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Hydrodynamic Assessment of a Porcine Small Intestinal Sub-Mucosa Bioscaffold Valve for Pediatric Mitral Valve Replacement

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HYDRODYNAMIC ASSESSMENT OF A PORCINE SMALL INTESTINAL SUB-MUCOSA BIOSCAFFOLD VALVE FOR PEDIATRIC MITRAL VALVE REPLACEMENT

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To: Interim Dean Ranu Jung
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This thesis, written by Omkar Mankame, and entitled Hydrodynamic Assessment of a Porcine Small Intestinal Sub-Mucosa Bioscaffold Valve for Pediatric Mitral Valve Replacement, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Omkar Mankame is approved.

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Interim Dean Ranu Jung
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ABSTRACT OF THE THESIS

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Florida International University, 2017

Miami, Florida

Professor Sharan Ramaswamy, Major Professor

Valve replacement for critical heart valve diseases is in many cases not an option. Our clinical experience in pediatric compassionate care has shown robust function of porcine small intestinal submucosa (PSIS) valves. We assessed functional effectiveness of 4ply (~320μm) and 2ply (~166μm) PSIS mitral valves under pediatric-relevant hemodynamic pulsatile conditions. Key conclusions: (i)PSIS valves demonstrated statistically similar acute functionality in comparison to a commercially available valve. (ii)Energy losses were similar ($p>0.05$) under pediatric conditions which was not the case under adult aortic conditions. (iii)2ply valves were observed to be superior to 4ply, based on the robust hydrodynamic data, the mechanical properties suitable for pediatric applications and *de-novo* tissue replacement potential with less demand on the body. Demonstrating somatic growth, valve tissue filling matching PSIS degradation and PSIS-valve fatigue assessment are critical endeavors that need to be carried out to ensure mid to long term function of these bioscaffold mitral valves.

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CHAPTER 1 Introduction

Heart valves perform a very important role in regulating the forward flow of blood initiating from the four chambers of the heart (Fig. 1). Heart valves help in coordinating blood flow during the cardiac cycle. They have a direct effect on the overall efficiency of the cardiovascular system. Tissue leaflets in the valves open and close with each beat of the heart and in healthy states, permit robust hemodynamic conditions. Critical heart valve disease, a serious health issue, often requires valve replacement with a prosthetic valve. In the young, critical congenital valve anomalies have been a life-threatening condition and has frequently resulted in mortality [1]. In the United States, there are an estimated 650,000 to 1.3 million children and adults living with congenital heart disease [2]. A common cardiac abnormality occurs with the cardiac valves, which accounts for 30% of all congenital heart problems [3].

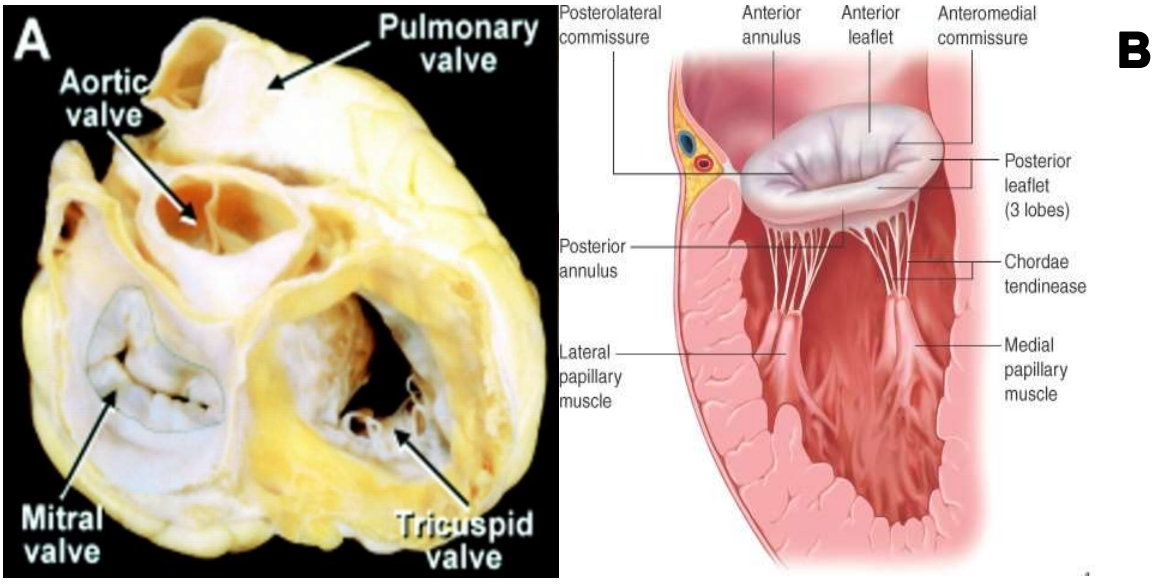

Figure 1: (a) Anatomy of heart valves (Ho S. Y, 2002). (b) The mitral apparatus: Continuity of the mitral apparatus and the left ventricular myocardium (Otto C.M, 2001).

Heart valves open and close due to the pressure difference between either side of the valve. Heart valve disease occurs if one or more of the valves function abnormally. The complications that can develop with these valves are mainly of two types: (a) stenosis and (b) regurgitation and prolapse [4, 5, 6]. Stenosis occurs when there is obstruction to the flow of blood in the forward direction. This may occur if the valves do not open fully or narrowing of the orifice area of the valve [7]. Alternatively, there may a narrowing of the vessel (e.g. aorta) at the site of the valve due to congenital causes. Stenosis can result in increased work load on the heart as the heart needs to pump harder to drive the blood through the stenosed region [8]. On the other hand, if the valve is diseased, such that it does not close completely or form a tight seal between leaflets, then there will be leakage that will cause the back-flow of blood [9]. This defect is called regurgitation. Regurgitation also puts an extra strain on the heart to pump the necessary volume of blood in the right direction. Congenital defects include atresia, which occurs if the heart valves are not formed properly or are partially or fully absent, causing loss of sufficient local control to permit unidirectional blood flow. All four valves can be prone to congenital problems and/or disease, but the aortic and mitral valves which are commonly affected [10] can lead to death very quickly owing to high hemodynamic blood pressures on the left side of the heart.

Depending on the severity of the diseased heart valve condition, the valve is either treated with vasodilators, repaired or is replaced completely [11]. For mild valvular disease, clinicians may prescribe vasodilators or diuretics to assist in promoting enhanced blood flow. However, if the valves are severely damaged, valve repair/replacement surgeries are usually recommended [11]. Some of the valve repair procedures that a surgeon can perform

are decalcification, patching, balloon valvuloplasty, reshaping and leaflet extension techniques. Valve replacement surgeries are typically performed when the valves are beyond repair. Aortic and mitral valve replacement surgeries are common-place today with reasonably good outcomes. However, some segments of the population are not suitable candidates for valve replacement either because valve sizing/growth issues exist (e.g. in pediatric patients) or because of complications that can occur with the surgery and/or specific prosthetic being used (e.g. women who wish to have children cannot have a mechanical valve since it requires lifelong anticoagulant therapy).

Heart valves are surgically replaced when they fail to operate physiologically. Heart valve prostheses, like mechanical valves (caged ball, tilting disc, hinged bileaflet) which are made of artificial materials, chemically fixed animal tissue bioprosthetic valves (porcine or bovine tissue) and homografts (human cadaver valves) are generally used for valve replacement surgeries. Mechanical valves are very strong and durable and are functional for longer durations, in some patients they have lasted as long as 25 years without problems [12]. However, as previously mentioned, these valves constantly require continuous use of anticoagulants since the mechanical valves is prone to thrombus formation. Glutaraldehyde-fixed bioprosthetic valves or tissue valves have shorter life-span as compared to the mechanical valves, generally a mean life-span of 10 years, but on the other hand, these valves do not require anticoagulant treatment. Bioprosthetic valves are similar to native human valves and also have better hemodynamic characteristics than mechanical valves. Bioprosthetic valves are also a preferred candidate among the elderly (> 65 years of age) as it can also be implanted in a minimally invasive manner (e.g. transcatheter aortic valve replacement/TAVR). Typically, current available prostheses can

be utilized in adults, but for replacement procedures in infants and young patients they are not suitable.

Utilization of currently available tissue and mechanical prosthetic valve substitutes in infants and children have restrictions in terms of the relatively large size of available prosthetic devices, somatic growth, durability, thrombogenicity and endocarditis or susceptibility to infection. All the mechanical valves involve permanent use of anticoagulation. This may present greater risk to infants and children who are prone to accidental injury during play [13]. Bioprosthetic valves degrade quickly in young patients and are prone to accelerated calcification. However, above all, sizing limitations and an inability to support valvular growth are the two major drawbacks of prosthetic heart valves.

Over the past few years, better surgical approaches have aided children, suffering with congenital heart defects, to survive into adulthood [14]. Regardless of these developments, significant number of patients require multiple operations [14]. Of late it has been shown that after implantation, acellular porcine small intestinal submucosa (PSIS) scaffolds have potential capacity to support hemodynamic functions and permit PSIS bioscaffold replacement by organized collagen and colonization of cells similar to the endothelial cells [15, 16].

PSIS is a biodegradable, naturally occurring scaffold material which degrades with time promoting *in vivo* tissue growth. These scaffolds support cell infiltration and tissue remodeling after implantation. Cardiovascular use of PSIS patches *in vivo* in animal models has been promising demonstrating restoration and integration [17, 18, 19]. PSIS valves have the potential to address the complications posed by conventional heart valve substitutes and thus, offer a possibility that they may be used as a permanent approach for

replacing defective heart valves, since somatic growth can in theory, be facilitated. Our collaborators at Joe DiMaggio Children's Hospital (Hollywood, FL) have implanted custom-made PSIS valves in 4 infants with critical valve disease unable to receive standard valves, as compassionate care measures. The follow-ups (post-1 year) have been promising and have reported that the valves have performed well, with low transvalvular pressure gradients and no need for anti-coagulant therapy [15]. These are remarkable observations given that these patients would have died within a few days to weeks if left untreated and also again in recognizing that no current treatment option exists for these patients; if successful PSIS valves may offer the potential to revolutionize the treatment of critical congenital valve diseases.

Although PSIS material possesses desirable cardiovascular traits, before this technology can be used on a wider scale for valve applications, an organized evaluation of PSIS valves functionality is needed so that any long-term complications beyond a few years post-implant can be identified. It is important to recall that the valve needs to function and grow for 18 years till adulthood. One way in which functionality can be evaluated is through hydrodynamic assessment with a pulse duplicator (Vivitro System, Vivitro Laboratories, Victoria, Canada).

Previously we evaluated PSIS prosthetic bioscaffold valves in the aortic position *in vitro* by utilizing a pulse duplicator system (Vivitro Laboratories, Victoria, Canada) [20]. We analyzed the hydrodynamic functionality of PSIS trileaflet bio scaffold valves (CorMatrix Cardiovascular, Inc., Roswell, GA, USA) by comparing these valves with bioprosthetic valves (Medtronic Freestyle, Medtronic, Minneapolis, MN) under physiological adult conditions at a heart rate of 70 bpm and stroke volume of 80 ml/beat.

Here we chose Medtronic bioprosthetic valves as a control. Hydrodynamic testing results exhibited robust functionality of the PSIS valves and the valves preformed very close to the commercially available Medtronic freestyle bio-prosthetic valve [20]. However, the PSIS aortic valves exhibited a cause of concern in that significantly higher ($p < 0.05$) energy losses were observed, which could lead to longer term complications due to increased workload on the heart.

The focus of this thesis was to evaluate the acute functionality of mitral 4ply PSIS valves (~ 320μm) under varying pediatric physiological conditions and compare the results with those of commercially available bioprosthetic valves (Edwards Lifesciences, Irvine, CA). The series of experiments were performed to determine the effects of varying physiologically-relevant pediatric heart rates and stroke volumes on in vitro hydrodynamic functionality of manually-assembled PSIS mitral valves. Subsequently we moved on to the thinner 2ply valves (~ 166μm) in order to test if the thinner PSIS valve maintained its hydrodynamic functionality. The end-goal of this work was to identify hydrodynamic metrics of PSIS bioscaffold mitral valves under pediatric conditions and thus contribute towards the optimization of these constructs for the potential treatment of critical valvular diseases.

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CHAPTER 2 Specific Aims

Currently there are no valve replacement strategies that can completely replace the defective human mitral valve, having the ability to grow with the patient. Commercially available prosthetic valves also have other shortcomings in terms of durability, thrombogenicity and endocarditis. This problem is more evident in infants and small children, as they grow with time but prosthetic valves remain the same size, thus requiring multiple reoperations. Tissue engineered heart valve (TEHV) development has the capability to offer growth, resistance to infection, self-repair and a permanent attempt for substituting diseased and defective heart valves and treat critical congenital valve disease in pediatric patients, which the current valve substitutes lack [1]. Nevertheless, a major challenge in this concept is to discover a scaffold which is biocompatible with in vivo hemodynamic conditions and biodegradable, which can provide for somatic growth in the longer term.

In 2015, the Food and Drug Administration (FDA) approved valve replacement procedures of trileaflet PSIS valves (Cormatrix: April 2015) in up to 15 patients. The overall purpose of this work was to propose a simple and novel approach for the treatment of critical pediatric congenital heart valve disease by using an extracellular porcine small intestinal submucosa (PSIS) bioscaffold construct for valvular replacement. Our collaboration with our clinical colleagues at Joe DiMaggio Children's Hospital (Hollywood, FL) has shown promising results with PSIS scaffolds working flawlessly in pediatric compassionate care patients, the longest follow up being around 2 years. PSIS has exhibited the ability to recruit endogenous cardiovascular cells, leading to phenotypically-matched tissue replacement when the scaffold degrades completely [2,3].

This property to permit implantation of the scaffold without in vitro cell seeding and culturing is attractive specially for pediatric valvular applications and has a huge potential, where somatic growth is crucial. However, there are mixed clinical reports regarding PSIS bioscaffold with the key apprehensions being enduring inflammatory response and the absence of a tri-layered valve structure after some months of implantation [4, 5]. Nevertheless, no large-scale, organized valvular study to evaluate PSIS valve capability has been performed till date.

The immediate objective of this study was to assess in vitro hydrodynamic functionality of PSIS bioscaffolds in mitral valve replacement application, especially for infants and small children. Our work combined assessing the hydrodynamic functionality of PSIS bioscaffold valve in an in-house pulse duplicator system (Vivitro Laboratories, Victoria, BC) under varying native pediatric conditions (aim 1) and then optimizing the PSIS bioscaffold dimensions to the best selected pediatric environment in the same simulator (aim 2) in order to establish the in vitro functionality of PSIS-valve as a strategy for the treatment of pediatric, critical congenital valve diseases. Our two specific aims are as follows:

Specific Aim 1: Identifying the biofluid dynamics of novel 4ply PSIS bileaflet valves (~320μm) for functional assessment in the mitral position at varying pediatric conditions. By biofluid dynamics we aim to establish certain fluid mechanical parameters that will aid us towards understanding if the valves function appropriately. These include but are not limited to pressure gradient across the valve, forward flow, effective orifice area, regurgitation fraction and the systolic energy

losses. Insights into these parameters for PSIS valves will provide both acute as well as prediction on mid to long term performance.

We accomplished this by using a left heart simulator in our laboratory [6, 7]. This study was conducted by suturing a bi-leaflet valve configuration in the mitral position in the pulse duplicator system (Vivitro Laboratories, Victoria, BC) and exposing the valves to pediatric native mitral valve hemodynamic conditions in order to predict potential long-term complications. Pediatric conditions had two sets of testing parameters; one at constant heart rate and other at constant stroke volume. Our current experience with PSIS bioscaffolds is based on constructs measuring 320 μm in mean thickness (n = 5 leaflets) and a leaflet radius of ~ 8mm, using lobe-shaped leaflets. The leaflet width was scaled to fit into the vascular conduit into which the valve is sutured.

Specific Aim 2: Establish the feasibility of 2ply PSIS mitral valves (~166μm) with ~47% reduction in thickness and ~54% reduction in volume as compared to the 4ply valves (~320μm) in aim 1. The underlying goal of this aim is to reduce the burden on the body to produce de novo tissues since less biomaterial will be used.

Owing to the unique material properties of PSIS, our hypothesis is that PSIS bioscaffold valvular dimensions can be further minimized, ideally by 50% of current bioscaffold thickness, while still allowing for robust functionality, and in addition, will support de novo valvular tissue formation and somatic growth in vivo From the hydrodynamic metrics measured, the leaflet thickness was fine-tuned through an iterative process to establish robust functionality for the mitral position with PSIS bioscaffold material. Thus, when implanted, this in turn will reduce the burden on the host since less

tissue is required to fill a smaller scaffold space. These valves were subjected to the best selected pediatric condition from aim 1 within the same pulse duplicator system.

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CHAPTER 3 Literature Review

3.1. Anatomy and Physiology of Heart Valves:

The mitral valve is located on the left side of the heart connecting the left atrium and the left ventricle [1, 2]. On the contrary, tricuspid valve is located between the right atrium and right ventricular chambers. Together these valves are referred to as "atrioventricular valves". The mitral valve has two leaflets unlike the other cardiac valves while the tricuspid valve has three cusps [2]. Similarly, there are two "semilunar valves" namely aortic and pulmonary valves and are correspondingly positioned in the left and right ventricular tract. Both these valves are tri-leaflet valves consisting of three semilunar cusps. The mitral and the aortic valves are structured close to each other whereas the pulmonary and the tricuspid valves are separated by myocardium. Of specific interest to this study is the mitral valve. The mitral valve leaflets control the flow of blood at the orifice between the left atrium (LA) and the left ventricle (LV) and open during diastole to allow the blood flow from the LA to the LV. During ventricular systole, the left ventricle muscle contracts causing the mitral valve leaflets to close and prevents back flow to the LA. Blood is ejected through the open aortic valve. The mitral valve is a complex structure that depends on its 6 components, which are the left atrial wall, the annulus, the leaflets, the chordae tendineae, the papillary muscles, and the left ventricular wall [3, 4]. Any congenital or acquired disorder as a result of rheumatic fever or degenerative change of individual components can disturb the synchronized mechanisms of the mitral valve and result in a malfunctioning heart valve [3, 5, 6].

A single heart beat comprises of a cardiac cycle which can be thought to initiate with blood ejection from the left ventricle to the systemic circulation and is completed when the

heart is filled again with blood. There are two sub-phases in a single cardiac cycle; systole and diastole. During the diastolic phase, the ventricles are relaxed and the atria contract. This leads to the opening of mitral and tricuspid valves. On the contrary, aortic and pulmonary valves are closed during the diastolic phase. The opening of the atrioventricular valves allows the flow of blood from the atria to the ventricles (Fig. 2) and the closing of the semilunar valves prevents back flow of blood into the atria. During the systolic phase, the ventricles are contracted as the impulse from SA node spreads to the ventricles. This contraction opens the semilunar valves to allow ejection of blood into the arteries. At the same time, mitral and tricuspid valves snap shut, blocking the flow of blood into left and right ventricles. However, blood continues to enter the atria though the vena cavae and pulmonary veins. Each cardiac cycle is crucial for the proper functioning of the cardiovascular system.

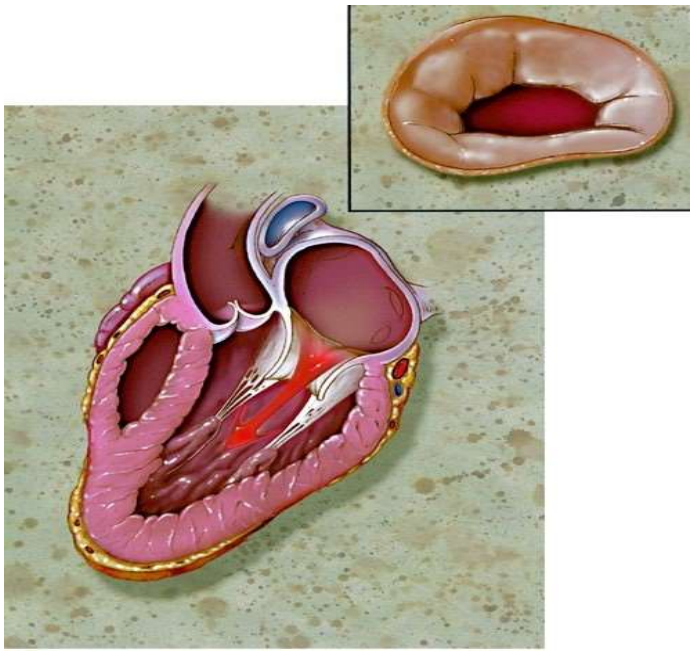


Figure 2: The heart in diastole (during relaxation of the left ventricle). In normal individuals, the heart valve opens to allow blood to flow into the left ventricle; notice the supporting structure of tendon and muscle (Turi A, 2004).

3.2. Mitral Valve Disease:

The mitral valve is the most complex amongst the four valves of the heart and is the most commonly associated with disease [7]. Mitral valve disease can be acquired due to rheumatic fever, infections or due to ageing or this disease may occur before birth as the heart is developing. Congenital mitral valve abnormalities are either observed in seclusion or with other congenital heart disease. There are 3 different types of irregularities related to the mitral valve: mitral stenosis, mitral regurgitation, mitral prolapse (Fig. 3).

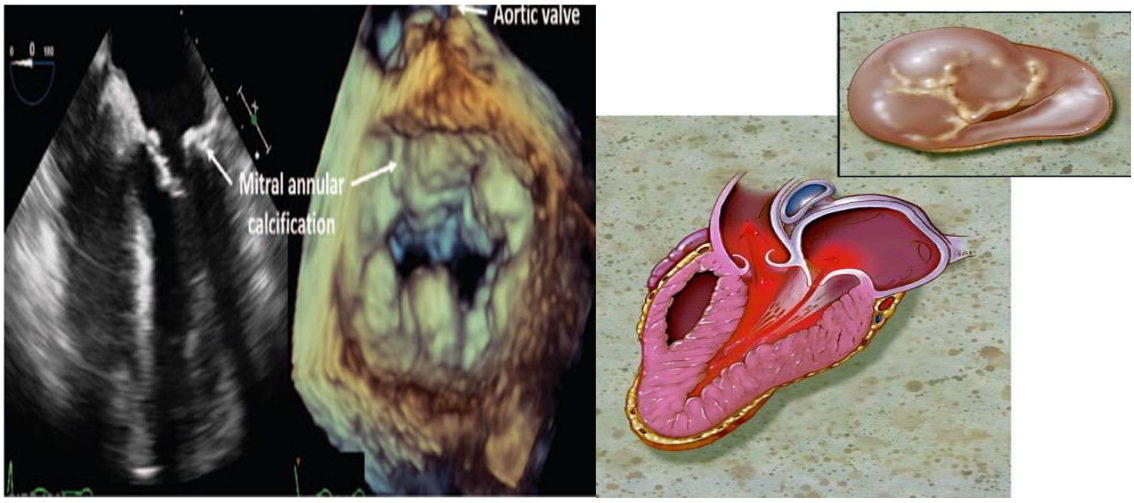


Figure 3: (a) Extensive calcification of the mitral annulus (Cherry A.D, 2016). (b) In the patient with both prolapse and mitral regurgitation, the valve does not close completely and part of a leaflet bulges back into the left atrium (Turi A, 2004).

3.2.A. Mitral Stenosis:

Mitral stenosis prevents the proper opening of the valves during diastolic phase thus filling the ventricles with inadequate blood volume. It is a structural malformation of the mitral valve which results in hardening of the leaflets which prevents leaflet movement, thus obstructing cleft ventricular inflow. Rheumatic carditis is the main cause of mitral stenosis. Rheumatic carditis and mitral stenosis are interrelated as stenosis of mitral valves

occurs in 40% of all patients with rheumatic heart disease [8]. Congenital mitral stenosis was noted to affect multiple mitral valve segments as observed by Ruckman and van Praagh in 1978 [9].

3.2.B. Mitral Regurgitation

Mitral regurgitation is divided into either primary (a structural or degenerative abnormality of the mitral valve apparatus) or secondary (a disease of the left ventricle, which interferes with the function and integrity of the mitral valve apparatus) mitral regurgitation [10]**.** This anomaly leads to backflow or leakage of blood from the left ventricular chamber to the left atrium through the mitral valve. During systole, the mitral valve suffering from regurgitation irregularity does not close properly and hence the contracting ventricles push blood back into the left atrium. Severe regurgitation increases the workload on the heart to maintain the forward flow and thus can lead to malfunction of the heart. Hemodynamically regurgitation is most common anomaly related to mitral valve as compared to mitral stenosis [9]

3.2.C. Mitral Prolapse

Bulging of mitral valves back into the left atrium is called as mitral valve prolapse. In mitral valve prolapse, during contraction of the left ventricle (systole), one or both the leaflets prolapse into the left atrium causing the leakage of blood back into the atrium through the open orifice area. The valve usually becomes floppy and does not close the valve tightly. This abnormality usually leads to palpitations, shortness of breath, pain in chest. Severe mitral prolapse raises the pressure in the left atrium to adjust the forward flow and thus can cause heart failure. Mild prolapse can be treated with medications which helps

to avert complications. Some people, however, need mitral valve repair or valve replacement surgeries if the problem is too severe.

Generally, for mitral stenosis, a balloon valvotomy procedure is usually prescribed. It is a minimally invasive procedure in which a catheter tube is inserted through an artery in the arm or groin and upon reaching the mitral valve location, the balloon at the tip of the catheter is inflated swiftly. This helps in separating the narrowed valves and ultimately increases the forward flow of blood. Other valvotomy techniques have also been developed for mitral valve regurgitation. Valve repair techniques provide better morbidity, mortality and long-term outcomes as compared to the valve replacement procedures [11]. On the contrary, valve replacement is must for severe valve damage where valve repair is not an option.

The European Association of Echocardiography and the American Society of Echocardiography Recommendations for Clinical Practice has defined the criteria for severity of mitral regurgitation and stenosis based on hemodynamics (Table 1).

Table 1: Severity of mitral valve stenosis based on hemodynamics.

Mitral Valve	Regurgitation Fraction (%) [12]	Mean transvalvular pressure gradient (diastole) (mmHg)* [13]
Mild	< 30	<5
Moderate	30-49	5-10
Severe	≥ 50	>10

*** At heart rates between 60 and 80 bpm.**

3.3 Prosthetic Heart Valves:

Heart valve replacement surgery was introduced in the early 1960s which improved the outcome of patients with valve related abnormalities [14]. Original Starr-Edwards prosthesis were used in 1960s and the few of the patients who underwent valve replacement

surgeries with these valves are reported to be alive [11]. As explained by a pioneer cardiac surgeon, Dr. Dwight Harken, a model prosthetic valve to be used in humans should have outstanding hemodynamic properties, high resistance to thrombosis, long-term durability and also excellent implantability [15]. These valves should work excellently in coordination with the entire circulatory system. It should imitate the characteristics of a normal human native valve.

3.3.A. Mechanical Valves:

Mechanical valves are made of robust materials, primarily of metal or carbon alloy, and are most durable valves among the replacement valves, most lasting at least 20 to 30 years [16]. However mechanical valves are thrombogenic in nature causing the blood to clot and thus involve long-term anticoagulant therapy to prevent valve associated thrombosis [17]. The blood clots can break off from one site and travel through the blood stream (embolism) where they may lodge in blood vessels and cause further heart related complications. Life-long warfarin therapy is necessary for the patients with mechanical valve prosthesis [8]. It helps to avoid blood to clot, as the mechanical valves promote clotting of blood which gets lodged in the flaps or hinges of the valves and thus cause malfunction. Mitral valve replacement by mechanical prostheses is associated with higher degrees of thromboembolism as compared to replacement in other valves [8, 16].

There are three types of mechanical valve designs that have been used in medical practice (Fig. 4): caged ball valves, monoleaflet or disc valves and bileaflet valves. These valves are described according to the shape of the occluder, that opens and closes the orifice area of the valve. The caged ball mechanical valves, the original Star-Edwards 6120 and 1260 models, were developed as mitral and aortic valves respectively. These valves

included a silastic ball with a circular sewing ring and a cage made of 3 metal curvatures [11, 14]. The ball moves forward into the cage when open and seats back when closed. The Starr-Edwards prosthesis has the longest implant history and durability of around 40 years. However, these valves when implanted in patients reported very high thrombogenesis [16]. The patients needed very intensive anticoagulant therapy more than that required in any other mechanical valves. The caged ball mechanical valves are no longer utilized for valve replacement procedures as they are hemodynamically inferior to their more recent counterparts.

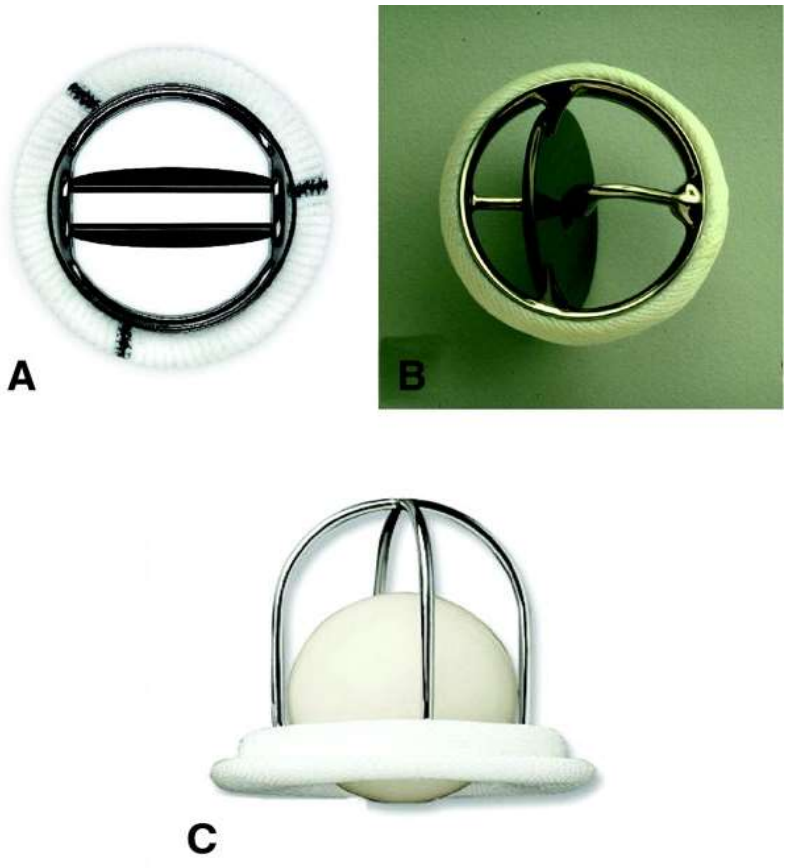


Figure 4. (a) St Jude bileaflet mechanical valve. (b) Medtronic Hall monoleaflet mechanical valve. (c) Starr-Edwards caged ball valve (Pibarot P, 2009).

A monoleaflet valve has a single circular graphite disc, hence the name disc valve. The disc leaflet is fixed by a lateral or central metal strut that tilts to open and closes to block the valve orifice area. When the disc tilts open, it results in two different orifice areas of different sizes. The opening angle of this singular disc in relation to the valve annulus ranges from 60° to 80°. Karl-Victor Hall, Arne Wolen and Robert Kaster developed a Hall-Medtronic single tilting-disc valves that was approved by US Food and Drug administration in 1977 [15]. This valve is one of the valves with the longest implant history. Hall-Medtronic valve prosthesis was later purchased by Medtronic, Inc.

St Jude bileaflet valve is also a type of mechanical valve which was approved by FDA and introduced in 1977 [15]. Bileaflet valves have two semicircular leaflets or discs connected by hinges to a stiff metal valve ring. During the forward flow of blood, both the leaflets tilt open forming three orifices, two larger orifices on outer regions of the two leaflets and one small orifice between these leaflets. Both the leaflets close when the forward flow stops, thus obstructing the backflow of blood. These valves have the lowest possibility of thromboembolism and thus require less use of anticoagulant agents [11]. Tilt disc valves and bileaflet valves have similar properties.

3.3.B. Bioprosthetic Valves:

Bioprosthetic heart valves (Fig. 5) or biological valves are the tissue valves that are constructed from animal tissues. Generally, low intensity anticoagulant therapy with warfarin is suggested during the first 3 months after the bioprosthetic valves are implanted [8, 16]. This is necessary as there is a risk of thromboembolism during the 3 months' time span. Nonetheless, compared to the mechanical valves, the bioprosthetic valves do not require long-term anticoagulant therapy. Bioprosthetic valves are fixed in glutaraldehyde

causing the crosslinking of collagen. This process masks the antigens and helps in chemical stabilization leading to lower the immunogenicity (immune response of the body). Bioprosthetic valves generally provide hemodynamic properties which are more similar to those of the native valves. These valves are less durable and can normally last for around 10-15 years.

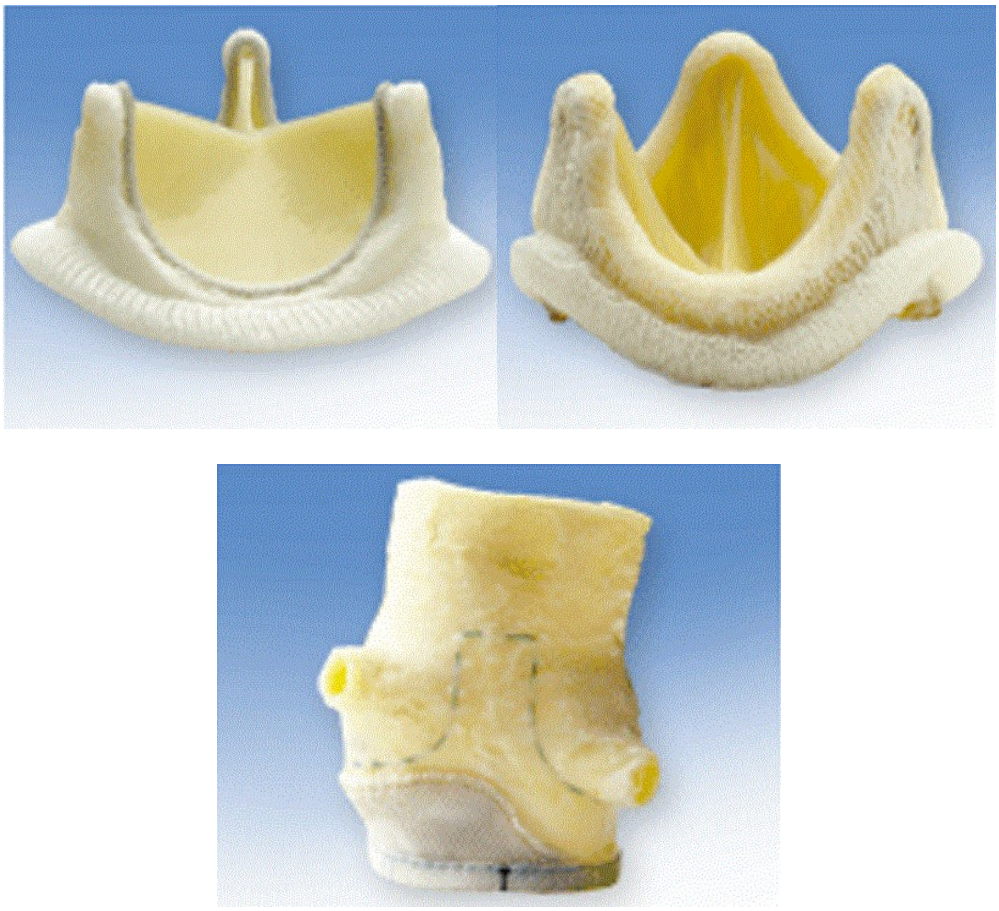


Figure 5: (a) Carpentier-Edwards Perimount Bovine Pericardial Tissue Valve. (b) Carpentier-Edwards S.A.V. Porcine Stented Valve. (c) Edwards Prima Plus Porcine Stentless Valve (Edwards Lifesciences®).

Autologous valves are constructed from the patient's own tissue derived from deep fascia, layer of dense connective tissue, of the thigh or pericardium. During 1970s valves

were shaped by hand from patient's own tissue during surgery [11]. However, these valves have been abandoned as they were technically challenging and had limited durability. Carpentier-Edwards Perimount pericardial prosthesis is an example of frame mounted valves created from the patient's own pericardium using a commercially available kit which has been developed lately.

Bioprosthetic valves obtained from human tissue valves are of two types: autografts and homografts. Autograft biological tissue valve replacement procedures generally involve acquiring patient's own valve from one site and implanting at the site where the disease transpires. Usually this procedure is aimed at aortic valve defects, as described by Donald Ross in 1967, where pulmonary valve is grafted at aortic site and the pulmonic valve is replaced by homograft. This procedure provides children suffering with aortic valve abnormality a hemodynamically superior valve in place of diseased valve and also helps in facilitating somatic growth [18]. However, this technique requires double valve replacement surgery and thus increased invasive risk. Conversely, homograft is a cadaveric aortic valve which is sterilized using an antibiotic solution and cryopreserved in liquid nitrogen till the time it is required. Homografts are directly implanted without using a stent at the site of valve defect.

Bioprosthetic heart valves constructed from animal tissue valves or from the tissue from pericardium of an animal are referred to as heterografts or xenografts. Most commonly, porcine aortic valves and bovine pericardial tissue are utilized to build the heterograft valves [19]. Before using the porcine valves, they are treated with varied concentration of glutaraldehyde which sterilizes the tissue and makes it biocompatible. The Medtronic Hancock II prosthesis and the St Jude Medical Biocor prosthesis are the example

of glutaraldehyde fixed porcine heterografts. The porcine heterograft valve tissue is mounted on metal stents (mostly cobalt-nickel alloy). These are called the stented valves where the valves are sewn on to a fabric which is in turn mounted on the stent ring. The Medtronic Freestyle valve is one of the stentless porcine bioprosthetic valves which are available more recently that have no supporting stents. The advantage of stentless bioprosthetic valve when compared to the stented one is that it has greater effective orifice area (EOA) improving hemodynamics and patient survival [20]. However, stentless valves are more prone to endocarditis, structural dysfunction and calcification and are also technically more complex to graft than the stented ones. Porcine heterografts with good durability can last for at least 10-15 years [18]. Pericardial valves are designed from bovine pericardium. These valves have similar design to that of porcine heterografts

3.4 Porcine Small Intestinal Submucosa Bioscaffold:

There have been recent developments in prosthetic valve design and surgical procedures in the past few years, but in spite of these improvements, there is no treatment option that can permanently relieve the patient suffering from severe heart valve defects. More specifically mechanical and bioprosthetic valves face major drawback when used as valve replacement options in atrioventricular section after valve failure specially in infants mainly due to size restrictions and inability to grow with the young patient [21]. Prosthetic valves used in children often require multiple reoperations. There is a much greater need for the development of prosthetic valves which can be used in infants and young population where valve repair techniques are not an option.

Extracellular matrix (ECM) derived from Porcine Small Intestinal Submucosa (PSIS) has shown successful results when used as a patch for tissue repair in valves, vessels and myocardium and also in the treatment of hernia, urinary system disease and refractory skin trauma [22, 23, 24]. The PSIS scaffolds in infants and young patients suffering from cardiac disorder has shown proof of possessing resorption properties, reendothelialization and also replacement of PSIS material with organized collagen with no evidence of pericardial effusions, calcification or intracardiac and intravascular thrombogenicity [24]. Tubular PSIS-ECM tricuspid bioprosthetic valves, in 7 out of 8 ovine models, demonstrated influx of mesenchymal cells, growth and cell-matrix configuration similar to that of mature native valves including normal valve function [22]. There was no evident inflammation or calcification. Lately, Cormatrix PSIS material (ECM®, CorMatrix Cardiovascular, Inc., Roswell, GA, USA), has obtained recognition by utilizing the scaffold in a variety of cardiovascular surgical procedures due to its simplicity, suitable mechanical properties, restoration properties, lower immunogenicity, absorbability and potential to promote native tissue growth [23, 24].

PSIS contains collagen, elastin, glycosaminoglycans, proteoglycans, and growth factors which helps in growth and healing [26]. PSIS material demonstrates FGF-2 and TGF- β associated activities [27]. FGF-2 is a fibroblast growth factor that is linked with limb and nervous system development, and wound healing. TGF-β belongs to the transformation growth factor family and assists in inducing transcription of different target genes that serve in differentiation, chemotaxis, proliferation and activation of numerous immune cells. Both these growth factors express tissue development and differentiation in

PSIS-ECM scaffolds and causes wound healing and tissue remodeling. PSIS closely mimics the normal ECM of the human dermis [26, 28].

The submucosa layer is present between the two layers, i.e. the mucosal and muscular layers of the small intestine. Submucosa provides strength to the small intestine through intricate fibrous matrix formed by collagen. The Cormatrix scaffold is constructed from porcine small intestinal submucosa (PSIS) which is extracted from the intestine by removing all the cells but keeping the complex extracellular matrix together. The scaffold is processed and manufactured in a way that maintains the fibrous nature and porous nature of the matrix. This helps in providing space for cell migration, proliferation, differentiation and growth, and also helps in safe implantation. Once manufactured the acquired material is carefully disinfected and finally decellularized. At the last phase, all the scaffolds undergo sterilization.

In our previous study, in the Tissue Engineered Mechanics, Imaging and Materials Laboratory (TEMIM Lab) at Florida International University (FIU), involved utilization of Cormatrix PSIS bioscaffold valves (CorMatrix Cardiovascular, Inc., Roswell, GA, USA) in the aortic position. This study aimed at testing tri-leaflet PSIS bioscaffolds to evaluate their functional effectiveness by comparing them with the porcine bioprosthetic control valves (Medtronic Freestyle, Medtronic, Minneapolis, MN) in an in-house pulse duplicator system (Vivitro Laboratories, Victoria, Canada). Both the PSIS and bioprosthetic aortic valves were subjected to physiological flow conditions of that of an average adult human. The heart rate was set at 70 bpm and the stroke volume at 80 ml/beat. The results (Fig 6) showed physiologically shaped flow and pressure data (ventricular and aortic pressure) with no significant differences in flow and pressure when compared to that of the control

bioprosthetic valves [29]. However, the tri-leaflet PSIS valves demonstrated higher forward energy losses (Table 1), i.e. during the systolic phase of cardiac cycle when the aortic valves are open for the blood to flow, as compared to the control valves [29]. The PSIS scaffold presented some promising data however further in vitro and in vivo studies are required to validate the complete effectiveness of the PSIS bioscaffold.

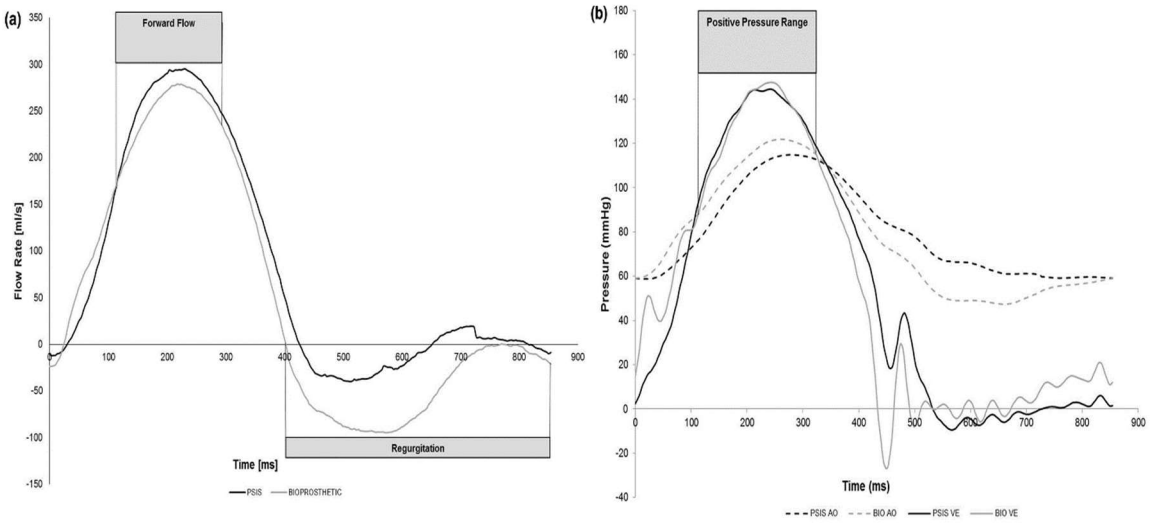
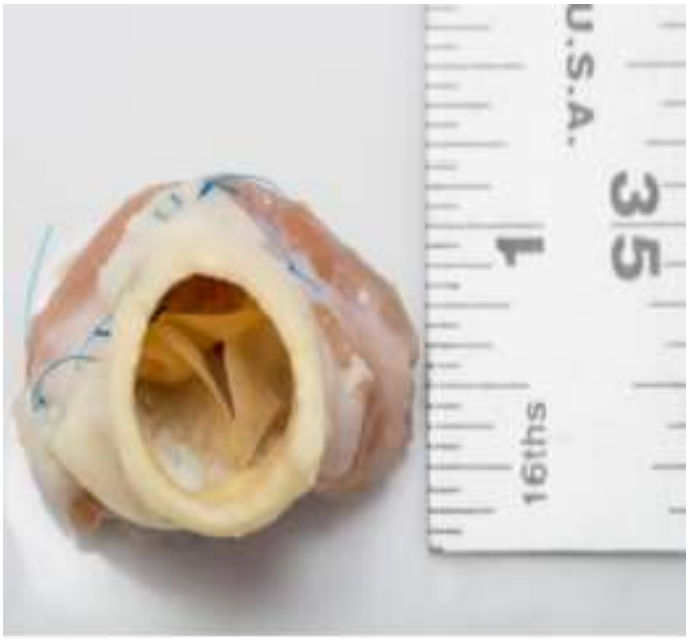


Figure 6: (a) Flow and (b) Pressure waveforms (Ramaswamy S, 2016).

Table 2: Mean ± SEM of Hydrodynamic Parameters of Aortic PSIS Valves (n=3) and Bioprosthetic Valves (n=2) during the Systolic Phase of the Cardiac Cycle (Ramaswamy S, 2016).

Aortic Valves	**ΔP (mmHg)**	**Q_{rms} (ml/s)**	**EOA (mJ)**	**Forward Flow Energy Loss* (mJ)**	**RF* (%)**
PSIS (n=3)	24.0 ± 2.4	239.9 ± 44.4	0.96 ± 0.21	286.4 ± 16.7	13.8 ± 9.8
Bioprosthetic (n=2)	18.1 ± 1.9	244.4 ± 3.6	1.12 ± 0.08	174.9 ± 17.4	51.3 ± 0.8

(A)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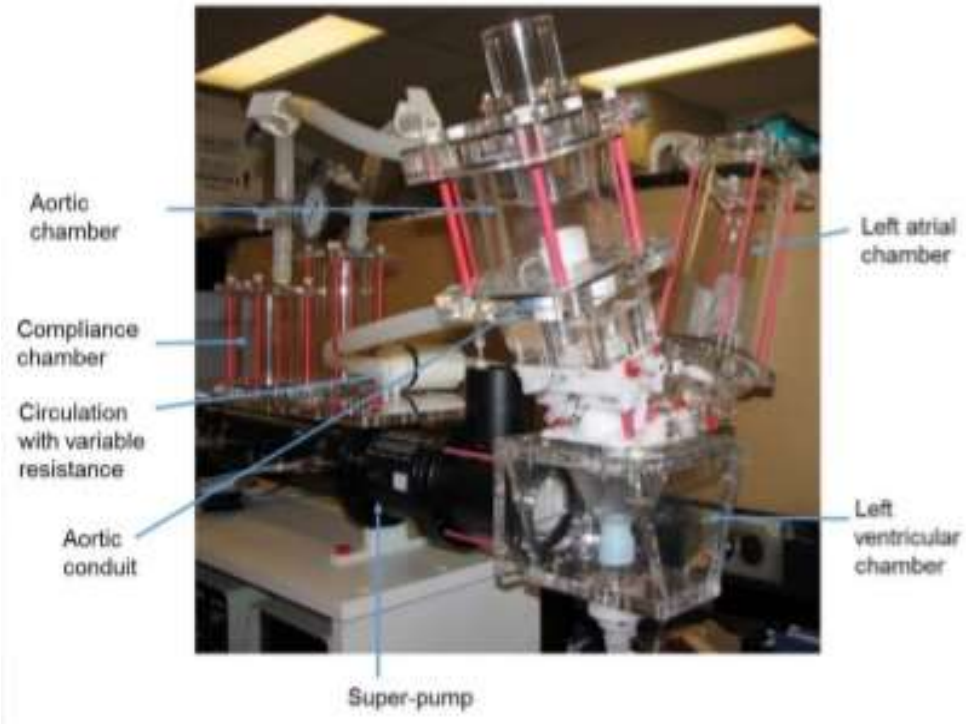
(B)


Aortic chamber
Compliance chamber
Circulation with variable resistance
Aortic conduit
Super-pump
Left atrial chamber
Left ventricular chamber

(C)

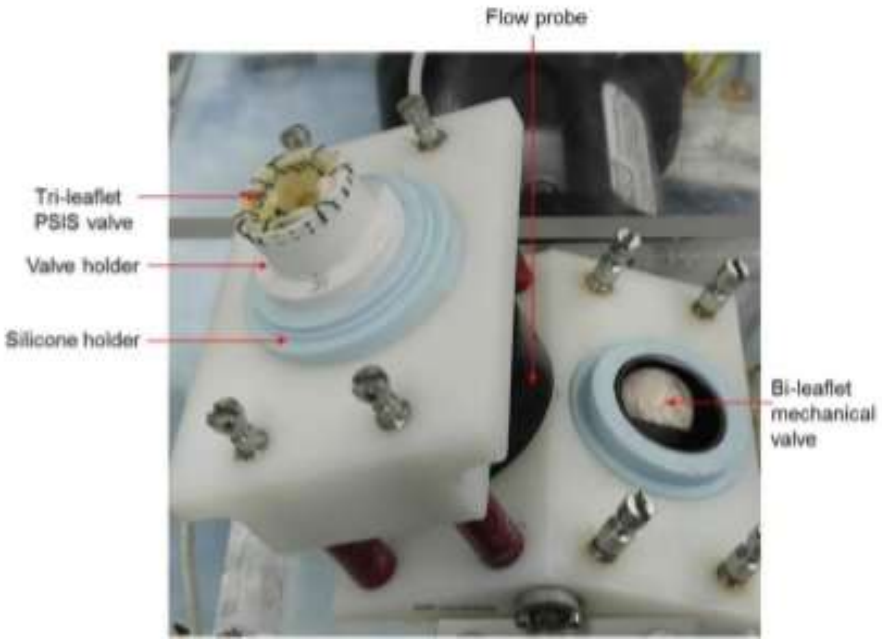


Figure 7: (a) Trileaflet PSIS valves sutured into freshly collected porcine aortic root in place of native valves (Ramaswamy S, 2016). (b) Pulse Duplicator System (Vivitro Laboratories, Victoria, Canada) for assessing hydrodynamic characteristics of the valves (Ramaswamy S, 2016). (c) Positioning of aortic and mitral valves inside the duplicator system (Ramaswamy S, 2016).

PSIS has demonstrated to be biocompatible and has no adverse response in cross-species transplantations [30, 31]. PSIS has been approved by the Food and Drug Administration (FDA) for a variety of medical applications [32]. The PSIS bioscaffold valves have the potential to deliver a solution for replacement of diseased valves in adults and specially in infants and young patients. Nonetheless long-term effects of PSIS material for tissue repair and replacement is still unknown and needs further examination Tissue engineering technique in heart valves is challenging as bioscaffolds are required possessing material properties similar to those of the native valvular tissues. Tissue engineered materials are subjected to complex hemodynamic forces, high shear stresses and large

transvalvular pressures during the cardiac cycle. Thus, it is of utmost importance that the PSIS bioscaffolds are functionally optimized for the mitral valve position when critical mitral valve disease is present, in order for valve replacement to be facilitated.

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CHAPTER 4 Methods

An ideal heart valve substitute should have excellent hemodynamic functionality. It should possess the following operational qualities: 1) adequate forward flow with reasonably small transvalvular pressure drop when open; 2) prevent backward flow with clinically acceptable small regurgitation values; 3) resist embolization 4) avoid thrombus formation; 5) resist hemolysis 6) be biocompatible; 7) be compatible with in vivo diagnostic techniques; 8) is deliverable and implantable in the targeted population; 9) continues to be secured once implanted; 10) permits reproducible function; 11) retains its functionality for an acceptable duration; 12) maintains sterility for a reasonable shelf life prior to implantation [1].

In this particular study, as our first aim, we sutured the current clinically used geometry of acellular PSIS bioscaffold valves in the analogous mitral position within the pulsatile simulator. The valves were contained within Dacron conduits. We then evaluated the hydrodynamic functionality of PSIS bioscaffold mitral valves at various native pediatric physiological hemodynamic conditions. This was achieved in vitro in a left heart simulator in our laboratory. Subsequently, in aim 2, the thickness of PSIS bioscaffold was fine-tuned to optimize the following hydrodynamic metrics: 1) valve pressure gradient, 2) forward flow, 3) energy losses and 4) regurgitation volume, with minimal PSIS material usage. Specifically, a thinner PSIS mitral valve (2ply) was evaluated as compared to the PSIS mitral valve assessed in aim 1 (4ply)

4.1 Valve Preparation:

PSIS bi-leaflet bioscaffold valves (n=6) and porcine conduitless tri-leaflet bioprosthetic control valves (Edwards Lifesciences, Irvine, CA) (n=4) were tested in an in-house pulse

duplicator system (Vivitro Laboratories, Victoria, Canada). Bi-leaflet PSIS valves assembled from sheets of PSIS bioscaffold (Cormatrix, Roswell, GA) were manually sutured in Dacron conduits by referring to the procedure previously utilized in heart valve repairs [2] (Fig. 9). In short, to construct a bi-leaflet valve, a patch of extracellular matrix (Cormatrix, Roswell, GA) was folded over the top edge of the sheet to form a cuff. A dilator of the preferred size was then used to wrap the same sheet around it. The folded-over annulus of the bioscaffold valve was attached with a running stitch to create a sewing cuff, and the free edges of the wrapped material were cut to size over a dilator. The PSIS material was then sewn together in a continuous fashion to form a tube. Following this, a running 5-0 Prolene suture was used to suture the bioscaffold to the proximal end of the Dacron tube.

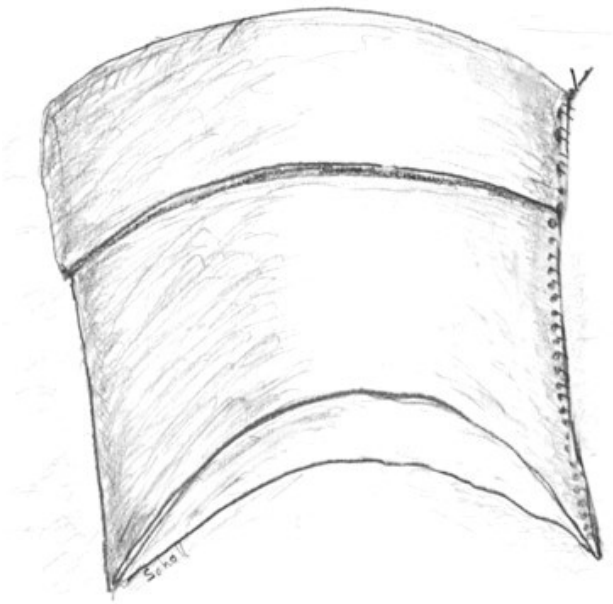

Figure 8: Appearance of the custom made bileaflet PSIS valve after construction (Bibevski S, 2015).

These constructs were subsequently immersed in a protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO), with phosphate buffer solution (PBS) as the solvent. Valve holders were designed (Solidworks, Waltham, MA) and customized for each PSIS valve to improve the solidity of the valves during *in vitro* hydrodynamic testing (Fig. 11). Precise measurements of the dimensions of each valve were recorded and the computer-aided drawing (CAD) of the valve holder was custom-made to accurately accommodate the valves. The valve holders were then fabricated using white-colored, Poly-L-lactic acid raw filament material (Dynamism, Chicago, IL) via 3-dimensional printing (Makerbot replicator series, Makerbot Industries LLC, Brooklyn, NY). Once the holders were printed, the valves were secured compactly to the holders by suturing around the annulus and sino-tubular junction (STJ) of the valve. The secure valve was then press-fitted into the mitral location within the pulse duplicator system.

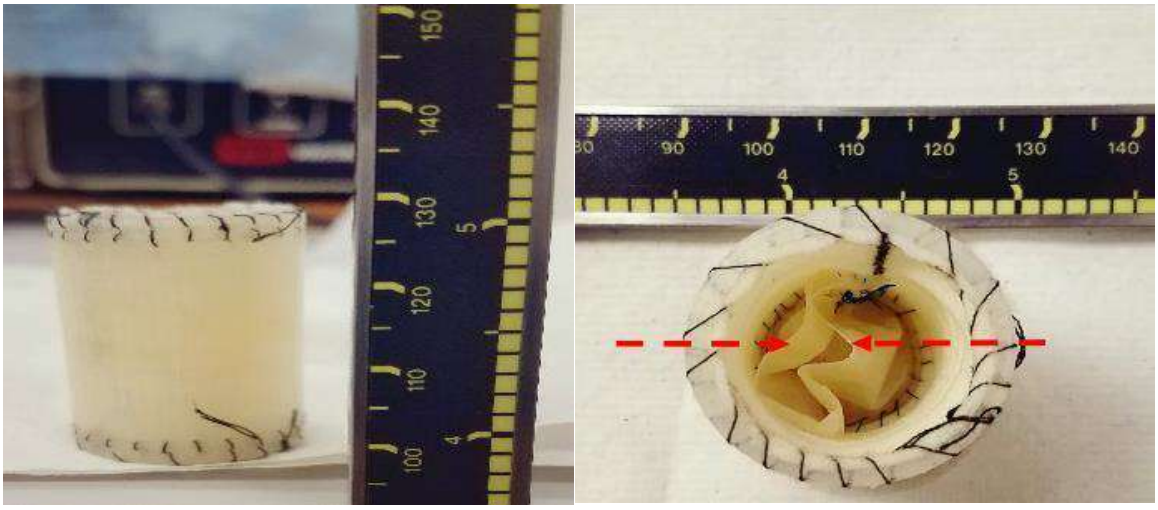

Figure 9: Bileaflet Porcine Small Intestine Submucosa valve for mitral valve testing. The two leaflets are indicated by the arrows.

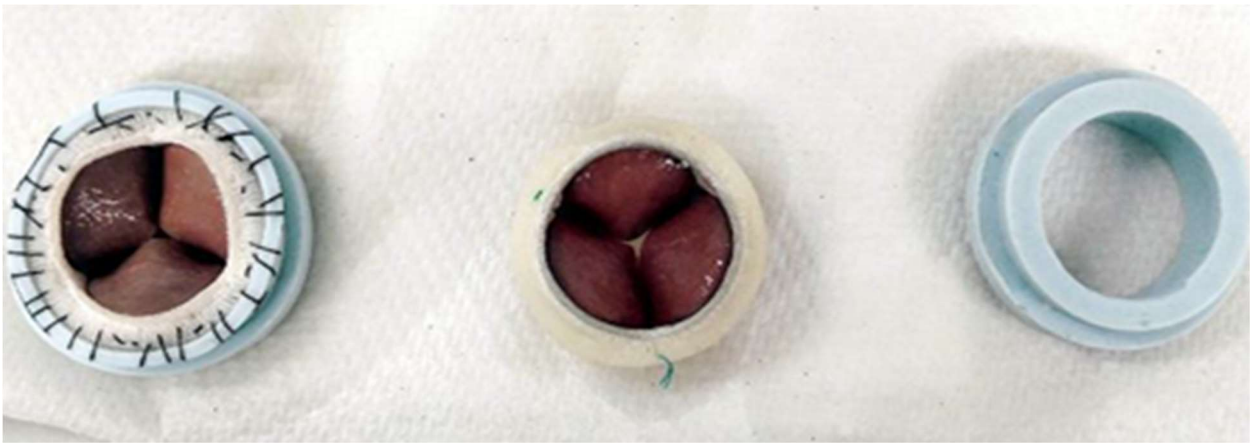

Figure 10: Edwards Lifesciences Bioprosthetic Valve serving as control valve.

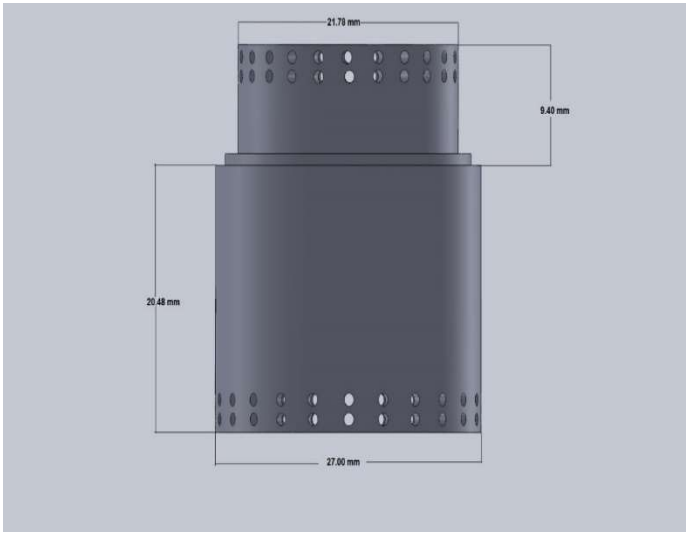

Figure 11: Valve Holder designed in SOLIDWORKS (Ramaswamy S, 2016).

4.2 Hydrodynamic Assessment:

We have utilized a commercially available pulse duplicator unit (Vivitro System, Victoria, BC) in our previous experiments to evaluate flexible-leaflet valve implants and to assess functionality of PSIS tri-leaflet aortic valves [3, 4] (Fig. 12). We used the same pulse duplicator system which accommodates bi-leaflet and tri-leaflet valve constructs and in-turn evaluates mitral and aortic valve hydrodynamic metrics respectively (e.g. energy

losses, regurgitation volume, etc.). The mechanical component of the pulse duplicator system comprises of atrial, ventricular and aortic units, which mimic those to left side of the heart, a piston pump, and a computer-based user interface (ViViTest Software, Vivitro Laboratories) for waveform specification and data acquisition/analysis. The system simulates physiologically-relevant circulatory environments defined by the user. To enable the measurement of flow and pressure data, a flow probe was positioned at the inlet of the mitral valve and two piezoresistive transducers (Model 6069, Utah Medical Products, Inc., Midvale, UT) pressure transducers at atrial and ventricular location in the system. To measure instantaneous flow rate, we placed an electromagnetic-based flow probe, connected to a flow meter (Carolina Medical Electronics), between the atrial and the ventricular chamber of the pulse duplicator just proximal to the inlet of the mitral valves. The spatial placement of the flow probe and pressure transducers was the same for all the valves tested.

In the current study, for both the aims, PSIS valves were mounted in the mitral position within the test unit, whereas a bi-leaflet mechanical valve was mounted in the aortic position to complete the loop (Fig. 13). A bi-leaflet mechanical valve (St. Jude Medical, St. Paul, MN) was mounted in the aortic position for all valve tests conducted (Fig. 13). Finally, a 0.9% saline solution was introduced through the atrial chamber, to fill the loop with fluid, in accordance with hydrodynamic valve testing industrial practice using international Organization for standardization guidelines (ISO 5840). The system was checked to confirm there were no observable leaks. Before commencing the experiment, we calibrated the entire system.

Calibration was performed for accurate pressure and flow output readings, as per ISO 5840 specifications for prosthetic valves [5]. The calibration process is a step-by-step procedure. It is very important to calibrate the entire system before using it every time. There are four different calibration steps. These steps involve zero offset measurements, pump calibration, calibration of the flow meter and flow probe, and calibration of the pressure sensors. The ViViTest software is used to accomplish this task leading the calibration values shown in the sensor calibrations panel. Before starting the calibration procedure, an open spacer ring was placed in the mitral position and the opposite side (aortic position) was closed using a solid plug. No valves were introduced during the calibration phase. We introduced the flow probe in the mitral location, i.e. site of interest. Once the system was set-up, we filled the assembly with 0.9% saline solution through the atrium chamber and the pump control was set using ViViTest software to desired levels (Heart rate = 70bpm and S50 waveform).

The first step was to zero any offset of the sensor values. Zero values for the sensors were calculated for the pressure, pump and flow sensors on a static system open to atmosphere. After zeroing the values, pump calibration was carried out in order to scale the stroke volume displayed in the software to match the stroke volume displayed on the SuperPump Controller. This was done by dialing up the stroke volume on the SuperPump through the desired testing range. Once the pump was calibrated, we proceeded towards the calibration of the flow probe and flow meter. It involved two steps, an external adjustment of the flow meter prior to flow calibration in ViViTest and tuning of the flow probe using the ViViTest. The flow meter tuning was executed by switching ON the flow meter and then adjusting the ZERO, NULL, BALANCE, and + or – switches respectively

as per the system guidelines. After this step the flow probe was calibrated by pumping the saline solution through the probe lumen at heart rate of 70bpm and stroke volume of 80 ml/beat. With this calibration, the flow rate through the transducer was made directly proportional to the piston displacement signal differentiated with respect to time (dL/dt). The dL/dt signal is an output from the SuperPump Controller. The last step was to calibrate the pressure sensors. The pressure transducers were mounted on a manifold and first exposed to the atmosphere to establish the lower end of the pressure range. The low and high pressures were chosen to span the expected working range of the transducers, in this case 0 to 200 mmHg. Then a large syringe and a digital manometer was used to apply and measure the upper range pressure, i.e. 200 mmHg. Subsequently the pressure sensors were introduced into the system in their specified location and were introduced to the static flow before saving the values. After all the calibration processes were completed, the calibration parameters were saved and used for data acquisition.

A pulsatile waveform representing a physiological flow profile (S50 waveform, Vivitro Laboratories) and which has been previously utilized for hydrodynamic studies using the same pulse duplicator system [4, 6] was selected for all tests conducted. After switching on the flowmeter, the amplitude of the pump regulator was slowly increased until the stroke volume reached 80 mL/beat.

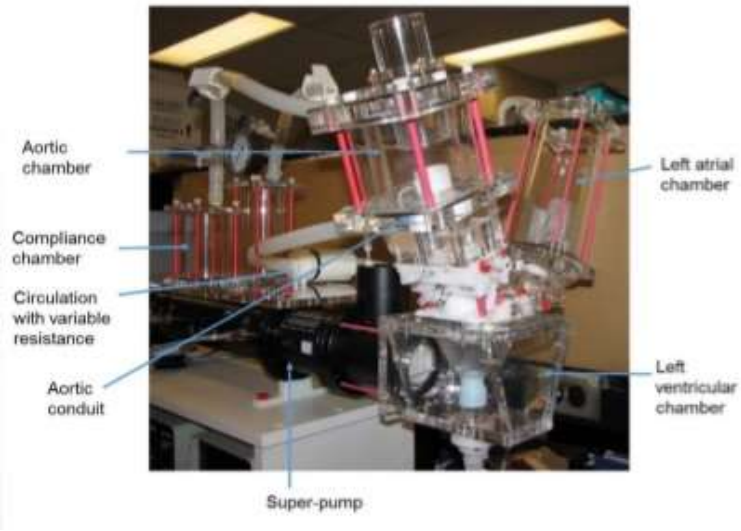

Figure 12: Pulse duplicator system (Vivitro Laboratories, Victoria, Canada).

Figure 13: Mitral valve configuration in the Pulse Duplicator System.

4.2.1 Aim 1:

Our study was specifically designed to provide potential valve replacement alternatives for infants and small children. PSIS material has the ability to provide somatic growth along with biodegradation of the material, which is an important property for pediatric

populace. However, it was important to prove the hydrodynamic functionality of this bioscaffold.

For **Aim 1**, we were interested in testing the PSIS bioscaffold mitral valves (n=5) at pediatric conditions as the main goal of this study is to potentially develop a valve replacement solution for infants and children. The established parameters incorporated two sets of flow profiles depicting left circulatory conditions in the pediatric (Table 2(a) & (b)). A physiological waveform representative of the pumping action of the left ventricle was selected (in the case of the Vivitro system the S50 waveform was chosen for all hydrodynamic tests) to drive flow through the loop [6]. At the end of each run, data was recorded over 10 continuous cycles

Table 3(a): Varying heart rates at constant stroke volume = 40 ml/beat.

Pediatric Hemodynamic Conditions at Constant Stroke Volume	Heart Rate (bpm)
	110
	125
	140

Table 3(b): Varying stroke volumes at constant heart rate = 145 bpm.

Pediatric Hemodynamic Conditions at Constant Heart Rate	Stroke Volume (ml/beat)
	20
	25
	30

4.2.2 Aim 2:

Motivation: The rationale behind this aim was to decrease the demand on body for native tissue replacement once the PSIS bioscaffold degrades. We conducted this study to test the efficacy of these 2ply PSIS valves against 4ply PSIS and bioprosthetic valves

Similar to Aim 1, the study for **Aim 2** was conducted by suturing a bi-leaflet valve configuration in the mitral position in the simulator and exposing the valves to the best selected pediatric mitral valve hemodynamic conditions. For our first aim, we tested 4-ply PSIS mitral valves (n=5) which were at 100 % thickness. The leaflet thickness was fine-tuned to establish robust functionality for the mitral position with minimal use of bioscaffold material. The thickness of the valves was reduced by half, that is we used 2-ply valves as other set of valves (thickness reduced from 100% to 50%). This approach has the potential to reduce the burden on the host since less tissue is required to fill a smaller scaffold space. It is important to maintain robust functionality of PSIS-valves but with minimal use of material thereby reducing the demand for de novo replacement tissue growth in the patient. Therefore, it was critical to conduct functional hydrodynamic testing of these valves using the same pulsatile simulator that is used to evaluate clinically-approved, flexible bio-prosthetic valves, to account for measurement differences that may be system dependent.

4.3 Data Analysis:

As mentioned before, we analyzed the data over 10 cycles for each PSIS and bioprosthetic valves. We investigated 40 runs (r = 40) for 4ply PSIS valves (n = 4), 40 runs (r = 40) for bioprosthetic control valves (n = 4) and 20 runs (r = 20) for 2ply PSIS valves (n = 2). Our pulse duplicator system (Vivitro Laboratories, Victoria, Canada) currently allows us to capture 10 cycles at a time. We have presented a table of mean +/- SEM of atrial, ventricular and forward flow values at one of the conditions (140 bpm, 40 ml/beat), over the above-mentioned runs (Table).

Table 4: Mean ± SEM of ventricular, atrial and forward flow values for the entire cycle at heart rate of 140 bpm and stroke volume of 40 ml/beat for 2ply valves (r = 20 runs), 4ply (r = 40 runs) and bioprosthetic valves (r = 40 runs).

Valves	Ventricular Pressure (VE) (mmHg)	Atrial Pressure (AT) (mmHg)	Flow (ml/s)
2ply	67.8 ± 0.42	10.9 ± 0.3	83.9 ± 1.53
4ply	55.2 ± 1.02	9.46 ± 0.49	78.9 ± 1.89
Bioprosthetic	28.6 ± 0.87	6.5 ± 0.43	78.8 ± 1.64

The mean pressure and flow calculations observed here are robust with very small errors. Therefore, we believe for short term predictions of hydrodynamic functionality across the valve, 10 cycles are adequate.

We calculated the following hydrodynamic metrics for each of the valves tested; pressure gradient (ΔP; mmHg), root mean square flow rate (Qrms; mL/s), effective orifice area (EOA; cm^2), closing energy loss, (mJ) and regurgitation fraction (% RF). Pressure gradient was directly computed from the mean pressure profiles during the diastolic phase when the mitral valve is open. Similarly, forward flow was computed during diastolic phase, directly from flow profiles of the mitral valves. However, RF and closing energy loss were computed during the systolic phase when the mitral valve is closed. The forward flow (Q_{rms}), effective orifice area (EOA) [7, 10] and closing energy loss for the mitral valves [8, 9, 10] was defined as follows:

Equation 1

$$Q_{rms} = \sqrt{\frac{Q_1^2 + Q_2^2 + \cdots + Q_n^2}{n}}$$

Equation 2

$$EOA = \frac{Q_{rms}}{51.6 * \sqrt{\Delta P}}$$

Equation 3

$$Systolic\ Energy\ Loss = \int_{t0}^{t1} \Delta p(t) * q(t) dt$$

where t0 = beginning and t1 = end respectively of the systolic phase of the cardiac cycle, Δp(t) is the pressure gradient between atrial chamber and ventricular chamber over the cardiac cycle and q(t) is the pulsatile flow rate of the fluid. Ten cycles were recorded for each PSIS and bioprosthetic valve tested and data were averaged for each group.

4.4 Statistics:

The t test was used to establish any significant differences in hydrodynamic parameters between the 4ply PSIS bioscaffold group and the Control group in aim 1, while an ANOVA test was used for aim 2 where we compared 2ply PSIS bioscaffold valves vs 4ply PSIS bioscaffold valves vs bioprosthetic valves. A difference between the two groups was considered statistically significant when $p<0.05$. All hydrodynamic metrics were presented in Mean ± Standard error of the mean (SEM).

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CHAPTER 5 Results and Discussion: Aim 1

The mitral valvular system is an intricate anatomical and functional entity. Different congenital malformations may affect the mitral valve and can occur as an isolated defect or in combination with other complex left sided lesions [1]. In one echocardiographic study, congenital mitral valve malformation was detected in almost 0.5% of the 13,400 subjects [2]. Mitral valve repair is always desirable when possible over replacement [3,4]. Generally critical congenital mitral valve pathology in infants and children poses frequent challenges to pediatric cardiac surgeons, as mitral valve replacement (MVR), being inevitable, increases the rate of morbidity in the pediatric population significantly [5]. When reconstruction fails or is not feasible, valve replacement becomes unavoidable.

The pediatric and neonatal population with mitral valve disease has limited choices for prosthetic valve replacement because of the unavailability of suitable prosthetic valve alternatives and restrictions with respect to patient to prosthetic dimension [7]. The major drawback with the current prosthetics is their inability to grow with the patient, thus requiring multiple valve replacement surgeries as the child grows. Implantation of large prosthetic mitral valves in the supra annular position and Ross II procedure are the two available options for infants and children suffering from mitral valve defect. However, these options have their drawbacks specially in pediatric patients as use of prosthetic valves is associated with a high rate of thromboembolic adverse events. Mechanical prosthetic valves are durable but lead to clotting of the blood, thus requiring life-long anticoagulation treatment. Bioprosthetic valves and Homografts on the other hand do not require life-long anticoagulation, but are less durable as compared to mechanical valves. Also, these valves do not provide for somatic growth and thus there is always a need of multiple valve

replacement surgeries thus leading to further complications. The Ross II procedure also have limitations in terms of somatic growth [8, 9].

For pediatric application, an ideal valve should support somatic growth, i.e., grow with the patient, have low risk of thrombosis and should fit into the small annular sizes of infants and children. In this study, we proposed a technique of mitral valve replacement with porcine small intestinal sub-mucosal bioscaffold (PSIS) valve, which is custom handmade and has the potential to be implanted in an infant and a small child. As previously stated, compassionate care cases involving neonates suffering with congenital critical valve disease, our collaborators have already employed this bioscaffold for valvular replacement. According to our understanding we are the only group to have utilized this technique in pediatric patients for the management of congenital heart valve disease in infants and children. In Vivo studies have reported that the valves have performed well, with lowered transvalvular pressure gradients and not necessitating anti-coagulant therapy [10, 11].

PSIS bioscaffold as described before is an extracellular matrix having acellular, biodegradable soft tissue material properties which has the potential to remodel itself after implantation and leave in its place a healthy and organized native tissue. PSIS possesses the molecular make-up of native cardiovascular structures, such as collagen and elastin. It also possesses fibronectin and laminin adhesion glycoproteins, glycosaminoglycans (GAGs) and matricellular proteins (e.g. thrombospondins, osteopontin, and tenascins). These properties make it suitable for cardiovascular regenerative applications especially in infants and small children.

Although some results have been positive, substantial uncertainties still remain. There are reports that show adverse effects of PSIS bioscaffold. The first adverse results were

published by Weber and colleagues. The results exhibited pseudoaneurysm formation with disorganized collagen in patients who had undergone carotid artery patch angioplasty with PSIS bioscaffold [12]. In the other study, it has been reported presence of hemodynamically significant lesions at the site of the PSIS implantation which required reoperation, in 6 of 25 patients receiving PSIS patches during cardiac operations [13]. Explanted specimens established an intense inflammatory response characterized by numerous eosinophils, histiocytes, and plasma cells, accompanied by granulation tissue and fibrosis [13]. Few studies have reported the inflammatory response to CorMatrix PSIS bioscaffold. In couple of studies PSIS scaffold inflammation was observed in the graft application. Similar findings of an intense inflammatory response to the material was noticed in the aortic patch along with early critical disintegration and aneurysm formation [14].

In one of the pediatric studies by Zaidi et al, histologic evaluation of 11 Cormatrix PSIS aortic and mitral valves was presented [15]. These valves were explanted due to fixation, thickening, prolapse of the leaflets, dehiscence and folded PSIS patch. The 9 months implantation results showed no remodelling in analogous to native valves having three layers along with the inflammatory response. In other study, implants obtained from 11 pediatric patients relating to 2 mitral valves, 2 aortic valves, 8 outflow septal or conduit patches, tissue necrosis was observed in 5 cases along with chronic inflammation [16]. One of the surgical procedures reported delamination of the bioscaffold patches which caused continued washing of the PSIS material [17]. PSIS valves are scaffolds that are designed by laminating multiple layers together. The delamination can also lead to blood flow loss due to blood being potentially captured within the multiple laminations, resulting in failure of the valves.

Cardiovascular applications of CorMatrix PSIS patch is comparatively new. There are currently mixed reviews about this bioscaffold. However, these studies have certain limitations mainly with regards to lack numbers of subjects and organized reporting. In spite of these, the growth prospects and mid to long term potential has not been systematically, nor scientifically explored. Here our aim was to assess the functionality of PSIS bioscaffolds in mitral valve replacement application. We understand and recognize the significance of performing a systematic assessment of PSIS mitral valve functionality in terms of its opening and closing properties, before this expertise can be made available widely for all the patients as a first choice and not just used for compassionate care cases.

To achieve this purpose, PSIS bioscaffold valves were examined under the physiologically expected hydrodynamic conditions of native mitral valve from pediatric population. To best assess these bioscaffold valves in vitro under physiological conditions we utilized a Vivitro pulse duplicator available in our laboratory (Vivitro Laboratories, Victoria, BC). It has been modified to facilitate these testing and used before to test flexible leaflet valve and PSIS aortic bioscaffold valve implants [18, 19]. Thus, with this device, we could assess hydrodynamic functionality of sutured PSIS bioscaffold valves which were contained within a Dacron conduit.

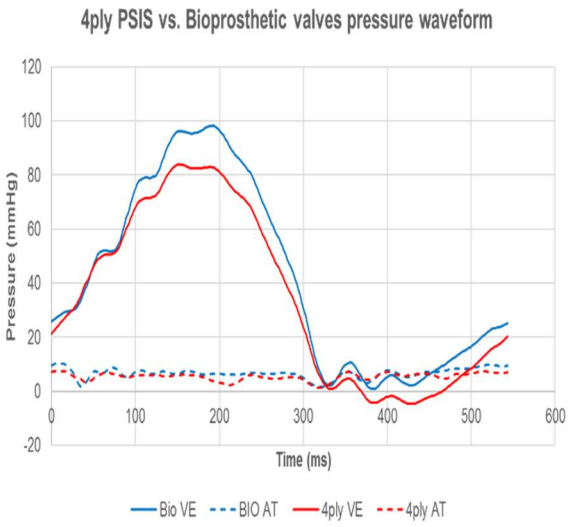
Bi-leaflet PSIS bioscaffold valve templates (Cormatrix, Roswell, GA) were assembled manually into the Dacron conduit as described in the methods section and which has been used to treat clinical cases for critical congenital mitral and aortic valve diseases respectively. The valves were then secured to the custom-made valve holder by progressing with suturing along the circumference of the holder. The holder with the sutured PSIS-valve was mounted in the mitral position within the test unit, whereas a bi-leaflet

mechanical valve was mounted in the aortic position, within the pulse duplicator system (Vivitro Laboratories, Victoria, BC). A 0.9% saline solution was introduced through the atrium chamber, to fill the loop with fluid. To enable the measurement of flow and pressure data, a flow probe was positioned at the inlet of the mitral valve and two pressure transducers at atrial and ventricular location in the system. Calibration was performed to warrant for accurate pressure and flow output readings. After the system was checked for any observable leaks, testing was initiated. The established parameters (heart rate, stroke volume) incorporated two sets of flow profiles depicting left circulatory conditions in the pediatrics.

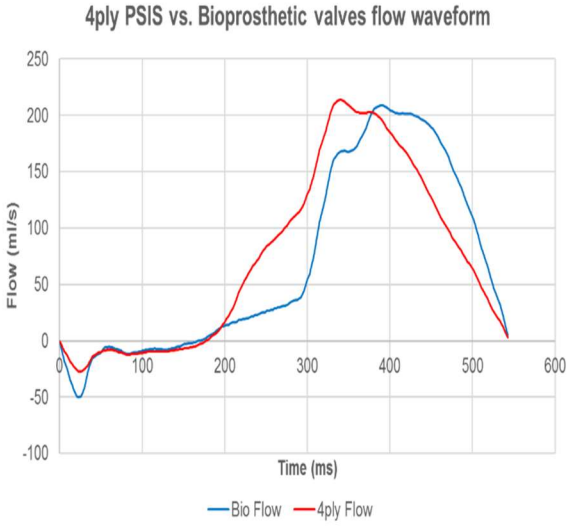
A physiological waveform representative of the pumping action of the left ventricle was selected (in the case of the Vivitro system the S50 waveform was chosen for all hydrodynamic tests) to drive flow through the loop [19]. At the end of each run, data was recorded over 10 continuous cycles.

Results and Discussion:

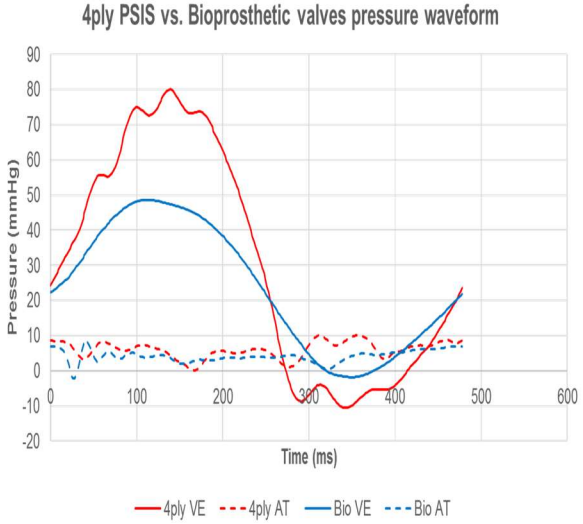
Results were compared to commercially available porcine bioprosthetic mitral heart valves (control group).


4ply PSIS vs. Bioprosthetic valves pressure waveform
Pressure (mmHg)
Time (ms)
Bio VE
BIO AT
4ply VE
4ply AT

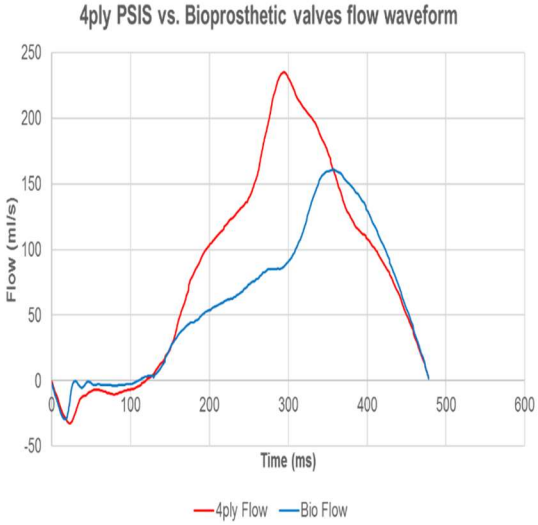
(A)


4ply PSIS vs. Bioprosthetic valves flow waveform
Flow (ml/s)
Time (ms)
Bio Flow
4ply Flow

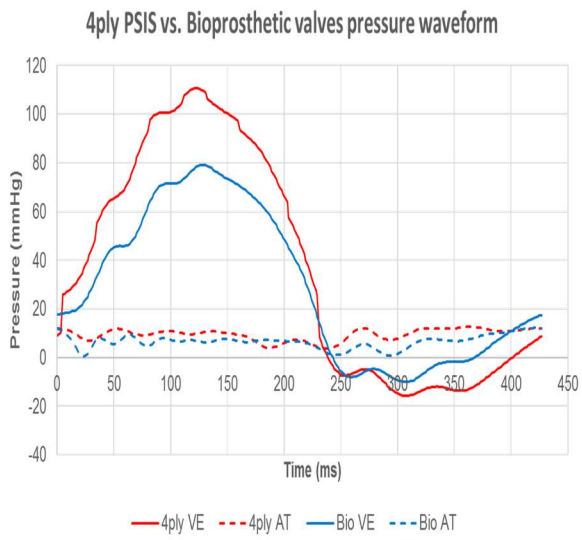
(B)


4ply PSIS vs. Bioprosthetic valves pressure waveform
Pressure (mmHg)
Time (ms)
4ply VE
4ply AT
Bio VE
Bio AT

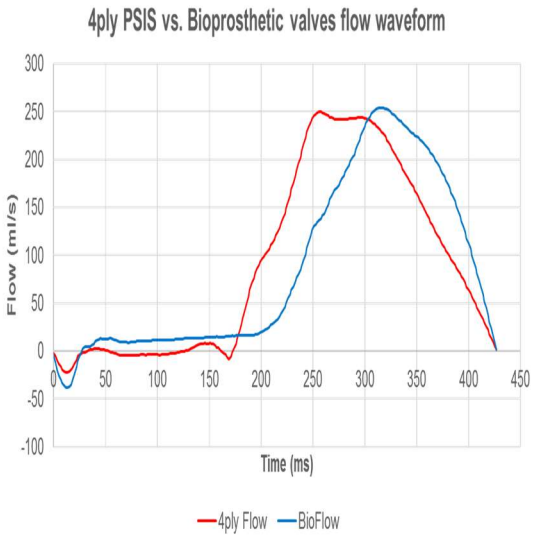
(C)


4ply PSIS vs. Bioprosthetic valves flow waveform
Flow (ml/s)
Time (ms)
4ply Flow
Bio Flow

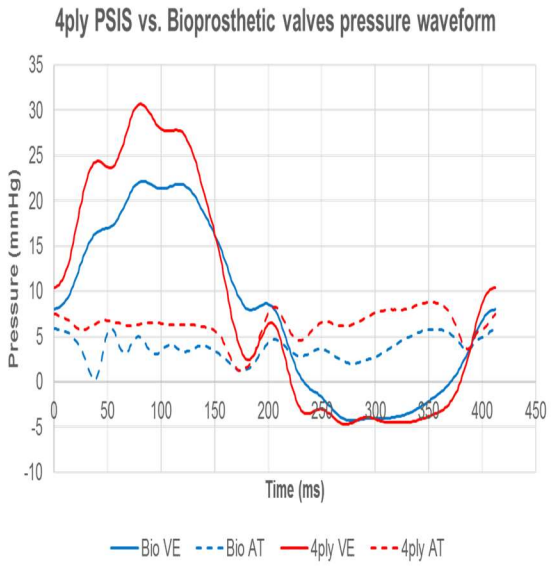
(D)


4ply PSIS vs. Bioprosthetic valves pressure waveform
Pressure (mmHg)
Time (ms)
4ply VE
4ply AT
Bio VE
Bio AT

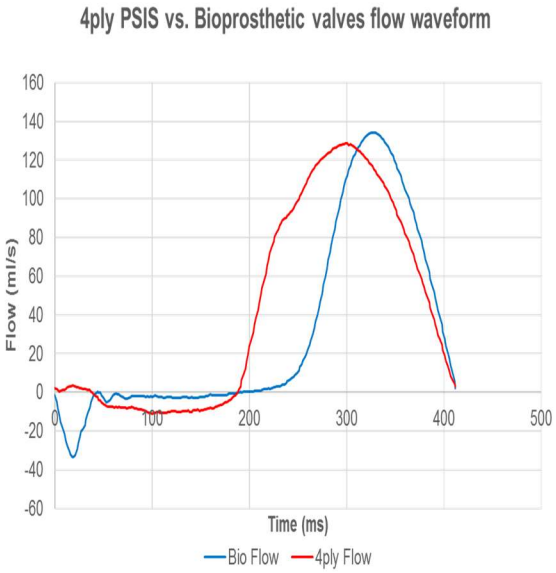
(E)


4ply PSIS vs. Bioprosthetic valves flow waveform
Flow (ml/s)
Time (ms)
4ply Flow
BioFlow

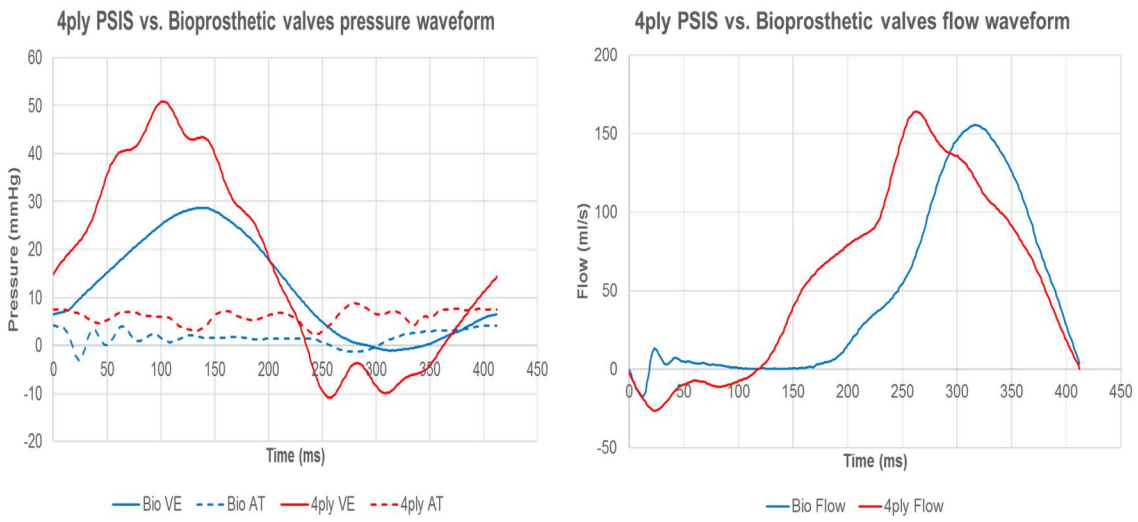
(F)


4ply PSIS vs. Bioprosthetic valves pressure waveform
Pressure (mmHg)
Time (ms)
Bio VE
Bio AT
4ply VE
4ply AT

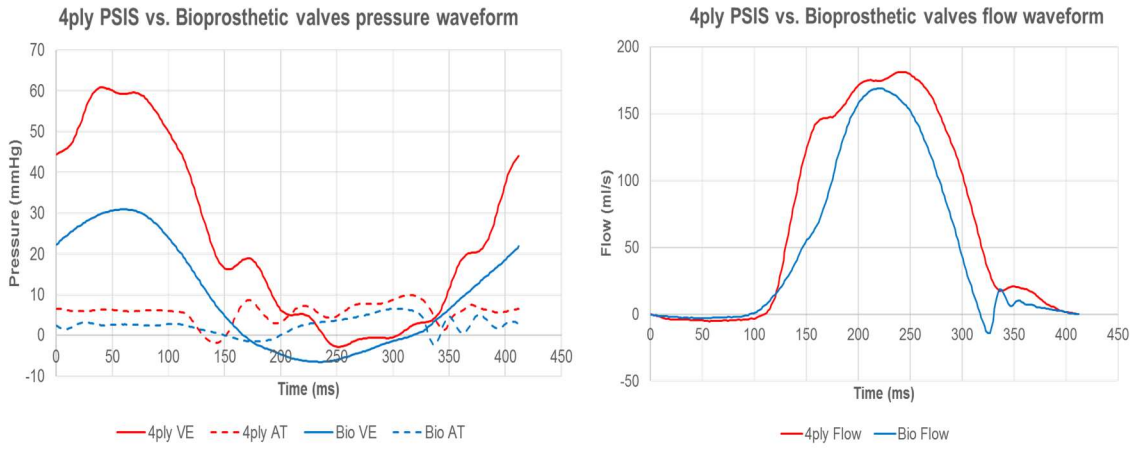
(G)


4ply PSIS vs. Bioprosthetic valves flow waveform
Flow (ml/s)
Time (ms)
Bio Flow
4ply Flow

(H)



(I) (J)



(K) (L)

Figure 14: Mean temporal (A, B) pressure and flow waveforms at 110 bpm and 40 ml/beat, (C, D) pressure and flow waveforms at 125 bpm and 40 ml/beat, (E, F) pressure and flow waveforms at 140 bpm and 40 ml/beat, (G, H) pressure and flow waveforms at 145 bpm and 20 ml/beat, (I, J) pressure and flow waveforms at 145 bpm and 25 ml/beat, (K, L) pressure and flow waveforms at 145 bpm and 30 ml/beat measured for 4ply PSIS valves (n=4) and Bioprosthetic valves (Edwards Lifescience) (n=4) tested in the mitral position in our pulse duplicator system (Vivitro Laboratories).

Upon completion of flow and pressure profiles we computed the following hydrodynamic metrics: pressure gradient, forward flow, effective orifice area of the valve, systolic energy losses and regurgitation volumes. Our findings were as following results. Results are summarized in Table 3(a), 3(b), 4(a) and 4(b).

Table 5(a): Mean ± SEM of Hydrodynamic Metrics of PSIS Mitral Valves (n=4) at constant stroke volume of 40 ml/beat.

Heart Rate (bpm)	**$\Delta P_{(diastolic)}$ (mmHg)**	**Peak $\Delta P_{(systolic)}$ (mmHg)**	**Q_{rms} (ml/s)**	**EOA (cm^2)**	**Systolic Energy Loss (mJ)**	**RF (%)**
110	4.64 ± 0.45	78.5 ± 3.19	135.6 ± 17.3	1.22 ± 0.08	20.6 ± 4.1	4.25 ± 1.8
125	6.02 ± 0.3	75.4 ± 3.46*	148.6 ± 6.88	1.26 ± 0.03	29.3 ± 5.87	3.54 ± 0.64
140	6.17 ± 0.75*	115.9 ± 3.74*	159 ± 7.89	1.31 ± 0.2	39.1 ± 3.12	4.38 ± 0.25

Table 5(b): Mean ± SEM of Hydrodynamic Metrics of porcine trileaflet Bioprosthetic (Edwards Lifescience) (n=4) at constant stroke volume of 40 ml/beat.

Heart Rate (bpm)	**$\Delta P_{(diastolic)}$ (mmHg)**	**Peak $\Delta P_{(systolic)}$ (mmHg)**	**Q_{rms} (ml/s)**	**EOA (cm^2)**	**Systolic Energy Loss (mJ)**	**RF (%)**
110	4.32 ± 0.15	91.8 ± 5.47	127.1 ± 4.21	1.19 ± 0.07	14.5 ± 2.27	3.44 ± 0.72
125	5.92 ± 0.32	44.6 ± 4.15*	157.4 ± 2.42	1.35 ± 0.05	19.2 ± 4.8	3.2 ± 0.37
140	5.34 ± 0.24*	72.8 ± 4.4*	178.2 ± 6.52	1.56 ± 0.09	29.8 ± 3.76	3.71 ± 0.68

Note: '*' indicates statistical significance.

Table 6(a): Mean ± SEM of Hydrodynamic Metrics of PSIS Mitral Valves (n=4) at constant heart rate of 145 bpm.

Stroke Volume (ml/beat)	$\Delta P_{(diastolic)}$ (mmHg)	Peak $\Delta P_{(systolic)}$ (mmHg)	Q_{rms} (ml/s)	EOA (cm^2)	Systolic Energy Loss (mJ)	RF (%)
20	4.24 ± 0.27	28.4 ± 2.12*	99.4 ± 2.80	0.92 ± 0.04	8.88 ± 1.67	3.04 ± 0.38
25	4.99 ± 0.3	44.9 ± 3.07*	119.4 ± 3.21	0.99 ± 0.07	17.2 ± 4.11	3.59 ± 0.51
30	5.6 ± 0.38	54.6 ± 2.66*	137.3 ± 1.35	1.12 ± 0.09	30.82 ± 2.77	4.14 ± 0.4

Table 6(b): Mean ± SEM of Hydrodynamic Metrics of porcine trileaflet Bioprosthetic (Edwards Lifescience) (n=4) at constant heart rate of 145 bpm.

Stroke Volume (ml/beat)	$\Delta P_{(diastolic)}$ (mmHg)	Peak $\Delta P_{(systolic)}$ (mmHg)	Q_{rms} (ml/s)	EOA (cm^2)	Systolic Energy Loss (mJ)	RF (%)
20	4.05 ± 0.7	17.7 ± 2.56*	103.2 ± 4.6	1.01 ± 0.1	6.29 ± 0.08	2.65 ± 0.5
25	4.4 ± 0.32	27 ± 2.79*	120.9 ± 2.85	1.03 ± 0.06	11.8 ± 1.1	2.9 ± 0.49
30	5.14 ± 0.41	28.2 ± 4.3*	145 ± 3.56	1.2 ± 0.04	20.68 ± 3.9	3.08 ± 1.7

Note: '*' indicates statistical significance

As stated, we assessed the PSIS bi-leaflet bioscaffold valves for acute functionality in the mitral position at 6 different pediatric physiological conditions of stroke volume and heart rate. We compared PSIS mitral valves with the bioprosthetic control group at all conditions. For pediatric conditions results demonstrated similar physiological pressure

and flow data when compared with trileaflet bioprosthetic valves. However, we see some discrepancies in the ventricular pressure between the two group as we increase the heart rate.

At lower pediatric relevant heart rate of 110 bpm, the pressure and flow waveforms were very similar, but as we increased the heart rate keeping the stroke volume constant at 40 ml/beat, we observed differences particularly in the ventricular pressure. In the case of varying stroke volume at the constant heart rate of 145 bpm, even at the lower stroke volume we saw some key difference in both the ventricular pressure and the flow rate. As we increase the stroke volume, we noticed these differences continued. Thus, at higher heart rates and higher stroke volume, the differences in the ventricular pressure and some differences in the flow rate were noticeable. Peak systolic pressure gradient for 4ply PSIS valves during constant stroke volume ranged from 78.5 mmHg to 115.9 mmHg and for bioprosthetic valve it ranged from 44.6 mmHg to 91.8 mmHg. At constant heart beat the systolic pressure gradient range for 4ply PSIS valves was from 28.4 mmHg to 54.6 mmHg and for bioprosthetic valve 17.7 mmHg to 28.2 mmHg.

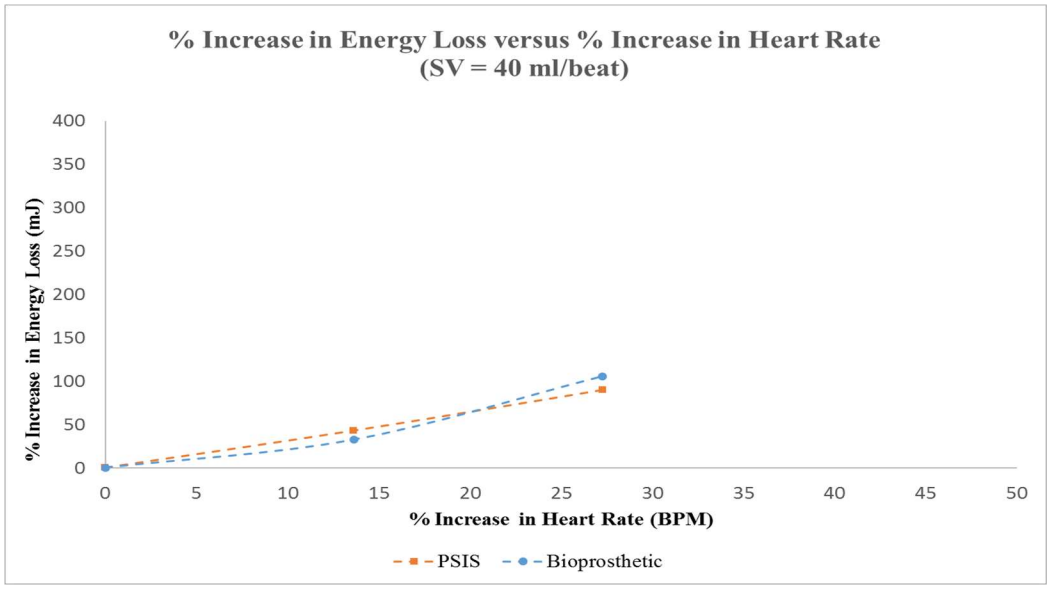
The normal systolic pressure for adult is 100-140 mmHg [20] and for child before adolescent is 60-120 mmHg which gradually increases as the child grows [21]. We observed that peak systolic pressure gradient for PSIS valves increased progressively with the increase in the cardiac output under pediatric conditions and were close to the reference values. Conversely, for the bioprosthetic valves we noticed that the peak systolic pressure gradient was relatively low. The peak systolic gradient for bioprosthetic valve was statistically lower than that of the PSIS valves at all conditions, except at 110 bpm at 40 ml/beat. However, the mitral valves in both the groups were efficiently closed during the

systolic phase as can be observed from the RF (%), which was low for both PSIS and bioprosthetic valves. The low peak pressure gradient during the systolic phase may be due to the material properties of the bioprosthetic scaffold. In our previous publication [19], we reported mean Young's modulus of both the PSIS and chemically-fixed bioprosthetic values. Mean Young's modulus for bioprosthetic valves was ~43 MPa [24], whereas PSIS valve tissues were comparatively stiffer circumferentially with modulus of elasticity of 101.99 ± 58.24 MPa (but only about 9.18 ± 1.81 MPa radially) [25, 26]. We speculate that the bioprosthetic valves being less stiff circumferentially may have potentially led to a lower peak systolic gradient.

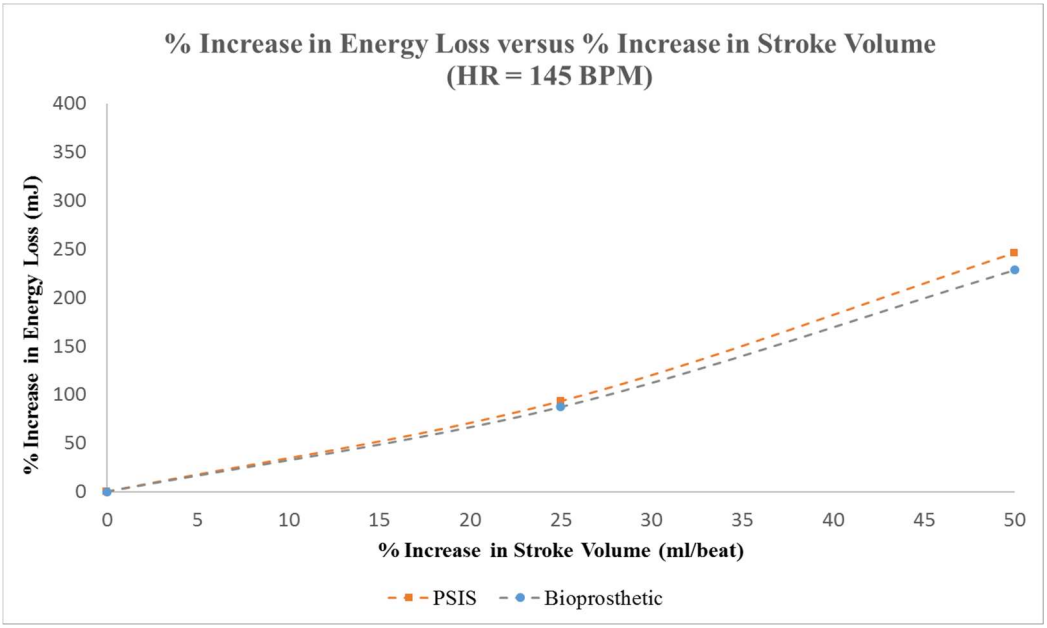
EOAs were comparable at all 6 different conditions ($p<0.05$) which permitted an objective comparison in acute hydrodynamic functionality between the two groups. ΔP was almost similar in both the groups and statistically insignificant in all the cases, expect at 140 bpm and 40 ml/beat. Even though the ΔP was significantly higher at this condition, it was very close to the acceptable clinical value [22]. Forward flow was dominant in the diastolic phase when the mitral valve is open with relatively low to no forward flow during systole. Forward flow (Q_{rms}) was found to be not significantly different ($p>0.05$) between the two groups thus resulting in robust flow data. We noticed slightly greater regurgitation fraction (RF) in PSIS group. However, RF was statistically insignificant ($p>0.05$) in comparison to the bioprosthetic group and also in the acceptable clinical range [23].

Although the systolic energy losses observed in both groups at 6 different conditions were not statistically significant, the PSIS-valves exhibited higher energy losses during the systolic period compared to the bioprosthetic controls. We noticed that as we increase the stroke volume or the heart rate (Fig 15 (a), (b)), the net effect of the energy loss was the

same in both the 4ply PSIS valves and the bioprosthetic valves. When we computed the cardiac output, and looked at the percent increase in the energy loss (Fig 15 (c)), the net effect of the energy loss in 4ply PSIS valves was again consistent as that of the bioprosthetic control group, i.e. increase by approximately 3.5 times with doubling of the cardiac output in both groups.



(a)



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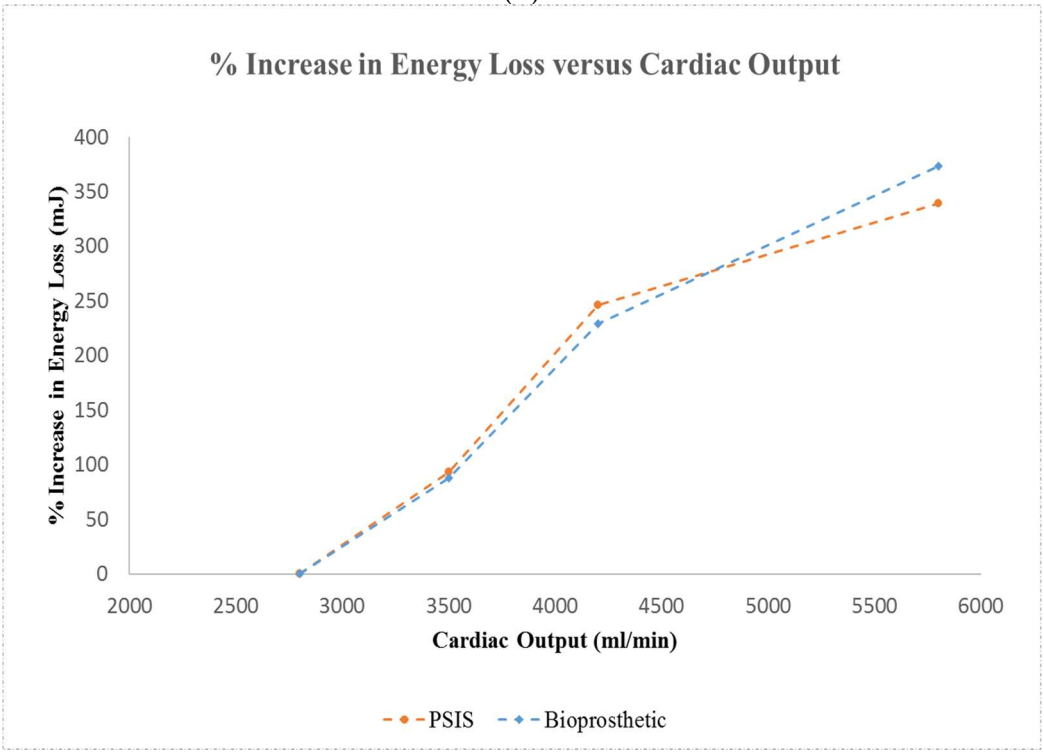

(c)

Figure 15: % increase in PSIS and Bioprosthetic energy loss during systolic phase vs. (a) % increase in heart rate at constant stroke volume of 40 ml/beat. (b) % increase in stroke volume at constant heart rate of 145 bpm. (c) cardiac output (ml/min).

Nevertheless, it is obligatory that the PSIS valves grow longitudinally and have symmetric growth after implantation. This accurate somatic growth is the must to mitigate or to support the normal rise in the energy losses. However, if the growth is inadequate and random or if there is improper growth of the PSIS bioscaffold, this will adversely affect the energy loss parameter, resulting in greater energy losses and subsequent failure of the PSIS mitral valve, ultimately causing increased workload on the heart.

We evaluated energy loss during the systolic phase as most of the mitral valve disease is associated with mitral regurgitation or prolapse as compared to mitral stenosis. As

Energy loss may serve as a biomarker indicating improper closing of the mitral valve during the systolic phase and potentially long-term detriment to heart function. Sufficient energy applied by the contraction of the left ventricular chamber helps in the proper closing of the mitral valves, thus avoiding regurgitation or prolapse. The mitral valves that do not close aptly may lead to more vicious pumping of the left ventricle, thus resulting in higher energy losses. This may eventually lead to heart failure.

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CHAPTER 6 Results and Discussion: Aim 2

It is important for infants and children that a tissue engineered or biocompatible heart valve prosthesis possess appropriate hemodynamic profile, low thrombogenic potential, provide somatic growth and an integral part of patient's native tissue over time [2]. One promising bioscaffold material for the assembly of such a valve is an acellular bioscaffold composed of non-crosslinked extracellular matrix (ECM) derived from porcine small intestinal submucosa (PSIS). This material has shown bioresorbable capabilities of in vivo native cellular recruitment and repopulation in a broad range of tissues, including cardiac tissue [3-7]. Promising in vivo growth, both circumferential and longitudinal, has also been reported in a study that utilized PSIS material as an interpositional vena cava graft in a growing piglet. The longitudinal growth of the PSIS graft was reported to be around 147% and circumferential around 184% over a 3-month time period [8].

When implanted in the body, it has been observed that the degradation of the ECM scaffold by immunomodulatory, pro-remodeling macrophages produces biochemical cues that attract progenitor cells. and, in concert with the surrounding biomechanical and fluid-dynamic environment, induce progenitor-cell proliferation and phenotypic maturation [5, 9, 10]. Products of matrix degradation also confer antimicrobial properties on the ECM material [11] and lack of crosslinking reduces its propensity for inflammation and calcification [5]. With respect to the physical architecture of a bioprosthetic heart valve, experimental and clinical studies over the past 2 decades have demonstrated the superiority of a tubular design in terms of restoring normal transprosthetic flow dynamics and stress distribution on the valve leaflets, regardless of the type of tissue or other material used to construct such tubular valves [12].

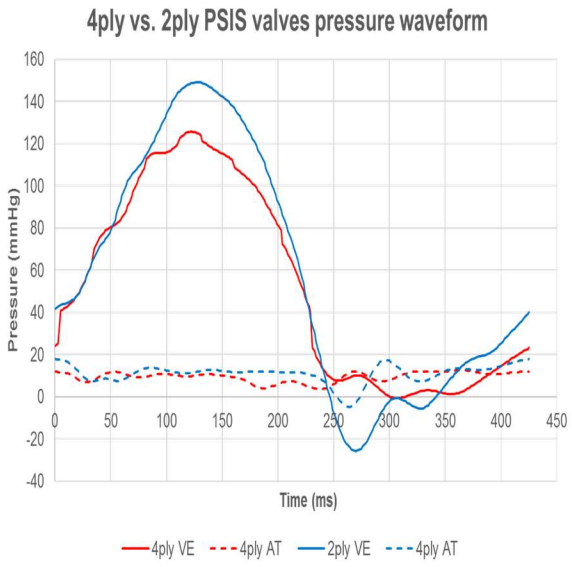
As stated in the previous chapters in this thesis, the main focus of this study was the determine hydrodynamic function of a PSIS mitral valve design for valve replacement in infants and children suffering from severe congenital heart valve disease, which can accommodate somatic growth. PSIS is a decellularized bioscaffold from the submucosal layer of the porcine small intestine. A key attractive feature of PSIS bioscaffold is its biodegradability with de novo replacement of tissues exhibiting the cardiovascular phenotype [13, 14]. The specific objective of aim 2 in this investigation was to reduce the thickness of the PSIS-assembled mitral valves from ~ 320 μm (4 ply) to ~ 166 μm (2 ply), which results in an overall percent volumetric reduction of ~ 54 %. In theory, this reduction contributes towards reduction in burden on the body to produce newer valvular tissues. Moreover, another potential benefit of a thinner valve would be a decreased risk of leaflet delamination which would be detrimental in the context of increased thrombus risk and interfering with unidirectional blood flow.

Nonetheless, an important aspect of thickness reduction, is the critical need to continue to improve upon or at least maintain robust hydrodynamic functionality of the bioscaffold with minimal use of PSIS material. To accomplish this task, it is important to conduct functional testing of 2ply bioscaffold mitral valves using the same pulse duplicator system (Vivitro Laboratories, Victoria, BC) that was used to evaluate PSIS bioscaffold of 4 ply mitral valve thickness in aim 1, which is routinely used in the FDA approval process for commercially-available mechanical and bioprosthetic valves. All the steps carried out were the same as mentioned before for the 4ply valves. However, the parameters selected were only 2; (i) Heart rate of 140 bpm and stroke volume of 40 ml/beat. (ii) Heart rate of 145 bpm and stroke volume of 20 ml/beat. We sutured the 2ply PSIS valves into custom

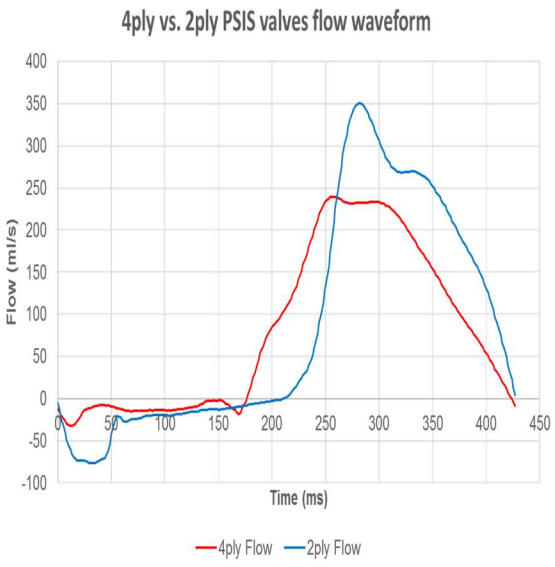
adapters similar to that of the 4ply valves. The adaptor with the secured valves was then press-fit into the mitral position into the pulse duplicator system for subsequent testing.

The testing was performed similarly as in first aim and 10 cycles were recorded for each valve and at each set of parameters. Figure 15 (A-D) depicts the pressure and flow waveforms for 4ply PSIS vs. 2ply PSIS mitral valves. Figure 15 (A, B) are the pressure and flow waveforms for the PSIS valves tested at heart rate of 140 bpm and stroke volume of 40 ml/beat. Likewise, pressure and flow plots for PSIS valves tested at heart rate of 145 bpm and stroke volume of 20 ml/beat are illustrated by figure 15 (C, D).

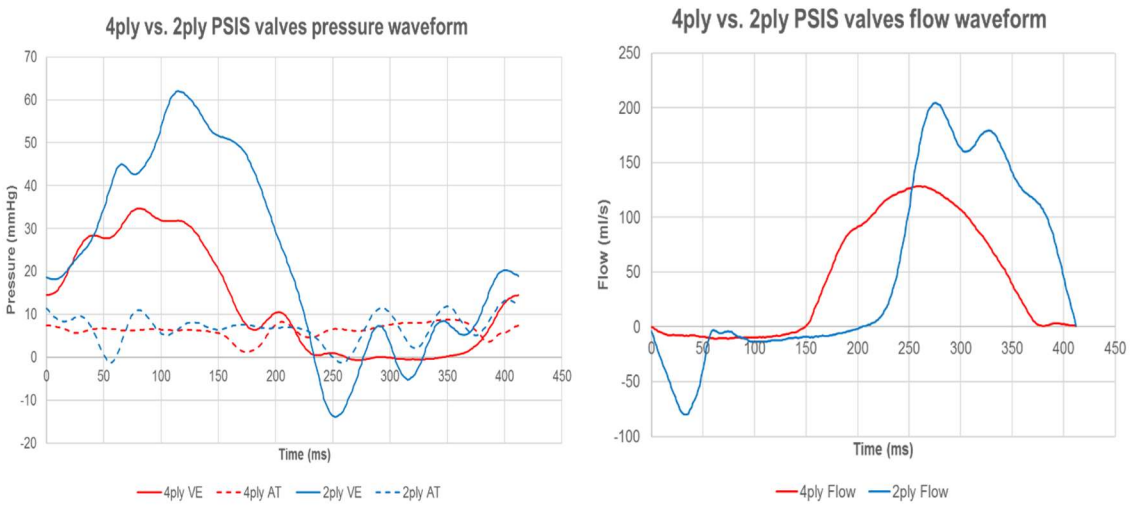
Results and Discussion:



(A)



(B)



(C) (D)

Figure 16: Mean temporal (a) pressure waveforms at 140 bpm and 40 ml/beat, (b) flow waveforms at 140 bpm and 40 ml/beat, (c) pressure waveforms at 145 bpm and 20 ml/beat and (d) flow waveforms at 145 bpm and 20 ml/beat measured for 4ply PSIS valves (n=4) and 2ply PSIS valves (n=2) tested in the mitral position in our pulse duplicator system (Vivitro Laboratories).

Table 7(a): Mean ± SEM of Hydrodynamic Metrics of 2ply PSIS mitral valve (n=2) vs 4ply PSIS mitral valve (MV) (n=4) vs porcine trileaflet Bioprosthetic valve (BPV) (Edwards Lifescience) (n=4) at heart rate of 140 bpm and stroke volume of 40 ml/beat.

MV	**$\Delta P_{(diastolic)}$ (mmHg)**	**Peak $\Delta P_{(systolic)}$ (mmHg)**	**Q_{rms} (ml/s)**	**EOA (cm^2)**	**Systolic Energy Loss (mJ)**	**RF (%)**
2ply	6.56 ± 0.34	137.4 ± 1.59*	262.3 ± 29.8*	1.97 ± 0.18	25.2 ± 2.81	7.03 ± 0.82*
4ply	6.17 ± 0.75	115.9 ± 3.74	159 ± 7.89	1.31 ± 0.2	39.1 ± 3.12	4.38 ± 0.25
BPV	5.34 ± 0.24	72.8 ± 4.4	178.3 ± 6.52	1.56 ± 0.09	29.8 ± 3.76	3.71 ± 0.68

Note: '*' indicates statistical significance.

Table 7(b): Mean ± SEM of Hydrodynamic Metrics of 2ply PSIS mitral valve (n=2) vs 4ply PSIS mitral valve (MV) (n=4) vs porcine trileaflet Bioprosthetic valve (BPV) (Edwards Lifescience) (n=4) at heart rate of 145 bpm and stroke volume of 20 ml/beat.

MV	$\Delta P_{(diastolic)}$ (mmHg)	Peak $\Delta P_{(systolic)}$ (mmHg)	Q_{rms} (ml/s)	EOA (cm^2)	Systolic Energy Loss (mJ)	RF (%)
2Ply	4.27 ± 0.96	55.1 ± 1.4*	165.4 ± 14.2*	1.49 ± 0.21*	6.18 ± 3.11	5.87 ± 0.75*
4Ply	4.24 ± 0.27	28.4 ± 2.12	99.4 ± 2.80	0.92 ± 0.04	8.88 ± 1.67	3.04 ± 0.38
BPV	4.05 ± 0.7	17.7 ± 2.56	103.2 ± 4.6	1.01 ± 0.1	6.29 ± 0.08	2.65 ± 0.5

Note: '*' indicates statistical significance.

Hydrodynamic assessment of 2ply PSIS bioscaffold mitral valves also revealed hydrodynamically robust data both in terms of flow and pressure in both the cases. In the first set of data at heart rate of **140 bpm** and stroke volume of **40 ml/beat**, when compared with the 4ply PSIS mitral valves and the control group we noticed that the Q_{rms} through the 2ply PSIS valve was significantly greater ($p<0.05$) than the other two groups. Moreover, we found that the RF was significantly higher ($p<0.05$) in 2ply valves when compared to 4ply and bioprosthetic valves but within clinical range [15].

In the other data set at heart rate of 145 bpm and stroke volume of 20 ml/beat, we again observed significantly higher Qrms ($p<0.05$) in 2ply valves as compared to the 4ply valves and bioprosthetic valves. Likewise, similar to the first case, RF was significantly higher ($p<0.05$) in 2ply valves. Moreover, EOA was seen to be significantly larger ($p<0.05$) in 2ply bioscaffold valves at 145 bpm and at 20 ml. More flow through 2ply valves as compared to 4ply valves may indicate more opening of the bileaflet valves during the

forward flow. Even though RF is greater, but when compared to clinical values it's still trivial [15].

Similar to aim 1, we again noticed significant differences in the peak systolic pressure gradients between 2ply and 4ply valves. The peak systolic pressure gradient for 2ply valves was significantly higher ($p < 0.05$) as compared to the 4ply valves and closer to the reference values [16, 17]. At heart rate of 140 bpm and stroke volume of 40 ml/beat peak systolic pressure gradient was 137.4 ± 1.57 mmHg and at 145 bpm and 20 ml/beat it was 55.1 ± 1.4 mmHg. Both 2ply and 4ply valves had sufficient systolic pressure gradients to keep the mitral valve closed effectively. It is possible however that the higher systolic pressure drop across the 2-Ply PSIS valve compared to 4-ply PSIS valve may maintain adequate valve sealing and closure while longitudinal somatic growth and remodeling occurs.

The key factor behind the selection of these two set of parameters was considering the closing energy losses in 4-Ply PSIS mitral valves. Energy loss was highest for 4ply PSIS valves at heart rate of 140 bpm and stroke volume of 40 ml/beat and lowest at heart rate of 145 bpm and stroke volume of 20 ml/beat. The closing energy losses for 2ply valves were observed to be less as compared to 4ply and bioprosthetic valves, although there was no statistical significance in both cases ($p=0.083$ for HR: 140 bpm and SV: 40 ml/beat; $p > 0.05$). A limitation in our comparison of thinner PSIS mitral valve is the small sample size ($n = 2$ valves). We believe that testing more 2ply valves will give us statistical confidence regarding less closing energy losses in 2ply PSIS mitral valves as compared to the 4ply PSIS valves, in a statistically significant manner. The work presented here on acute PSIS

bioscaffold mitral valve hydrodynamic functionality results are early findings and more valves need to be assessed to obtain a tighter data set.

With regards to 4ply PSIS valves we observed that 2ply valves performed efficiently, however since less material is used in 2ply valves, it is important to check its structural integrity under *in vivo* high press regions. The mechanical properties of these bioscaffolds will play an important role when implanted in patients, in the way they intend to remodel and handle stresses and strains. The collagen fibers present in cardiovascular tissues are oriented, rendering the mechanical behavior of these tissues nonlinear and anisotropic [18]. Structural analysis of the PSIS bioscaffold showed that the fibers are aligned in a single preferred direction along the longitudinal axis, however rarely fibers were aligned at ±30° to the longitudinal axis [19, 20, 21]. Biaxial testing performed on these scaffolds signified the mechanically anisotropic behavior of the material showing stiffness and strength [21, 22].

It was observed that the mechanical behavior of a single layer of PSIS material is insufficient for most load bearing applications, however the strength of the material increases with the increase in the number of layers or by using multiple laminations [23]. In one of the studies assessing the ball-burst strength of multilaminate PSIS scaffolds, it was observed that increasing the PSIS layers from 2 to 4 resulted in an increase in failure load of nearly 150%, the strength of 2ply PSIS material being 42 ± 9 N [23, 24]. In other study using same techniques there was an increase in strength by 81 % when the number of layers of PSIS scaffold were increased from 2 to 4 [25]. A 25.4mm smooth steel ball was directed through the PSIS material at a constant rate of 25.4 mm/min. The mean ball-burst strength of 2ply PSIS scaffold showed maximum loading of 73.67 ± 7.66 N and that

of 4ply was 133.53 ± 21.31 N [25]. Likewise, the 2 ply materials displayed higher stresses at equivalent strains compared to the thicker 4ply laminates. In the same study, prior to rupture, it was noted that 2ply and 4ply materials required comparable forces to deflect to similar indentations [25].

However, the 2ply Cormatrix PSIS material is proposed to be used in neonates, infants and children due to its handling and remodeling characteristics [26]. The performance assessment of these valves in the present study confirmed that the 2ply materials possesses adequate material properties and can endure the tension, suture tension, and hemodynamic forces exerted on the material when used for pediatric cardiovascular repair in the acute timeframe [26].

In one of the papers, the 2ply PSIS bioscaffold was utilized for tricuspid valve replacement in an ovine model [27]. Echocardiography results exhibited normal forward flow with excellent coaptation of the bioscaffold leaflets from the day of implantation till the day of explant. When explanted, the 2ply PSIS valves showed distinctive trileaflet structure similar to native tricuspid valve. Considerable host-cell infiltration, structural reorganization of the bioscaffold, elastin generation at the annulus was confirmed upon histopathologic investigation by 3 months, and by 5 months' time span, amplified collagen organization and glycosaminoglycan presence were detected in the leaflets.

The ball-burst studies confirmed that 2ply material is weaker in comparison to the 4ply material. However, in one of the studies following implantation as a body wall scaffold in canine model, it was shown that approximately at day 45, after implantation, there was increase in the strength of the bioscaffold which was greater than the native tissue [28]. For pediatric applications, the 2ply valves are capable of enduring the hemodynamic forces and

tensions effectively, as mentioned above. For pediatric application, the performance testing established that 2ply material surpasses the required biomechanical properties [26]. But, if the degradation and the remodeling of the 2ply substrates is slow, it can be a concern for the proper functioning of the valves. Thus, it is necessary that the 2ply valves enable tissue infiltration at a faster rate as compared to 4ply valves and that the growth is appropriate once implanted.

Nevertheless, we need to perform extensive mechanical and fatigue testing of both 2ply and 4ply material intended to be used as a mitral valve substitute. It is important that the 2ply PSIS material maintain its integrity during initial period under high stress environment.

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CHAPTER 7 Conclusion and Future Direction

Our current study involving extracellular matrix PSIS bioscaffold mitral valves, has the potential to solve the limitations related to the conventional prosthetic mitral valves. PSIS in recent times has been reported as a candidate material for valve replacement and repair due to its capacity to support, bioscaffold degradation and allow tissue ingrowth [1, 2]. In this study, we describe in vitro results of acute hydrodynamic functionality of PSIS bioscaffold assembled mitral valvular constructs compared to commercially available trileaflet porcine bioprosthetic valves and relate findings to potential long-term complications. This study was designed particularly for pediatric populace as the need for alternate valve replacement substitutes providing somatic growth is more in them.

Our two aims have investigated hydrodynamic functionality and characteristics of 4ply and 2ply PSIS bioscaffold mitral valves as compared to the porcine trileaflet bioprosthetic control group (Edwards Lifescience). Both these approaches had robust data sets and were physiologically similar to the control group, with some inconsistencies in the ventricular pressure between the two group, especially during the systolic phase, as we increase the heart rate.

The major conclusions of this study were:

- PSIS bileaflet valves appear to demonstrate acute functionality for treatment of critical mitral valve disease. The PSIS valves performed hydrodynamically similar to the bioprosthetic valves with regards to the opening and closing functionality in short term. This can potentially predict mid-term to long-term. However, we observed differences in the peak systolic pressure gradients between the two groups, potentially due to the material

properties. Nevertheless, both the valves closed effectively which is supported my minimal RF (%).

- Energy losses were not significantly different ($p > 0.05$) between PSIS and a commercially available bioprosthetic valve for mitral valve pediatric application, but was for aortic valve under adult physiological conditions [3]. For the pediatric conditions, we observed that the percentage increase in the energy losses in the PSIS valves were similar to that of the bioprosthetic valves when we increased the heart rate or the stroke volume. However, doubling of cardiac output from child-to-adulthood increased the energy losses by more than 3.5 times for both the groups, which appears to be a natural increase with age, since this also occurred in the bioprosthetic valve control group. On the other hand, even though the PSIS valves have the ability to grow with the patient, it needs to be longitudinal as well as symmetric. Any abrupt changes to the growth factor may affect the PSIS valves adversely as well as the energy loss metric which could increase rapidly, thus overworking the heart.
- 2ply PSIS valves have shown early promising results by maintaining the pressure gradient across the valves, in the clinically acceptable range [4], during the forward flow when compared to the 4ply valves. There was increase in the forward flow in these valves providing greater flow rate. Here we observed that the forward flow during the diastolic phase when the mitral valve is open was significantly higher than both the 4ply PSIS valves and Edwards Lifescience bioprosthetic valves. However, we noticed significantly higher RF (%) ($p<0.05$), though clinically unremarkable [5]. Also, 2ply valves are attractive in terms of less usage of biomaterials. This will potentially reduce demand for de nevo tissue replacement when implanted in patients. Correspondingly, 2ply valves have the potential

to remodel faster when compared to their thicker counterparts, provided the growth is consistent with time and there is gradual degradation of the material along with the symmetric growth of the native tissue.

- From the literature study of ball burst strengths of 2ply and 4ply PSIS material, we observed that the 2ply material is weak in comparison to the 4ply material. However, 2ply PSIS valves are intended to be used in infants and small patients having low cardiac pressure regions. Biomechanical testing and studies of the 2ply Cormatrix PSIS valves demonstrated adequate supporting material properties like tensile strength and endurance of hemodynamic forces exerted on the material when used in infants and children [6]. Also, *in vivo* study in ovine model demonstrated excellent coaptation of the 2ply leaflets along with proper growth.

Limitations of our study:

- Our study had certain limitations. One of them was the sample size in each group. We believe that by testing more valves we will get more robust data. Thus, we will be able to compare each hydrodynamic parameter more effectively.
- The other limitation was considering the anatomy of the mitral valve. The mitral valve is a complex structure consisting of several components which function in harmony with each other in order to open and close in high pressure environment, during diastolic and systolic phase respectively [7]. Any changes in one or more of these components can have adverse effect on the leaflet closure thus resulting in regurgitation and more energy being lost during the systolic phase. Thus, normal mitral valve relies on each component for the efficient hydrodynamic functionality. However, the PSIS valve design in our study

does not reflect these complexities including the chordae tendinae and the papillary muscles and solely focuses on the leaflets.

- Also, we didn't compare the durability (i.e., fatigue properties) of the three groups, especially between the PSIS valves and the bioprosthetic valves. Durability is an important component of the valve design, since these scaffolds would perform *in vivo* under high pressure regions when used for valve replacement procedures. It is essential to observe the fatigue properties of 2ply and 4ply PSIS valves to reach more effective conclusion.
- Lastly, we didn't look at the growth component of the PSIS valves when implanted in high pressure region, in human body. Even though, remodeling of the implanted PSIS bioscaffold by fibrosis has been documented in the literature [8], but we are still unclear on the mechanism through which this bioscaffold degrades and permits tissue filling, and in this case valvular matrix tissue filling to be precise s. Correspondingly we didn't look at the immune response of the PSIS bioscaffold valve when used as valve replacement *in vivo.*

Future Directions:

- Both the 2ply and 4ply valves still need to be further characterized at pediatric conditions. Our immediate focus after this work is to test more valves; 2ply, 4ply and bioprosthetic valves. Testing more valves will give us statistical confidence and will help us to complete our *in vitro* study more effectively.
- After this we will like to conduct durability test of these 2ply and 4ply valves to show how the PSIS bioscaffold leaflets would survive hundreds of millions of cycles under physiological pulsatile conditions. This will help us to predict the long-term consequences of *in vivo* conditions on these bioscaffolds, i.e. if the valves will remain functional from a

structural standpoint, over several years and also aid us in determining at what time point the valves are prone to fatigue.

- Our long-term goal is to see how these PSIS valves grow and its immune response *in vivo* under physiologically dynamic conditions. CorMatrix PSIS material is biodegradable and has the potential to act as a remodelling scaffold. Further investigation is required to determine how the PSIS material is being cellularized after it is implanted into the patient along with the rate of its degradation to warrant somatic growth. To achieve this knowledge, in vivo evaluation in an appropriate animal model is essential. We have proposed non-human primates for this study. Because of the anatomical and physiological closeness to humans, we have proposed Baboons as our in vivo model. This will give us an insight on longitudinal somatic growth, immune response, extent of PSIS biodegradation, de novo tissue regeneration and biochemical structure and phenotype of tissue growth. Thus, will help us interpret the long-term effectiveness of PSIS bioscaffold valves *in vivo* for critical congenital heart valve defects in infants and children.

In conclusion, PSIS bi-leaflet valves appear to exhibit equivalent short term hydrodynamic functionality to standard bioprosthetic valves in the mitral position and also the 2ply PSIS valves have shown early promising results. Thus, on the basis of our hydrodynamic assessment, the supporting claims of adequate biomechanical properties of the 2ply materials for pediatric applications, the *in vivo* growth in ovine model and the potential of biodegradation of the material and tissue replacement with less demand on the human body, we can conclude that 2ply PSIS mitral valves are better when compared to the 4ply valves when used in infants and children. Nonetheless, we need to assess how the bioscaffold degrades and need to check if the growth is rapid, sufficient and symmetric.

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