of 120 mmHg (hypertensive pressure) caused a significant reduction in leaflet extensibility in the circumferential and radial directions at both time intervals. These findings highlight an inverse relationship between extensibility and the increase in collagen content in leaflets exposed to hypertensive pressure when compared to those at static atmospheric pressure. The results are in agreement with a previous study investigating the effects of cyclic and static pressure on aortic valve leaflets that showed that hypertensive pressure caused a proportional increase in collagen synthesis in leaflet tissue [85]. Elevated pressure exerts compressive force on the leaflets [83]. As collagen is the main load bearer of the force created by pressure, it is plausible that higher pressure causes a greater need for collagen in order to adapt to harsher mechanical conditions. Multiple mechanisms have been implicated in the aortic valve's response to pressure. Elevated pressure may cause

changes collagen content in the leaflet by direct mechanotransduction. In addition, pressure may affect the ECM synthesis indirectly by utilizing paracrine factors released by endothelial cells on the leaflet surface [85].

After 48 hours, leaflets incubated at the cyclic pressure of 80 mmHg (normotensive pressure) had similar extensibility to those incubated at static atmospheric pressure in both directions. These results follow the trend of collagen content findings under the same conditions. After 24 hours, the extensibility of leaflets incubated at normotensive pressure was less than those incubated at static atmospheric pressure in the circumferential direction. These results do not agree with collagen content findings. The collagen content in leaflets incubated at normotensive pressure was statistically similar to those incubated at static atmospheric pressure for 24 hours. However, in the radial direction, leaflets incubated at normotensive pressure were significantly similar in extensibility and collagen content when compared to static atmospheric pressure after 24 hours. While these results imply that collagen content may not be the only factor in the leaflets' response to a change in cyclic pressure, it is important to note that the collagen assay used in the current study only accounts for pepsin soluble collagen. Salt soluble, acid soluble, and insoluble collagen [88] may play a role in leaflet tissue extensibility. In addition, the findings introduce the possibility of cyclic pressure playing specific roles in the extensibility of tissue in individual layers of the leaflet.

The combination of Ang II and hypertensive pressure significantly reduced extensibility in the circumferential and radial directions after 24 and 48 hours. These results inversely relate to collagen content findings under the same conditions. In addition, the extensibility of leaflets exposed to the combination of Ang II and

hypertensive pressure did not significantly differ from those exposed to hypertensive pressure alone in either direction. In the circumferential direction, the extensibility of leaflets incubated with Ang II at normotensive pressure was statistically similar to those incubated without Ang II at static atmospheric pressure at both time intervals. These findings are in agreement with results that showed Ang II had no effect on extensibility in the circumferential direction. These findings also agree with results that indicated normotensive pressure had little effect on the extensibility of leaflets when compared to those at static atmospheric pressure.

In the radial direction, leaflets incubated at normotensive pressure with Ang II showed less extensibility than those at static atmospheric pressure without the presence of Ang II at both time intervals. These results are in agreement with findings that show Ang II alone was capable of decreasing leaflet tissue extensibility in the radial direction. Leaflets incubated at normotensive pressure with Ang II were statistically similar to those at hypertensive pressure with Ang II at both time points in the radial direction. These findings indicate that the presence of Ang II had more of an effect on leaflet tissue extensibility in the radial direction than hypertensive pressure when used in combination. Normotensive pressure in combination with Ang II had no effect on extensibility in the circumferential direction while it did produce a reduction in extensibility in the radial direction. This finding further suggests a side-specific response to Ang II. The collagen content of leaflets incubated at normotensive pressure with the addition of Ang II was significantly greater than those at static atmospheric pressure without Ang II at both time points. These findings further imply that disorganized collagen fibers and collagen of

different stages may play a role in the leaflets' response to cyclic pressure and Ang II [35,88].

Ang II proved to have more influence on the extensibility of leaflet tissue than hypertensive pressure in the radial direction. However, there was no significant difference in collagen content between leaflets incubated at static atmospheric pressure in the presence of Ang II verses those incubated at hypertensive pressure without Ang II. These findings suggest that Ang II may affect more than collagen synthesis alone in aortic valve leaflets. Previous studies have reported that Ang II can elicit contractions in arterial smooth muscle cells [59,66] and equine aortic valve leaflets [94]. However, it is unclear if interstitial cell contractions could be responsible for a significant increase in extensibility after the prolonged periods 24 and 48 hours.

Overall, peak stretch was statistically similar after 48 hours when compared to 24 hours in both directions. With the exception of leaflets exposed to hypertensive pressure without the presence of Ang II, the collagen contents of leaflets incubated for 24 hours were statistically similar to those incubated for 48 hours under the same conditions. The net collagen content of the leaflet tissue is regulated by the balance between newly synthesized and degraded collagen. The amount of collagenases such as MMPs and cathepsin L in the ECM has been shown to be affected by hypertensive pressure [95]. In leaflets incubated with Ang II, the increase after the initial 24 hour incubation may be due to collagen synthesis occurring at a faster rate than degradation. After 48 hours of incubation, MMPs may increase in an attempt restore homeostasis in the ECM resulting in a similar net amount of collagen.

The VICs of aortic leaflets play a major role in the remodeling and organization of the ECM. Altered mechanical and/or biochemical conditions on the leaflet to the VICs may cause a series of changes in the composition and structure of the ECM, potentially modifying the functionality of the leaflet [96,97].

Cell elasticity is primarily the result of the intrinsic properties of the cell membrane and cytoskeleton. Intracellular components of the cytoskeleton include actin fibers, intermediate fibers, and microtubules. The reorganization of cell organelles and these structural components each play a role in cell stiffness [98,99]. The Young's modulus of VICs incubated under hypertensive pressure was significantly lower than those incubated at static atmospheric and normotensive pressures. These results contrast with those stating that cell stiffness increases in proportion to the level applied mechanical stress [15,100]. However, little is known regarding aortic VICs response to altered TVP. The current results also contrast with a study by Merryman et al. that reported VICs from the pulmonary valve were less stiff than those isolated from the aortic valve [15]. The stiffness of the cells showed a positive correlation to the transvalvular pressures of the valves. These findings suggest that an increase in TVP causes increased VIC stiffness. In the current study, the VICs were exposed to altered mechanical and biochemical conditions for 24 hours. The suddenly altered environment may have caused different responses in the cytoskeleton than those of extended time in normal hemodynamic environments. In addition, the multiple cell types found in the ECM of the aortic valve and their individual responses to pressure may also play a critical role in the overall VIC stiffness [98] under normotensive and hypertensive pressure conditions.

Following a trend similar to hypertensive pressure alone, VICs incubated with Ang II at hypertensive pressure also displayed a lower modulus than those incubated with Ang II at static atmospheric pressure. However, the stiffness of cells incubated with Ang II was greater than those incubated without the vasoactive agent under the same pressure conditions. These results imply that the addition of biochemical hypertensive conditions may have altered the intracellular components and/or cell membrane of the VICs to a configuration that provided greater cell stiffness.

VICs did not follow the general trends of the biaxial and collagen content results. The VICs exposed to static atmospheric pressure showed a decrease in stiffness when compared to hypertensive pressure alone and in combination with Ang II. The results suggest an inverse relationship between leaflet stiffness and VIC stiffness. Studies have also shown that apoptosis occurs more in statically cultured conditions than in ex vivo organ culture systems that simulate aortic valve hemodynamic environments [101]. In addition, studies have suggested that apoptosis occurs proportional to the level of applied stress in cells [102]. These results imply that a lack of native mechanical stimulation may be detrimental to health of VICs. An apoptotic response to increased cyclic pressure with and without the presence of Ang II may play a role in the stiffness of VIC.

Another important factor to consider in these findings is the lack of ECM and VECs in VIC culture. VECs on the surface of the leaflet are responsible for detecting variations in the mechanical and biochemical environments of the aortic valve. The cells translate mechanical and biochemical stimuli into signals to communicate with VICs in the ECM. The communication between VECs and VICs plays a critical role in the

homeostasis of the ECM [14,35,103]. A disruption in normal cell-to-cell communication may affect VIC response to hypertensive mechanical and biochemical conditions.

A major limitation to this study was the lack of additional mechanical forces such as stretch and shear that are normally experienced by the aortic valve hypertensive conditions. Stretch has been shown to induce cell proliferation, apoptosis, collagen synthesis and changes in valve biomechanical properties [43]. Shear stress regulates common features of aortic valve disease such as inflammation and calcification [2]. These mechanical forces affect the composition and homeostasis of the ECM.

CHAPTER VI

CONCLUSIONS AND FUTURE STUDIES

Conclusion

The focus of this study was on the effects of elevated cyclic pressure and Ang II on the biomechanical properties of aortic valve leaflets. Changes in aortic valve biomechanical properties may give insight to the ability to withstand altered biochemical, mechanical, and hemodynamic forces associated with hypertension. In the current study, Ang II and elevated pressure have affected the biomechanical properties and ECM composition of the aortic valve leaflet. Results show that elevated cyclic pressure reduced the extensibility of leaflet tissue. Ang II produced direction-specific changes in leaflet extensibility, implying that different mechanisms are utilized in the layers of the leaflet. Elevated pressure and Ang II produced increases in collagen content in the ECM when used independently and in tandem. A decrease in VIC stiffness was noted when exposed to elevated pressure and Ang II. These findings may aid in the understanding of adaptive responses of aortic valve biomechanical properties and provide insight to create more successful pharmacological approaches to prevent and treat aortic valve disease.

Future Studies

The current study shows that elevated pressure and Ang II affect the Young's modulus of VICs. However, the science as to how hypertensive conditions affect cell

stiffness remains unclear. A further approach might be to determine the effects of elevated pressure and Ang II on the cytoskeleton of aortic VICs. This information may provide a better understanding of how hypertensive conditions affect the Young's modulus of VICs. Further work may also be done on the understanding of the signaling pathways that are important in VEC to VIC mechanotransduction. VECs on the surface of the leaflet are sensitive to hemodynamic and mechanical changes in their environment. Their primary functions are to sense and communicate stimuli to the VICs. A better understanding of VEC-VIC communication in in response to elevated pressure and Ang II may provide a better understanding of the cells' role in calcific aortic valve disease.

In addition, future work on the signaling pathways of Ang II associated with aortic valve calcification would be beneficial to understanding the direction specific response in extensibility of Ang II.

REFERENCES

[1] Misfeld M., and Sievers H.-H., 2007, "Heart valve macro- and microstructure.," Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, **362**(1484), pp. 1421–36.

[2] Balachandran K., Sucosky P., and Yoganathan A. P., 2011, "Hemodynamics and mechanobiology of aortic valve inflammation and calcification.," International Journal of Inflammation, **2011**, p. 263870.

[3] Roger V. L., Go A. S., Lloyd-Jones D. M., Adams R. J., Berry J. D., Brown T. M., Carnethon M. R., Dai S., de Simone G., Ford E. S., Fox C. S., Fullerton H. J., Gillespie C., Greenlund K. J., Hailpern S. M., Heit J. a, Ho P. M., Howard V. J., Kissela B. M., Kittner S. J., Lackland D. T., Lichtman J. H., Lisabeth L. D., Makuc D. M., Marcus G. M., Marelli A., Matchar D. B., McDermott M. M., Meigs J. B., Moy C. S., Mozaffarian D., Mussolino M. E., Nichol G., Paynter N. P., Rosamond W. D., Sorlie P. D., Stafford R. S., Turan T. N., Turner M. B., Wong N. D., and Wylie-Rosett J., 2011, "Heart disease and stroke statistics--2011 update: a report from the American Heart Association.," Circulation, **123**(4), pp. e18–e209.

[4] Roberts W. C., and Ko J. M., 2005, "Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation.," Circulation, **111**(7), pp. 920–5.

[5] Mihaljevic T., Sayeed M. R., Stamou S. C., and Paul S., 2008, "Pathophysiology of Aortic Valve Disease," Cardiac Surgery in the Adult, **3**(2008), p. 825.

[6] Anderson R. H., 2000, "Clinical anatomy of the aortic root.," Heart (British Cardiac Society), **84**(6), pp. 670–3.

[7] Sacks M. S., Smith D. B., and Hiester E. D., 1998, "The aortic valve microstructure: effects of transvalvular pressure," Journal of Biomedical Materials Research, **41**(1), pp. 131–41.

[8] Bronzino J. D., ed., 2006, "Biomedical Engineering Fundamentals," Biomedical Engineering Fundamentals, CRC/Taylor & Francis, pp. 552–557.

[9] Tseng H., and Grande-Allen K. J., 2011, "Elastic fibers in the aortic valve spongiosa: a fresh perspective on its structure and role in overall tissue function.," Acta Biomaterialia, **7**(5), pp. 2101–8.

[10] El-Hamamsy I., Chester A. H., and Yacoub M. H., 2010, "Cellular regulation of the structure and function of aortic valves," Journal of Advanced Research, **1**(1), pp. 5–12.

[11] Stella J. a, and Sacks M. S., 2007, "On the biaxial mechanical properties of the layers of the aortic valve leaflet.," J Biomech Eng, **129**(5), pp. 757–66.

[12] Sacks M. S., David Merryman W., and Schmidt D. E., 2009, "On the biomechanics of heart valve function.," Journal of Biomechanics, **42**(12), pp. 1804–24.

[13] Simmons C. a, Grant G. R., Manduchi E., and Davies P. F., 2005, "Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves.," Circulation Research, **96**(7), pp. 792–9.

[14] Butcher J. T., Tressel S., Johnson T., Turner D., Sorescu G., Jo H., and Nerem R. M., 2006, "Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences: influence of shear stress.," Arteriosclerosis, Thrombosis, and Vascular Biology, **26**(1), pp. 69–77.

[15] Merryman W. D., Youn I., Lukoff H. D., Krueger P. M., Guilak F., Hopkins R. A., Sacks M. S., David W., Krueger M., and Michael S., 2006, "Correlation between heart valve interstitial cell stiffness and transvalvular pressure□: implications for collagen biosynthesis," American Journal of Physiology-Heart and Circulatory Physiology, **290**, pp. 224–231.

[16] Marron K., Yacoub M. H., Polak J. M., Sheppard M. N., Fagan D., Whitehead B. F., de Leval M. R., Anderson R. H., and Wharton J., 1996, "Innervation of Human Atrioventricular and Arterial Valves," Circulation, **94**(3), pp. 368–375.

[17] Rabkin-Aikawa E., Farber M., Aikawa M., and Schoen F. J., 2004, "Dynamic and reversible changes of interstitial cell phenotype during remodeling of cardiac valves.," The Journal of Heart Valve Disease, **13**(5), pp. 841–7.

[18] Davies P. F., 1997, "Mechanisms involved in endothelial responses to hemodynamic forces," Atherosclerosis 131 Suppl. S-15-S17.

[19] Li C., Xu S., and Gotlieb A. I., 2013, "The progression of calcific aortic valve disease through injury, cell dysfunction, and disruptive biologic and physical force feedback loops.," Cardiovascular pathology□: The Official Journal of the Society for Cardiovascular Pathology, **22**(1), pp. 1–8.

[20] Taylor P. M., Batten P., Brand N. J., Thomas P. S., and Yacoub M. H., 2003, "The cardiac valve interstitial cell.," The International Journal of Biochemistry & Cell Biology, **35**(2), pp. 113–8.

[21] Engelmayr G. C., Hildebrand D. K., Sutherland F. W. ., Mayer J. E., and Sacks M. S., 2003, "A novel bioreactor for the dynamic flexural stimulation of tissue engineered heart valve biomaterials," Biomaterials, **24**(14), pp. 2523–2532.

[22] Thubrikar M., Piepgrass W. C., Deck J. D., and Nolan S. P., 1980, "Stresses of natural versus prosthetic aortic valve leaflets in vivo," The Annals of Thoracic Surgery, **30**(3), pp. 230–239.

[23] Vesely I., 1998, "The role of elastin in aortic valve mechanics.," Journal of Biomechanics, **31**(2), pp. 115–23.

[24] Vesely I., and Noseworthy R., 1992, "Micromechanics of the fibrosa and the ventricularis in aortic valve leaflets.," Journal of Biomechanics, **25**(1), pp. 101–13.

[25] Merryman W. D., Huang H.-Y. S., Schoen F. J., and Sacks M. S., 2006, "The effects of cellular contraction on aortic valve leaflet flexural stiffness.," Journal of Biomechanics, **39**(1), pp. 88–96.

[26] Mirnajafi A., Raymer J. M., McClure L. R., and Sacks M. S., 2006, "The flexural rigidity of the aortic valve leaflet in the commissural region.," Journal of Biomechanics, **39**(16), pp. 2966–73.

[27] Vesely I., and Noseworthy R., 1990, "Functional Interactions Between the Fibrosa and the Ventricularis of Aortic Valve Leaflets," Annual International Conference of the IEEE Engineering in Medicine and Biology Society, **12**(5), pp. 2080–2081.

[28] Sacks M. S., and Yoganathan A. P., 2007, "Heart valve function: a biomechanical perspective.," Philosophical transactions of the Royal Society of London. Series B, Biological Sciences, **362**(1484), pp. 1369–91.

[29] Christie G. W., and Barratt-boyes B. G., 1995, "Biaxial mechanical leaflets of explanted," Ann Thorac Surg, **60**, pp. S160–4.

[30] Billiar K. L., and Sacks M. S., 2000, "Biaxial Mechanical Properties of the Native and Glutaraldehyde- Treated Aortic Valve Cusp□: Part II — A Structural Constitutive," J Biomech Eng, **122**, pp. 327–335.

[31] Billiar K. L., and Sacks M. S., 2000, "Biaxial Mechanical Properties of the Native and Glutaraldehyde- Treated Aortic Valve Cusp-Part I: Experimental Results," Journal of Biomechanical Engineering, **122**(1), pp. 23–30.

[32] Anssari-Benam A., Bader D. L., and Screen H. R. C., 2011, "Anisotropic time-dependant behaviour of the aortic valve.," Journal of the Mechanical Behavior of Biomedical Materials, **4**(8), pp. 1603–10.

[33] Carew E. O., Garg A., Barber J. E., and Vesely I., 2004, "Stress relaxation preconditioning of porcine aortic valves.," Annals of Biomedical Engineering, **32**(4), pp. 563–72.

[34] Stella J. a, Liao J., and Sacks M. S., 2007, "Time-dependent biaxial mechanical behavior of the aortic heart valve leaflet," Journal of Biomechanics, **40**(14), pp. 3169–77.

[35] Chen J.-H., and Simmons C. a, 2011, "Cell-matrix interactions in the pathobiology of calcific aortic valve disease: critical roles for matricellular, matricrine, and matrix mechanics cues.," Circulation Research, **108**(12), pp. 1510–24.

[36] Mohler E. R., Gannon F., Reynolds C., Zimmerman R., Keane M. G., and Kaplan F. S., 2001, "Bone formation and inflammation in cardiac valves," Circulation, **103**(11), pp. 1522–1528.

[37] Butcher J. T., and Nerem R. M., 2007, "Valvular endothelial cells and the mechanoregulation of valvular pathology.," Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, **362**(1484), pp. 1445–57.

[38] Dasi L. P., Sucosky P., de Zelicourt D., Sundareswaran K., Jimenez J., and Yoganathan A. P., 2009, "Advances in cardiovascular fluid mechanics: bench to bedside.," Annals of the New York Academy of Sciences, **1161**, pp. 1–25.

[39] Yoganathan A. P., He Z., and Casey Jones S., 2004, "Fluid mechanics of heart valves.," Annual Review of Biomedical Engineering, **6**, pp. 331–62.

[40] Weston M. W., LaBorde D. V., and Yoganathan a P., 1999, "Estimation of the shear stress on the surface of an aortic valve leaflet.," Annals of Biomedical Engineering, **27**(4), pp. 572–9.

[41] Butcher J. T., Tressel S., Johnson T., Turner D., Sorescu G., Jo H., and Nerem R. M., 2006, "Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences: influence of shear stress.," Arteriosclerosis, Thrombosis, and Vascular Biology, **26**(1), pp. 69–77.

[42] Rabkin S. W., 2005, "The association of hypertension and aortic valve sclerosis.," Blood Pressure, **14**(5), pp. 264–72.

[43] Balachandran K., Konduri S., Sucosky P., Jo H., and Yoganathan A. P., 2006, "An ex vivo study of the biological properties of porcine aortic valves in response to circumferential cyclic stretch.," Annals of Biomedical Engineering, **34**(11), pp. 1655–65.

[44] Thubrikar M., Aouad J., and Nolan S., 1986, "Comparison of the in vivo and in vitro Mechanical Properties of Aortic Valve Leaflets," The Journal of Thoracic and Cardiovascular Surgery, **92**(1), pp. 29–36.

[45] Otto C. M., 2008, "editorials Calcific Aortic Stenosis — Time to Look More Closely at the Valve," N Engl J Med, pp. 1395–1398.

[46] Chuangsuwanich T., Warnnissorn M., Leksrisakul P., Laksanabunsong P., Thongcharoen P., and Sahasakul Y., 2004, "Pathology and etiology of 110 consecutively removed aortic valves.," Journal of the Medical Association of Thailand- Chotmaihet Thangphaet, **87**(8), pp. 921–34.

[47] Tzemos N., Therrien J., Thanassoulis G., Tremblay S., Jamorski M. T., Webb G. D., and Siu S. C., 2008, "With Bicuspid Aortic Valves," American Medical Association, **300**(11), pp. 1317–1325.

[48] Butcher J. T., Mahler G. J., and Hockaday L. a, 2011, "Aortic valve disease and treatment: the need for naturally engineered solutions.," Advanced Drug Delivery Reviews, **63**(4-5), pp. 242–68.

[49] O'Brien K. D., 2006, "Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more).," Arteriosclerosis, Thrombosis, and Vascular Biology, **26**(8), pp. 1721–8.

[50] O'Brien K. D., 2006, "Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more).," Arteriosclerosis, Thrombosis, and Vascular Biology, **26**(8), pp. 1721–8.

[51] Rajamannan N. M., Subramaniam M., Rickard D., Stock S. R., Donovan J., Springett M., Orszulak T., Fullerton D. a, Tajik a J., Bonow R. O., and Spelsberg T., 2003, "Human aortic valve calcification is associated with an osteoblast phenotype.," Circulation, **107**(17), pp. 2181–4.

[52] Fondard O., Detaint D., Iung B., Choqueux C., Adle-Biassette H., Jarraya M., Hvass U., Couetil J.-P., Henin D., Michel J.-B., Vahanian A., and Jacob M.-P., 2005, "Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors.," European Heart Journal, **26**(13), pp. 1333–41.

[53] Bosman F. T., and Stamenkovic I., 2003, "Functional structure and composition of the extracellular matrix.," The Journal of Pathology, **200**(4), pp. 423–8.

[54] Mohler E. R., 2004, “Mechanisms of aortic valve calcification.,” The American Journal of Cardiology, **94**(11), pp. 1396–402, A6.

[55] Chester A. H., 2011, “Molecular and cellular mechanisms of valve calcification,” Aswan Heart Centre Science & Practice Series, **2011**(1), p. 4.

[56] Stewart B. F., Siscovick D., Lind B. K., Gardin J. M., Gottdiener J. S., Smith V. E., Kitzman D. W., Otto C. M., The F. O. R., and Ealth C. A. H., 1997, “Clinical Factors Associated With Calcific Aortic Valve Disease,” Journal of the American College of Cardiology, **29**(3), pp. 630–634.

[57] Rizzoni D., Muiesan M. L., Porteri E., Salvetti M., Castellano M., Bettoni G., Tiberio G., Giulini S. M., Monteduro C., Garavelli G., and Agabiti-Rosei E., 1998, “Relations between cardiac and vascular structure in patients with primary and secondary hypertension.,” Journal of the American College of Cardiology, **32**(4), pp. 985–92.

[58] Carretero O. a., and Oparil S., 2000, “Essential Hypertension□: Part I: Definition and Etiology,” Circulation, **101**(3), pp. 329–335.

[59] Reid I. A., 1998, “The Renin-Angiotensin System□: Physiology, Pathophysiology, And Pharmacology The Renin-Angiotensin System,” Advances in Physiology Education, **20**(1), pp. S236–245.

[60] Touyz R. M., 2012, “New insights into mechanisms of hypertension.,” Current Opinion in Nephrology and Hypertension, **21**(2), pp. 119–21.

[61] Chobanian A. V., Bakris G. L., Black H. R., Cushman W. C., Green L. a, Izzo J. L., Jones D. W., Materson B. J., Oparil S., Wright J. T., and Roccella E. J., 2003, “Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.,” Hypertension, **42**(6), pp. 1206–52.

[62] Mathieu P., Poirier P., Pibarot P., Lemieux I., and Després J.-P., 2009, “Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease.,” Hypertension, **53**(4), pp. 577–84.

[63] Onusko E., 2003, “Diagnosing secondary hypertension.,” American Family Physician, **67**(1), pp. 67–74.

[64] Fielitz J., Hein S., Mitrovic V., Pregla R., Zurbrügg H. R., Warnecke C., Schaper J., Fleck E., and Regitz-Zagrosek V., 2001, “Activation of the cardiac renin-angiotensin system and increased myocardial collagen expression in human aortic valve disease.,” Journal of the American College of Cardiology, **37**(5), pp. 1443–9.

[65] Helske S., Lindstedt K. a, Laine M., Mäyränpää M., Werkkala K., Lommi J., Turto H., Kupari M., and Kovanen P. T., 2004, "Induction of local angiotensin II-producing systems in stenotic aortic valves.," Journal of the American College of Cardiology, **44**(9), pp. 1859–66.

[66] Mehta P. K., and Griendling K. K., 2007, "Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system.," American Journal of Cell Physiology, **292**(1), pp. C82–97.

[67] Rajamannan N. M., 2008, "Update on the pathophysiology of aortic stenosis," European Heart Journal Supplements, **10**(Suppl E), pp. E4–E10.

[68] Brecher P., 1996, "Angiotensin II and Cardiac Fibrosis.," Trends in Cardiovascular Medicine, **6**(6), pp. 193–8.

[69] Tharaux P.-L., Chatziantoniou C., Fakhouri F., and Dussaule J.-C., 2000, "Angiotensin II Activates Collagen I Gene Through a Mechanism Involving the MAP/ER Kinase Pathway," Hypertension, **36**(3), pp. 330–336.

[70] Kupfahl C., Pink D., Friedrich K., Zurbrügg H. R., Neuss M., Warnecke C., Fielitz J., Graf K., Fleck E., and Regitz-Zagrosek V., 2000, "Angiotensin II directly increases transforming growth factor beta1 and osteopontin and indirectly affects collagen mRNA expression in the human heart.," Cardiovascular Research, **46**(3), pp. 463–75.

[71] Ford C. M., Li S., and Pickering J. G., 1999, "Angiotensin II stimulates collagen synthesis in human vascular smooth muscle cells□: involvement of the AT1 receptor, transforming growth factor- , and tyrosine phosphorylation," Arteriosclerosis, Thrombosis, and Vascular Biology, **19**(8), pp. 1843–1851.

[72] Weber K. T., Swamynathan S. ., Guntaka R. V., and Sun Y., 1999, "Angiotensin II and extracellular matrix homeostasis," The International Journal of Biochemistry & Cell Biology, **31**(3-4), pp. 395–403.

[73] Ikonomidis J. S., Kratz J. M., Crumbley A. J., Stroud M. R., Bradley S. M., Sade R. M., and Crawford F. a, 2003, "Twenty-year experience with the St Jude Medical mechanical valve prosthesis.," The Journal of thoracic and cardiovascular surgery, **126**(6), pp. 2022–31.

[74] Schoen F. J., 2008, "Evolving concepts of cardiac valve dynamics: the continuum of development, functional structure, pathobiology, and tissue engineering.," Circulation, **118**(18), pp. 1864–80.

[75] Kouchoukos N. T., Masetti P., Nickerson N. J., Castner C. F., Shannon W. D., and Dávila-Román V. G., 2004, "The Ross procedure: long-term clinical and echocardiographic follow-up.," The Annals of Thoracic Surgery, **78**(3), pp. 773–81; discussion 773–81.

[76] Aslam A. K., Aslam A. F., Vasavada B. C., and Khan I. a, 2007, "Prosthetic heart valves: types and echocardiographic evaluation.," International Journal of Cardiology, **122**(2), pp. 99–110.

[77] Rahimtoola S. H., 2010, "Choice of prosthetic heart valve in adults an update.," Journal of the American College of Cardiology, **55**(22), pp. 2413–26.

[78] Cribier a., 2002, "Percutaneous Transcatheter Implantation of an Aortic Valve Prosthesis for Calcific Aortic Stenosis: First Human Case Description," Circulation, **106**(24), pp. 3006–3008.

[79] Webb J. G., 2008, "Percutaneous aortic valve replacement will become a common treatment for aortic valve disease.," JACC: Cardiovascular Interventions, **1**(2), pp. 122–6.

[80] Heinze H., Sier H., Schäfer U., and Heringlake M., 2010, "Percutaneous aortic valve replacement: overview and suggestions for anesthestic management.," Journal of Clinical Anesthesia, **22**(5), pp. 373–8.

[81] Toggweiler S., Gurvitch R., Leipsic J., Wood D. a, Willson A. B., Binder R. K., Cheung A., Ye J., and Webb J. G., 2012, "Percutaneous aortic valve replacement: vascular outcomes with a fully percutaneous procedure.," Journal of the American College of Cardiology, **59**(2), pp. 113–8.

[82] Yeow W. L., Roberts-Thomson P., Shetty S., and Yong G., 2012, "Expanding role for transcatheter aortic valve replacement: successful transfemoral implantation of a medtronic corevalve for severe aortic regurgitation.," Heart, Lung & Circulation, pp. 3–7.

[83] Yap C. H., Kim H.-S., Balachandran K., Weiler M., Haj-Ali R., and Yoganathan A. P., 2010, "Dynamic deformation characteristics of porcine aortic valve leaflet under normal and hypertensive conditions.," American Journal of Physiology-Heart and Circulatory Physiology, **298**(2), pp. H395–405.

[84] Warnock J. N., Gamez C. a P., Metzler S. a, Chen J., Elder S. H., and Liao J., 2010, "Vasoactive agents alter the biomechanical properties of aortic heart valve leaflets in a time-dependent manner.," The Journal of Heart Valve Disease, **19**(1), pp. 86–95; discussion 96.

[85] Xing Y., Warnock J. N., He Z., Hilbert S. L., and Yoganathan A. P., 2004, "Cyclic pressure affects the biological properties of porcine aortic valve leaflets in a magnitude and frequency dependent manner.," Annals of Biomedical Engineering, **32**(11), pp. 1461–70.

[86] Ikhumetse J. D., Konduri S., Warnock J. N., Xing Y., and Yoganathand A. P., 2006, "Cyclic aortic pressure affects the biological properties of porcine pulmonary valve leaflets.," The Journal of Heart Valve Disease, **15**(2), pp. 295–302.

[87] Grashow J. S., Yoganathan A. P., and Sacks M. S., 2006, "Biaixal stress-stretch behavior of the mitral valve anterior leaflet at physiologic strain rates.," Annals of Biomedical Engineering, **34**(2), pp. 315–25.

[88] Chow M.-J., and Zhang Y., 2011, "Changes in the mechanical and biochemical properties of aortic tissue due to cold storage.," The Journal of Surgical Research, **171**(2), pp. 434–42.

[89] Lijnen P. J., Prihadi J. S., Pelt J. F. V., and Fagard R. H., 2011, "Modulation of reactive oxygen species and collagen synthesis by angiotensin II in cardiac fibroblasts," The Open Hypertension Journal, pp. 1–17.

[90] Rosenkranz S., 2004, "TGF-beta1 and angiotensin networking in cardiac remodeling.," Cardiovascular Research, **63**(3), pp. 423–32.

[91] Funck R. C., Wilke A., Rupp H., and Brilla C. G., 1997, "Regulation and role of myocardial collagen matrix remodeling in hypertensive heart disease.," Advances in Experimental Medicine and Biology, **432**, pp. 35–44.

[92] Sadoshima J., and Izumo S., 1993, "Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype," Circulation Research, **73**(3), pp. 413–423.

[93] Chua C. C., Hamdy R. C., and Chua B. H. L., 1996, "Angiotensin II induces TIMP-1 production in rat heart endothelial cells," Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, **1311**(3), pp. 175–180.

[94] Bowen I. M., Marr C. M., Chester A. H., Wheeler-Jones C. P. D., and Elliott J., 2004, "In-vitro contraction of the equine aortic valve.," The Journal of Heart Valve Disease, **13**(4), pp. 593–9.

[95] Platt M. O., Xing Y., Jo H., and Yoganathan A. P., 2006, "Metalloproteinases and cathepsin activity in porcine aortic valves," The Journal of Heart Valve Disease, **15**(5), pp. 622–629.

[96] Butcher J. T., Simmons C. A., and Warnock J. N., 2008, "Review□: Mechanobiology of the aortic heart valve," Journal of Heart Valve Disease.

[97] Butcher J. T., and Nerem R. M., 2004, "Porcine aortic valve interstitial cells in three-dimensional culture: comparison of phenotype with aortic smooth muscle cells.," The Journal of Heart Valve Disease, **13**(3), pp. 478–85; discussion 485–6.

[98] Kuznetsova T. G., Starodubtseva M. N., Yegorenkov N. I., Chizhik S. a, and Zhdanov R. I., 2007, "Atomic force microscopy probing of cell elasticity.," Micron (Oxford, England□: 1993), **38**(8), pp. 824–33.

[99] Huang H., Sylvan J., Jonas M., Barresi R., So P. T. C., Campbell K. P., and Lee R. T., 2005, "Cell stiffness and receptors: evidence for cytoskeletal subnetworks.," American Journal of Cell Physiology., **288**(1), pp. C72–80.

[100] Mofrad M. R., and Kamm R. D., eds., 2010, Cellular Mechanotransduction: Diverse Perspectives from Molecules to Tissues, Cambridge University Press.

[101] Konduri S., Xing Y., Warnock J. N., He Z., and Yoganathan A. P., 2005, "Normal physiological conditions maintain the biological characteristics of porcine aortic heart valves: an ex vivo organ culture study.," Annals of Biomedical Engineering, **33**(9), pp. 1158–66.

[102] Balachandran K., Sucosky P., Jo H., and Yoganathan A. P., 2009, "Elevated cyclic stretch alters matrix remodeling in aortic valve cusps□: implications for degenerative aortic valve disease," American Journal of Physiology-Heart and Circulatory Physiology, **0535**, pp. 756–764.

[103] El-Hamamsy I., Balachandran K., Yacoub M. H., Stevens L. M., Sarathchandra P., Taylor P. M., Yoganathan A. P., and Chester A. H., 2009, "Endothelium-dependent regulation of the mechanical properties of aortic valve cusps.," Journal of the American College of Cardiology, **53**(16), pp. 1448–55.

APPENDIX A

PRESSURE SYSTEM SCEMATIC AND REPRESENTATIVE CURVES

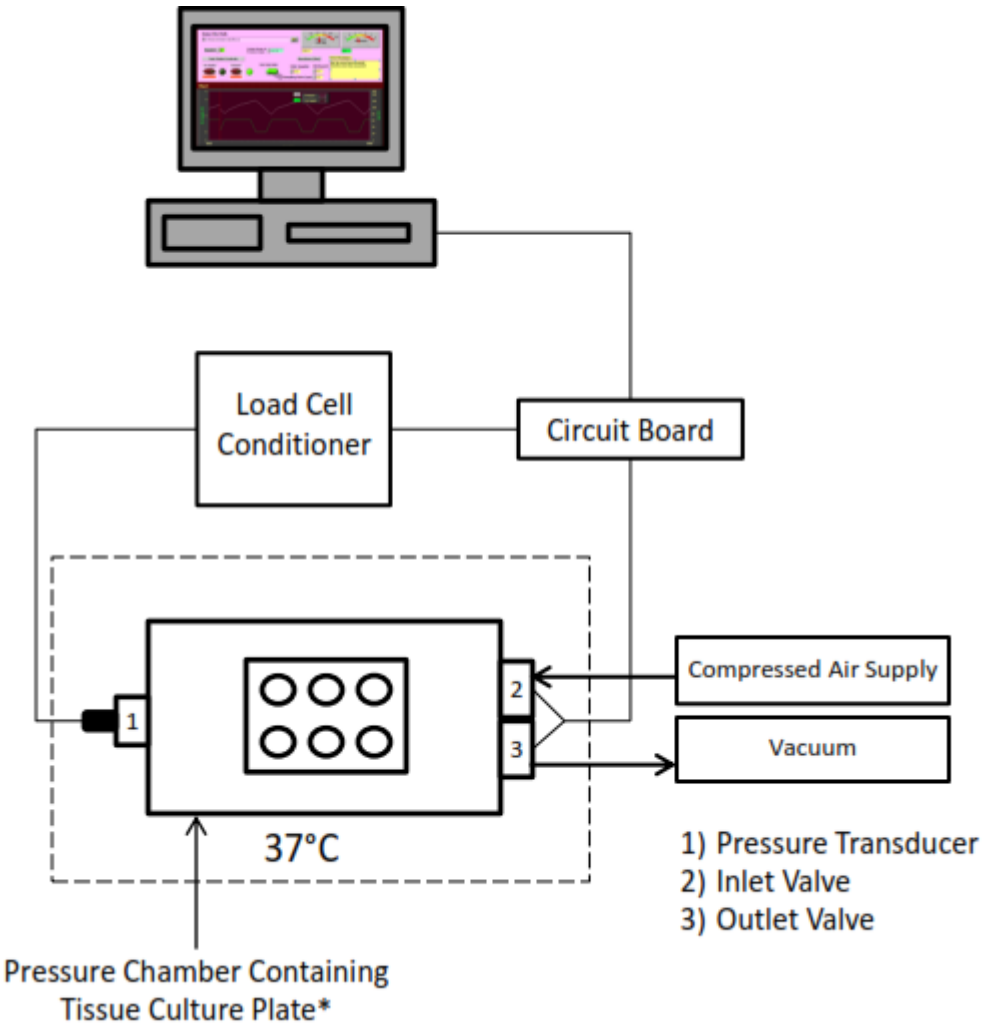

Figure A.1 Pressure System Schematic.

*Average temperature inside pressure chamber, 35.9°C.

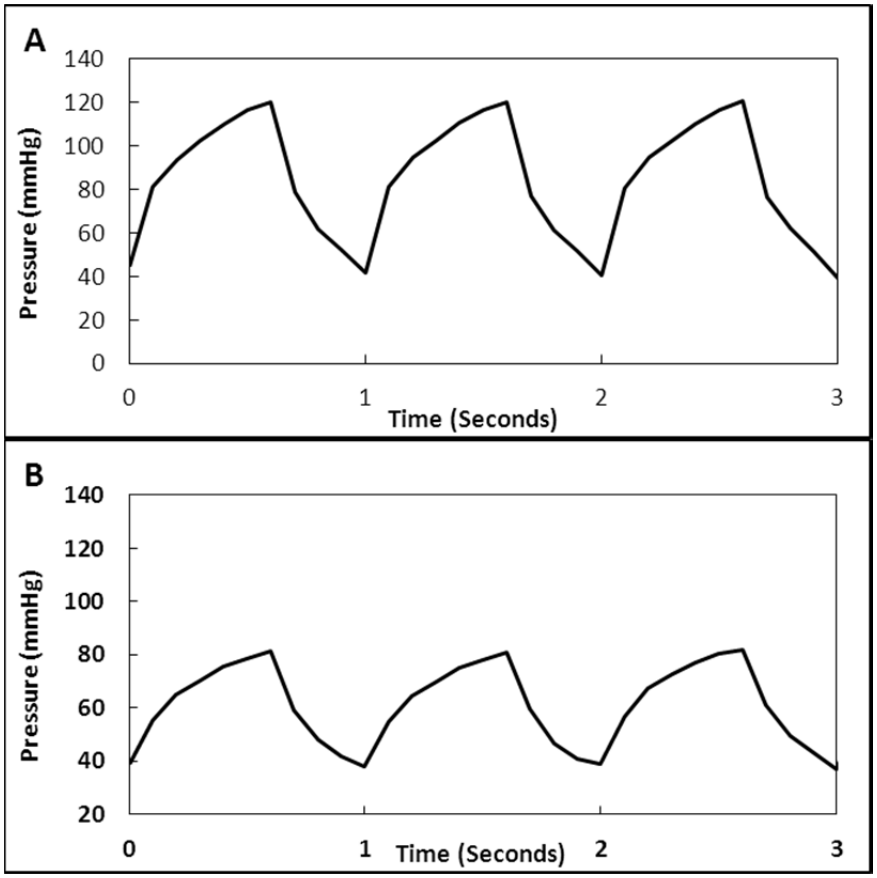

Figure A.2 Graph of pressure simulation within bioreactor

A) normal and B) hypertensive pressure conditions.

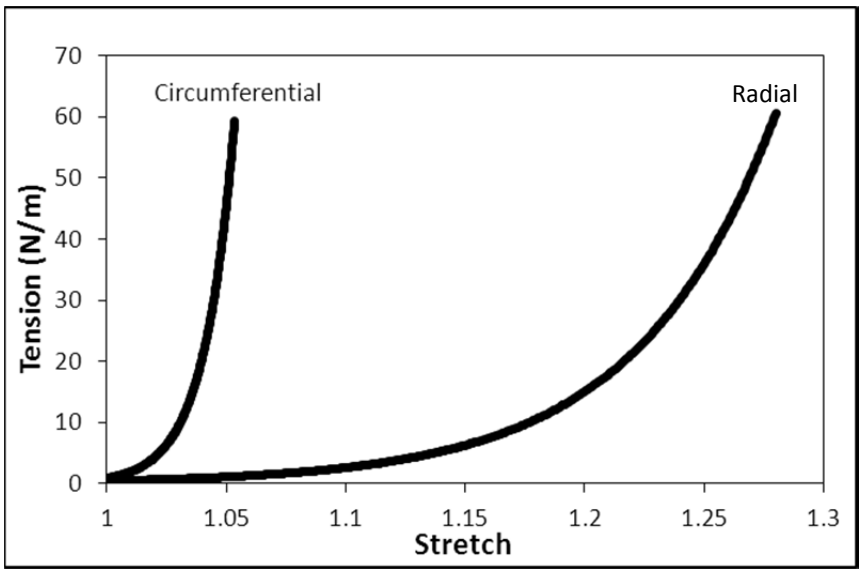

Figure A.3 Representative tension vs. stretch biaxial mechanical testing curve.

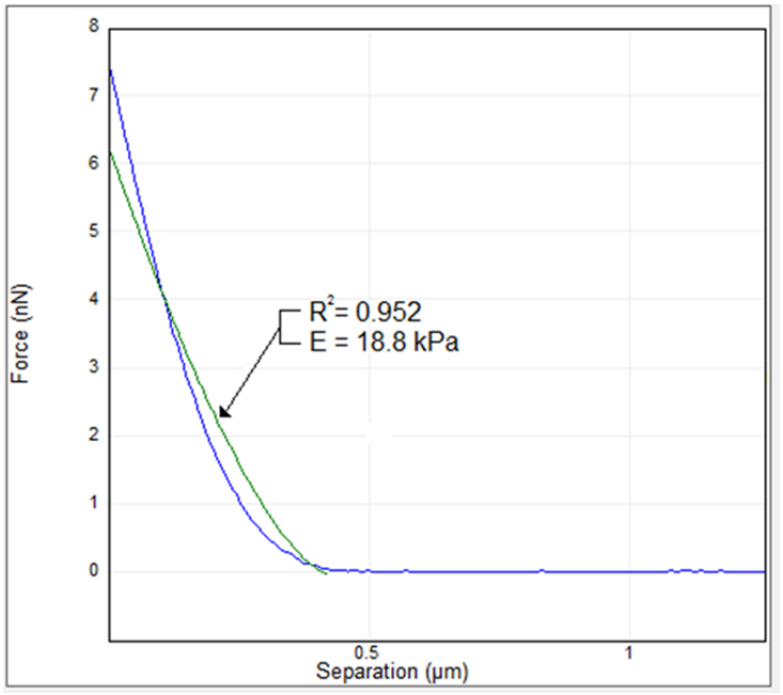

Figure A.4 Force vs. separation (indentation depth) (blue curve) from a single indentation of an aortic valve interstitial cell with AFM.

APPENDIX B

PROTOCOLS

VIC Cryopreservation and Recovery

Cell Freezing

1. Remove old culture medium and rinse with sterile PBS.
2. Add Trypsin to the flask and incubate for 5 minutes.
3. Add an equal amount of culture medium and place in a centrifuge tube.
4. Centrifuge for 5 minutes at 1000 RPM
5. Remove the supernatant from the centrifuge tube.
6. Re-suspend pellet in 10% DMSO in FBS to give desired freezing cell concentration.
7. Aliquot suspension into cryo vials.
8. Place vials in pre-chilled Nalgene Mr. Frosty and place in -80°C freezer overnight.
9. Remove vials from Mr. Frosty and place in -80°C liquid nitrogen.

Cell Recovery

1. Remove vials from -80°C and place them in a 37°C water bath until semi thawed.
2. Gently re-suspend cells in solution.
3. Place solution into a centrifuge tube.
4. Add 9ml of DMEM/ 10% FBS / 10% ABAM.
5. Re-suspend cells in solution.
6. Centrifuge for 5 min at 1000 RPM.
7. Remove the supernatant from the centrifuge tube.
8. Re-suspend in culture medium and place in T-25 flask.
9. Replace media within 24 hours.

Table B.1 Reagents for Cell Culture and Cryopreservation

Reagent	Company	Catalog Number
Dulbecco's Modified Eagle's Medium (DMEM)	Sigma	D5796
Fetal Bovine Serum (FBS)	Invitrogen	10437-028
Antibiotic/Antimycotic (ABAM)	Gibco	15240
Trypsin EDTA	Gibco	25300
T-12.5 Flasks	BD Falcon	353107
T-25 Flasks	BD Falcon	353109
T-75 Flasks	BD Falcon	353136
Swabs	Texwipe	TX761
Syringe Filters	VWR	28145-477
Syringes	BD Falcon	309654
Collagenase Type II	Gibco	17101-015
Filter Bottles	Corning	430769
Dulbecco's Phosphate Buffered Saline (PBS)	Sigma	D5652
Dimethyl Sulfoxide (DMSO)	Sigma	D2650
Freezer Vials	VWR	89094-810
Nalgene Mr. Frosty	Sigma	C1562

Sircol Collagen Assay

Procedure:

1. Digest tissue in pepsin dissolved in 0.5M acetic acid (buffer) for 48 h at 50°C at concentration of 0.1ml/mg of 0.5M acetic acid with occasional agitation.
2. Add 1ml of samples to 100µl acid neutralizing reagent.
3. Add 200µl of cold isolation and concentration reagent to each tube.
4. Mix tube contents by tube inversions.
5. Place tubes in ice-water mix and incubate overnight at 4°C.
6. Centrifuge tubes at 12,000 rpm for 10 minutes.
7. Use a micropipette to remove 1000µl of supernatant from each tube.
8. Prepare reagent blanks (100 µL deionized water) and collagen standards (duplicates containing 15, 30, 60 µg).
9. Add 1 mL Sircol Dye reagent to each tube.
10. Place tubes in a mechanical shaker for 30 min.

11. Transfer tubes to a microcentrifuge and spin at 12,000 rpm for 10 minutes.
12. Invert tubes to drain the unbound dye.
13. Add 750µl of ice-cold acid-salt wash reagent to each tube to remove unbound dye from the surface of the pellet and inside of the tube.
14. Centrifuge and spin at 12,000 rpm for 10 minutes.
15. Add 250µl alkali reagent and mix.
16. Transfer 200µL aliquots of samples from tubes to a 96-well multi-well plate.
17. Place the plate in a microplate reader, read the absorbance at 555 nm.

Table B.2 Reagents for Collagen Assay

Reagent	**Company**	**Catalog Number**
Pepsin	Sigma	P7012
Acetic acid 0.5 M	Sigma	A8976
Sircol collagen assay kit	Accurate Chemical	CLRS1000